

Journal of Agricultural Entomology

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THE JOURNAL OF AGRICULTURAL ENTOMOLOGY

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Burns, D. A. 1957. Title: same rules for subtitles – don't forget lowercase. Publisher, city, state or province (spell out), 346 pp.

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Mitchell, E. R. [Ed.]. 1981. Management of insect pests with semiochemicals: concepts and practice. Plenum, New York, 514 pp.

Article or Chapter in a Book

Myler, A. 1985. Article or chapter title, pp. 00-00. *In* I. S. Burke, Jr. and L. B. Armstrong [Eds.], Book title. Publisher, city, state, 233 pp.

Reynolds, H. T., P. L. Adkisson & R. F. Smith. 1975. Cotton insect pest management, pp. 379-443. *In* R. L. Metcalf and W. H. Luckmann [eds.], Introduction to insect pest management. Wiley, New York, 587 pp.

Royer, T. A., J. V. Edelson & B. Cartwright. 1988. Onion thrips control, 1987, p. 129. *In* Insecticide and acaricide tests, vol. 13. Entomological Society of America, College Park, Maryland, 459 pp.

Proceedings

Reynolds, H. T. 1985. Pesticides: a dependable component of IPM, pp. 21-24. *In* Proceedings, Regional workshop on pesticide management, Nairobi, Kenya, 128 pp.

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Reports

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- Colorado Agricultural Experiment Station. 1989. Annual report. Colorado State University, Ft. Collins, 62 pp.
- Webster, J. A. & D. H. Smith, Jr. 1983. Developing small grains resistant to the cereal leaf beetle. United States Department of Agriculture Technical Bulletin 1673, Washington, D.C., 12 pp.
- Young, D. A. 1986. Taxonomic study of the Cicadellinae (Homoptera: Cicadellidae). Part 3: Old World Cicadellinae. North Carolina Agricultural Experiment Station Technical Bulletin 281, Raleigh, 639 pp.

In Press

- Rogers, L. E. & J. F. Grant. In press. Infestation levels of dogwood borer (Lepidoptera: Sesiidae) larvae on dogwood trees in selected habitats in Tennessee. *J. Entomol. Sci.*

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- Department of Agriculture. 1985. Insects of eastern forests. United States Department of Agriculture Forest Service Miscellaneous Publication 1426, Washington, D.C., 608 pp.
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Abstracts and Translations

- Barker, S. 1989. Toxicity of XXX. *Chem. Abstr.* 18: 193a.
- Hooker, M. W. & E. M. Barrows. 1989. Clutch sizes and sex ratios in *Pediobius*. *Ann. Entomol. Soc. Am.* 82: 460 (abstr.)
- Shenderovskaya, L. P. 1979. Introduced insect enemies and microorganisms. *Zash. Rast. (Kiev)* 3: 52-56 (in Russian).
- Shenderovskaya, L. P. 1979. Introduced insect enemies and microorganisms. *Zash. Rast. (Kiev)* 3: 52-56. (translated in OTS 61: 31267), U. S. Department of Commerce, Washington, D.C.

Magazine Articles

- Headley, J. C. 1979. Economics of pest control. *Chem. Eng. News*, Jan. 15, pp. 55-57.

Other

Code of Federal Regulations. 1986. Title. 7 CFR Chapter III, Section 318.13-46, pp. 128-129.

SAS Institute. 1985. SAS user's guide: statistics, version 5 ed. SAS Institute, Cary, North Carolina, 956 pp.

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Pyrethroid Resistance Levels in Two Generations of Soybean Looper (Lepidoptera: Noctuidae) on Soybean in Mississippi¹

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J. Agric. Entomol. 14(1): 9–15 (January 1997)

ABSTRACT Resistance to permethrin in soybean looper, *Pseudoplusia includens* (Walker) (Lepidoptera: Noctuidae), larvae from generations one and two on soybean, *Glycine max* (L.) Merrill, was monitored in the central Delta area (Holmes County) of Mississippi in 1992. Dose responses were determined by topical treatment of third instars. Populations of first- and second-generation soybean looper had LD₅₀s (95% confidence limits [CL]) expressed as micrograms of insecticide per larva of 0.0028 (0.0018–0.0040) and 0.0096 (0.0050–0.0154), respectively, which were significantly higher than the LD₅₀ (CL) of the susceptible laboratory strain (0.0002 [0.0001–0.0003]). Resistance level in the second generation was 3.4-fold higher than that in the first generation. Similar data were obtained by Portillo et al. (1993) in recording 3.3- and 2.2-fold higher soybean looper resistance levels in second-generation than in first-generation soybean looper in 1989 and 1991, respectively. These data corroborate earlier reports that levels of soybean looper resistance to pyrethroid insecticides significantly increased in cotton-soybean production areas as the crop growing season progressed. It appears that mid-season pyrethroid insecticide applications on cotton increase selection pressure for pyrethroid resistance in soybean looper populations in cotton-soybean agroecosystems.

KEY WORDS Soybean, soybean looper, pyrethroid, resistance

Pyrethroid insecticides were first used on soybean, *Glycine max* (L.) Merrill, in Mississippi for control of soybean looper, *Pseudoplusia includens* (Walker) (Lepidoptera: Noctuidae), in 1982. Control failures with recommended rates were suspected in 1985 and 1986 in northern Holmes County, Mississippi (Felland et al. 1990). Pyrethroid resistance in soybean looper in the southern United States was confirmed in 1987 (Felland et al. 1990, Leonard et al. 1990, McPherson & Herzog 1990). Furthermore, soybean looper resistance to pyrethroids has been shown to be

¹ Accepted for publication 25 July 1996.

² Present address: BASF Corporation, Agricultural Research Station, 103 BASF Road, Greenville, Mississippi 38701.

higher in areas where soybean and cotton, *Gossypium hirsutum* L., are grown in close proximity (Felland et al. 1990, Leonard et al. 1990). Soybean loopers infest cotton earlier than soybean (often several weeks); therefore, one generation of loopers may occur on cotton before loopers infest soybean. Because few or no pyrethroid applications are made on soybean, it has been speculated that selection for insecticide resistance in the soybean looper is increased on cotton where multiple pyrethroid applications for bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), control are made (Felland et al. 1990, Leonard et al. 1990, Luttrell et al. 1990, Portillo et al. 1993).

In this study, first- and second-generation soybean looper larvae on soybean were collected in the Delta area of Mississippi and monitored for resistance to permethrin in 1992. Soybean looper insecticide resistance data obtained by Felland et al. (1990) and Portillo et al. (1993) are presented for discussion.

Materials and Methods

Soybean looper strains were established by collecting larvae from natural populations on soybean in 1992. The laboratory (LAB-MS) strain maintained at the Southern Field Crop Insect Management Laboratory at Stoneville, Mississippi, was used as the susceptible strain. This colony originated in 1981 with adults from a South Carolina laboratory colony and was not exposed to insecticides in the laboratory. However, wild insects collected on untreated soybeans in the Delta (Holmes County) in Mississippi had been introduced into this strain in 1981, 1986, 1987, 1989, and 1991. These insects were collected in an area where pyrethroid-resistant soybean loopers have been documented since 1987 (Felland et al. 1990).

First- and second-generation larvae on soybean were identified and separated according to larval size within sample collections (e.g., only late instars of the first and second field infestations were collected) (Felland et al. 1990, Portillo et al. 1993). Fields were sampled weekly prior to infestation by the first-generation soybean looper on soybean. Two hundred larvae representing the first generation on soybean were collected during the week of 24–28 August, and an equal number representing the second generation (or possibly new immigrants) was collected during the week of 14–18 September in a field adjacent to cotton in Holmes County (same fields reported by Felland et al. 1990 and Portillo et al. 1993). The soybean fields had not been sprayed with insecticides before the insect collections in 1992. Larvae and soybean foliage were placed in 29.6-ml plastic cups (1 larva per cup), capped with cardboard lids, and held inside a box containing BLUE ICE ice substitute (Rubbermaid Incorporated, Wooster, Ohio) during transport to the laboratory. Larvae and adults were reared and handled as reported by Portillo et al. (1993). Approximately 50 adults (males plus females) were obtained from each field collection of larvae. Larvae of the first laboratory generation resulting from each field generation were used for insecticide assays.

The standard bioassay procedure recommended by the Entomological Society of America for measuring insecticide resistance in bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), was used (Anonymous

1970). Early third instars (weight not determined, but larval size was similar to populations tested in previous studies in which they averaged 12.66 ± 2.93 mg) were topically treated on the thoracic dorsum with 1- μ l aliquots of acetone (control) or a solution containing one of five serial concentrations of technical grade permethrin (FMC Corporation, Philadelphia, Pennsylvania 19103) dissolved in acetone. Treated larvae were held in cups (29.6 ml) containing nutri-soy flour/wheat germ diet (Raulston & Shaver 1970), and mortality data were recorded 48 h after treatment. Total mortality was recorded as the number of dead and moribund larvae.

Dose-mortality regressions and the 95% confidence limits (CL) for the LD₅₀ (based on micrograms of permethrin per larva) were obtained by probit analysis by using the microcomputer software Polo (Robertson et al. 1980). Differences between strains were considered significant based on overlap of 95% CL. Resistance ratios (RR) were calculated by dividing the LD₅₀ of each field strain by that of the LAB-MS susceptible strain.

Results and Discussion

Pyrethroid resistance levels in soybean looper larvae representing the first and second field generations on soybean were 15.7- and 53.6-times greater, respectively, than that in the susceptible LAB-MS strain in 1992 (Table 1). The LD₅₀s (95% CL) of the field strains were significantly higher than that of the LAB-MS strain. Resistance levels increased 3.4-fold in the second field generation as compared with the first field generation (Table 1). Portillo et al. (1993) reported higher resistance levels in the first and second field generations of soybean looper as compared with the LAB-MS strain in 1989 and 1991 (Table 1). In these earlier studies resistance levels increased 3.3- and 2.2-fold from first to second field generation in 1989 and 1991, respectively (Table 1). Felland et al. (1990) reported a significant 23.6-fold higher resistance level in the second field generation in Mississippi as compared with the LAB-MS strain; the level of resistance was not determined for the first field generation (Table 1). This study corroborates the results of a previous study (Portillo et al. 1993) indicating that pyrethroid resistance levels in soybean looper increased from generation one to generation two on soybean in a given year. This is of importance because it is the second soybean looper generation on soybean that usually reaches economic infestation levels and warrants the need for control with insecticides.

Because pyrethroids were not applied to soybean prior to collection of the larvae in any year except 1987, selection for resistance had to occur other than on soybean. Multiple applications of pyrethroids were made on cotton, but they were made prior to soybean looper infestations on soybean (Felland et al. 1990, Portillo et al. 1993). Because cotton is the only other major host for soybean looper in the area, it is reasonable to assume that adults bred on cotton are responsible for soybean looper populations found on soybean. Thus, observations support the hypothesis that selection for pyrethroid resistance in soybean looper occurs on cotton. Mark and recapture studies with soybean looper are currently being conducted by the authors to further test this hypothesis.

Table 1. Toxicity of permethrin to several strains of soybean looper larvae collected on soybean in the Mississippi Delta (topical bioassays).

Year	Field Generation ^a	Laboratory Generation ^b	<i>n</i> ^c	Slope ± SE	LD ₅₀ (95% CL) ^d	RR ^e
1987 ^f						
Lab-MS	—	1	356	2.27 ± 0.24	0.0076 (0.0057-0.0105)	—
Soybean	2	1	146	1.45 ± 0.27	0.1797 (0.1204-0.3186)	23.6
1989 ^g						
Soybean	1	2	71	1.61 ± 0.33	0.0157 (0.0097-0.0278)	2.1 ^h
	2	2	107	1.63 ± 0.28	0.0520 (0.0363-0.0823)	6.8
1991 ^g						
Lab-MS	—	1	500	1.55 ± 0.15	0.0021 (0.0015-0.0027)	—
Soybean	1	1	120	1.10 ± 0.36	0.0121 (0.0002-0.0345)	5.7
	2	1	476	1.24 ± 0.15	0.0264 (0.0043-0.0551)	12.4
1992						
Lab-MS	—	1	419	1.61 ± 0.20	0.00018 (0.0001-0.0003)	—
Soybean	1	1	248	1.38 ± 0.18	0.00283 (0.0018-0.0040)	15.7
	2	1	411	1.13 ± 0.12	0.00965 (0.0050-0.0154)	53.6

^aRepresents generations of larvae collected as they appear in the field through time.

^bRepresents generations of larvae after transferring to the laboratory.

^cNumber of subjects, excluding controls.

^dDoses reported in micrograms of insecticide per larva.

^eResistance ratios (RR) = LD₅₀ of the field strain / LD₅₀ LAB-MS strain.

^fFrom Felland et al. 1990. Original data presented as micrograms of insecticide per gram of larval weight.

^gFrom Portillo et al. 1993. Original data presented as micrograms of insecticide per gram of larval weight.

^hLD₅₀ and RR values for 1989 were compared and calculated, respectively, by using data from the LAB-MS in 1987.

When the dose-mortality lines of the second field-generation strain of soybean loopers on soybean in all years (1987 strain reported by Felland et al. 1990; 1989 and 1991 strains reported by Portillo et al. 1993) and the 1992 strain of the present study were compared, we observed lower levels of resistance each year from 1987 to 1992 (Fig. 1). The 1987 strain had a significantly higher LD_{50} than strains of the other years (Table 1). Two factors differed in the 1987 study compared with studies in the other years: higher tobacco budworm infestation levels on cotton, resulting in increased use of pyrethroids on the crop in 1987, and the application of one pyrethroid treatment on soybean prior to collection of the second-generation strain on soybean (Felland et al. 1990, Portillo et al. 1993). The 1989 second-generation strain had a higher resistance level than the 1991 and 1992 second-generation strains (2.0- and 5.4-fold, respectively); the difference in resistance level was significant only in comparison with the 1992 strain (Table 1). The 1991 second-generation strain had 2.8-fold increase in resistance level as compared with the 1992 second-generation strain; however, their confidence limits overlapped (Table 1). There were fewer pyrethroid applications on cotton in 1991 than in 1987 (Felland et al. 1990) and 1989 (Portillo et al. 1993), which apparently contributed to the lower levels of insecticide resistance in 1991 than in 1987 and 1989. Pyrethroids were applied to a limited extent for bollworm/tobacco budworm control on cotton in this area in Mississippi in 1992, but the number of applications is not known.

Higher insecticide RR values in second-generation soybean looper strains from 1989 (6.8) to 1992 (53.6) (Table 1) might imply that resistance levels in the field populations increased from 1989 to 1992. Resistance ratios appeared to increase as a result of lower LD_{50} s in the susceptible LAB-MS strain through time. The LAB-MS strain was, however, infused with wild insects every 2 yr since 1987, which introduced some insecticide resistance alleles into the colony. Nevertheless, the frequency of insecticide resistance alleles in the wild soybean looper populations decreased from 1987 to 1989, as observed by lower LD_{50} s in first and second generations during this time (Table 1). Thus, the pyrethroid RR values were not good criteria to explain changes in pyrethroid resistance levels in soybean looper populations through time.

Pyrethroid resistance levels increased from first- to second-generation soybean looper as the growing season progressed in 1989 and 1992. Because the use of pyrethroids on cotton early in the growing season is known to increase the selection pressure for resistance in soybean looper on cotton (Portillo et al. 1993), which directly affects resistance levels in soybean looper populations on soybean, the selection and use of insecticides for insect pest management in a cotton-soybean production system should be given careful consideration. Results of the present study support the concept of restricted use of pyrethroids on the early stages of the cotton crop. The mid-South insect resistance management plan recommends that the use of pyrethroids be restricted on cotton during early to mid-season (e.g., June to mid-July) (Anonymous 1986); nevertheless, in Mississippi these materials are recommended and extensively used from 1 July to 15 August for bollworm/tobacco budworm control (Mississippi Cooperative Extension Service 1992, 1995). Therefore, one or two generations of soybean looper may be

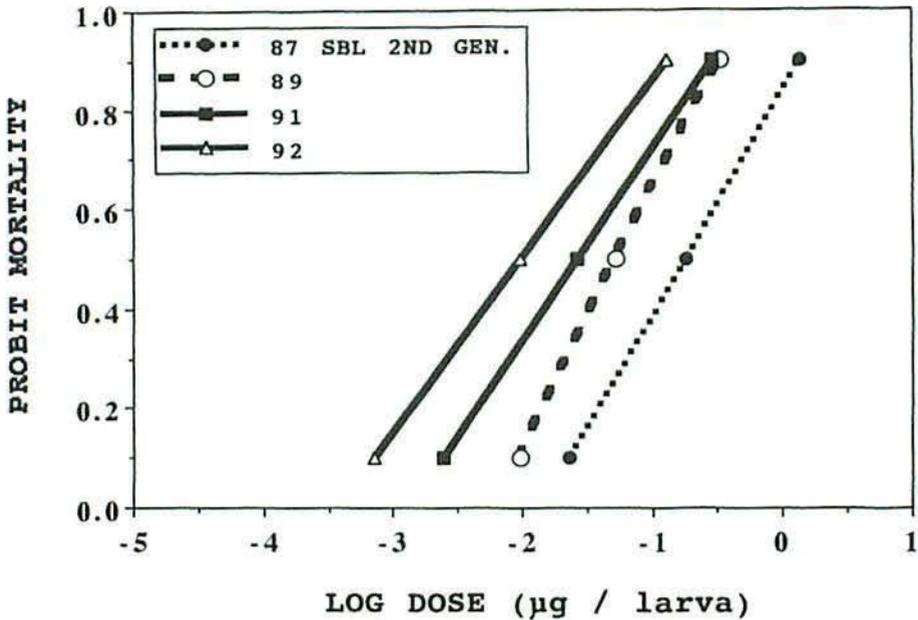


Fig. 1. Dose-mortality lines for permethrin on second-generation field strains of soybean looper (SBL) collected on soybean in the Mississippi Delta in 1987, 1989, 1991, and 1992.

exposed to pyrethroids in cotton resulting in development of resistance in soybean looper populations that later move into soybeans. Limiting the use of pyrethroids on cotton in mid-season should benefit soybean looper control on soybean, particularly where soybean is planted in the proximity of cotton. Biological insecticides based on crystal proteins synthesized by the bacterium *Bacillus thuringiensis* Berliner (Bt) have been widely used as a substitute for pyrethroids in the control of soybean looper in soybean. Bt insecticides also are used as a resistance management tool for control of tobacco budworm in cotton. Furthermore, it is estimated that about 81,000 ha of transgenic cotton varieties capable of producing their own Bt toxins will be planted in the United States in 1996 (Anonymous 1996), with dramatic increases in cotton acreage planted to these varieties in subsequent years. Therefore, an increased selection pressure for resistance to Bt insecticides in soybean looper populations in cotton should be expected.

Results from this study indicate the potential benefit of monitoring soybean looper resistance levels to pyrethroids as well as to the various Bt toxins used on cotton and soybean in areas where soybean is grown in proximity to cotton. Alternative insecticides for control of soybean loopers that are not affected by

selection pressure in cotton appear to be the best long-term control measure for this insect pest on soybean in a cotton-soybean agroecosystem.

Acknowledgement

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Suppression of Mating by Beet Armyworm (Noctuidae: Lepidoptera) in Cotton With Pheromone^{1,2}

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ABSTRACT Two cotton fields of 14.1 and 15 ha, respectively, received a single treatment of Yoto-con-S[®] twist-tie rope dispensers containing a 70:30 blend of (Z,E)-9,12-tetradecadien-1-ol acetate and (Z)-9-tetradecen-1-ol acetate, two components of the sex pheromone of the female beet armyworm, *Spodoptera exigua* (Hübner). The rope dispensers, each containing 160 mg of total pheromone blend, were applied at the rate of 1,000 units/ha when the cotton was in the 8-10 leaf stage. The pheromone treatments suppressed trap captures of male beet armyworm moths and mating by sentinel female moths for >100 d. On average, fewer beet armyworm egg masses (57%) and larvae (95%) were recorded in the pheromone-treated fields compared with the control field. These results suggest that pheromone can be used to protect cotton from a strong flying insect like beet armyworm, even in relatively small fields.

KEY WORDS *Spodoptera exigua*, mating disruption, *Cotesia marginiventris*, pheromone

The beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), is believed to have originated in southern Asia. It thrives in semitropical climates but may survive in temperate areas during mild years. Mitchell (1979) reviewed the information relating to the overwintering capabilities of the beet armyworm in the United States. Because the beet armyworm has no diapause mechanism, the overwintering range presumably is determined by the occurrence of frost that kills host plants. Using Cooperative Economic Insect Reports from 1974-1978, Mitchell (1979) showed beet armyworm larvae occur as early as May in Ohio; September in

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² This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or the recommendation for its use by USDA.

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West Virginia, Virginia, and Delaware; and October in New York. This ability to rapidly migrate long distances along with its apparent adaptation to cotton led Mitchell (1979) to conclude that beet armyworm could become a serious and regular pest of the crop in the southeastern United States.

A serious outbreak of beet armyworm in southeastern cotton occurred in 1977. The cotton crop was planted late, and hot, dry weather persisted throughout much of the growing season. To compound the situation, growers used multiple applications of insecticides with poor coverage techniques. These factors plus a high level of resistance to insecticides in use at the time apparently led to the outbreak or aggravated the situation (Sprenkel & Austin 1994). Since 1977, beet armyworm has become a chronic pest of cotton in the southeastern United States with serious outbreaks occurring in 1980, 1981, 1988, and 1993 (Smith 1989, Smith & Freeman 1994). Outbreaks of beet armyworm in cotton were especially severe in 1993, at which time this moth was rated the number one pest in cotton from Mississippi to Georgia.

Brady & Ganyard (1972) identified one of the sex pheromone components of beet armyworm as (*Z,E*)-9,12-tetradecadien-1-ol acetate. However, Mitchell & Doolittle (1976) showed that this compound had no attractant activity for beet armyworm by itself. Tumlinson et al. (1981, 1990) reinvestigated the sex pheromone components, identified 11 compounds from virgin female secretions, and showed that (*Z*)-9-tetradecen-1-ol was an essential component for male attraction. Mitchell et al. (1983) reported an effective formulation for attraction, a mixture of 0.1 mg of (*Z,E*)-9,12-tetradecadien-1-ol acetate and 0.01 mg (*Z*)-9-tetradecen-1-ol dispensed on a rubber septum.

Mitchell (1976) investigated the feasibility of using (*Z,E*)-9,12-tetradecadien-1-ol acetate as a sex communication disruption agent for beet armyworm when evaporated in small plots as a background odor surrounding traps baited with virgin female moths. Captures of male beet armyworm moths in traps located in plots treated with (*Z,E*)-9,12-tetradecadien-1-ol acetate were reduced 96% over a 3-wk period compared to control areas. Subsequently, Wakamura & Takai (1992) showed that, when a 7:3 mixture of (*Z,E*)-9,12-tetradecadien-1-ol acetate and (*Z*)-9-tetradecen-1-ol was dispersed into a 155-ha field, attraction of male beet armyworm moths to sex pheromone traps was completely inhibited, and densities of egg masses and young larvae on Welsh onion were reduced significantly relative to those in an untreated field about 9 km away. In their study, the Welsh onion fields totaled only about 25 ha; the remainder of the pheromone-treated area included rice fields, greenhouses, orchards, home gardens, and forests.

The present study was conducted to evaluate Yoto-con-S[®] twist-tie rope dispensers containing beet armyworm pheromone for season-long suppression of mating by beet armyworm in cotton. Unlike the trials by Wakamura & Takai (1992), only individual fields were treated with pheromone.

Materials and Methods

Experimental location. The cotton fields used in this study were located near the township of Houston in the northeastern corner of Suwannee County, Florida. A total of 61 ha of dryland cotton was planted in fields of various sizes

between 1 May and 1 June, 1994. The three fields selected for the trial were the last planted (week of 27 May), and all were about the same size (14–15 ha) and shape (Fig. 1). Cultivars Stoneville[®] 474 and 887 were planted in the pheromone-treated fields, A and B, and control field, respectively. The grower exercised the usual fertilization and cultural practices recommended for producing cotton in north-central Florida (Sprenkel 1996).

Pheromone treatment. The fields were treated with the the Yoto-con-S[®] (Shin-Etsu Chemical Co., Ltd., Tokyo, Japan) dispenser, a polyethylene tube with an aluminum wire containing a 70:30 blend of (*Z,E*)-9,12-tetradecadien-1-ol acetate and (*Z*)-9-tetradecen-1-ol acetate (Lot no. 65010). The dispensers, typically called twist ties or ropes, were brown, 20 cm long, and contained about 160 mg of total pheromone blend.

The pheromone treatments were applied when the cotton was in the 8–10 leaf stage (field A, 14 July; field B, 8 July). The ropes were tied to the stem of plants at a height of 20–25 cm above ground level; 2 ropes were applied at each location in a 5.23-m grid throughout the field (1,000 dispensers/ha). Field A was about 0.2 km from the control field and field B was 4.0 km from field A and 1.6 km from the nearest cotton field located to the north of the test site (Fig. 1).

Evaluation of treatment effects. Treatment efficacy was measured by reductions in capture of beet armyworm males (hereafter referred to as trap shutdown) in Universal Moth Traps[®] traps baited with beet armyworm sex pheromone (Tumlinson et al. 1990, Mitchell & Tumlinson 1994), reductions in mating frequency by sentinel beet armyworm females positioned on mating tables (hereafter referred to as mating shutdown), and weekly counts of beet armyworm egg masses and larvae in each field versus the control field. Pheromone traps and mating tables, two of each per field, were positioned a third of the way in from each corner of the field. The mating tables were located diagonally across the field from the pheromone traps (see inset, Fig. 1). This allowed continuous operation of the pheromone traps, even on the nights that sentinel female moths were set out on mating tables. Trap capture data were recorded 3–4 times/wk; always on the day that sentinel females were put out, and the following morning when they were recovered. Pheromone baits (rubber septa, Trece, Inc., Salinas, California) were changed every 2 wk throughout the season. A small piece of Vapona[®] insecticide strip (Vaportape II, Hercon Environmental Co., Emigsville, Pennsylvania) was placed in each trap to kill captured moths.

The mating tables were constructed from white plastic trays measuring 52 cm × 39 cm × 8 cm. A cylinder (8 cm diam. × 18 cm high) made of fine mesh hardware cloth and capped with a white styrofoam plate (23 cm diam.) was positioned in the center of each table to provide shade and protection of the moths from birds. Each mating table was positioned 1.5 m above ground level on a piece of electrical conduit (Fig. 2).

Laboratory-reared beet armyworm females emerged in screened cages held under ambient light in a greenhouse and were fed a 10% honey-water solution. Six to 8 virgin females 2–3 d old were placed on each mating table 3–4 h before sunset. One forewing of each moth was clipped to prevent escape by flight. The moths were collected the following morning and returned to the laboratory where they were dissected to establish mating status as

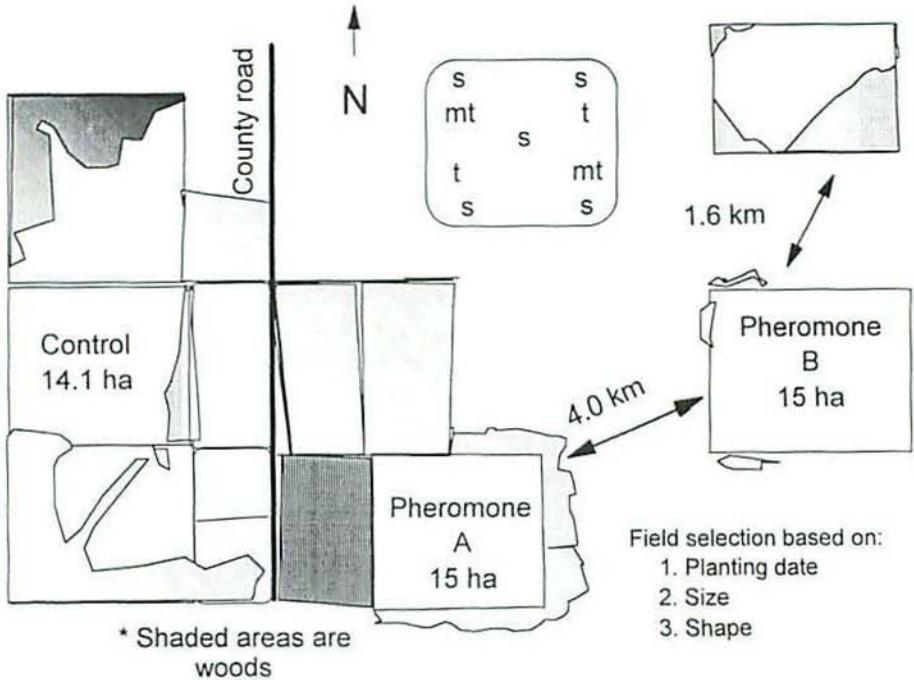


Fig. 1. Schematic of the experimental site near Houston in northeast Suwannee County, Florida. The enlarged inset shows the relative position of mating tables (mt), pheromone traps (t), and plant sampling sites (s) in each field. All fields shown were planted to cotton.

determined by the presence or absence of a spermatophore in the bursa copulatrix.

Beet armyworm populations were assessed weekly from 29 June through 12 September by examining whole cotton plants at each of five sites in each field (Fig. 1). Initially, 25 plants were examined at each site, but as the plants increased in size and began to square and set bolls, the number examined per site was reduced to 15 (9, 15, and 22 August) or 10 (29 August and 6, 12 September). The fields were sampled more intensely on 26 September and 5 October. On these dates, a total of 121.6 m of row (eight randomly selected sites of 15.2 m each) was examined for beet armyworm larvae, egg masses, and cocoons of *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae), a solitary larval parasitoid of many Lepidoptera, including beet armyworm (Tingle et al. 1994). Where appropriate, the data were analyzed by analysis of variance using sample sites in each field as replicates. Differences between the means were separated with Duncan's multiple range test (SAS Institute 1990).

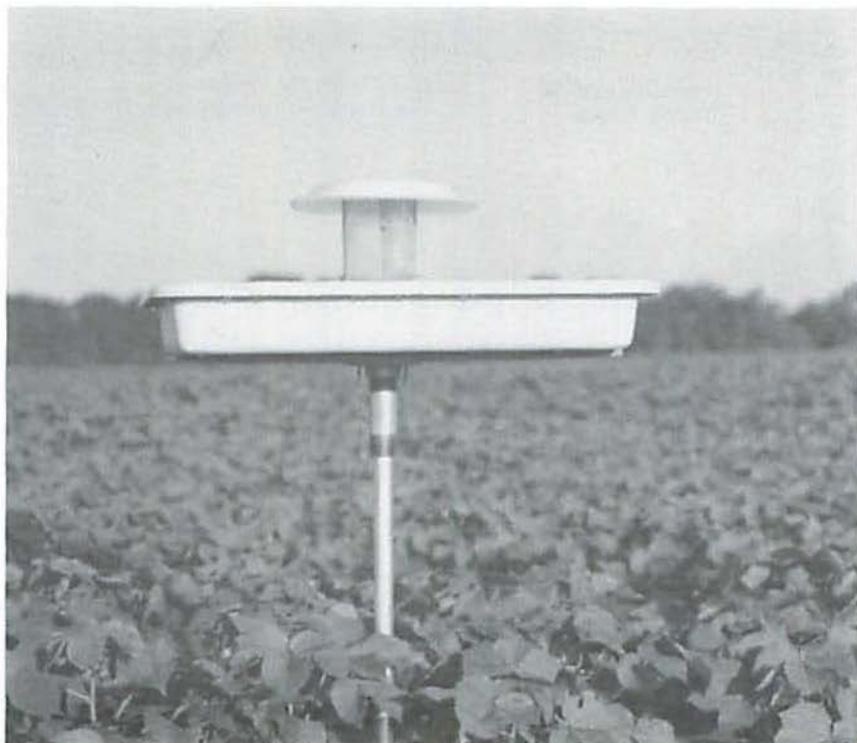


Fig. 2. Table used to assess the effect of pheromone on mating of sentinel female moths by wild beet armyworm males in pheromone-treated fields and control fields.

Results and Discussion

The beet armyworm population in the area was low initially, as indicated by trap capture data, but began to increase rapidly by the end of August (Fig. 3). However, the frequency of mating in the pheromone-treated fields showed only a slight increase during September and October when the beet armyworm population peaked in the control, which averaged 100 males per trap per night on 26 and 30 September. Captures of moths in traps in the pheromone-treated fields showed only a slight increase during this period. As trap captures of moths in the control declined in early October, moth captures in pheromone fields A and B quickly returned to zero or nearly so.

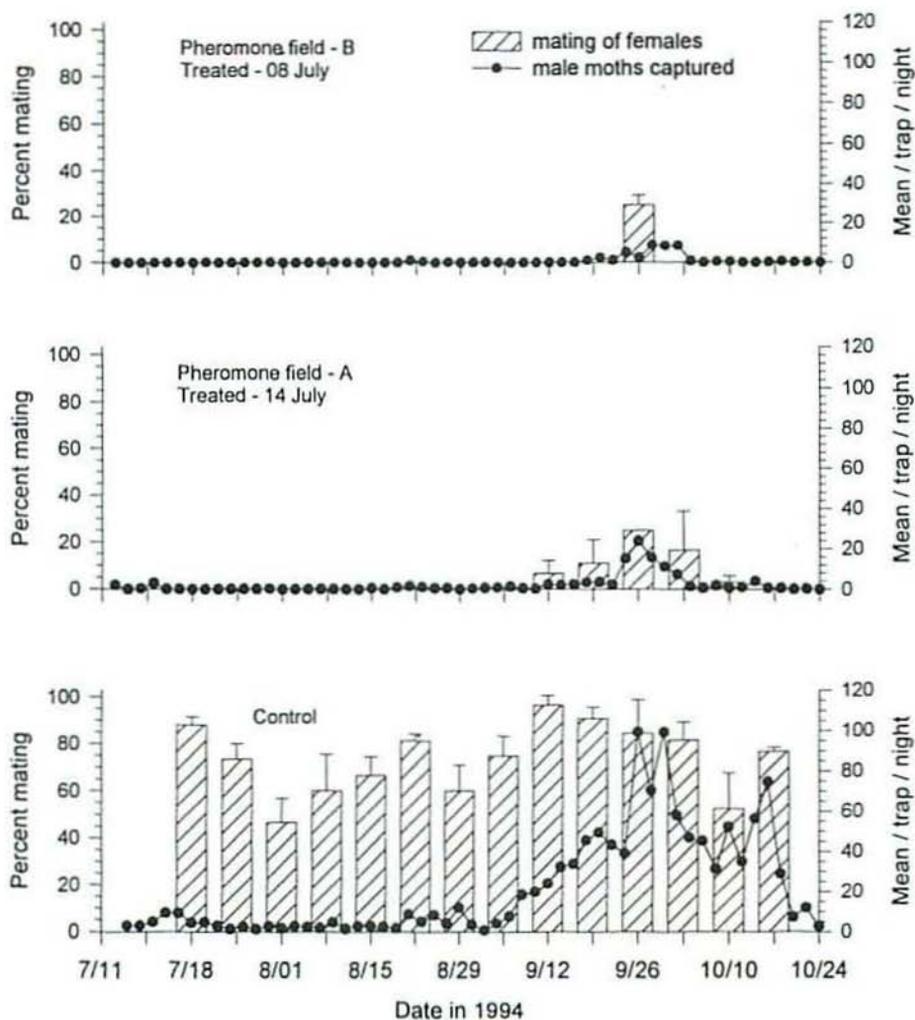


Fig. 3. Mating disruption of sentinel beet armyworm female moths set out in cotton fields treated with pheromone dispensed from Yoto-con-S[®] twist-tie ropes (1,000 ha).

As might be expected, matings by sentinel females showed an increase during late September when the beet armyworm population was at its peak (Fig. 3). However, the level of mating by sentinel females in the pheromone-treated fields remained very low compared to the control field. Thus, the pheromone treatment continued to be effective at shutting down mating in spite of the huge flight of beet armyworm in the area as indicated by captures of male moths in the control field (Fig. 3). As observed for trap captures, the frequency of matings by sentinel females in the pheromone treatments declined quickly following the period of peak of moth activity the last week of September. Thus, the effects of the pheromone on moth captures and mating by beet armyworm persisted for >100 d. The release rate of pheromone from the dispensers over time and the quantity remaining at the end of the test are unknown. The manufacturer did not provide release rate data for fresh pheromone dispensers or for samples collected from the field at weekly intervals throughout the cotton-growing season and subsequently forwarded to Japan for analysis.

Beet armyworm egg masses and larvae also were reduced in the pheromone-treated fields compared to the control area (Fig. 4). The pheromone-treated fields were sprayed three times each for larvae and whiteflies, and the control field was sprayed five times (Table 1). Infestation counts did not indicate that the cotton crop in the pheromone-treated fields was ever in danger from beet armyworm; however, the sharp increase in the number of beet armyworm larvae in the control field in mid-September (Fig. 4) prompted the cooperator to apply pesticide to all fields on 25 September. Larval counts made the following day and again on 5 October (Table 2) confirmed our season-long observations that the pheromone-treated fields had significantly fewer beet armyworm than did the control field.

We also recovered fewer *C. marginiventris* cocoons in the pheromone-treated fields than in the control field (Table 2). *Cotesia marginiventris* is a highly effective parasitoid of beet armyworm and other noctuid pests (Tingle et al. 1994). The fact that we recovered significantly fewer cocoons in pheromone fields A and B than in the control field does not indicate an adverse effect of pheromone on this parasitoid. On the contrary, these numbers tend to substantiate the effects of the pheromone treatment on beet armyworm; quite simply, fewer *C. marginiventris* cocoons were recovered in the pheromone-treated fields because there were fewer beet armyworm larvae to be parasitized.

For mating disruption technology to be most effective, the pheromone treatment should be applied early in the season when pest numbers are low. Trap capture data indicated that the beet armyworm population was quite low at the start of the season and increased in typical fashion as the cotton-growing season progressed (Fig. 3). Our results are supportive of the findings by Wakamura & Takai (1992) who controlled beet armyworm in Welsh onion plots by using mating disruption technology. In contrast to our study, however, Wakamura & Takai (1992) treated a large buffer zone (130 ha) with beet armyworm pheromone surrounding the Welsh onion fields (25 ha) they were trying to protect. The following year, they reduced the pheromone-treated area to 50 ha of which Welsh onion plots comprised 24 ha. In both years, trap

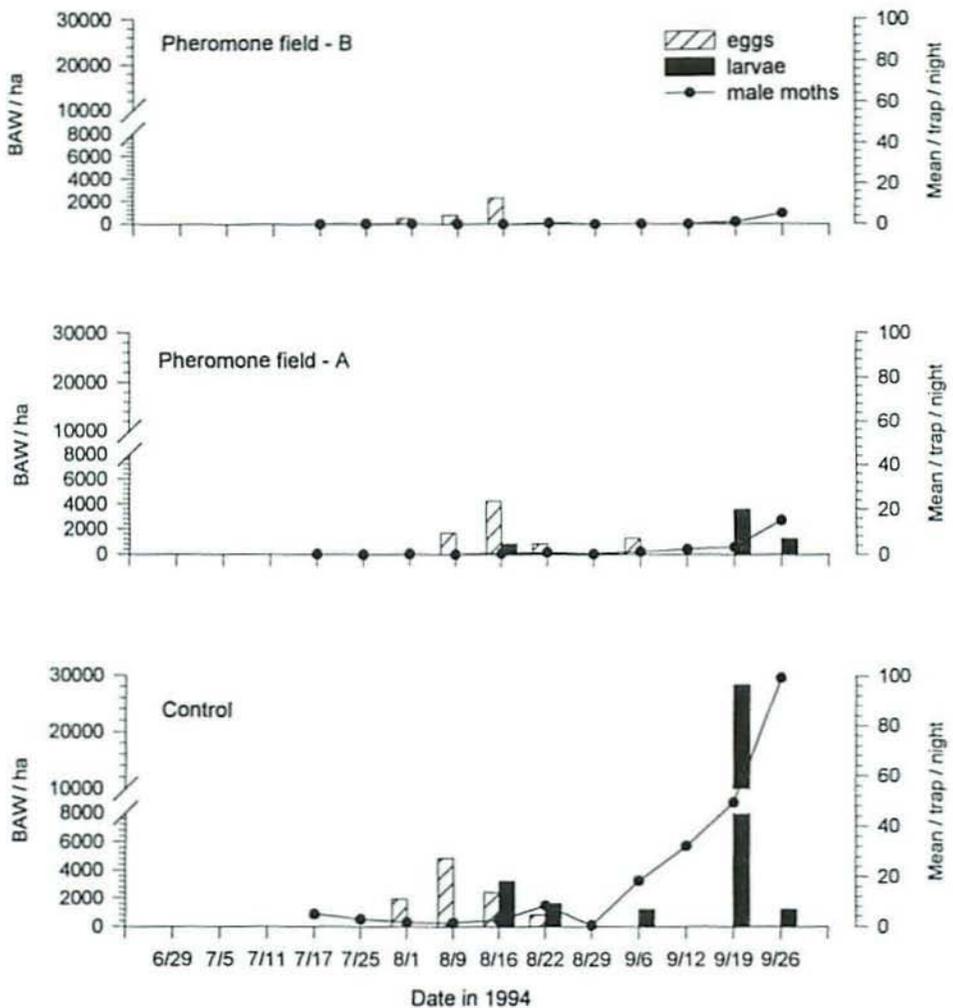


Fig. 4. Effect of pheromone treatment on beet armyworm (BAW) egg deposition, larval numbers and capture of male moths in pheromone traps in cotton. Yoto-con-S[®] twist-tie ropes were applied at the rate of 1,000 ha in fields A (14 July) and B (8 July).

Table 1. Pesticides used for insect control in pheromone-treated and control cotton fields at Houston, Florida. 1994.

Date	Material	Rate/ha
Pheromone field A		
19 July	Karate	0.38 liter
03 August	Karate	0.35 liter
	Orthene	0.56 kg
25 August	Lorsban	2.33 liters
	Orthene 90	0.56 kg
Pheromone field B		
20 July	Karate	0.38 liter
03 August	Karate	0.35 liter
	Orthene 90	0.56 kg
25 August	Lorsban	2.33 liters
Control field		
19 July	Karate	0.38 liter
02 August	Karate	0.35 liter
	Orthene 75	0.28 kg
19 August	Karate	0.35 liter
25 August	Lorsban	2.33 liters
	Orthene 90	0.56 kg
29 September	Orthene 90	0.56 kg

Table 2. Mean number of beet armyworm larvae and cocoons of *Cotesia marginiventris* per plant (\pm S.E.) in pheromone and conventionally treated cotton fields at Houston, Florida. 1994.

Field	September 26	October 05
	Beet armyworm larvae ^a	
Control	0.62 \pm 0.17a	0.13 \pm 0.14a
Pheromone A	0.06 \pm 0.03b	0 b
Pheromone B	0 b	0.01 \pm 0.01b
	<i>Cotesia marginiventris</i> cocoons ^a	
Control	0.82 \pm 0.17a	0.41 \pm 0.07a
Pheromone A	0.33 \pm 0.06b	0.13 \pm 0.04b
Pheromone B	0.19 \pm 0.02b	0.06 \pm 0.01b

^aMeans in the same column with different letters are significantly different ($P < 0.004$, analysis of variance, Duncan's Multiple Range Test).

captures of beet armyworm males in pheromone-treated zones were completely eliminated and densities of egg masses and young larvae were reduced >95% over control areas located about 9 km away. In fact, the beet armyworm population in the pheromone-treated area was so low the second year that Wakamura & Takai (1992) concluded that the low initial density was a possible effect of the sex pheromone treatment the previous year. The present study is especially meaningful because it demonstrates that mating by a strong flying insect like beet armyworm can be suppressed with pheromone in relatively small fields even when large moth populations are present. In spite of the lack of field isolation, the relatively small field size, and the confinement of pheromone treatments to the fields proper, there was no significant difference in the percentage of matings by sentinel females, the number of beet armyworm males captured in pheromone traps, or larval populations in the pheromone-treated fields. However, pheromone fields A and B showed significant reductions in all categories over the control field.

This is the third demonstration on the use of mating disruption technology to suppress mating by a noctuid pest in a row crop on a field basis. Mitchell & McLaughlin (1982) reduced the number of egg masses deposited by fall armyworm, *S. frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), 75%–95% in corn treated with (Z)-9-tetradecen-1-ol acetate formulated in hollow fibers, with corresponding reductions in whorl damage in the pheromone-treated corn versus corn plants in untreated fields located nearby. Dunkelblum et al. (1992) suppressed mating of the spiny bollworm, *Earias insulana* (Boisduval) (Lepidoptera: Noctuidae), in cotton in Israel by more than 90% by using a blend

of the species' pheromone formulated in Shin-Etsu rope dispensers. Dunkelblum et al. (1992) also recorded significant reductions in captures of male moths in pheromone traps and lower infestations of cotton bolls in the pheromone-treated plot compared with the control plot. Although some insecticidal treatments were necessary for protection of the cotton crop, fewer were required in the pheromone-treated area than in the control. In addition, McLaughlin et al. (1994) successfully controlled *Plutella xylostella* (L.) (diamondback moth, Lepidoptera: Plutellidae) in a 8.1-ha plot of cabbage for the entire growing season with a single pheromone treatment applied shortly after the crop was planted. Although the effectiveness of mating disruption as a control strategy would be enhanced greatly by treating larger areas, our results and those of Mitchell & McLaughlin (1982), Dunkelblum et al. (1992), and McLaughlin et al. (1994) strongly suggest that mating disruption technology can be used on a field basis with favorable results.

Acknowledgment

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Evaluation of a Low-input On-farm Disposal System for Trifluralin, Cyfluthrin, and Mancozeb¹

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ABSTRACT The rate of loss of three pesticides, trifluralin, cyfluthrin, and mancozeb, in evaporative containers and in compost units was studied over a 3-yr period. The rinsate disposal facility evaluated included an evaporative containment tank (pilot tank) used to receive rinsate during the season of use and a compost pile. Tank rinsate mixtures were pumped onto the compost pile at the conclusion of the spray season. Small experimental containers and compost units that mimicked the large-scale system were used to quantify loss rates. Trifluralin, cyfluthrin, and mancozeb rinsates, which approximated rinsate concentrations, were established, and the loss rates of the three parent compounds were followed. Rinsate-compost mixtures were examined for the presence of these compounds. Trifluralin was recovered by solid-phase extraction and quantified using gas chromatography. Without mechanical agitation, trifluralin in the multipesticide rinsates (pilot tanks) settled to the bottom of the tank. Trifluralin remaining in experimental containers, maintained in covered and unaerated containers, was reduced to approximately 40% active ingredient [AI] on day 80. Uncovering the containers resulted in a drop in the concentration of trifluralin to 10%. Uncovering the containers and aerating the rinsate provided optimum breakdown of trifluralin; on day 80, less than 1% of the original concentration remained. Cyfluthrin, extracted with dichloromethane and concentrated by evaporation, was quantified using gas chromatography. Cyfluthrin remaining in rinsates was decreased to 0.5% of the original concentration on day 80. Mancozeb was recovered by decomposition and distillation and was quantified using spectrophotometry at 435 nm. On day 80, 0.6% of the original mancozeb concentration remained.

KEY WORDS Trifluralin, Treflan[®], cyfluthrin, Tempo 2[®], mancozeb, Manzate[®], evaporative containers, compost pile, pilot tanks

Contamination of potable water supplies from pesticides used in agriculture is not uncommon (Rothschild et al. 1983, Spalding et al. 1980, Zaki et al. 1982). Pesticide wastes that are generated on farm include unwanted, noncurrent chemicals, used

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pesticide containers, and rinsates from tanks and bins that have been used for pesticide applications. Pesticides are often intensively used in agricultural production systems in North Dakota, and intensive use magnifies the attendant pesticide waste disposal problems. Because groundwater supplies are used as the potable water supply for most of rural North Dakota and the rural United States, workable solutions to pesticide disposal problems encountered by farmers are needed.

A system currently being evaluated at Carrington, North Dakota, consists of a concrete catch pad with an open 2,280-L fiberglass holding tank at each end (tanks A & B). The catch apron is a 15-cm thick reinforced concrete slab, made with a high cement to water ratio for added strength. The slab is 6 m wide by 15 m long and slopes to each end. Each end of the apron has two drains. One diverts rain water to the side of the apron onto a grassy area, and the other one leads to one of the holding tanks. If the catch apron is being used for washing or loading sprayers, the drain used to divert rain water is plugged, so the water flows into the holding tank. Tanks are located below ground level and nested inside a larger 3,800-L tank to catch any pesticide that may leak or overflow from the inner tank (Figs. 1, 2). For safety, the tanks are covered with welded wire panels.

Railroad ties form a retaining wall around the tanks to keep soil out of the tanks. The pesticide rinsate is held up to, but not exceeding, 90 d in the open tanks in compliance with guidelines for operation of a temporary waste site (Hofman & Gardner 1989). Pesticide rinsate from both tanks is pumped onto the compost pile at the end of the field season and is expected to be broken down by chemical and biological degradation. The compost (crop residues and residues from the station livestock unit) pile is located on a concrete pad with three concrete side walls approximately 1 m high. This mixture of compost and pesticide rinsate is turned using a front-end loader and is stored for subsequent use as fertilizer.

The objective of this study was to quantify the fate of the herbicide trifluralin (Treflan®), the insecticide cyfluthrin (Tempo 2®), and the fungicide mancozeb (Manzate®) disposed in the evaporative and compost system. Results demonstrated the rate at which these pesticides degraded over time and identified problems with the system as designed. The system as constructed could be a low-cost, viable method for North Dakota farmers to dispose of rinsates of pesticides.

Materials and Methods

Pilot disposal unit. The pilot tanks (2,280-L fiberglass holding tanks as previously described) received rinsate from the sprayer after cleaning. Samples from pilot tanks were collected in early July, the end of August, and mid-September, 1992. Samples were collected 2 cm from the bottom and 2 cm from the surface on each sampling date. At each depth, four 25-ml samples were taken using a disposable syringe attached to Tygon® tubing. Samples were transferred to 100-ml closed glass containers, returned to the laboratory, and kept refrigerated (4°C) prior to analysis.

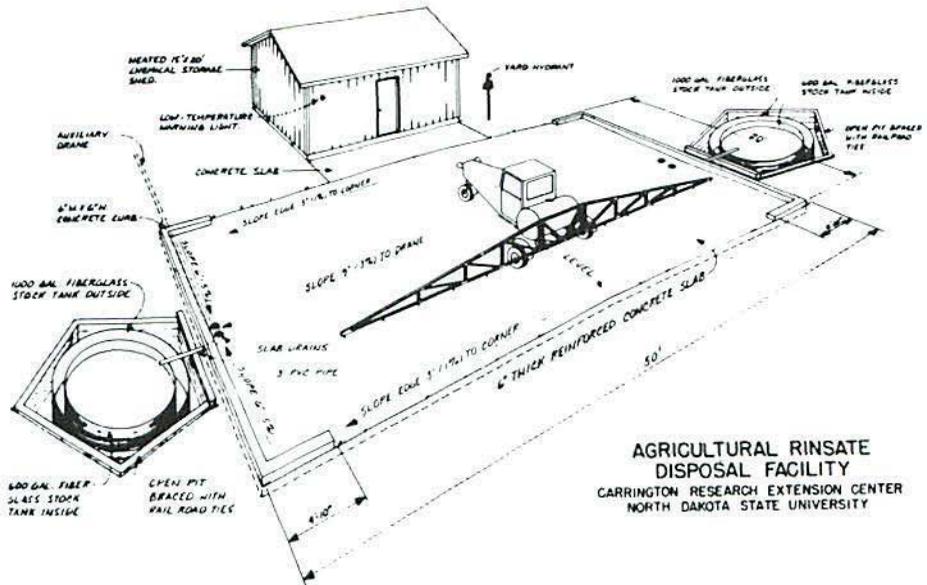


Fig 1. The Agricultural Rinsate Disposal Facility at the Carrington Research Extension Center, Carrington, North Dakota (courtesy of NDSU, Extension Circ. AE-977) (measurements are not presented in metric units).

Experimental disposal unit. At North Dakota State University in Fargo, 12 19-L experimental containers of trifluralin at 159.4 μg [AI]/ml of water, 4 19-L containers of cyfluthrin at 12.58 μg [AI]/ml of water, and 4 19-L containers of mancozeb at 96.25 μg [AI]/ml of water were prepared. These concentrations approximated the starting concentration of rinsates resulting from tank cleaning.

The experimental design for trifluralin was a two-factor factorial arrangement with three replications. Treatments included covered containers with rinsate aerated by an airflow supplied by an aquarium air pump, covered containers without aeration, uncovered containers with aeration of rinsate, and uncovered containers without aeration. Cyfluthrin and mancozeb rinsate containers were uncovered and not aerated.

Sixteen composting units were constructed from 209-L polyethylene barrels. The compost was mixed from animal pen scrapings (cattle) and crop residues (wheat and corn) without regard to quantification of components before mixing in composting units. This compost material is similar to that likely to occur from a typical combined crop-livestock operation. Approximately 34 kg of the compost was added to each composting unit. Three liters of pesticide rinsates and a water control were added to separate compost units at the following concentrations: trifluralin, 159.4 $\mu\text{g}/\text{ml}$; cyfluthrin, 12.9 $\mu\text{g}/\text{ml}$; and mancozeb, 96.3 $\mu\text{g}/\text{ml}$. Three liters of rinsate was previously established as the amount required to wet 34 kg of compost without runoff. Each treatment was replicated in four compost units.

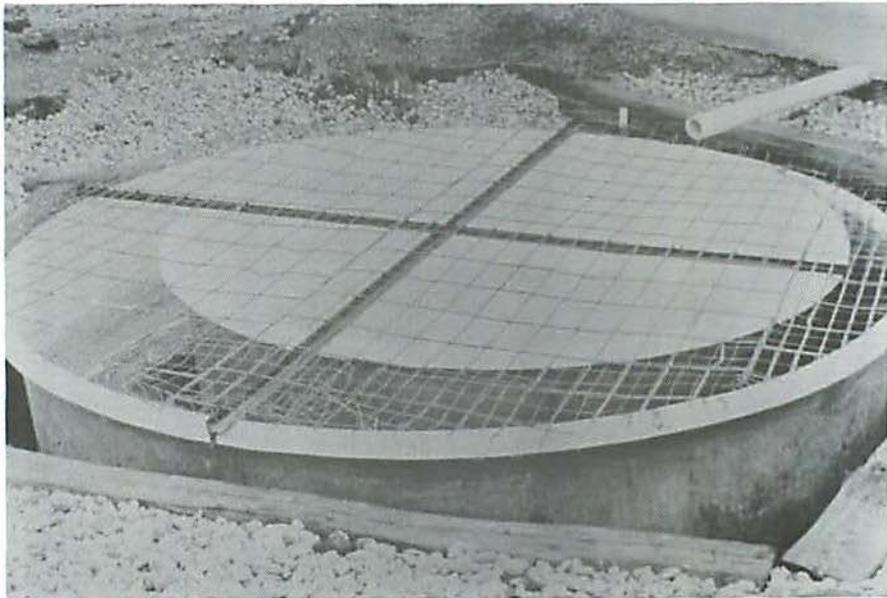


Fig 2. One of two 2,280-L inner fiberglass holding tanks with a 3,800-L tank to catch any pesticide that may leak from the inner tank. The tank top is at ground level and is protected by a five-sided railroad tie wall surrounding the hole (courtesy of J. Gardner, Carrington Research Extension Center).

Glass containers were used to collect rinsate samples from experimental containers at 2-wk intervals during June through August, 1992. Evaporation of water from the containers was compensated by addition of water to rinsates. Samples were returned to the laboratory and kept refrigerated (4°C) prior to analysis. Samples were analyzed within 1 wk.

Five milliliters of trifluralin rinsate was collected at each sampling date, and data were analyzed using an analysis of variance (Snedecor 1980). The volume of samples collected from mancozeb rinsate differed during the experimental period. Manzate® is effective for about 10 d under field conditions. On day 15, 25 ml of mancozeb rinsate was collected; larger volumes of rinsate were collected for subsequent sampling dates, depending on the recovery from the previous sampling date. On day 80, 250 ml of mancozeb rinsate was collected. Cyfluthrin samples (100 ml) were collected during the sampling period.

Four-liter glass containers were used to collect the leachate (material eluted through the compost) from each composting unit during the experimental period. Leachate from the compost units was collected on day 20, 45, and 70 after addition of the rinsate. All of the leachate was collected each time. The volume of leachate collected between sampling periods also was recorded. One milliliter of leachate containing trifluralin and 100 ml of leachate containing

cyfluthrin were used for laboratory analysis. One hundred milliliters of mancozeb leachate collected on day 20 and 45, and 200 ml collected on day 70 was used for further analysis.

Compost was sampled by collecting 50 g of compost at the beginning of the experiment (June 1992), and on day 40 and 70 after addition of the rinsate. Samples from the four blank-control composting units also were analyzed for the presence of trifluralin, cyfluthrin, and mancozeb.

Recovery of trifluralin from trifluralin-water rinsate. An analytical standard trifluralin (99.5% purity) was purchased from Chem Service, West Chester, Pennsylvania, and Treflan® (41.2% [AI]) was from Elanco, Indianapolis, Indiana. The recovery of trifluralin from known rinsate concentrations was compared with analytical standards of trifluralin.

Solid-phase extraction columns (C₁₈ Bond Elut cartridges) were used for the simultaneous isolation and concentration of trifluralin (Swineford & Belisle 1989). This was followed by quantitative analysis by using gas chromatography (GC). A C₁₈ column was attached to a filtering flask and conditioned by drawing 5 ml of methanol under vacuum, followed by 10 ml of 0.5% glacial acetic acid in distilled water (vol/vol). After 1 ml of unfiltered rinsate sample was eluted through the column, the glass container was rinsed with 3 × 10 ml of 0.5% glacial acetic acid in distilled water, and each rinsate also was eluted through the column.

The column was air-dried for 5 min by drawing air through the column with suction. The rinsate residues were eluted from the column into an Erlenmeyer collection flask with 20 ml of acetone and transferred to a 50-ml volumetric flask. The collection flask was rinsed three times with 10-ml washes of acetone:ethyl acetate (3:1 vol/vol) containing 0.2% glacial acetic acid, and the rinses were added to the volumetric flask, with the final adjustment to 50 ml. A 3- μ l aliquot of recovered trifluralin in acetone:ethyl acetate was injected into a GC equipped with an electron capture detector (ECD). About 98% of trifluralin was recovered when 1 ml of unfiltered rinsate sample was eluted through the column.

Recovery of trifluralin from the compost. The compost sample was homogenized in a blender. Fifty grams of compost was transferred to 1-L Erlenmeyer flasks; 300 ml of acetonitrile-water (99:1) was added to immerse the 50-g compost sample. There were four replications. Erlenmeyer flasks were agitated for 4 h on a orbit-shaker at 200 rpm; the long shaking period optimized recovery of trifluralin. After 4 h of agitation, a 20-ml aliquot of clear supernatant was removed, filtered through Whatman No. 1 filter paper, and transferred to a 125-ml evaporating flask. Thirty milliliters of acetonitrile was added to the evaporating flask to help evaporate traces of water, and the contents were evaporated to about 1 to 2 ml on a rotary vacuum evaporator at 45°C. Twenty-five milliliters of dichloromethane was added to remove remaining acetonitrile without compound loss, and the contents were evaporated to about 1 ml. A 4-ml volume of acetone was added to the flask to dissolve the recovered trifluralin. A 3- μ l aliquot of recovered trifluralin in acetone was injected into a GC equipped with an ECD (West et al. 1988).

A Hewlett Packard 5794A GC equipped with ECD was used for sample analysis. The analysis was performed using a 15 m × 0.53 mm OV-11 capillary

column with an oven temperature of 150°C. Helium (with a flow rate 20 ml/min) was used as the carrier gas, and an argon:methane mixture (95:5) at a flow rate of 45 ml/min was used as the detector gas. The retention time of trifluralin was 4.85 min.

Analytical methods for cyfluthrin. An analytical grade cyfluthrin (92.77% purity) and Tempo 2® (24.3% [AI]) were obtained from Mobay Corporation, Kansas City, Missouri. The recovery of cyfluthrin from known rinsate concentrations of Tempo 2® was compared with that of analytical standards of cyfluthrin.

Cyfluthrin recovery from the rinsate and compost was attempted using a confidential method for analysis provided by Metabolism and Methodology Research and Development Department, Miles Corporation, Stillwell, Kansas. A Hewlett Packard 5794A GC equipped with an ECD was used to analyze the samples. The analysis was performed using a 10 m × 0.53 m OV-101 capillary column with an oven temperature of 260°C. The detector temperature was 300°C. Helium (with a flow rate of 13 ml/min) was used as the carrier gas, and an argon:methane mixture (95:5) (with a flow rate of 57 ml/min) was used as the detector gas. The injection volume was 3 µl, and the retention time of cyfluthrin was 2.95 min.

Analytical method for mancozeb. Heating mancozeb (Manzate®, 80% purity) with a solution of stannous chloride and hydrochloric acid yields carbon disulphide (CS₂) that can be distilled, purified, and collected in an ethanolic solution of cupric acetate and diethanolamine. Two yellow cupric-N,N-bis dithiocarbamate complexes are formed that can be measured by spectrophotometry (Thier & Zeumer 1987).

Evaluation of the residue levels of mancozeb was performed by preparing a calibration curve by using the method of Thier & Zeumer (1987). The residue level of mancozeb is related to CS₂. The calibration curve was derived by plotting the micrograms of CS₂ per mixture on the abscissa versus absorbance value on the ordinate. The equation of the regression line determined, using a Shimadzu UV-160 spectrophotometer, was $y = 0.0037X - 0.105$, and the coefficient of correlation was 0.999. The conversion factor 1.776 was used to relate the recovered amounts of CS₂ to the active ingredient mancozeb. Mancozeb recovery from the rinsate and compost was accomplished using a decomposition and distillation apparatus (Thier & Zeumer 1987).

Results and Discussion

Recovery of parent compounds from the pilot tanks. Trifluralin was detected at a higher level in the bottom of the multirinsate pilot tanks (Fig 3). Tank A had a consistent low concentration of trifluralin throughout the sampling period. Trifluralin was detected at a higher level in Tank B. During the latter part of the sampling period, the concentration of trifluralin increased at the bottom of Tank B.

According to entries in the log book record maintained at the pilot disposal facility, trifluralin rinsates were washed into the pilot disposal system 11 times during the growing season, and more rinsate was allowed to flow into tank B. Mixing due to addition of the rinsates to the pilot tanks was frequent between

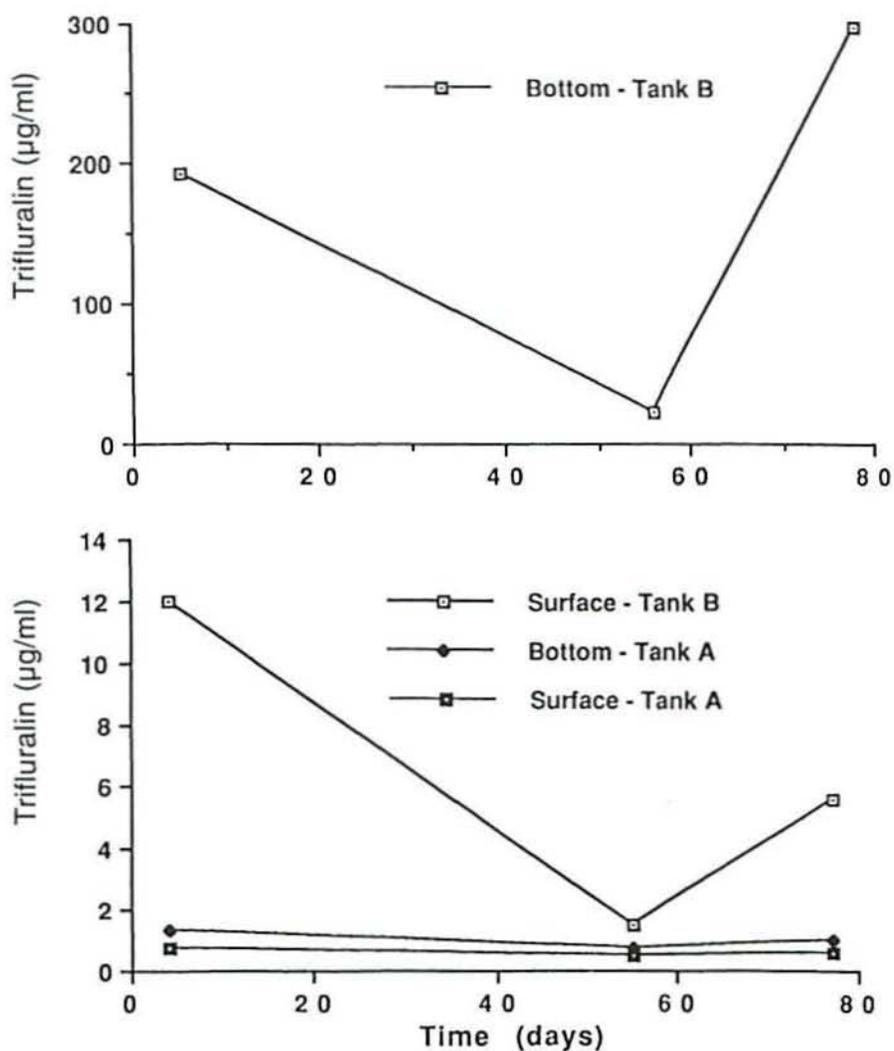


Fig 3. Recovery of trifluralin from the pilot tanks (Carrington, North Dakota).

the first and second sampling dates, thus preventing stratification and enhancing photodecomposition of trifluralin.

Between the second and third sampling dates, the rinsate was undisturbed, allowing trifluralin to settle to the bottom. Therefore, during the latter part of the sampling period, the concentration of trifluralin increased at the bottom of Tank B. Also, the pilot tanks depended solely on rainfall for dilution when rinsates were no longer being added to the tanks. Low water solubility, differences in density of the formulated trifluralin, evaporation of water, and inefficiency of sunlight in penetrating through the accumulated multiple rinsates caused an increased concentration of trifluralin.

With the exception of one sample on 4 July 1992, no evidence of mancozeb or cyfluthrin was found in the pilot tanks in 1991 (Vethanayagam 1993) and in 1992. The 4 July sample showed a concentration of 93 $\mu\text{g}/200\text{ ml}$ for dithiocarbamate and/or thiuram disulphide, the indicator compound for mancozeb.

Recovery of trifluralin from Treflan[®]-water rinsate. During our initial studies, evaporation of water from the containers resulted in an increase in trifluralin concentration that obscured the actual loss that resulted from breakdown of trifluralin in the containers (Vethanayagam 1993).

The effects of aeration and exposure to sunlight on the loss of trifluralin are illustrated in Fig. 4. When the containers were neither exposed to sunlight nor aerated, about 40% trifluralin remained on day 80. When the containers were exposed to sunlight, but not aerated, about 10% [AI] remained on day 80. Containers exposed and aerated had only 1% of the trifluralin remaining on day 80. Analysis of variance for trifluralin in samples analyzed on day 15, 32, and 48 showed that both exposure to sunlight and aeration had a significant effect on the amount of trifluralin remaining. There was no interaction between the additive main effects (exposure to sunlight and aeration) until day 66.

Recovery of cyfluthrin from Tempo 2[®]-water rinsate. The average recovery of cyfluthrin was $10.5 \times 0.5\ \mu\text{g [AI]/ml}$ of rinsate at time 0. This 84% of average recovery was based on three replications of known concentration (12.58 $\mu\text{g cyfluthrin/ml}$ rinsate) of cyfluthrin. During the 1991 studies, which were carried out at the Carrington Research and Extension Center, 56% of cyfluthrin was degraded within 14 d, and about 3% remained on day 56 (Vethanayagam 1993). Sixty-three percent of cyfluthrin was degraded within 15 d, and about 0.57% remained on day 80 during the 1992 experimental period. The recoveries of cyfluthrin from samples for 1991 (Carrington) and 1992 (Fargo) were compared because Carrington and Fargo have different water sources (Fig. 5). Cyfluthrin remained at a low residue level on day 80.

Recovery of mancozeb from Manzate[®]-water rinsate. Rinsate volumes analyzed during this research varied. The average recovery of mancozeb containing known CS_2 concentrations between 50 to 250 μg was $83.2\% \pm 3.12$ at time 0. About 76% of mancozeb was degraded within 15 d, and 0.58% remained on day 80 (Fig. 6). Degradation of mancozeb does not necessarily mean that toxicity is eliminated because its metabolite ethylene thiourea (ETU) is a carcinogen. The ethylenebis-dithiocarbamates (EBDCs) degrade rapidly in soil into ETU, which is quite soluble and mobile under certain soil conditions and pose a significant threat to groundwater (Neil & Williams 1988).

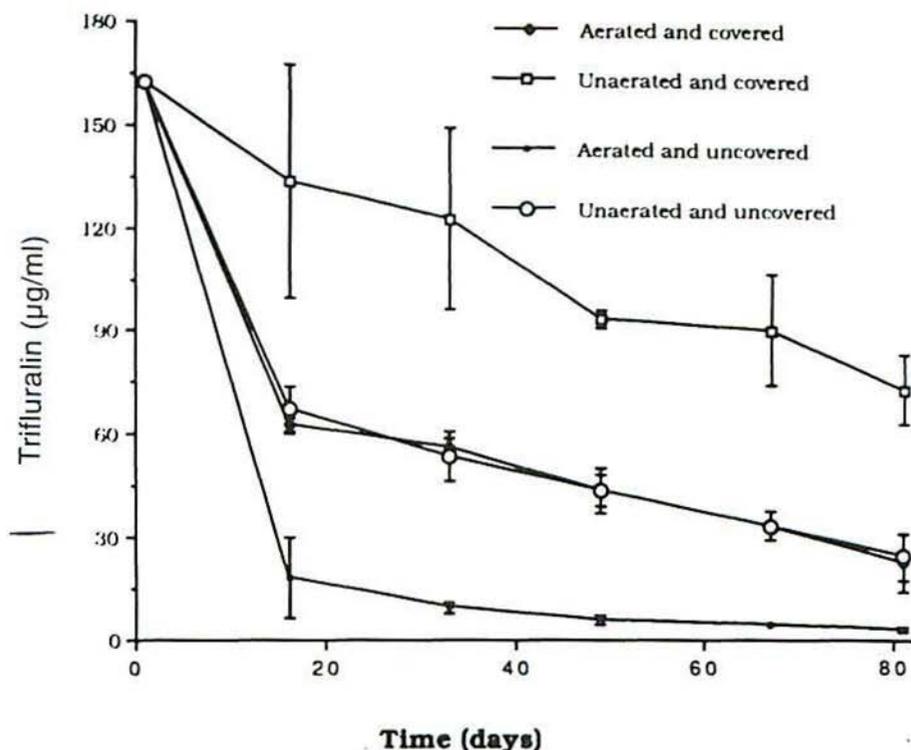


Fig 4. Effect of aeration and exposure to sunlight on the loss of trifluralin in experimental containers.

Recovery of parent compounds from the compost. During our initial studies, rinsate exposed to sunlight during the summer in replicated containers was added to the compost at the end of the season. This methodology is analogous to the way rinsate is pumped from pilot tanks onto the larger compost pile at the pilot disposal facility. Compost samples that were collected in the following spring had no detectable trifluralin.

Significant amounts of trifluralin were recovered from the four composting units when samples were collected on day 1, 40, and 70 after addition of the rinsate. Recoveries ranged from 49%–99% (Table 1). An average recovery of trifluralin from compost was not determined because the percentage of organic matter and other constituents in each compost sample was unknown and differed among the four composting units. This finding also was true for the farm pilot system. Even though the compost was turned, the mixture was heterogeneous. The difference in the percentage recovery could be due to the heterogeneous composition of the compost.

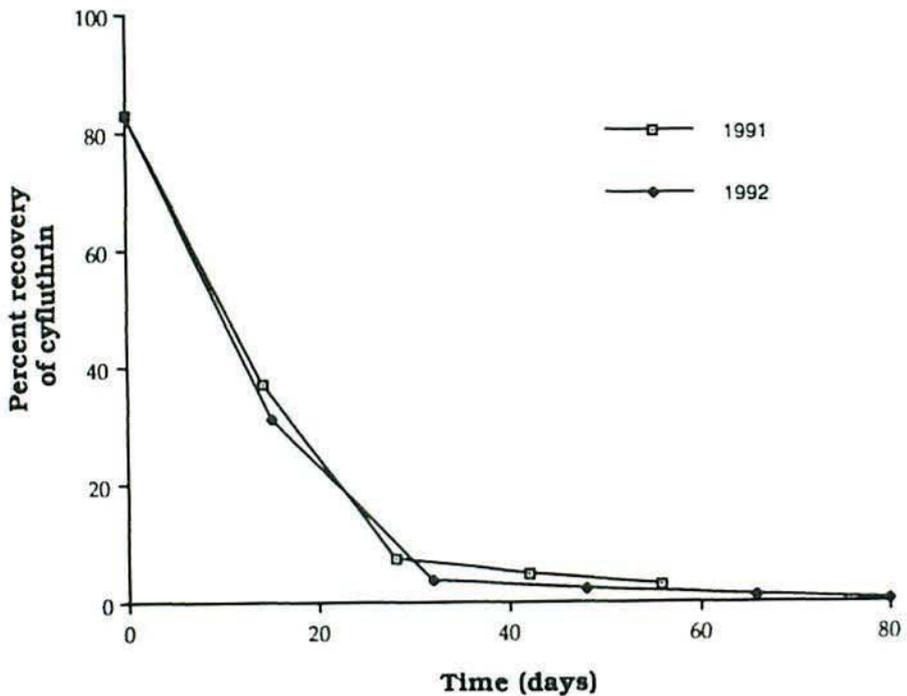


Fig 5. Comparison of recoveries of cyfluthrin from rinsate samples for 1991 (Carrington, North Dakota) and 1992 (Fargo, North Dakota).

Cyfluthrin was not recovered from the compost samples. Cyfluthrin is degraded in the compost by microbial enzymes. In general, the initial degradation in soil is the split of the ester bond. Demoute (1989) concluded that all pyrethroids are readily degraded in soil by microorganisms.

A significant percentage of the mancozeb was recovered from the four composting units. At day 1, recoveries ranged from 76%–91% (Table 2). On day 70, they ranged from 3.4%–4.9%.

Temperatures recorded in the compost bins were higher than the ambient temperatures during the season and about the same as the ambient temperatures at the end of the season. The average temperature of composting units was 30°C on day 40, which was 9°C higher than the ambient temperature of 21°C. It is not necessarily that microbial activity is directly related to temperature, although it generally occurs. The average temperature was 17°C on day 70, which is only 3°C higher than the ambient temperature. This lower temperature suggests a low level of microbial activity at the end of the season.

Recovery of parent compounds from leachate. The volume of leachate collected and the percentage of trifluralin eluted through the compost between sampling dates are presented in Table 3. Volumes of leachate collected from

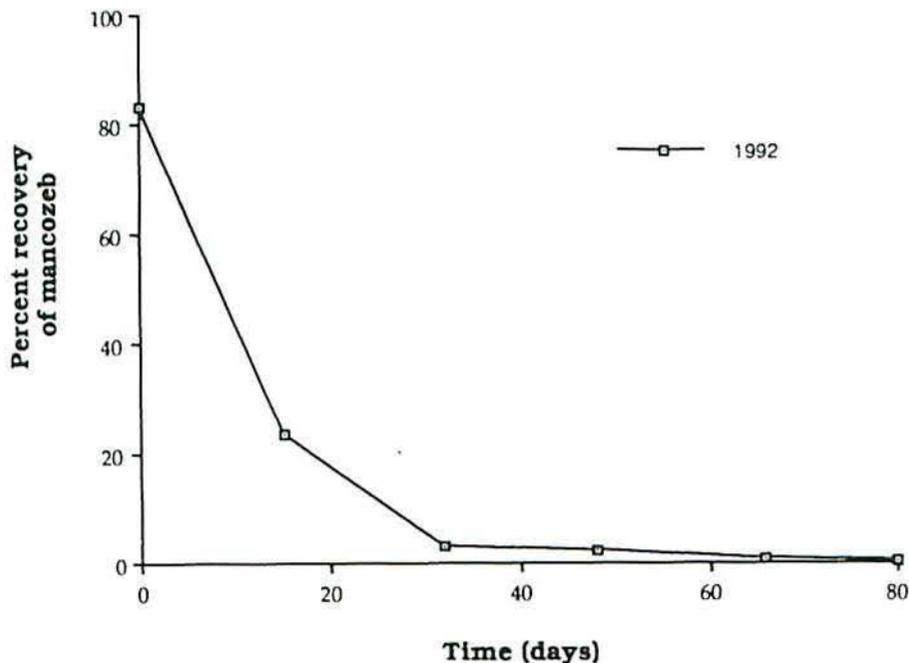


Fig 6. Percentage recovery of mancozeb from Manzate®-water rinsate.

composting units and the percentage of cyfluthrin eluted are given in Table 4. A minimal amount of cyfluthrin was found in leachate on day 20 and 45. Hill (1989) concluded that several pyrethroids in field soils are adsorbed to soil surfaces and that these products are substantially less bioavailable from the adsorbed phase than from the aqueous phase. Frequent rainfall during the experimental period and infiltration probably led to the presence of cyfluthrin in the leachate. The volume of leachate collected and the percentage of mancozeb eluted are given in Table 5. Four composting units received 3 L of water without any rinsate as a blank control. Pesticide compounds were not recovered from these units.

Sunlight transformation is an important degradation pathway for many pesticides. The rate of direct photodecomposition of a pesticide is a function of its absorption of sunlight. Photoreduction, photohydrolysis, and rearrangements also are important for pesticide degradation (Miller & Herbert 1987). The quantum energy available at longer wavelengths (above 400 nm) is insufficient to break most kinds of chemical bonds commonly found in pesticides (Crosby 1972). The ultraviolet region is the most important portion of the sunlight spectrum because pesticides generally absorb sunlight most readily at these wavelengths (Miller & Herbert 1987). Trifluralin is decomposed in water under sunlight wavelengths to form a multitude of products. The dinitroanilines are susceptible to ultraviolet decomposition.

Table 1. Recovery of trifluralin from 50 g of compost.

Compost bins Replication	Recovery of trifluralin in micrograms (% recovery) from 50 g of compost		
	time 0	after 40 d	after 70 d
1	505.1 (71.8)	347.5 (49.4)	-
2	697.5 (99.2)	533.3 (75.9)	214.7 (30.5)
3	582.5 (82.9)	420.8 (59.9)	-
4	345.0 (49.1)	230.0 (32.7)	-

Table 2. Recovery of mancozeb from 200 g of compost established at Fargo, North Dakota.

Composting units	Recovery of mancozeb in micrograms (% of recovery) from 200 g of compost		
	Day 1	Day 40	Day 70
1	1,537 (91)	355 (21)	84 (5)
2	1,328 (79)	199 (9)	69 (4)
3	1,278 (76)	682 (40)	66 (4)
4	1,344 (80)	220 (13)	57 (3)

Table 3. Leachate collected and the percentage of trifluralin eluted through the compost between two sampling periods.

Compost bins - replication	Volume collected ^a			Percentage of trifluralin eluted ^b		
	Day 20	Day 45	Day 70	Day 20	Day 45	Day 70
1	500	50	4	0.12	0.009	-
2	-	450	4	-	0.078	-
3	-	50	4	-	0.013	0.468
4	-	-	4	-	-	-

^aThe volume collected between two sampling periods is presented in milliliters for Days 20 and 45 and in liters for Day 70 (containers were filled due to rainfall).

^bAmount of trifluralin eluted through the compost between sampling dates.

Table 4. Leachate collected and the percentage of cyfluthrin eluted through compost established at Fargo, North Dakota.

Compost bins - replication	Volume collected ^a			Percentage of cyfluthrin eluted ^b		
	Day 20	Day 45	Day 70	Day 20	Day 45	Day 70
1	215	-	4	0.005	-	-
2	800	290	4	0.003	0.007	-
3	50	330	4	0.006	0.001	-
4	240	390	4	0.006	-	-

^aVolumes collected between two sampling periods. Volumes are in milliliters for Days 20 and 45 and in liters for Day 70.

^bThe amount of cyfluthrin eluted through the compost between sampling periods.

Table 5. Leachate collected and the percentage of mancozeb eluted through the compost established at Fargo, North Dakota.

Composting units	Volume collected ^a			Percentage of mancozeb eluted		
	Day 20	Day 45	Day 70	Day 20	Day 45	Day 70
1	300	260	4	0.14	0.09	<i>b</i>
2	540	940	4	0.27	0.33	<i>b</i>
3	200	130	4	0.08	0.03	<i>b</i>
4	900	500	4	0.44	0.68	<i>b</i>

^aVolumes in milliliters for Days 20 and 45 and in liters for Day 70.

^bWhen carbon disulphide was quantified, the concentration did not fall in the linear range of the calibration curve between 50 to 250 µg.

Stratification would inhibit photodecomposition in the pilot disposal tanks and thereby increase the initial amount of pesticide residues incorporated into the compost. The introduction of aeration as a means of circulation in the experimental containers helped to disperse trifluralin in water and probably promoted volatilization. Dispersion led to efficient absorption of sunlight, which ultimately leads to degradation.

Based on the results both for the pilot tanks and replicated experimental containers, it was concluded that the pesticide disposal system at Carrington has potential for trifluralin-rinsate disposal. The ideal situation for use under North Dakota conditions would be to reduce the amount of trifluralin initially incorporated into the compost to a minimum. The time required to break down pesticides such as trifluralin remaining in the rinsate is dependent on weather conditions and the initial amount of pesticides transferred to the pile, together with the chemical stability of compounds remaining in the tanks.

If high concentrations of trifluralin are transferred to the compost pile, serious effects on the microflora may result. The effects of trifluralin and acifluorfen on the size of the microbial biomass in soil under permanent pasture and on respiration were studied by Dumontet & Perucci (1992). In their study, the herbicides were applied to the soil at the rate commonly used in the field and also at a 10-fold higher rate to test the response of microflora to higher than normal concentrations. Trifluralin demonstrated a marked toxic effect on both the respiration and the size of microbial biomass. Both doses of trifluralin exerted a strong biocidal effect and the toxic effect continued after the end of the incubation period. The extremes of North Dakota weather conditions make it important to reduce the amount of trifluralin initially incorporated into the compost.

Pumping the rinsate onto compost during the middle of the growing season, but after the period of rapid disappearance of pesticides in the rinsate, would enable microbial activity to take place over a long time period and would coincide with the peak microbial activity in the compost. Prolonged storage of pesticides such as trifluralin that have very low solubility in water would lead to stratification in the pilot tanks without further degradation.

Double-lined, surface-level holding disposal tanks provide a safe method of storing farm pesticide rinsates, but without special treatment, do not allow a fast and efficient breakdown of trifluralin. Due to the presence of the multipesticide rinsates, soil from equipment washing, and some plant debris, the tank water does not act as an efficient transmitting medium for light. Low, shallow tanks could be used to increase photodecomposition by providing a large surface area for UV light penetration. Any form of mechanical agitation of the multirinsate could be used to disperse the pesticides to increase exposure at the surface.

For the most efficient use, the Carrington evaporative tanks and compost site would require some monitoring. However, this pilot disposal system that combines physical degradation processes in the evaporative tank and chemical and biological degradation processes in the compost pile could be effective. The system could be readily adaptable to most farming systems if the concentration of multipesticide residues could be brought to a minimum at the end of the season. Importance should be given to the water level in the pilot tanks. Care

also should be taken that both tanks receive equal amounts of rinsates during the holding periods. Mechanical agitation of the rinsates could be used to prevent stratification of the pesticides mainly for low water-soluble pesticides.

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Comparative Effectiveness of Coumaphos Treatments Applied by Different Methods for the Control of *Boophilus microplus* (Acari: Ixodidae)^{1, 2}

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ABSTRACT Effectiveness of coumaphos applied by three different treatment methods (dipping vat, spray-dip, and power spray) to cattle infested with parasitic stages (adult, nymphal, and larval) of *Boophilus microplus* (Canestrini) was studied. Both the number of ticks per calf and the index of reproduction (IR) of engorged females recovered from untreated calves were significantly higher ($P < 0.05$) than from treated calves, regardless of the developmental stage of the ticks or the method of applying the acaricide, indicating that coumaphos had a dramatic adverse effect on ticks. When adult ticks were subjected to the dipping vat method of treatment, both the number of females recovered (54 ticks/calf) and the IR (0.0) were significantly lower ($P < 0.05$) than those of adult ticks subjected to spray-dip (146 ticks/calf; IR = 118,253) or power spray treatments (199 ticks/calf; IR = 289,198). The trend was repeated in ticks that were in the nymphal stage at the time of treatment, indicating that the dipping vat method (1 tick/calf; IR = 0.0) was more effective than either the spray-dip (54 ticks/calf; IR = 38,014) or power spray treatments (96 ticks/calf; IR = 115,945). Likewise, the dipping vat method was more effective (0 ticks/calf; IR = 0.0) than the spray-dip (23 ticks/calf; IR = 7,426) or the power spray (57 ticks/calf; IR = 23,848) when ticks were in the larval stage of development at the time treatments were applied. Although all three treatment methods were successful, the dipping vat method reduced tick numbers and reproduction more effectively than spray-dip or power spray. Because the objective of any eradication program is the complete elimination of ticks, treatment in a dipping vat would be the most desirable method.

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²Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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The United States eradication program against *Boophilus* spp. has eliminated these serious pests from an area of more than 1,813,000 km² (Graham & Hourrigan 1977), primarily through the systematic treatment of all livestock with the organophosphorus acaricide coumaphos (Drummond et al. 1967, 1968, Drummond & Utterback 1979, Ahrens et al. 1982, Davey & Ahrens 1982). The eradication program still maintains an active, permanent quarantine barrier in eight counties in deep south Texas along the Texas-Mexico border. In addition to the U.S. program, until very recently, a federally funded eradication program existed in the Commonwealth of Puerto Rico where *Boophilus microplus* (Canestrini) still occurs. Systematic treatment of livestock with organophosphorus acaricides continues to be the principal method for eradicating outbreaks and preventing dispersal of cattle ticks into the continental United States and back into tick-free areas of Puerto Rico.

Application of acaricides to livestock for the elimination of cattle ticks can be accomplished by total immersion dipping vat, power spray, spray-race, and more recently, by using spray-dip equipment (box with a series of high pressure nozzles through which the acaricide is applied to a restrained animal within the box). For the eradication program in the continental United States, the immersion of cattle in a dipping vat has long been the preferred treatment method for eliminating cattle ticks. During the first eradication program in Puerto Rico (1937 – 1953), total immersion dipping vats also were the preferred treatment method (Tate 1941). However, since the second eradication program was initiated in 1979, power sprays have been the treatment method used most extensively (Garris & George 1985). Although spray-dip treatments also have been used in both the United States and Puerto Rico, this method of application is only used on an intermittent basis. The use of spray-race as a treatment method has never been used in any U.S. funded eradication program.

Some investigators have reported that dipping vats (total immersion plunge vats) are the most effective method of applying acaricides to cattle for control of cattle ticks (Powell 1977, Kearnan et al. 1982), although others have reported that there is no difference in effectiveness between dipping and spraying of animals (Drummond et al. 1966). In one study, it was reported that careful hand spraying was slightly more effective than dipping, and that dipping of animals was markedly more effective than spray-race treatments (Wharton et al. 1970). No quantitative data exist on the overall effectiveness of spray-dip as a means of applying acaricides. The differing results of these investigations suggest that there is no real consensus as to the superiority of one application method over another. However, it is precisely this inability to establish a clear difference between the effectiveness of different treatment methods that indicates the critical need for determining whether the methods of application (dipping vat, spray-dip, or power spray) used in the official cattle tick eradication programs implemented by the United States Department of Agriculture (USDA) are equally effective.

The purpose of the present study was to compare the effectiveness of three different application methods for treating cattle infested with all parasitic stages of *B. microplus*. Information from this investigation will be valuable to the eradication program in refining procedural requirements to be followed in the treatment of tick-infested livestock.

Materials and Methods

The study was conducted at the USDA, Agricultural Research Service (ARS), Cattle Fever Tick Research Laboratory (CFTRL), at Mission, Texas. Three different methods of applying acaricide (coumaphos) to tick-infested cattle were evaluated to determine the comparative effectiveness of each technique in reducing the repletion rate and reproductive potential (index of reproduction, IR) of ticks that were in different parasitic developmental stages (adult, nymphal, and larval) at the time treatments were applied.

Experimental design. Twelve Hereford heifer calves weighing ca. 175 kg each were randomly assigned to four equal groups (three animals per group). Each calf was individually stanchioned in a 3.3 m × 3.3 m stall in an open-sided barn under ambient environmental conditions, except that no direct sunlight or rainfall reached the cattle. Twenty days before treatments were applied, each calf was infested with ca. 10,000 *B. microplus* larvae that were 2–3 wk of age. Additional infestations with larvae (ca. 10,000 per infestation) were made at 13 and 6 d prior to treatment. The infestation pattern provided ticks that were in the adult stage (20-d pretreatment infestation), nymphal stage (13-d pretreatment infestation), and larval stage (6-d pretreatment infestation) at the time treatments were applied (Hitchcock 1955, Davey et al. 1982, 1984).

Every day for 21 d after treatment, engorged female ticks that detached from each calf were collected from the stall floor and counted. Random samples of 10 females per day per calf (whenever possible) were saved to obtain data on the ovipositional capabilities of the surviving ticks. Females within each sample (≤ 10) were weighed collectively, placed in coded 9-cm diam Petri dishes, and stored in an incubator at $27 \pm 2^\circ\text{C}$, 92.5% RH, under a 12:12 (L:D) h photoperiod and allowed to oviposit for 20 d. After oviposition was complete, females were discarded and the eggs produced in each sample were weighed and placed in coded 25 mm × 95 mm (8 dram) shell vials, stoppered with a cotton plug, and returned to the incubator. After 4 wk the percentage hatch of each sample group was visually estimated by comparing the proportion of larvae to the proportion of unhatched eggs within the vial.

When data on daily tick counts and oviposition of the saved females was complete (21 d), the daily IR for each of the three calves within each treatment or control group was calculated by the following formula (Drummond et al. 1967):

$$\text{number of } \text{♀} \text{♀} \times \frac{\text{weight of eggs (g)}}{\text{number of saved } \text{♀} \text{♀}} \times \text{egg hatch (\%)} \times 20,000 = \text{IR}$$

The 20,000 is a constant that reflects the approximate number of eggs contained in 1 g (Davey et al. 1980). Thus, the IR value provided an estimate of

the number of larvae that would be produced from a known number of engorged females laying a known weight of eggs with a known hatching level.

Treatment procedures. On the day prior to treatment, a concrete dipping vat (total immersion plunge vat) was filled to capacity (11,335 liters) with water and thoroughly mixed with 37.2 liters of coumaphos (CoRal[®] 42% active ingredient [AI] Flowable; Bayer Corp., Kansas City, Missouri) to produce a finished concentration of 0.165% AI as one of the methods of application. After the dipping vat was charged and mixed, 757 liters of the coumaphos solution was removed and placed in the reservoir tank of a spray-dip machine for evaluation as the second method of acaricide application. An additional 38 liters of the dipping vat solution was removed and placed in a large plastic container for use in a power sprayer as the third method of applying the acaricide. This procedure ensured that the coumaphos solution applied to the tick-infested cattle by the three treatment methods originated from the same source and thus, produced the same coumaphos concentration (0.165% AI). All procedures for applying the coumaphos by the three treatment methods were conducted according to standards required by the tick eradication program under operational conditions (USDA 1978).

On the day of treatment, one group of calves was removed from stanchions and each calf was totally immersed in the dipping vat with special care taken to ensure that each calf was completely submerged in the acaricide solution. After treatment, calves were returned to their respective stanchions. A second group of cattle was removed from stanchions and each calf was individually placed in the enclosed box container of a spray-dip machine for treatment. The treatment consisted of three separate bursts of the coumaphos solution from pressurized nozzles inside the enclosed box container. Each burst lasted 5 sec with a 10-sec interval between each burst to allow the animal time to breathe. After treatment, the cattle were returned to their respective stanchions. A third group of calves was removed from stanchions and each calf was treated individually with 10 liters of the acaricide solution by using a model 61 Bean[®] power sprayer at 827 KPa calibrated to deliver 7.125 liters/min. After treatment, calves were returned to their original stanchions. The fourth group of calves remained untreated and served as a negative treatment control.

Data analysis. The timing of pretreatment infestations (20, 13, and 6 d before treatment), the interval between infestations (7 d), and the known parasitic development and detachment patterns of *B. microplus* provided a means for classifying and analyzing the effect of the treatments on tick numbers and IR values by individual parasitic developmental stages. Hitchcock (1955) also reported that ca. 95% of all ticks infested at a given time will detach 21–27 d after infestation. Therefore, by using this information, a system was devised for classifying the engorged females collected after treatments were applied. Females collected 1–7 d after treatment were considered to be adults at the time of treatment and were detaching at 21–27 d after they were infested. Females collected 8–14 d after treatment were classified as nymphs at the time of treatment and were detaching at 21–27 d after they were infested; females recovered 15–21 d after treatment were classified as larvae at the time of treatment and were again dropping at 21–27 d after they were infested. This classification system allowed for evaluation of

the various treatment methods against each parasitic developmental stage on an individual basis.

Once the daily tick numbers and calculated IR values of each calf within each treatment or control group over the 21-d study period were obtained, the numbers and values were summed across each of the three developmental stages for each individual calf to provide the total tick number and IR value. Data were transformed to $\log(x + 1)$ prior to analysis to equalize the variance, then the transformed variables were subjected to a general linear model (GLM), one-way analysis of variance (ANOVA) to determine the treatment effects on tick number and IR value (SAS Institute 1987). A Fisher's least significant difference (LSD) was calculated to determine differences among the treatment means ($P < 0.05$) for each variable. For ease of interpretation, treatment means were reported in the form of actual values; however, the LSD values were reported in $\log(x + 1)$ form. To make the comparison of differences using the LSD value, the actual values must be converted to $\log(x + 1)$.

Results

The mean number of ticks per calf and the total IR of the ticks in all three developmental stages are presented in Table 1. For each of the stages, both the number of ticks per calf and the total IR values of the untreated group were significantly higher ($P < 0.05$) than any of the coumaphos-treated groups, regardless of the treatment method. Thus, the presence of coumaphos had a dramatic adverse effect on the survival and reproductive capacity of the ticks, irrespective of the method by which the acaricide was applied.

Among the three treatment methods applied to calves when the ticks were in the adult stage of development, there was a significant effect ($F = 31.85$; $df = 3, 8$; $P < 0.0001$; $LSD = 0.406$) on the mean number of ticks per calf recovered from the various treatment groups. The cattle treated in the dipping vat produced significantly fewer ($P < 0.0001$) females per calf (54 ticks/calf) than the other treated groups (Table 1). Although calves treated in the spray-dip machine produced fewer ticks (146 ticks/calf) than those treated by power sprayer (196 ticks/calf), there was no significant difference ($P > 0.9$) between these two means. The total IR of the ticks treated as adults followed the same trend as the mean number of ticks per calf ($F = 252.93$; $df = 3, 8$; $P < 0.0001$; $LSD = 0.616$). The IR value for females treated in the dipping vat (IR = 0.0) was significantly lower ($P < 0.0001$) than females subjected to spray-dip (IR = 118,253) or power spray (IR = 289,198), where differences were not significant ($P > 0.3$).

Treatment of cattle while ticks were in the nymphal stage of development also resulted in significant differences ($F = 95.31$; $df = 3, 8$; $P < 0.0001$; $LSD = 0.418$) in the number of ticks per calf among the three treatment groups. Cattle treated in the dipping vat produced significantly fewer ($P < 0.0001$) ticks per calf (1 tick/calf) than either of the other treatment methods (Table 1). Calves treated by spray-dip machine produced a mean of 54 ticks per calf, and cattle subjected to the power spray treatment produced 96 ticks per calf, although there was no significant difference ($P > 0.4$) between these two means. The total IR of ticks treated during the nymphal stage of development also resulted in

Table 1. Mean number of engorged females and index of reproduction (IR) of *Boophilus microplus* in different parasitic developmental stages at the time various treatment methods of coumaphos (0.165% AI) were applied to infested cattle.

Developmental stage at treatment	Method of treatment	Mean no. of females/animal ^a	Index of reproduction (IR) ^a
Adult	Untreated	2,279a	6,763,449a
	Power Spray	199b	289,198b
	Spray-dip	146b	118,253b
	Dipping Vat	54c	0c
	LSD:	0.406	0.606
Nymph	Untreated	2,109a	5,493,684a
	Power Spray	96b	115,945b
	Spray-dip	54b	38,014b
	Dipping Vat	1c	0c
	LSD:	0.418	0.715
Larva	Untreated	1,406a	3,109,876a
	Power Spray	57b	23,848b
	Spray-dip	23b	7,426b
	Dipping Vat	0c	0c
	LSD:	0.391	0.646

^a Means in the same column for each stage followed by the same letter are not different ($P > 0.05$); Fisher's least significant difference (LSD = $\log \{x+1\}$).

significant differences ($F = 168.39$; $df = 3, 8$; $P < 0.0001$; $LSD = 0.715$) among the treatment groups. The IR of ticks treated in the dipping vat ($IR = 0.0$) was significantly lower ($P < 0.0001$) than that of ticks subjected to either the spray-dip machine ($IR = 38,014$) or power sprayer ($IR = 115,945$). There was no significant difference ($P > 0.6$) in IR values of the two latter means.

When the coumaphos treatment methods were applied to ticks in the larval stage of development, differences ($F = 114.84$; $df = 3, 8$; $P < 0.0001$; $LSD = 0.391$) in number of ticks per calf among means resulted (Table 1). Significantly fewer ($P < 0.0001$) ticks were recovered from cattle treated in the dipping vat (0 ticks/calf) than either the spray-dipped calves (23 ticks/calf) or the power sprayed cattle (57 ticks/calf), where differences were not statistically significant ($P > 0.1$). As for the adults and nymphs, the IR values of larval-treated ticks resulted in significant differences ($F = 182.82$; $df = 3, 8$; $P < 0.0001$; $LSD = 0.646$) among treatment groups. Ticks treated in the dipping vat had a significantly lower ($P < 0.0001$) IR value ($IR = 0.0$) than either of the other coumaphos treatment methods. The IR value of ticks treated in the larval stage by the spray-dip was 7,426, and the IR of ticks subjected to power spray was 23,484; differences were not significant ($P > 0.5$).

Another factor that resulted in significant differences ($F = 1082$; $df = 2, 6$; $P < 0.01$; $LSD = 1.0$) was the analysis of the total number of days after treatment on which engorged females were recovered from each calf within each treatment group. Engorged female ticks were recovered on each of the 21 d after treatments were applied in the untreated, spray-dipped, and power sprayed treatment groups. In contrast, however, engorged females were recovered on significantly fewer ($P < 0.05$) days after treatment in the dipping vat ($\bar{x} = 6$ d), with the last female collection occurring on the 10th day after treatment.

Discussion

The dipping vat, spray-dip, and power spray treatment methods evaluated in this study produced excellent results when coumaphos was applied to cattle infested with all parasitic life stages of *B. microplus*. Both the number of females and reproductive potential (IR) of ticks recovered from coumaphos-treated cattle, regardless of the method of treatment, were vastly reduced as compared to ticks recovered from untreated calves. Consequently, the level of control achieved by any of the three treatment methods could be expected to be very high. Even though each of the treatment methods would drastically reduce tick numbers and reproductive capacity, results of this study strongly indicated that the dipping vat method of application was significantly more effective than the use of either a spray-dip machine or power sprayer as a means of eliminating cattle ticks.

The statistical differences between the dipping vat method and the other two treatment methods are of biological and regulatory importance. It is evident from the results of the study that sufficient numbers of engorged female ticks treated while in the adult, nymphal, and larval stages of development subsequently detached from cattle treated by spray-dip (146, 54, and 23 ticks/calf, respectively) and power spray (199, 96, and 57 ticks/calf, respectively)

to warrant the exercise of caution in the use of these methods of treatment of infested cattle. Likewise, the IR values of ticks (all developmental stages) obtained from cattle treated by spray-dip (IR = 7,426 – 118,253; range) and power spray (IR = 23,484 – 289,198; range) could hardly be considered inconsequential in an eradication program where the measure of success or failure is judged on the ability of any ticks to successfully reproduce. Although some ticks were recovered from cattle treated in the dipping vat, none of the ticks were capable of reproduction. From an eradication perspective, the dipping vat method of treatment came closer to achieving the ultimate goal of complete elimination than either of the other treatment methods, even though immediate and complete elimination of ticks is highly unlikely, regardless of the acaricide used or the method by which it is applied.

The results of this study generally compare favorably with other investigations, although not always. Drummond et al. (1964) reported that the control of female *B. microplus* infested on cattle that were dipped in coumaphos (0.125% AI) was 90%–100% during the first 7 d after treatment. Roulston & Wharton (1967) stated that *B. microplus*-infested cattle sprayed with coumaphos produced replete females capable of oviposition for up to 16 d after treatment, which is consistent with the results in our study. Wharton et al. (1969) reported that tick counts of 6–8 ticks per week were obtained from infested cattle following a spray-dip treatment application, although it should be noted that coumaphos was not the acaricide used to treat the cattle. In contrast to our findings, Wharton et al. (1970) stated that control achieved against *B. microplus* was significantly higher when spray applications of coumaphos were made than when infested cattle were dipped.

The dynamics of the duration of detachment of ticks from treated cattle is of critical importance in determining the effectiveness of a given treatment method from an eradication perspective. If viable ticks continue to develop to repletion for extended periods after treatment, then there is the inherent risk that ticks will be dispersed to tick-free areas if cattle are allowed to move. Such inadvertent movement of viable ticks could have devastating effects on an eradication program. Within the eradication program, movement of cattle within or out of the quarantine zone is based on the careful inspection of the cattle to determine the presence or absence of ticks. If ticks are discovered on cattle, they must be treated multiple times (3 times at 7-d intervals) before movement is allowed. However, if no ticks are discovered, the cattle are treated once and allowed to move. Detection of ticks on the cattle is a function of the number of ticks present and the size (developmental stage) of the ticks. Because adult ticks are relatively large, it is generally easy to detect them on the animal. However, because nymphs and larvae are very small, it is often difficult or impossible to detect their presence on the animal. The fact that engorged females were recovered from cattle treated by spray-dip and power spray on each of the 21-d after treatment indicates that these treatment methods failed to completely eliminate ticks that were in the nymphal and larval stage of development at the time of treatment. Conversely, animals treated in the dipping vat produced no engorged females after the 10th day posttreatment, indicating that this treatment was essentially 100% effective against ticks that were in the nymphal and larval stage of development at the

time of treatment. Therefore, spray-dip and power spray equipment should be used with extreme caution as methods of applying acaricide to certify the movement of cattle because neither method provides complete control of immature ticks, which could subsequently lead to establishment of a cattle tick population in areas outside of the quarantine zone.

The most important objectives of an eradication program are to eliminate ticks and prevent reproduction as quickly as possible after treatments are applied and to prevent possible dispersal of ticks to uninfested areas. Based on the results of this study, the use of the dipping vat method of application will achieve these goals more rapidly and reliably than a spray-dip or power spray application. Thus, it is evident that treatment of cattle in a dipping vat should always be chosen, unless circumstances prevent the use of this application method. If spray-dip or power spray methods are the only means of treatment available, extreme caution is warranted to prevent failure of the program from occurring.

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NOTE

Parasitism of Adult *Ceutorhynchus assimilis* (Coleoptera: Curculionidae) by *Microctonus melanopus* (Hymenoptera: Braconidae) in northern Idaho and eastern Washington¹

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The cabbage seedpod weevil, *Ceutorhynchus assimilis* Paykull (Coleoptera: Curculionidae), is a serious pest of canola and rapeseed (*Brassica napus* L. and *Brassica rapa* L., respectively) throughout much of North America (Buntin & McCaffrey 1995, McCaffrey 1992) and Europe (Dmoch 1965). The weevil is not a native of North America, and its presence in the United States was first recorded in Washington in 1936 (Baker 1936). In northern Idaho, weevil larvae can reduce yields of winter (fall-planted) rapeseed 15 to 35% in fields not treated with insecticides (McCaffrey et al. 1986). Several hymenopteran parasitoids of cabbage seedpod weevil larvae are known in Europe (Dmoch 1975) and North America (Doucette 1948, McLeod 1953, Waltz 1957). In northern Idaho, larval parasitism may result in mortality rates approaching 15% (Harmon & McCaffrey, unpublished data).

In 1991 we discovered a parasitoid of the adult cabbage seedpod weevil in Latah County, Idaho. It was later identified as *Microctonus melanopus* Ruthe (Hymenoptera: Braconidae), a known parasitoid of *C. assimilis*, *C. quadridens* (Panzer), and *C. pleurostigma* Marsh in Europe (Jourdheuil 1960). We were unable to find any previous reports of this parasitoid in North America. Preliminary studies were begun in 1992 to determine the distribution and parasitism rates of seedpod weevil adults by *M. melanopus* in northern Idaho and eastern Washington.

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The biology of *M. melanopus* has been described in detail by Jourdheuil (1960). The parasitoid attacks the new generation of *C. assimilis* and overwinters as a first instar within the adult weevil. The parasitoid larva emerges from the adult weevil the following spring and pupates in the soil; the new generation parasitoids attack the same overwintering generation of weevils. The next generation of parasitoids will attack the new overwintering weevil generation. Thus, there are two generations of the parasitoid and one generation of the weevil (Jourdheuil 1960).

To compare parasitism rates among different sites in northern Idaho and nearby eastern Washington, weevils were collected by sweep net at three winter rapeseed sites in 1992 (4 km northwest of Potlatch, Idaho; 1 km east of Moscow, Idaho; 8 km south of Deary, Idaho), and at five sites in 1993 (3 km north of Potlatch, Idaho; 1 km east of Moscow, Idaho; 8 km east of Genesee, Idaho; 9 km north of Culdesac; Idaho; 13 km east of Walla Walla, Washington). To intensively monitor parasitism rates throughout the growing season, weevils were collected every 2 wk in 1992 and weekly in 1993 at fields near Potlatch, Idaho. We chose to monitor this area because we knew there was an established population of the parasitoid. Twenty cages (185 cm³) of 10 unsexed weevils each were set up in a laboratory growth chamber at 20°C ± 2°C and 15:9 (L:D) h photoperiod for each collection date and site. Weevils were provided rapeseed pods for feeding. Cages were inspected daily for *M. melanopus* larvae and their dead hosts were removed. The sex of weevil hosts was recorded. Dead weevils were dissected if no parasitoid larvae emerged to observe if they were parasitized. The numbers of dead weevils with unemerged parasitoid larvae were very low, and those numbers were included in the calculations of percent parasitism.

Adult *M. melanopus* collected from sweep net samples (38.1 cm net, 180°) were recorded at the Potlatch sites in 1992 (80 sweeps) and 1993 (50 sweeps). In addition to adult parasitoids, adult *C. assimilis* collected in the sweep samples at the Potlatch site were recorded in 1993.

Parasitism rates of weevils collected from three survey sites on 29 April, 1992 were 2.0% ($n = 198$), 2.1% ($n = 199$), and 27.4% ($n = 197$) for the Moscow, Deary, and Potlatch fields, respectively. In 1993, the parasitism rates of weevils from five sites were 0.0% ($n = 194$), 0.0% ($n = 197$), 0.5% ($n = 189$), 1.5% ($n = 194$), and 7.0% ($n = 197$) for the Walla Walla (collected 27 April), Culdesac (collected 13 May), Genesee (collected 12 May), Moscow (collected 12 May), and Potlatch (collected 12 May) fields, respectively. Potlatch fields had the highest rates of parasitism both years. No parasitism by the wasp was detected outside of Latah County, Idaho, i.e., Culdesac (Nez Perce County, Idaho) and Walla Walla (Walla Walla County, Washington). The reason for the wide variation in parasitism rates among the sites is unknown. Most sites have had winter rapeseed or winter canola grown locally for many years. However, more intensive sampling across several collection periods may be necessary to fully ascertain if such differences reflect phenological differences in parasite activity or if parasitoid populations were low.

Parasitism rates and adult parasitoid populations estimated from sweep net collections at Potlatch fields are shown in Tables 1 and 2. The biology of *M. melanopus* appeared to be similar to that reported by Jourdheuil (1960).

Table 1. Parasitism rates of *Ceutorhynchus assimilis* adults and sweep numbers of *Microctonus melanopus* (1992, Potlatch, Idaho).

Date	No. parasitoids/ 80 sweeps	% weevils parasitized (n)	% male weevils parasitized (n)	% female weevils parasitized (n)
15 April		25.9 (108)		
18 April	0			
29 April		27.4 (197)	29.4 (163)	17.6 (34)
6 May	0			
13 May		7.8 (192)	9.4 (117)	5.3 (75)
27 May	6	34.0 (197)	32.7 (104)	35.5 (93)
3 June	238			
10 June	27	71.4 (196)	53.1 (64)	80.3 (132)
17 June	18			
24 June	0	17.6 (17)	12.5 (8)	22.2 (9)
1 July	0	1.6 (129)	1.9 (53)	1.3 (76)
8 July	1			
13 July	0	0.0 (195)	0.0 (109)	0.0 (86)

Overwintering weevils were attacked by two generations of *M. melanopus*. A second generation of parasitoids emerged in late July to early August to attack the new weevil generation that eventually overwintered. The rate of parasitism in the overwintering generation of weevils reached 70% during both years. Parasitism rates reported by Jourdeuil (1960) in France were considerably lower. We observed that parasitized female weevils did not oviposit, and this is consistent with the observations of Jourdeuil (1960). Also, Jourdeuil (1960) reported that field collection of adult parasitoids were biased towards female parasitoids. We confirmed this sex ratio bias at the Potlatch site (1992) where we obtained 3 males and 287 female parasitoids in field sweeps. However, we observed a much greater representation of male parasitoids (60% males; $n = 93$) emerging from field-collected weevils maintained in laboratory cages.

This note represents the first documentation of this European parasitoid in North America. The parasitoid has considerable potential as a biological control agent of the seedpod weevil as indicated at the Potlatch sites. As noted earlier, it is not clear why the parasitoid is so abundant in Potlatch, but present in low numbers or absent in other production areas within the region. The production practices, including the insecticide regimes, used for rapeseed and canola in Potlatch are similar to those in other areas. Further research should

Table 2. Parasitism rates of *Ceutorhynchus assimilis* adults and sweep numbers of *Microctonus melanopus* (1993, Potlatch, Idaho).

Date	No. weevils/ 50 sweeps	No. parasitoids/ 50 sweeps	% weevils parasitized (n)	% male weevils parasitized (n)	% female weevils parasitized (n)
12 May	2,630	0	7.0 (197)	6.7 (150)	8.5 (47)
19 May	1,225	0	0.5 (195)	0.6 (162)	0.0 (33)
26 May	1,178	0	0.0 (199)	0.0 (131)	0.0 (68)
2 June	556	0	0.5 (155)	0.6 (155)	0.0 (42)
9 June	273	0	10.5 (191)	8.3 (144)	17.0 (47)
16 June	33	0	15.2 (33)	11.8 (17)	18.8 (16)
23 June	3	5	50.8 (191)	43.6 (123)	57.4 (68)
30 June	7	3	62.6 (198)	51.1 (88)	71.8 (110)
7 July	0	1	70.4 (125)	50.9 (57)	86.8 (68)
14 July	0	0	14.3 (28)	16.7 (6)	13.6 (22)
21 July	2	0	3.6 (195)	0.0 (84)	6.3 (111)
30 July	99	0	0.0 (191)	0.0 (101)	0.0 (90)
6 August	144 ^a	0 ^a			
13 August	630 ^a	3 ^a			

^aSweeps made in neighboring *Brassica rapa* field.

determine if the parasitoid can be established in other production regions and whether production practices can be modified to enhance the parasitoid's impact on the weevil.

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Esterase Activity in Green Peach Aphid (Homoptera: Aphididae) Clones Collected from the Field and Commercially Available Bedding Plants¹

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ABSTRACT Green peach aphid (*Myzus persicae* [Sulzer]) clones collected from the field and commercially available bedding plants throughout Idaho were assayed for total esterase activity to assess their potential for insecticide resistance. In grouped-aphid assays, four aphid clones collected from bedding plants had significantly higher activities than an insecticide-susceptible clone and appeared to be moderately resistant based on the assay results. However, in individual aphid assays, two of these clones showed significant heterogeneity indicative of intraclonal reversion in highly resistant aphids. These results suggest that resistant green peach aphids might enter potato seed production areas via infested bedding plants for sale in commercial outlets. A comparison of the esterase substrates 1-naphthyl butyrate and 1-naphthyl acetate indicated that results obtained from the more sensitive 1-naphthyl butyrate were accurate in predicting elevated esterase activities related to insecticide resistance.

KEY WORDS Homoptera, Aphididae, *Myzus persicae*, green peach aphid, esterase, insecticide resistance, bedding plants

The green peach aphid, *Myzus persicae* (Sulzer), has developed carbamate, organophosphorus, and pyrethroid insecticide resistance in populations throughout the world (Devonshire 1989). Green peach aphid resistance to these insecticide classes may have a common mechanism, the geometric increase of esterase E4, or its isozyme FE4, through gene amplification (Devonshire & Moores 1982, French-Constant et al. 1988b, Devonshire et al. 1992). Originally, detection and monitoring of resistance in field populations were accomplished by measuring total esterase activity in aphid homogenates through hydrolysis of naphthyl acetates (Needham & Sawicki 1971, Beranek 1974, Devonshire 1977). Separation of total esterases by electrophoresis to isolate E4 and FE4 identified their role in resistance (Devonshire 1989),

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and isolation of these isozymes allowed development of an immunoassay for E4/FE4 for detecting resistance in field-collected material (French-Constant et al. 1988b, Field et al. 1989a). However, because the esterases associated with resistance are so abundant that they are clearly detected despite background esterase activity, measuring total esterase activity is still very useful, especially in preliminary screening for green peach aphid resistance (Devonshire et al. 1992). In addition, the total esterase assay presents a more readily available technique for assessing green peach aphid resistance in field-collected populations.

The green peach aphid is rarely a direct pest of potatoes in Idaho but is a major concern because it is the principle vector of important potato viruses, especially potato leafroll virus (PLRV) (Beemster & de Bokx 1987). Holocyclic overwintering of the green peach aphid in Idaho potato seed production areas is limited because peach and apricot trees—the primary hosts of the aphid (Bishop & Guthrie 1964)—cannot withstand the severe winter conditions. Bishop & Guthrie (1964) implicated commercially available bedding plants imported from surrounding states as the primary source of *M. persicae* entering these growing areas. A survey conducted by Halbert & Mowry (1992) indicated that commercially available bedding plants remain a major source of the green peach aphid in all Idaho seed production areas, even though bedding plant producers are required to supply pest-free material to retail outlets. To meet the demand for pest-free material, insecticides are used intensively in greenhouse bedding plant production. This approach increases the selection pressure for resistance in isolated aphid populations (Devonshire 1989) and assures that only insecticide-resistant aphids survive on bedding plants. Introduction of these resistant green peach aphid populations into potato seed production areas will make aphid control more difficult and possibly increase the incidence and spread of viruses, ultimately threatening seed certification. The present study examined total esterase activity in clonal green peach aphids to determine if insecticide resistance might be present in populations originating from commercially available bedding plants.

Materials and Methods

Green peach aphids were collected from the field and from bedding plants in retail outlets from May 1989 through May 1992 (Table 1). Clonal cultures were started from aphids collected at each site by placing single, viviparous females on excised leaves of green pepper, *Capsicum annuum* L. After the cultures became established, they were transferred every 7–10 d by placing 10 adult aphids on new green pepper leaves. The leaves were maintained by inserting their petioles through closed-cell foam into distilled water and, following aphid infestation, covered with a plastic cylinder having a nylon fabric top. An insecticide susceptible clone (OUR; Abdel-aal et al. 1990) was maintained in the same manner, and all cultures were kept in an insectary room at $22 \pm 2^\circ\text{C}$, 40%–60% RH, and a photoperiod of 16:8 (L:D) h. Voucher specimens are deposited at the Parma Research and Extension Center, Parma, Idaho. All esterase assays were performed in August 1993.

Grouped-aphid esterase assays. Mixed-age aphids from each clone were homogenized on ice at 1 mg fresh weight/ml of PBS-Tween (0.02 M phosphate

Table 1. Sources of green peach aphid clones collected throughout Idaho and adjacent states.

Clone ^a	Date	Location ^b	Collection Host	
			Scientific Name	Common Name
BP10	5/27/92	Driggs	<i>Chrysanthemum</i> sp.	Chrysanthemum
BP15	5/27/92	Aberdeen	<i>Capsicum annuum</i> L.	Green Pepper
BP17	5/27/92	Burley	<i>Brassica oleracea</i> L.	Cabbage
BP19	5/28/92	Twin Falls	<i>Brassica oleracea</i> L.	Cabbage
BP2	4/21/92	Wilder	<i>Dahlia</i> sp.	Dahlia
BP20	5/28/92	Buhl	<i>Capsicum annuum</i> L.	Green Pepper
BP21	5/28/92	Hagerman	<i>Brassica oleracea</i> L.	Cabbage
BP22	5/28/92	Glens Ferry	<i>Capsicum annuum</i> L.	Green Pepper
BP23	5/28/92	Mountain Home	<i>Capsicum annuum</i> L.	Green Pepper
BP24	5/28/92	Boise	<i>Brassica oleracea</i> L.	Cabbage
BP3	4/21/92	Caldwell	<i>Brassica pekinensis</i> (Lour.) Rupr.	Chinese Cabbage
BP5	5/26/92	Rexburg	<i>Capsicum annuum</i> L.	Green Pepper
F12a	8/3/89	Moscow	<i>Silene</i> sp.	Silene
F14a	8/3/89	Moscow	<i>Myosotis sylvatica</i> Hoffm.	Forget-Me-Not
F14b	8/3/89	Moscow	<i>Myosotis sylvatica</i> Hoffm.	Forget-Me-Not
F15a	8/3/89	Moscow	<i>Dianthus caryophyllus</i> L.	Carnation
F20h	1/11/90	Hermiston, OR	<i>Antirrhinum majus</i> L.	Snapdragon
F2f	5/17/89	Parma	<i>Antirrhinum majus</i> L.	Snapdragon
F7a	7/19/89	Shoshone Falls	<i>Veronica anagallis-aquatica</i> L.	Veronica
F8e	7/22/89	Pullman, WA	<i>Ajuga reptans</i> L.	Bugleweed
P14	8/14/91	Parma	<i>Solanum tuberosum</i> L.	Potato
P2	8/14/91	Parma	<i>Solanum tuberosum</i> L.	Potato
P22	8/13/91	Elmore Co.	<i>Solanum tuberosum</i> L.	Potato
P35	8/21/91	Fremont Co.	<i>Solanum tuberosum</i> L.	Potato
P36	8/21/91	Fremont Co.	<i>Solanum tuberosum</i> L.	Potato
P37	8/22/91	Bonneville Co.	<i>Solanum tuberosum</i> L.	Potato
P4	8/14/91	Parma	<i>Solanum tuberosum</i> L.	Potato
P41	8/22/91	Power Co.	<i>Solanum tuberosum</i> L.	Potato
P44	8/22/91	Minidoka Co.	<i>Solanum tuberosum</i> L.	Potato
P46	8/23/91	Gooding Co.	<i>Solanum tuberosum</i> L.	Potato

^aBP clones were collected from bedding plants for sale; F clones were field collected from non-potato hosts; P clones were field collected from potato.

^bAll locations are in Idaho unless otherwise noted.

+ 0.14 M NaCl + 3 mM KCl + 0.05% [w/v] Tween-20, pH 7.4) in a glass Potter-Elvehjem tissue grinder. A portion of the homogenate was diluted 1:100 in PBS-Tween and 50 μ l was added to triplicate wells in a microtiter plate. A series of 1-naphthol standards (0, 2, 4, 6, 8, and 10 nmoles) in PBS-Tween was added in 50- μ l aliquots to the appropriate wells of all microtiter plates. A 0.1 M stock substrate solution of 1-naphthyl butyrate (Sigma Chemical Co., St. Louis, Missouri) was prepared in acetone and kept at -25°C until diluted 1:100 in 0.02 M phosphate buffer, pH 7.4, just prior to use. Fifty microliters of substrate was added to all wells and the microtiter plate incubated at room temperature for 30 min in the dark. Finally, 100 μ l of 0.1% tetrazotized *o*-dianisidine (Fast Blue B; Sigma) in 3.5% sodium dodecyl sulfate was added to all wells, and the plate was incubated for 20 min at room temperature in the dark. The plate was read at 650 nm in an EL-312 microplate reader (Bio-Tek Instruments, Winooski, Vermont). This experiment was replicated three times in a randomized complete block design. Newly prepared aphid homogenates were used in each replicate.

Total protein was measured in the remaining undiluted homogenates of each clone based on a modification of the Lowry et al. (1951) assay by using reagents in the Protein Assay Kit No. P5656 (Sigma). The assay was performed by adding 50 μ l of each aphid homogenate and a series of bovine serum albumin (BSA) standards (0, 6, 12, 18, 24, 30 μ g) to triplicate wells in a microtiter plate. After adding 50 μ l of Lowry's reagent to all wells, the plate was incubated for 30 min at room temperature. Twenty-five microliters of phenol reagent from the kit was added to all wells, the plate incubated for 20 min, and then read at 650 nm in the EL-312 microplate reader.

Total esterase activity was expressed as μ moles 1-naphthol hydrolyzed/mg protein (hydrolysis time was constant) and transformed to $\log_{10}(x + 1)$ to stabilize variances. The transformed data were subjected to ANOVA with microplate well and clone as factors followed by Student-Newman-Keuls mean separation test (CoHort 1995).

Individual aphid esterase assays. Clones that covered the range of activities measured in the grouped-aphid assays were selected for individual esterase assays. Fourth instar and adult aphids were placed individually into the wells of a microtiter plate that contained 50 μ l of PBS-Tween. The aphids were homogenized by using a motorized pestle that had been fashioned to conform to the shape of the well, and each was examined under a binocular microscope to ensure complete homogenization. Each homogenate was sequentially transferred through PBS-Tween to produce a 1:100 dilution. Three aliquots of each final dilution were transferred to corresponding wells of three separate microtiter plates, and the esterase assay was carried out as described for grouped aphids. Frequency distributions of esterase activity were fit to a normal distribution to determine if heterogeneity was present in any clone by using the Kolmogorov-Smirnov test (Sokal & Rohlf 1969, Fox et al. 1995).

Esterase substrate comparison. In the above assays, 1-naphthyl butyrate was used as the substrate because of its relative sensitivity (Abdel-aal et al. 1990). However, Devonshire et al. (1992) stated that 1-naphthyl butyrate could not be used in a microassay for esterase activity related to resistance in the green peach aphid because it might be hydrolyzed by esterases other than those responsible for resistance. Therefore, individual fourth instar and adult

aphids from selected bedding plant clones were assayed for esterase activity by using 1-naphthyl acetate as substrate and following exactly the procedure described by Devonshire et al. (1992). Results from these assays were compared with those from assays with 1-naphthyl butyrate by using correlation analysis (Fox et al. 1995).

Results and Discussion

Grouped-aphid esterase assays. The only green peach aphid clones that had significantly higher esterase activities (Table 2; $P < 0.05$, Student-Newman-Keuls test) than the susceptible OUR clone were collected from commercially available bedding plants (Fig. 1). Of the 11 clones with higher esterase activity than OUR, nine were collected from bedding plants. Two of the six clones that had significantly lower esterase activity than OUR were bedding plant clones. All field-collected clones had esterase activities equal to or lower than the activity for OUR.

These results indicate that green peach aphids originating from commercially available bedding plants may be a source for establishing insecticide resistance in the field. Green peach aphids originating on infested bedding plants planted in backyard gardens are a threat as PLRV vectors to nearby potato fields (Bishop & Guthrie 1964, Bishop 1965, 1967). Growers are dependent upon insecticides for aphid control to limit the spread of PLRV within the crop, and resistant green peach aphids immigrating into potato fields could prove disastrous when inoculum levels are high.

Table 2. Analysis of variance of total esterase activities obtained from grouped-aphid assays of green peach aphid clones collected from the field and commercially available bedding plants.

Source	df	MS	F	P
Microplate Well	2	0.0006	0.0998	0.9051
GPA clone	29	0.2463	35.9644	0.0000
Well x Clone	58	0.0002	0.0312	1.0000
Error	178	0.0068	—	—

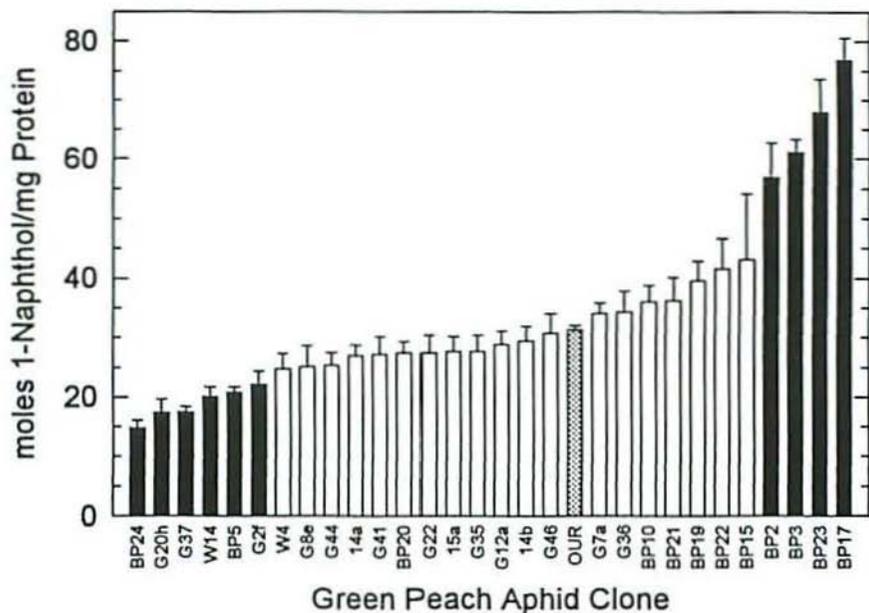


Fig. 1. Esterase activity in mass homogenates of green peach aphid clones assayed with 1-naphthyl butyrate as the esterase substrate. OUR (gray bar) is an insecticide-susceptible clone. BP clones were collected from commercially available bedding plants and all others were field collected. Black bars represent clones with significantly higher or lower esterase activity than OUR clone ($P < 0.05$; Student-Newman-Keuls test).

Individual aphid esterase assays. Figure 2 shows the frequency distributions of esterase activity for individuals from selected clones spanning the range of activities measured in the grouped-aphid assays. Individual esterase activities for some clones with significantly higher activities than OUR in the grouped assays did not fit a normal distribution, indicating that these clones were heterogeneous and that they may have originally had higher resistance levels (French-Constant et al. 1988a). In grouped assays, the esterase activities for BP2, BP3, BP17, and BP23 were not significantly different from each other (Fig. 1). However, the frequency distributions show that BP2, BP3, and BP23 were homogeneous clones relative to esterase activity whereas BP17 was heterogeneous (Fig. 2; Table 3). Indeed, some individuals in BP17 had lower activities than most of the individuals of OUR clone whereas others had the highest activities measured. BP15 also showed esterase activity heterogeneity, but the activity for this clone in grouped assays was not significantly higher than OUR. BP24 and P4 also showed heterogeneity, but this was due to both clones having many individuals with very low esterase activities resulting in skewed distributions.

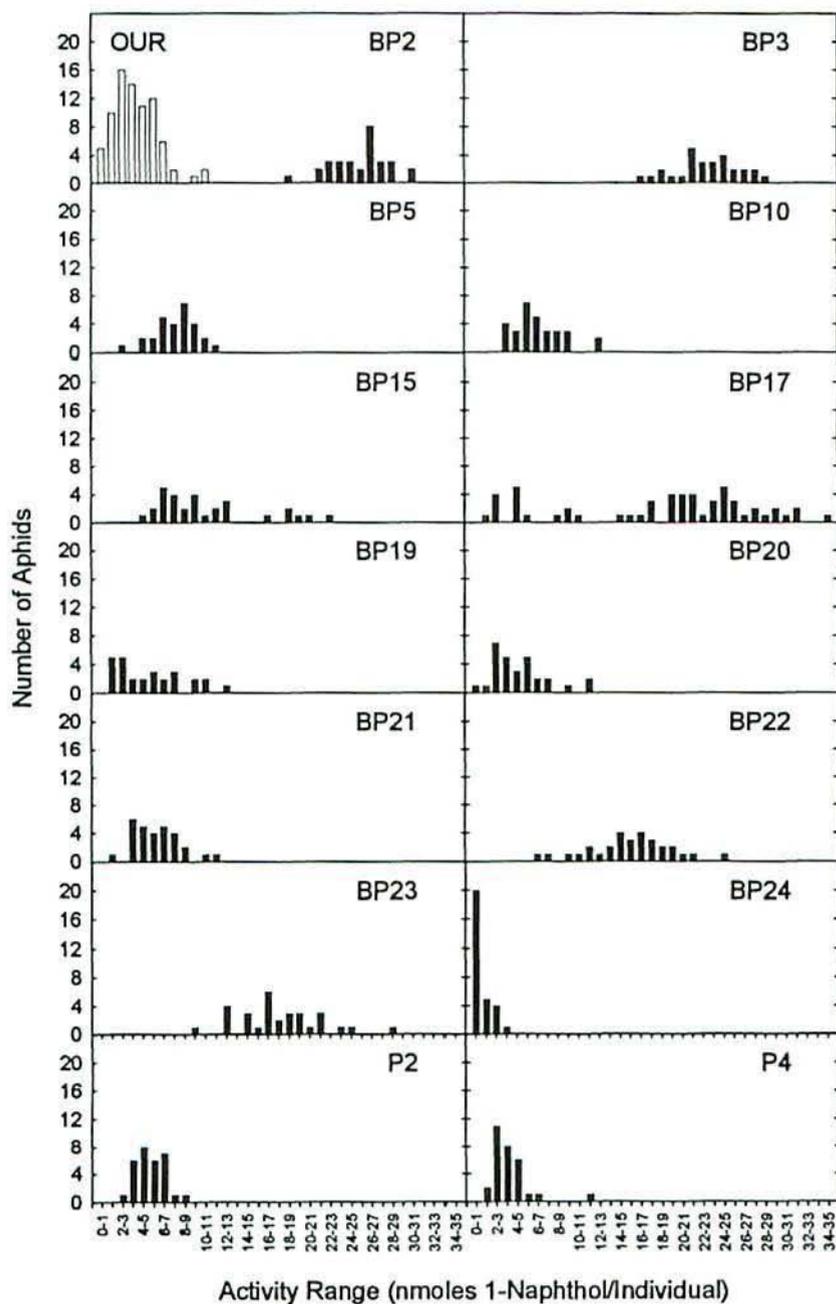


Fig. 2. Frequency distributions of esterase activities for individual green peach aphids from bedding plant- (BP) and field-collected clones from potato (P). OUR is an insecticide-susceptible clone.

Table 3. Fit of the observed esterase activity frequencies to a normal distribution for individual green peach aphids from bedding plant- and field-collected clones.

Clone ^a	Mean ^b	SD	K-S Distance ^c	P ^c
OUR	3.90	2.15	0.0819	0.208
BP2	25.60	2.80	0.1224	0.290
BP3	22.88	3.09	0.0722	0.864
BP5	7.70	2.01	0.1326	0.230
BP10	6.57	2.33	0.1192	0.326
BP15	10.71	5.01	0.1662	0.034
BP17	18.36	9.15	0.1640	0.000
BP19	5.25	3.30	0.1499	0.120
BP20	4.76	2.76	0.1217	0.319
BP21	6.62	5.10	0.2823	0.000
BP22	15.39	4.01	0.0773	0.824
BP23	17.50	4.08	0.0750	0.840
BP24	0.81	0.90	0.1840	0.011
P2	5.22	1.38	0.0973	0.607
P4	3.72	1.78	0.2018	0.002

^aOUR is an insecticide-susceptible clone; BP clones were collected from bedding plants for sale; P clones were collected from potato in the field.

^bnmoles 1-naphthol/individual aphid.

^cKolmogorov-Smirnov test for normality (Fox et al. 1995).

The esterase activity frequency distributions of BP2, BP3, BP22, and BP23 are indicative of slightly to moderately resistant clones whose esterase activity is relatively stable in the absence of insecticide selection pressure. The heterogeneity of BP15 and BP17 may indicate extensive intraclonal reversion that is characteristic of highly resistant clones not subjected to insecticides (French-Constant et al. 1988a, Field et al. 1988, 1989a,b, Devonshire 1989). If so, then it is reasonable to conclude that highly resistant green peach aphid clones may enter potato-producing areas on commercially available bedding plants, and this level of resistance might be maintained under normal insecticide pressure within the crop.

Esterase substrate comparisons. Assays performed in this study indicate that the substrate 1-naphthyl butyrate provides results highly correlated ($r = 0.732$) to those with 1-naphthyl acetate (Fig. 3), especially for green peach aphid clones with elevated esterase activities. Of the six clones compared, only BP22 appeared to show relatively high esterase activity in 1-naphthyl butyrate assays that was not reflected in 1-naphthyl acetate assays. The susceptibility of BP21 and BP24 as well as the resistance of BP17 and BP23 were confirmed in the 1-naphthyl acetate assays.

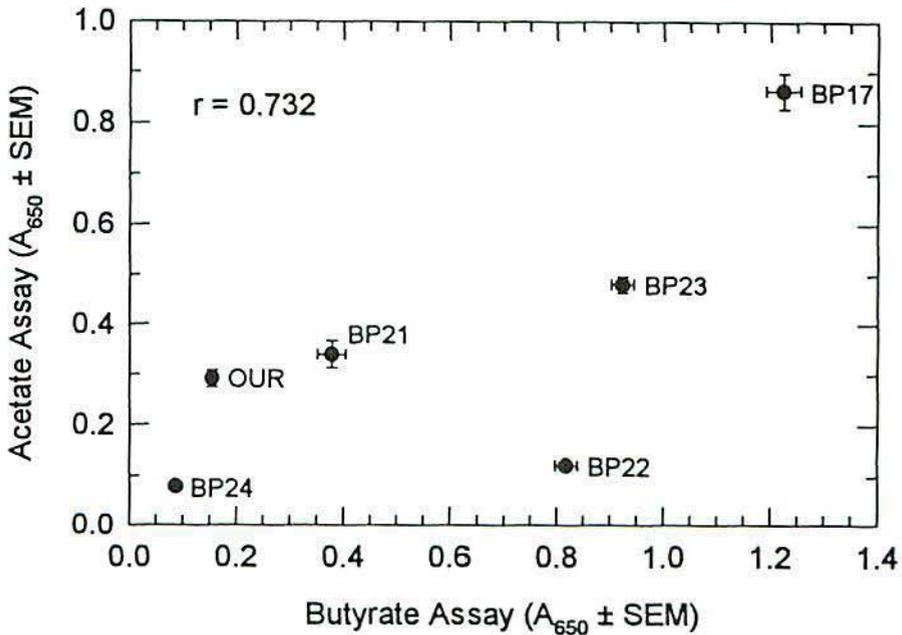


Fig. 3. Comparison of substrates 1-naphthyl acetate and 1-naphthyl butyrate used in microplate assays for detecting esterases linked to insecticide resistance. Both assays were performed by using homogenates of individual aphids.

In conclusion, commercially available bedding plants are known sources of green peach aphids (Bishop & Guthrie 1964, Halbert & Mowry 1992), and these aphids exhibit high esterase activities linked to insecticide resistance. Therefore, aphids escaping from bedding plants can exacerbate insecticide resistance problems in the local crops they infest. This is particularly troubling for seed potato production where virus infection may prevent certification. In years when the inoculum load is low, resistant aphids may go unnoticed due to minimal virus spread. However, when inoculum is prevalent, resistant aphids may cause sufficient viral infection to render the crop uncertifiable in spite of insecticide use to prevent within-crop virus spread. At this time, the contribution of resistant green peach aphids to the often cyclical epidemics of PLRV is unknown. Moreover, it is important to confirm green peach aphid insecticide resistance in the field before exercising regulatory pressure on the bedding plant industry.

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Bermudagrass Somaclone Resistance to Fall Armyworm (Lepidoptera: Noctuidae)¹

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ABSTRACT Somaclonal variants were produced from bermudagrass germplasm and evaluated for increased resistance to fall armyworm, *Spodoptera frugiperda* (J. E. Smith). A mass screening method was developed to rapidly evaluate these somaclonal lines for fall armyworm resistance in the greenhouse. Over 1,500 somaclones were produced from stolon- and immature inflorescence-derived callus cultures from six bermudagrass cultivars and three germplasm lines. Cuttings were made from 1,255 of these somaclones for testing in the mass greenhouse screenings. Individual somaclones were tested multiple times, depending on their level of fall armyworm resistance. The amount of somaclonal variation observed for fall armyworm resistance varied among germplasm sources. The ability to screen large numbers of plants resulted in the identification of several bermudagrass lines with increased resistance to fall armyworm. Somaclonal variation can provide a source of bermudagrass germplasm resistant to fall armyworm.

KEY WORDS Lepidoptera, Noctuidae, *Cynodon dactylon*, *Spodoptera frugiperda*, somaclonal variation, insect resistance, forage quality

The least expensive pest management tactic to control an insect pest below economic injury levels is through genetic resistance in plants. The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is a pest of bermudagrass, *Cynodon dactylon* (L.) Pers., in the southeastern United States. Bermudagrass cultivars and lines with fall armyworm resistance have been identified by using standard screening techniques (Leuck et al. 1968, Lynch et al. 1983, Quisenberry & Wilson 1985, Jamjanya & Quisenberry 1988). However, the number of cultivars and lines identified with fall armyworm resistance by using standard screening techniques is limited.

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Regeneration of plants from tissue culture and the production of somaclonal variants has been proposed as an approach for germplasm enhancement (Skirvin et al. 1993), including to enhance insect resistance. Croughan & Quisenberry (1989) reported two regenerated bermudagrass lines (Brazos-R3 and OSU LCB W26-R2) with increased resistance to fall armyworm. These somaclone plants were produced from stolon-derived callus of Brazos and OSU LCB W26. Brazos-R3 performed well in field trials and was released as germplasm (Reg. no. GP-1, PI 572566, Croughan et al. 1994) because of its increased resistance to fall armyworm, tolerance to the active metabolite produced by the fungus *Bipolaris cynodontis* (Marignoni) Shoemaker (causative agent for leaf spot disease), and its high forage yield and nutritive value.

The use of tissue culture to produce plants with increased pest resistance appears to have significant potential in bermudagrass. The research reported assesses fall armyworm in lines produced from stolon- and inflorescence-derived callus cultures.

Materials and Methods

Somaclones. Bermudagrass somaclones were developed from two cultivars and three germplasm lines from the Oklahoma State University bermudagrass breeding program (Hardie, Oklan, OSU 74x12-1, OSU 71x6-7, and OSU LCB-W26); two cultivars from Louisiana Agricultural Experiment Station (Grazer and Brazos); one cultivar from Cecil Greer Grass Farms, Edna, Texas (Alicia); and one cultivar from the Georgia Coastal Plain Experiment Station (Coastal). Cultures were initiated from surface-sterilized stolons or immature inflorescences. Immature inflorescences were cut into several pieces and plated onto Murashige and Skoog (MS) based medium (Murashige & Skoog 1962) with 1-2 mg/L of 2,4-dichlorophenoxyacetic acid (2,4-D) and 3% sucrose. Stolons were cut to expose the nodal area and cultured on the same medium. Once callus was obtained, it was transferred to MS based medium with lower 2,4-D and sucrose concentrations to regenerate plants. Somaclones were vegetatively maintained in flats in the greenhouse. Over 1,500 somaclones were developed, from which 1,255 were tested for fall armyworm resistance.

Fall armyworm colony. The fall armyworm colony originated from larvae collected on bermudagrass from East Feliciana Parish, Louisiana, and genetic variability was maintained by introducing larvae collected in bermudagrass fields in Baton Rouge Parish, Louisiana, into the laboratory colony annually. Larvae were reared on an artificial pre-mixed fall armyworm diet (Bio-Serv, Frenchtown, New Jersey; #F9179) and maintained in an incubator at 26°C, 14:10 (L:D) h photoperiod, and >50% relative humidity. For the greenhouse tests, neonate larvae were used on the same day they hatched from eggs.

Greenhouse tests. Replicate (6-10) cuttings of each somaclone and cultivar or breeding line from which the somaclones were derived were grown in 5.1-cm pots containing vermiculite. Plants were clipped and fertilized with N:P:K (13:13:13) fertilizer 2 wk before each test and then randomly arranged on plastic-lined greenhouse benches. Plants were watered until approximately 2.56 cm of water had collected in the plastic-lined bench. This provided water for the plants during the tests and reduced disturbance of the plants while the

fall armyworm larvae were feeding. The water also acted as a barrier to prevent larvae from moving from pot to pot. For infestation, approximately 8–10 neonates were placed on each plant by mixing neonates with corn cob grit, and dispensing with a Davis insect inoculator (Bio-Serv, Frenchtown, New Jersey) (Davis 1989, Minhm 1983). The bench then was covered with fine mesh netting and, after 5–10 d, each plant was evaluated for percentage of defoliation.

Greenhouse tests were repeated, each time evaluating a new set of somaclones, and repeating in the test any somaclone that showed promising fall armyworm resistance in previous experiments. Data were transformed to equate the defoliation rating of the parent line to 100%, and the somaclone lines to an equivalent value (Defoliation Rating = [defoliation rating of somaclone \times 100] \div untransformed defoliation rating of the parent). Plants more susceptible to fall armyworm than the parent bermudagrass would have values higher than 100, and plants more resistant would have values lower than 100. Data from all screenings were combined and analysis showed no significant difference within a bermudagrass line repeated in several trials ($P > 0.05$). Pooled data were subject to analysis of variance with SAS GLM procedure (SAS Institute 1987), and significant means separated by using Dunnett's procedure (SAS Institute 1987).

Forage quality analysis. Somaclones with fall armyworm resistance, and the cultivar from which they were derived, were grown in flats in the greenhouse and clipped and fertilized approximately every 6 wk. Grass was harvested after 2 wk of regrowth and fresh and dry weights determined. Samples were ground to 1 mm and analyzed for crude protein (CP) colorimetrically (AOAC 1990), neutral detergent fiber (NDF), and acid detergent fiber (ADF) by using methods described by Robertson & van Soest (1981), and *in vitro* true digestibility (IVTD) (Goering & van Soest 1971). Forage quality results are presented on a dry matter basis.

Results and Discussion

No significant ($P < 0.05$) differences in resistance to fall armyworm were observed in 299 somaclones derived from OSU 74x12-1, five somaclones derived from OSU 71x6-7, and eight somaclones derived from OSU LCB-W26. The somaclones tested from these breeding lines had an average defoliation rating within 20% of the parent.

The distribution of defoliation ratings for the 944 somaclones developed from the six cultivars are shown in Table 1. Twelve percent of the somaclones derived from the six cultivars tested were more resistant to fall armyworm than the parent. No somaclones were found with increased resistance in the 22 plants derived from Grazer or the 25 derived from Hardie. Only one of 69 somaclones was found with increased resistance in the Oklan-derived somaclones. Even though only 39 Alicia-derived somaclones were tested, 10 somaclones had lower defoliation ratings than Alicia. Ten percent (60 somaclones) of the Brazos-derived somaclones and 26% (45 somaclones) of the Coastal-derived somaclones were more resistant than the parent. Coastal had the highest percentage of significantly more resistant somaclones.

Table 1. Frequency of bermudagrass somaclones derived from six cultivars in five fall armyworm defoliation rating categories.

Rating	Cultivars					
	Alicia	Brazos	Coastal	Grazer	Hardie	Oklan
	—number of somaclones—					
More resistant:						
<60	0	4	12	0	0	0
61-80	10	56	33	0	0	1
Equal to parent:						
81-120	24	355	80	16	11	9
Less Resistant:						
121-160	0	182	23	6	13	57
161- >300	5	20	24	0	1	2
Total no.	39	617	172	22	25	69

Rating, percentage of defoliation where the control = 100%

Thirty-five percent of somaclones derived from the six cultivars tested were more susceptible than the parent to fall armyworm (Table 1). Fifty-two somaclones had significantly higher ($P < 0.05$) defoliation ratings than the parent (rating >161). Most of the somaclones derived from Oklan (86%) were less resistant than Oklan.

Fall armyworm feeding can be correlated with a number of nutritional parameters such as CP, or feeding deterrents and stimulants (Jamjanya et al. 1990, Lynch et al. 1986). The nutritional profile of 23 Brazos-derived somaclones representing the different defoliation rating categories are shown in Table 2. The data are from greenhouse-grown material; therefore, values are higher (CP and IVTD) or lower (NDF and ADF) than would be generally found in field-grown material. However, relative differences can be determined from the data. Overall, no nutritional component is correlated with differences observed in fall armyworm defoliation ratings. Bermudagrass somaclones developed earlier with increased resistance to fall armyworm (Croughan et al. 1994) did not show any decrease in nutritional parameters in field testing (Eichhorn et al. 1994). In addition, low levels of feeding stimulant were found in the fall armyworm-resistant somaclones (Mohamed et al. 1992). Because the

Table 2. Quality parameters of Brazos-derived somaclones by fall armyworm defoliation rating category.

Rating	Crude protein (%)	NDF (%)	ADF (%)	IVTD (%)
More resistant:				
<60	26.0 ± 0.7	54.3 ± 0.5	28.6 ± 0.88	3.6 ± 0.5
61-80	26.0 ± 0.3	54.5 ± 0.3	27.7 ± 0.38	5.6 ± 0.3
Equal to parent:				
81-120	24.7 ± 1.1	54.2 ± 0.4	28.0 ± 0.28	5.9 ± 1.9
Less resistant:				
121-160	25.7 ± 1.2	54.3 ± 0.9	27.1 ± 0.48	5.6 ± 2.1
161- >300	26.4 ± 0.3	53.9 ± 0.5	27.6 ± 0.38	5.3 ± 0.7

Rating, percentage of defoliation where the control = 100%; NDF, neutral detergent fiber; ADF, acid detergent fiber; and IVTD, in vitro true digestibility.

Table 3. Fall armyworm defoliation rating and quality parameters for two somaclones and their parent.

	Rating	Crude protein (%)	NDF (%)	ADF (%)	IVTD (%)
Hardie	100.0	22.2	56.0	29.6	83.6
B778	179.9	25.4	54.8	29.2	88.0
Oklan	100.0	19.8	56.1	28.4	80.7
B947	172.1	24.8	56.4	28.4	84.4

Rating, percentage of defoliation where the control = 100%; NDF, neutral detergent fiber; ADF, acid detergent fiber; and IVTD, in vitro true digestibility.

nutritional quality of bermudagrass is important to animal performance, a more desirable mechanism of host-plant resistance is one with secondary metabolites that do not affect animal performance.

Individual somaclones did show relative differences in nutritional parameters that were correlated with their resistance to fall armyworm. One somaclone derived from Hardie and one derived from Oklan were less resistant than the parent but had slightly better nutritional quality profiles (Table 3). The higher nutritional quality could account for why they were more susceptible to fall armyworm. Fall armyworm are known to prefer bermudagrass that is higher in nutritional quality (Lynch et al. 1986). If these lines maintain the higher-quality parameters in field tests, they may be useful in areas that do not have major infestations of fall armyworm.

Somaclonal variation has been suggested as a new source of breeding germplasm for many crops (Skirvin et al. 1993). The amount of variation observed between species and between germplasm sources can be highly variable. We found that derived somaclones of Alicia, Grazer, Hardie, and Oklan showed very little variation in fall armyworm resistance, whereas the derived somaclones of Coastal and Brazos were highly variable in levels of resistance observed. Somaclones can be easily produced from hybrid bermudagrass and the screening methods we used were effective in identifying fall armyworm-resistant somaclones, and thus, reduced the number that will be tested in field trials. Because armyworm can be easily reared in the laboratory, large numbers of plants could be screened in the greenhouse for fall armyworm resistance quickly. Although this method appears useful for identifying fall armyworm resistance, it also may be useful in identifying germplasm with higher nutritional quality parameters. Field test performance will determine the forage potential of the fall armyworm-resistant germplasm identified in this study.

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Arthropods in Litter of Poultry (Broiler Chicken and Turkey) Houses¹

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ABSTRACT Samples (110) of litter were collected from 26 commercial broiler chicken houses (18 farms) and 30 turkey (brooder and growout) houses (15 farms) in North Carolina, and the arthropods within the litter were recovered by using Tullgren funnels. Mites extracted from the litter samples belonged to two orders (Parasitiformes and Acariformes) and nine families (Macrochelidae, Urodynychidae, Trematuridae, Parasitidae, Digamasellidae, Laelapidae, Cheyletidae, Acaridae, Eremulidae). Predatory macrochelid mites (*Macrocheles muscaedomesticae* [Scopoli]), *M. merdaria* [Berlese], *Macrocheles* sp.) were collected in 45% and 49% of the litter samples from poultry houses in 1993 and 1994, respectively. Other predatory mites extracted were *Fuscuropoda marginata* (Koch) and *Cheyletus malaccensis* Oudemans. In addition, 110 duplicate litter samples were held with sentinel eggs of the darkling beetle *Alphitobius diaperinus* (Panzer) to detect mites preying on the eggs; the mite *Acarophenax mahunkai* Steinkraus & Cross (Acarophenacidae) was recovered from two broiler houses and one turkey house. Insects collected from the litter samples belonged to seven orders and 14 families. *Alphitobius diaperinus* was the dominant insect species and was found in all litter samples. The predatory histerid beetle *Carcinops pumilio* (Erichson) was found in 59% of litter samples. The pyralid moth pest *Pyralis farinalis* L. occurred in 10% of the litter samples.

KEY WORDS Arthropod fauna, Insecta, Acari, poultry litter

Numerous species of insects and related arthropods have been recorded from various types of manure in poultry and livestock production facilities from different parts of the world (Peck & Anderson 1969, Legner & Olton 1970, Pfeiffer & Axtell 1980, Rueda et al. 1990). Except for the limited reports of Lancaster et al. (1969) and MacCreary & Catts (1954), data are unavailable on the occurrence and

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abundance of arthropods in poultry litter in broiler and turkey houses in the United States. Litter refers to the floor covering consisting of wood shavings or other absorbent material contaminated with bird feces and spilled feed. We conducted a survey of the arthropod fauna associated with the litter in broiler chicken, turkey brooder, and turkey growout houses in North Carolina.

Materials and Methods

Sample sites and habitat descriptions. Litter samples were collected in five counties (Chatham, Duplin, Franklin, Sampson, Wayne) of North Carolina during November-December 1993 and May-June 1994. In 1993, litter samples were collected from 18 broiler farms (26 houses) and 15 turkey farms (15 brooder houses, 15 growout houses). In 1994, the same farms were visited to collect litter samples, except one additional growout turkey house and two fewer broiler houses were used.

The broiler chicken houses were 8-12 m wide and 71-146 m long with 9,000-26,000 birds. The litter (6-17 cm deep) on the dirt floor in the broiler houses was pine shavings, except in four farms having pine shavings topped with peanut hulls, two farms with sawdust topped with shredded newspaper, and three farms with pine shavings topped with shredded newspaper. The turkey brooder houses were 11-14 m wide and 88-133 m long, with 11,000-12,000 birds; growout houses were 13-16 m wide and 75-138 m long with 4,000-6,000 birds. The litter (5-18 cm deep) on the dirt floor in the turkey houses was pine shavings, except in one farm having pine shavings topped with peanut hulls.

Collection of arthropods. A total of 110 samples of litter (4 liters each) were collected from the poultry houses in 1993-1994. Samples were collected from the top 5-10 cm of accumulated litter because mites and other insects were observed to occur mostly in this upper layer. Each sample was a composite of eight subsamples from various subhabitats in a house (near walls, near feeders, near waterers, near middle wooden posts, open center). One-half (2 liters) of each sample was placed in a modified Tullgren funnel to extract the arthropods into alcohol. The other half of each sample was held with sentinel darkling beetle (*Alphitobius diaperinus* [Panzer]) eggs to detect the presence of predaceous mites, especially those in the family Acarophenacidae (see below).

Each 2-liter sample of poultry litter was placed in the funnel; a 60-watt bulb was suspended 10 cm above the upper surface of the litter. A 170-ml screw-capped plastic container (6.5 cm diam., 6.5 cm high), suspended at the bottom of the funnel, contained 100 ml of ethyl alcohol into which arthropods were collected. Funnel extraction lasted for 6 d, with the plastic container replaced on the third day of the extraction period. Recovered arthropods were counted under a dissecting stereo microscope. The alcohol containing the arthropods (ca. 25 ml each time) was poured into a Petri dish (15 cm diam., 2.5 cm deep) with the bottom marked into a grid of squares 20 mm on a side. Representative samples of mites and small insects were cleared in lactophenol for 2-3 d and were mounted in Hoyer's medium on a glass slide for examination by using a phase contrast microscope.

To detect acarophenacid mites, which prey on beetle eggs (Steinkraus & Cross 1993), the other 2-liter sample of litter collected from each poultry house was held in a mesh-topped 4-liter container. Two assemblies of papers were buried (5 cm deep) in the litter, each consisting of 10 pieces of 4 cm × 4 cm black construction paper held together at the center by a wire staple and containing eggs (ca. 1,000 eggs/assembly) retrieved from a culture of *A. diaperinus*. After 2 d of exposure, the paper assemblies were replaced with two new ones that were inserted in the litter for another 2 d. The exposed paper assemblies were examined for mites under a dissecting stereo microscope. Live mites were counted on the paper and then transferred to Petri dishes (9 cm diam., 1.5 cm deep) containing <1-d-old beetle eggs and observed for predation. The papers containing exposed beetle eggs were held for 4 or 5 d until all exposed beetle eggs hatched or failed to hatch due to destruction by the predatory mites. Aliquots of mites were preserved in 70% ethyl alcohol, cleared in lactophenol, and mounted in Hoyer's medium as described above.

Identification. Arthropods were identified with the aid of pertinent literature. The primary references for mites were: Baker & Wharton 1952, Hughes 1961, Axtell 1963, Summers & Price 1970, Krantz 1978, Hennessey & Farrier 1988, 1989, and Steinkraus & Cross 1993. Insects were identified by using Dillon & Dillon 1972, Pfeiffer 1978, Peterson 1979, 1982, Pfeiffer & Axtell 1980, and Borror et al. 1989. Nomenclature for classification of mites was patterned after Krantz (1978); otherwise, the work of Farrier & Hennessey (1993)—particularly on uropodina families—(Urodinychidae and Trematuridae) was used. For insects, Borror et al. (1989) nomenclature for classification was used. Identifications of mites and beetles were confirmed by comparing with reference materials previously identified by various taxonomists and available in the Medical Entomology Laboratory and the Entomology Museum at North Carolina State University, in Raleigh. *Acarophenax mahunkai* Steinkraus & Cross specimens were compared with identified mites on slides lent by D. Steinkraus, University of Arkansas, Fayetteville, Arkansas. *Pyrallis farinalis* L. specimens were identified by H. H. Neunzig (Department of Entomology, North Carolina State University).

Results

Broiler chicken litter. In broiler houses, mites collected from litter samples belonged to two orders (Parasitiformes and Acariformes) and 10 families (Table 1). The most frequently recovered mites in the litter samples were *Trichouropoda oribicularis* (Koch), which in much of the literature is named *Leiodynychus krameri* (G. & R. Canestrini) according to Farrier & Hennessey (1993). It was found in 96% and 92% of the samples in 1993 and 1994, respectively. *Cheyletus malaccensis* Oudemans was the second most abundant mite species and was found in 65% and 84% of the litter samples in 1993 and 1994, respectively. One or more macrochelid mites (*Macrocheles muscaedomesticae* [Scopoli], *M. merdaria* [Berlese], and *Macrocheles* sp.) were collected in 38% and 24% of litter samples in 1993 and 1994, respectively. *Macrocheles muscaedomesticae* was found in 12% and 4% of the samples in 1993 and 1994, respectively. *Macrocheles merdaria*, the most abundant species

Table 1. Mites recovered from litter (2-liter samples) collected from broiler chicken houses (51 samples, 26 houses, 18 farms), turkey brooder houses (30 samples, 15 houses, 15 farms), and turkey growout houses (29 samples, 15 houses, 15 farms) in North Carolina.

Mite Classification	Broiler chicken			Turkey brooder			Turkey growout		
	No.	No.	Mean No.	No.	No.	Mean No.	No.	No.	Mean No.
	Positive Samples ^a	Recovered	Per Positive Sample	Positive Samples	Recovered	Per Positive Sample	Positive Samples	Recovered	Per Positive Sample
PARASITIFORMES									
Macrochelidae									
<i>Macrocheles muscaedomesticae</i> (Scopoli)	4	59	14.8	2	6	3.0	2	39	19.5
<i>Macrocheles merdaria</i> (Berlese)	9	926	102.9	10	446	44.6	11	1,019	92.6
<i>Macrocheles</i> sp.	3	16	5.3	9	386	42.9	2	92	46.0
Urodinychidae									
<i>Fuscuropoda marginata</i> (Koch)	18	8,135	451.9	9	621	69.0	16	567	35.4
Trematuridae									
<i>Trichouropoda orbicularis</i> (Koch)	48	32,134	669.5	15	946	63.1	22	3,488	158.6
Parasitidae	5	309	61.8	11	114	10.4	4	11	2.8
Digamasellidae	17	3,049	179.4	11	772	70.2	14	1,731	123.6
Laelapidae	15	1,634	108.9	19	4,484	236.0	11	1,544	140.4
ACARIFORMES									
Cheyletidae									
<i>Cheyletus malaccensis</i> Oudemans	38	13,456	354.1	20	4,147	207.4	20	5,862	293.1
Acarophenacidae									
<i>Acarophenax mahunkai</i> Steinkraus & Cross	2	119	59.5	0	0	0	1	60	60.0
Acaridae	45	121,756	2,705.7	30	120,178	4,005.9	29	236,699	8,162.0
Eremulidae	1	27	27.0	6	86	14.3	1	18	18.0

^aNo. of positive samples is the number of samples (out of the total samples from each house type) from which one or more specimens of the mite species or family were recovered.

among the macrochelid mites, was found in 19% and 16% of the litter samples in 1993 and 1994, respectively. It should be noted that in much of the literature *M. merdaria* is named *M. merdarius* but Farrier & Hennessey (1993) changed the spelling of the trivial name to agree with the gender of the generic name. *Fuscuropoda marginata* (Koch) (= *F. vegetans* [De Geer] according to Farrier & Hennessey [1993]) (Urodinychidae) was collected in 15% and 56% of the samples in 1993 and 1994, respectively. Acarid mites (Acaridae) were abundant in litter, occurring in 85% and 92% of the samples in 1993 and 1994, respectively.

Insects collected in broiler chicken litter belonged to seven orders (Coleoptera, Lepidoptera, Diptera, Dermaptera, Hymenoptera, Psocoptera, and Collembola) and 8 families (Table 2). *Alphitobius diaperinus* was the dominant insect species, with larvae and adults collected from all litter samples in both years. The beetle *Carcinops pumilio* (Erichson) also was collected, with larvae and adults occurring in 50% of the samples in 1993 and in 42% (larvae) and 38% (adults) of the samples in 1994. Larvae of the lepidopteran *P. farinalis* were present in three litter samples (12%) in 1993 and in only one sample in 1994. Larvae of the house fly *Musca domestica* L. were found in two samples in 1993 and in only one sample in 1994. Earwigs (Dermaptera: Forficulidae) and ants (Hymenoptera: Formicidae) were present in three and two litter samples, respectively, in 1993. Only one sample contained booklice (Psocoptera: Liposcellidae) in 1993. Springtails (Collembola: Hypogastruridae) occurred in one and two samples in 1993 and 1994, respectively.

Turkey brooder litter. In turkey brooder houses, litter samples contained mites that belonged to two orders and nine families (Table 1). *Cheyletus malaccensis* was the most common mite species in the litter samples; it was found in 67% of the samples in both years. *Trichouropoda oribicularis*, the second most common species, occurred in 67% and 33% of the litter samples in 1993 and 1994, respectively. *Fuscuropoda marginata* was found in 20% and 40% of the litter samples and macrochelid mites were found in 60% and 80% of the litter samples in 1993 and 1994, respectively. *Macrocheles merdaria* was the most abundant species of macrochelid mite. Acaridae was the most frequently occurring mite family in the litter samples in both years.

Insects collected from litter samples in turkey brooder houses belonged to seven orders and 14 families (Table 2). *Alphitobius diaperinus* was the dominant species, with larvae and adults found in all litter samples, except in one sample with only adults in 1994. *Carcinops pumilio* was the second most abundant insect species in the litter; adults were found in 47% and 33% of the samples in 1993 and 1994, respectively, and larvae occurred in 53% and 33% of the samples in 1993 and 1994, respectively. Larvae of *P. farinalis* were found in 20% of the litter samples in both years. House fly larvae occurred in 13% and 27% of the litter samples in 1993 and 1994, respectively. Earwigs (Forficulidae) were found in 20% of the litter samples in 1993, and ants (Formicidae) occurred in 13% of the samples in both years. Booklice (Liposcellidae) were present in one and four litter samples in 1993 and 1994, respectively.

Turkey growout litter. In turkey growout houses, litter samples had mites that belonged to two orders and nine families (Table 1). *Trichouropoda*

Table 2. Insects recovered from litter (2-liter samples) collected from broiler chicken houses (51 samples, 26 houses, 18 farms), turkey brooder houses (30 samples, 15 houses, 15 farms), and turkey growout houses (29 samples, 15 houses, 15 farms) in North Carolina.

Insect Classification	Broiler chicken			Turkey brooder			Turkey growout		
	No. Positive Samples ^a	No. Recovered	Mean No. Per Positive Sample	No. Positive Samples	No. Recovered	Mean No. Per Positive Sample	No. Positive Samples	No. Recovered	Mean No. Per Positive Sample
COLEOPTERA									
Tenebrionidae									
<i>Alphitobius diaperinus</i> (Panzer), adults	51	4,962	97.3	29	1,193	41.1	29	1,557	53.7
<i>A. diaperinus</i> , larvae	51	20,408	400.2	30	5,275	175.3	29	6,809	234.8
<i>Palorus subdepressus</i> (Wollaston), adults	3	14	4.7	6	24	4.0	3	12	4.0
Unidentified spp., adults	2	17	8.5	0	0	0	0	0	0
Histeridae									
<i>Carcinops pumilio</i> (Erichson), adults	23	530	23.0	12	123	10.3	21	420	20.0
<i>C. pumilio</i> , larvae	22	737	33.5	13	227	17.5	22	485	22.1
<i>Dendrophilus xavieri</i> Marseul, larvae	1	14	14.0	0	0	0	0	0	0
<i>Gnathoncus nanus</i> (Scriba), adults	0	0	0	1	3	3.0	0	0	0
Rhizophagidae									
<i>Monotoma</i> sp., adults	0	0	0	1	2	2.0	2	5	2.5
Scarabaeidae									
<i>Trox</i> sp., adults	0	0	0	1	3	3.0	0	0	0
Staphylinidae, adults	0	0	0	8	49	6.1	1	2	2.0
Nitidulidae, adults	0	0	0	1	2	2.0	0	0	0
Anthicidae, larvae	0	0	0	1	3	3.0	0	0	0
Scolytidae, adults	0	0	0	5	7	1.4	2	3	1.5
LEPIDOPTERA									
Pyralidae									
<i>Pyralis farinalis</i> L., larvae	4	64	16.0	6	284	47.3	1	3	3.0
DIPTERA									
Muscidae									
<i>Musca domestica</i> L., larvae	3	17	5.7	6	300	50.0	9	137	15.2
DERMAPTERA									
Forficulidae	3	24	8.0	3	24	8.0	1	1	1.0
HYMENOPTERA									
Formicidae, adults	2	6	3.0	4	13	3.3	1	4	4.0
PSOCOPTERA									
Liposcellidae, adults	1	5	5.0	5	15	3.0	0	0	0
COLLEMBOLA									
Hypogastruridae, adults	3	538	179.3	1	504	504.0	1	2	2.0

^aNo. of positive samples is the number of samples (number of the total samples from each house type) from which one or more specimens of the insect species, stage or family were recovered.

oribicularis was the most common mite species and occurred in 71% and 86% of the litter samples in 1993 and 1994, respectively. *Cheyletus malaccensis*, the second most abundant mite species, was found in 57% and 86% of the litter samples in 1993 and 1994, respectively. *Fuscuropoda marginata* occurred in 29% and 86% of the samples in 1993 and 1994, respectively. Macrochelid mites occurred in 43% and 64% of the litter samples in 1993 and 1994, respectively. *Macrocheles merdaria* was the most common macrochelid from the litter samples. Acaridae was the most frequently occurring mite family and was found in all samples in both years.

In litter samples from turkey growout houses, the recovered insects belonged to seven orders and 13 families (Table 2). *Alphitobius diaperinus* was the most abundant insect species; larvae and adults occurred in all samples collected in both years. *Carcinops pumilio* larvae were present in 79% and 73% of the samples in 1993 and 1994, respectively, and adults in 79% and 67% of the samples, respectively. *Pyralis farinalis* larvae were present only in one litter sample in 1994. House fly larvae were found in 60% of the litter samples in 1994. Only one litter sample contained ants and earwigs in 1993, and also one sample had springtails in 1994.

Mites collected with sentinel beetle eggs. The predatory mite *A. mahunkai* was retrieved from paper assemblies with beetle eggs inserted in litter samples from a turkey growout house (Cripple Creek Farm, Kenansville, Duplin County; 15 November, 1993; Collection no. NC8-G), and two broiler houses (Rose Hill Farm, Nashville, Franklin County; 14 December, 1993; Collection no. NC24-6; and P. Breswell Farm, Nashville, Franklin County; 14 December, 1993; Collection no. NC25-1). The turkey growout house had a pine shaving litter floor. The broiler house on the Rose Hill Farm had pine shavings topped with peanut hulls, and the broiler house on the P. Breswell Farm had only pine shavings on the floor.

Aside from *A. mahunkai*, other mites that are nonparasitic on beetle eggs were retrieved from the litter samples by using the paper assembly with sentinel beetle eggs. They were *C. malaccensis*, *F. marginata*, *T. oribicularis*, and laelapid and acarid mites. These mites did not destroy or parasitize the beetle eggs. *Cheyletus malaccensis*, a predator of other mites (Hughes 1961), occurred in 40%, 31%, and 22% of litter samples collected from brooder, growout, and broiler houses, respectively. *Fuscuropoda marginata* occurred in 7% and 16% of brooder and broiler litter samples, respectively, and no specimens of this mite were retrieved from growout house litter. Acarid mites occurred in all litter samples from brooder and growout houses and in only 60% of samples from broiler houses. Laelapid mites occurred in only 1% of brooder litter samples and none from growout and broiler samples.

Discussion

Predatory and nonpredatory mites were commonly associated with poultry litter samples. Among predatory mites, *C. malaccensis* was the most dominant species in litter samples from broiler chicken, turkey brooder, and turkey growout houses. This species is mostly a predator of acarid mites (Hughes 1961) and occurs in feed trash, swine barns, and rodent cages (Summers &

Price 1970). Rueda (1985) observed *C. malaccensis* in association with house dust primarily from beds and carpets in houses of humans. Macrochelid mites, particularly *M. muscaedomesticae* and *M. merdaria*, were commonly found in broiler, turkey brooder, and turkey growout houses. Both species are known predators of house fly eggs and early instar housefly larvae (Axtell 1963, De Jesus & Rueda 1990). *Fuscuropoda marginata* also was common in litter samples from three types of poultry houses, and it feeds on house fly eggs and early instars (O'Donnel & Axtell 1965, Willis & Axtell 1968).

Acarophenax mahunkai, a potential biocontrol agent for *A. diaperinus*, was collected from floor litter in broiler and turkey growout houses by using the black paper assembly with sentinel beetle eggs; no *A. mahunkai* were obtained by using the Tullgren funnel procedure. These mites may have been present in the litter samples extracted by Tullgren funnels, but their small size made them impossible to recognize among litter debris under the dissecting microscope. In contrast, *A. mahunkai* were easily observed crawling on the beetle eggs attached to the black paper. We observed *A. mahunkai* destroying *A. diaperinus* eggs by sucking their contents. This mite acts like a parasitoid (Lindquist 1983) in killing host eggs and requiring only one beetle egg host to complete the development of mite progeny. Steinkraus & Cross (1993) observed the life history and "parasitism" by this mite under laboratory conditions. Female mites parasitized *A. diaperinus* egg masses and caused 76% reduction in egg hatch; male mites were not parasitic on beetle eggs.

Other mites that do not prey on *A. diaperinus* or *Musca domestica* were found in most litter samples from three types of houses. Although we collected a few unidentified species of Parasitidae, we did not find *Poecilochirus monospinosus* Wise, Hennessey and Axtell in the samples. This species is predaceous on house fly eggs and larvae inhabiting poultry manure (Wise et al. 1988).

Among the insect fauna, *A. diaperinus* was the dominant species in litter samples from all farms. Although this beetle occasionally preys on eggs of filth flies, it is considered a major pest in commercial poultry houses (Axtell & Arends 1990) rather than a beneficial species. The beetles harbor and potentially spread a variety of viral, bacterial, and fungal pathogens and serve as intermediate hosts of cestodes parasitizing poultry (Despins et al. 1994). The larvae often tunnel into building insulation materials for pupation sites and thus cause structural damage requiring expensive repairs (Safrit & Axtell 1984). Broiler chicks and turkey poults actively feed on beetle larvae and adults; this may result in abnormal growth and development of the birds as well as disease transmission to the birds (Despins & Axtell 1994, 1995).

Carcinops pumilio was common in litter samples from broiler, turkey brooder, and turkey growout houses. This species is a predator of house fly eggs and first instars of the house fly (Geden & Axtell 1988, Wilhoit et al. 1991). The pyralid moth *P. farinalis* was found in litter samples collected from all three types of poultry houses. This moth is an occasional pest of stored grain products (Curtis & Landolt 1992). We observed the larvae of this moth causing clumping of the pine shavings in poultry houses. Also, the moth larvae may cause clumping of the feed and interfere with the feed delivery system. When adult moths emerge in large numbers, they may become a nuisance

inside the poultry houses. House fly larvae also were found in litter samples from all three types of houses, particularly in slightly moist samples. Some parts of poultry house floors had slightly wet litter due to leaking water systems. Many of the house fly larvae collected were in the last instar; probably they moved to pupate in the drier portions of the litter where most of the samples were collected. A variety of other insects (ants, booklice, earwigs, springtails) was found associated with the litter from all three types of houses.

Our survey showed that the arthropod fauna of litter from broiler chicken and turkey houses included both predatory and nonpredatory species. Predatory arthropods, particularly histereid beetles and macrochelid mites, are potential biological control agents to suppress populations of house flies. Acarophenacid mites may suppress populations of *A. diaperinus*. Nonpredatory species may have some role as scavengers and serve as food for other arthropods in the microecosystem in poultry houses. Conserving the nonpest species of naturally occurring predatory and nonpredatory arthropods associated with floor litter should be considered in developing and implementing any pest management program in poultry broiler, brooder, and growout houses.

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Winter Survival of *Solenopsis invicta* and the *Solenopsis* hybrid (Hymenoptera: Formicidae) in Georgia¹

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ABSTRACT The hybrid imported fire ant (*Solenopsis invicta* Buren × *S. richteri* Forel) infests the northern half of Alabama and the Alabama border counties of northern Mississippi and northern Georgia in the United States. Its habitat in Georgia includes regions that were previously uninfested by imported fire ants. The winter temperatures in these regions were considered to be too cold for imported fire ant survival. The movement of the hybrid fire ant into previously uninfested northern counties of Georgia prompted this study to monitor the winter survival of the hybrid. Hybrid fire ant colonies were monitored for five consecutive winters, and their survival was compared with colonies of red imported fire ants (*S. invicta* Buren) in northern and southern Georgia during these five winters. Data showed no evidence that the hybrid can survive the cold winter temperatures of Georgia better than the red imported fire ant. Effect of location (southern or northern Georgia) on ant colonies was not significant, but the environmental conditions varying from one year to another numerically influenced colony survival of imported fire ants.

KEY WORDS Hymenoptera, Formicidae, *Solenopsis invicta*, overwinter survival, *Solenopsis* hybrid

At least two accidental importations of fire ants (*Solenopsis* spp.) into the United States have occurred in this century. The black imported fire ant, *Solenopsis richteri* Forel, and the red imported fire ant, *S. invicta* Buren, were probably introduced from their native homelands of Argentina and Brazil in the 1910s and 1930s, respectively. Their spread throughout the southern United States has been steady, with the most rapid spread occurring during the 1940s and 1950s (Lofgren et al. 1975). In South America, *S. invicta* appears to occupy an area from 10° to 34° south latitude, and *S. richteri* occupies an area from south latitudes 30° to 38° (Buren et al. 1974, Ross & Trager 1990). In the United States, *S. invicta* occupies areas from latitude 26° to 34°, and *S. richteri* occupies a very small area around 35° latitude (Diffie et al. 1988).

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The spread of imported fire ants within Georgia during the past 15 yr has been phenomenal. When *S. invicta* first arrived in Georgia in the early 1950s, it rapidly moved into regions considered prime habitat because the climate and terrestrial ecosystems were ideal for the establishment of colonies. Nonetheless, their most recent spread in Georgia has been into areas considered by some to be too cold for successful imported fire ant habitation: areas beyond the -12.2°C (10°F) minimum temperature isotherm (Maxwell 1976, Thorvilson et al. 1992). Others suggested a wider temperature range for the imported fire ants and set the lower limit at the -17.8°C (0°F) isotherm (Anonymous 1972, Pimm & Bartell 1980); however, this appeared exaggerated because the ants had yet to reach even the -12.2°C isotherm.

Morrill (1977) monitored overwintering survival of *S. invicta* along its northern range in Georgia and found low mortality during a normal winter in the central part of the state. According to this study, fire ants would be capable of extending their northern range if low temperatures were the only limiting factor. Three years after Morrill's (1977) study, fire ants were found in Floyd County, Georgia (County Extension Director Louie Canova, pers. comm.), about 130 km north of Morrill's test sites. The apparent rapid northward movement of imported fire ants in Georgia was found to be a consequence of the presence of hybrid (*Solenopsis invicta* \times *S. richteri*) fire ants in the northwestern part of the state (Diffie et al. 1988). Most likely, this purported northward movement was actually an eastward movement of the hybrid ants from Alabama instead of a northern movement of *S. invicta* from Morrill's location (County Extension Director Louie Canova, pers. comm.). Nevertheless, the question remained as to whether either of these imported fire ant forms (*S. invicta* or the *Solenopsis* hybrid) could survive in the northern regions of Georgia.

Because the *Solenopsis* hybrid is found in areas remote from imported fire ant research centers, very little research has been conducted and published on this ant. Diffie et al. (1988) first showed the approximate range of the hybrid extending from Mississippi to Georgia. Genetic studies conducted by Ross et al. (1987) and Ross & Robertson (1990) proved that the hybrid is a reproductively viable product of *S. invicta* \times *S. richteri* crosses and possesses characteristics of both parental lines. Trager (1991) described the phenotypic characteristics as similar to *S. richteri*; however, the hybrid can be distinguished from the parental forms morphologically (Ross et al. 1987). Obin & Vander Meer (1989) investigated species recognition and related it to hybrid zone dynamics. Diffie & Sheppard (1989) found no differences in the supercooling capacity of the hybrid relative to either parental species.

The hybrid's potential impact on uninfested land and also on the existing *S. invicta* population warrants additional research on the biology and control of the hybrid ant. The present 5-yr study was conducted to compare the survival ability of the *Solenopsis* hybrid and *S. invicta* through the winter months in Georgia.

Materials and Methods

Imported fire ant colonies were examined in the fall in randomly selected pastures in three Georgia counties (Fig. 1). Active colonies were marked in



Fig. 1 Map of Georgia indicating counties in which winter fire ant survival study was conducted from 1985–1990.

each county with orange surveyor stakes (Table 1). These colonies were reexamined for activity the following spring as described by Morrill (1977). Randomly selected hybrid colonies were identified by using morphological color pattern characters described by Trager (1991) and marked in pastures in Floyd County, Georgia. Pastures in Morgan County were used as the north Georgia *S. invicta* site of randomly selected colonies each year. These colonies represented the northernmost boundary of *S. invicta* in the state. Colonies in pastures in Tift County were used for the south Georgia site. These colonies represented well-established habitats for comparison with the two recently

Table 1. Number of marked active fire ant colonies recovered each spring following the winters of 1985–1990 in three Georgia locations.

	Number of active <i>Solenopsis</i> colonies					
	<i>S. hybrid</i>		(north) <i>S. invicta</i>		(south) <i>S. invicta</i>	
	pre	post	pre	post	pre	post
1985–1986						
1986–1986	80	78	91	88	82	78
1986–1987	50	44	61	59	71	63
1987–1988	44	43	69	65	46	41
1988–1989	45	33	77	70	64	59
1989–1990	70	70	67	64	64	61

infested areas of northern Georgia. Each year three different randomly selected fields were used and the sampling procedure was repeated. Survival was determined by comparing the number of active colonies recovered in the spring to the number of active colonies marked the previous fall. Colony movement was monitored by searching for active colonies within a meter of the original colony. Movement and natural mortality were assumed to be equal in the three locations. Assuming low temperatures to be the principal cause of mortality, the percentage of surviving colonies (number of active colonies/number of total colonies) was calculated for each area. This percentage was compared to the remaining two areas to determine if location (northern Georgia vs. southern Georgia) affected survival, and if hybrid colonies survived northern Georgia temperatures more effectively than did *S. invicta* colonies (Floyd Co. vs. Morgan Co.)

Ten-centimeter deep soil temperatures were obtained from the National Weather Service stations in Calhoun (near the hybrid area), Watkinsville (close to the northern Georgia *S. invicta* area), and Tifton (close to the southern Georgia *S. invicta* area) (Fig. 1). The number of days in each month with subfreezing temperatures at the 10-cm level was noted as an indicator of severity of cold weather.

Percent colony survival was computed and analyzed by two-way analysis of variance (SAS Institute, Proc GLM) to test the effect of location or ant form and year (SAS 1989). The percent survival data were transformed to arcsine values prior to analysis, but means were back transformed to percentage values for presentation. If significant, the PDIFF option of the PROC GLM (SAS 1989) was used to make pairwise comparisons between means by using Bonferroni *t*-test ($P = 0.05$). Collective yearly temperature data were analyzed similarly. Chi-square analyses also were used to assess the survival data in relation to ant form and year.

Table 2. Pooled colony survival of hybrid and red imported fire ants during the winters of 1985–1990 in three Georgia locations.

Location ^a	Ant Form ^b	Number of ant colonies		% Survival
		Fall	Spring	
Morgan	<i>S. invicta</i>	365	346	94.8
Floyd	<i>S. hybrid</i>	289	268	92.7
Tift	<i>S. invicta</i>	327	302	92.4

^aChi-square (df= 2) computed from 3 × 2 (location × no. of ant colonies) contingency table = 0.066, *P*= 0.968.

^bChi-square (df= 1) computed from 2 × 2 (ant form × no. of ant colonies) contingency table = 0.009, *P*= 0.923.

Results and Discussion

Location and ant form had no significant effect on the number of marked colonies surviving through the winter, as evidenced by the chi-square homogeneity test (Table 2). Thus, the number of marked ant colonies in the fall did not vary for the two ant forms or in northern or southern Georgia locations. The percent survival of these colonies differed little for the combined 5-yr study period. For southern and northern *S. invicta* populations, respectively, survival ranged from 92.4% (Tift County) to 94.8% (Morgan Co.) or for hybrid and *S. invicta* populations, respectively, it ranged from 92.7% to 94.8%, which indicates a very narrow range and lack of association between colony survival and ant forms.

Over individual years, the survival of hybrid ants was better numerically than *S. invicta* in 3 of the 5 yr. In one of the remaining years (1988–1989) the hybrid ants' survival (73.3%) was dramatically less than the survival of either *S. invicta* population (90.7% and 92.2%, Fig. 2). However, these differences were not statistically significant in either analysis at *P*= 0.05.

In both *S. invicta* locations, nearly 18% more colonies survived than did the hybrid colonies in 1988–1989 (Fig. 2) despite that the average monthly temperatures for the test period were almost identical for the two northern locations (Floyd Co. and Morgan Co.) and closely related to the third (Tift Co.) location (Fig. 3). The differences in survival rates between the two ant forms in other years were not notable. The *S. invicta* colonies in southern Georgia did not fare better numerically than the colonies in the two northern Georgia locations except in 1988–1989 (Fig. 2).

Analysis of variance of the survival over the entire test period indicated no significant effect due to location, ant form, or test years. When the three locations are combined, survival over the winter of 1988–1989 was noticeably

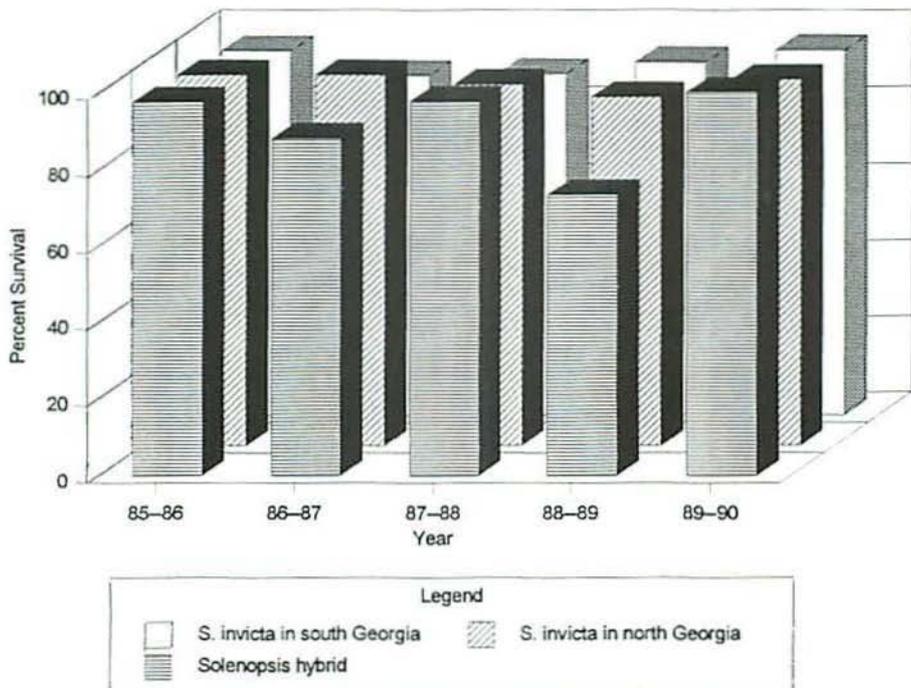


Fig. 2 Percent survival of hybrid imported fire ant and red imported fire ant colonies in northern Georgia, and red imported fire ants in southern Georgia following five winters.

lower than that over the winter of 1989-1990 (85.5% vs. 96.9%). However, the survival experienced during these two winters did not differ significantly from each other (ANOVA, $P < 0.05$) or from the other winters, and ranged from 91%-96%.

Hung & Vinson (1978) listed food, nest material, temperature, moisture, and relationship to other ants as the chief factors in limiting distribution. Explanations for the death of a colony during the winter months could include old age, starvation (Anonymous 1958), or lethal low temperatures. In this study, colonies were randomly selected and thus were assumed to range similarly in age and vigor in all three locations. Foragers are the only members of the colony to spend an appreciable amount of time above ground. Starvation could occur if extended periods of subfreezing ambient temperatures limited foraging and food availability, and severely stressed the colonies. Since foragers are the only exposed members of the colony, the death of the remainder of the colony in relation to freezing would be dependent upon soil temperature. Buren et al. (1974) speculated that winter kill is roughly

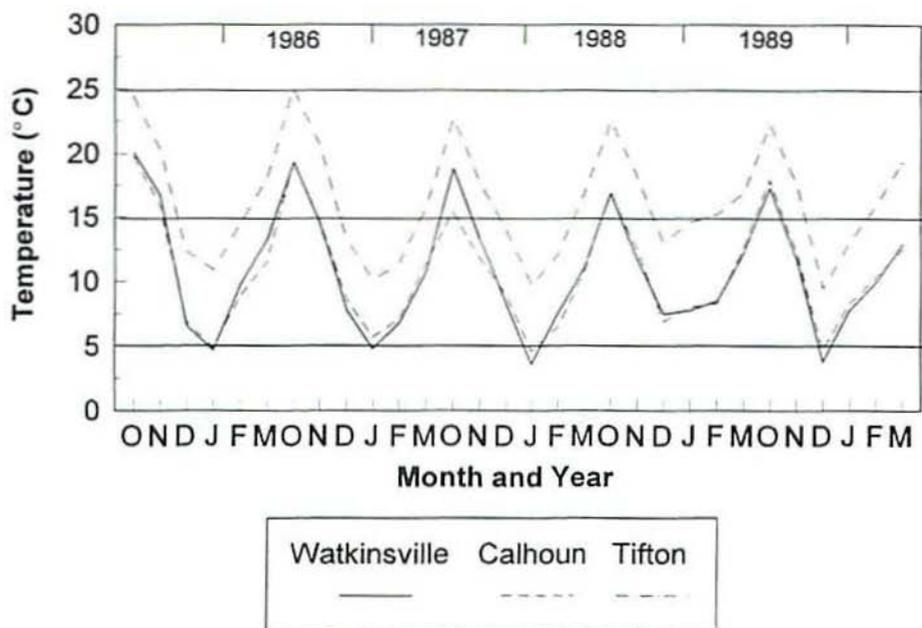


Fig. 3 Monthly average soil temperatures at 10 cm for three Georgia locations (1985–1990).

proportional to the depth at which the soil becomes frozen, and a depth of 10 cm would be sufficient to induce mortality. Data from the National Weather Service showed similar monthly average temperatures for the two northern Georgia locations. The southern Georgia location had a similar but warmer trend. During the test period, 1 d of subfreezing temperature at the 10-cm soil level was experienced during December 1985 and 4 d in January 1986 in Morgan County. Survival at that location was 96.7% in 1985–1986. Morgan County also experienced a total of 5 d of subfreezing temperatures during December 1989 and 1 d in January 1990. Survival in Morgan County was 95.5% during that year. Floyd County experienced 5 d of subfreezing temperatures at the 10-cm soil level in December 1989. Fire ant survival was 100% at that location during 1989–1990.

The poorest survival (73.3%) occurred in Floyd County (*S. hybrid* area) in 1988–1989. In February 1989, a Canadian front dropped temperatures 15.6°C (60°F) during the day in parts of the United States. This front barely crossed into Georgia but did reach Floyd County. The fire ants here may have suffered high mortality due to the rapid temperature drop. The minimum 10-cm soil temperature during this 5-d period fell from 8.3°C (47°F) on 22 February to 2.2°C (36°F) on 24 February 1989. The temperature remained at this level for 3 d before rising again. This occurrence during the otherwise most temperature-

stable winter of the test period suggests the ants were not acclimated to the sudden cold temperatures, and this may have been detrimental to their survival.

All colonies monitored in this study were randomly selected, established colonies. The effect of low winter temperatures on newly formed colonies (less than 1 yr old) could be more significant (Markin et al. 1973). If hybrid fire ant colonies are allowed to establish, they will be able to withstand Georgia winters underground. However, they do not appear to be superior to *S. invicta* in surviving cold temperatures, at least under the conditions of northern Georgia, corroborating the findings of Diffie & Sheppard (1989). The survival and eventual range expansion of *Solenopsis* hybrid colonies appear to be related to environmental conditions encountered rather than genetic constitution. The data suggest that the populations of *S. invicta* in northern and southern Georgia do not survive any better than the *Solenopsis hybrid* in northern Georgia. It is possible that subtle differences that may exist will be expressed as the populations continue to press northward. However, within Georgia, temperatures will likely not sufficiently challenge the ants in a manner that will allow this expression.

The movement of imported fire ants into previously uninfested areas of northern Georgia will result in the establishment of high numbers of colonies for several years. These numbers will decline as mature colonies compete for resources (Lofgren et al. 1975); however, due to the high number of mounds, the effect on the ecosystem may be more pronounced during this time as compared to later years when the numbers decrease (Porter 1992).

Movement of *S. invicta* is limited at the moment by the mountainous region of northeast Georgia. This area has less open pastureland than the western part of the state where the *Solenopsis* hybrid is found. Imported fire ants are typically found in open areas; thus, the densely wooded mountains appear to pose an obstacle to the establishment of fire ant colonies. The effect of winter temperatures in the mountains may already be limiting *S. invicta*'s spread. The *Solenopsis* hybrid in western Georgia should be able to continue its northward expansion until it meets the more severe winters of the Tennessee mountains.

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Green Cloverworm (Lepidoptera: Noctuidae) as an Alternate Host for Natural Enemies of Lepidopteran Pests of Soybean in South Carolina¹

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ABSTRACT Parasitoids, pathogens, and predators of lepidopterans were monitored during 1985 and 1986 in 14 fields of soybean, *Glycine max* (L.) Merrill, near Blackville, South Carolina. Chemical (permethrin at 0.0225 kg [AI]/ha) control of early-season green cloverworm, *Plathypena scabra* (F.), in soybean fields did not have an impact on predator population density later in the season when compared with untreated control fields. Chemical control of green cloverworm did not affect incidence of the entomopathogenic fungus *Nomuraea rileyi* (Farlow) Samson in subsequently occurring lepidopteran populations during 1985 when green cloverworm and soybean looper, *Pseudoplusia includens* (Walker), populations overlapped. However, its incidence was enhanced in soybean looper populations in untreated fields during September 1986 when green cloverworm did not occur in an overlapping generation with soybean looper. *Cotesia marginiventris* (Cresson) was the most prevalent parasitoid, and its parasitism of soybean looper was not dependent upon its prior occurrence in green cloverworm populations.

KEY WORDS Lepidoptera, Noctuidae, *Plathypena scabra*, soybean, *Nomuraea rileyi*, *Pseudoplusia includens*, *Helicoverpa zea*, *Anticarsia gemmatalis*, predaceous arthropods, *Cotesia marginiventris*

Green cloverworm, *Plathypena scabra* (F.) (Lepidoptera: Noctuidae), is one of the most common lepidopteran species in leguminous agroecosystems. Pedigo (1971) reviewed work in which green cloverworm was associated with various host plants, including alfalfa, red clover, soybean, vetch, cowpea, and beans. In South Carolina, it is abundant during the spring on vetch and clover; during the summer and fall, it occurs on alfalfa and soybean (G. S. M., unpublished data).

Green cloverworm usually completes two overlapping generations in soybean during the growing season in South Carolina (Shepard et al. 1977). Populations seldom reach levels high enough to cause damage resulting in economic loss. Some

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of the major natural enemies of green cloverworm also are effective against more economically important noctuids such as the soybean looper, *Pseudoplusia includens* (Walker); corn earworm, *Helicoverpa zea* (Boddie); and velvetbean caterpillar, *Anticarsia gemmatilis* (Hübner). Two to three outbreaks of these pests per growing season often require application of insecticides to prevent economic damage to the crop. When natural enemies are not disturbed by pesticides, they have the potential to delay the need for insecticide application during the growing season (G.S.M., unpublished data).

Green cloverworm may serve as an alternate host for natural enemies in South Carolina and other areas where it occurs early in a succession of lepidopterans. Each of the major lepidopterans (green cloverworm, corn earworm, soybean looper, and velvetbean caterpillar) in soybean is attacked by the parasitic wasp *Cotesia marginiventris* (Cresson) (McCutcheon & Turnipseed 1981) and the entomopathogenic fungus *Nomuraea rileyi* (Farlow) Samson (Gilreath et al. 1986). Gilreath et al. (1986) reported that a positive relationship existed between green cloverworm population density and subsequent incidence of *N. rileyi* in soybean looper ($r = 0.64$, $P < 0.10$) during 1975–1977 in South Carolina.

The most common predators of lepidopterans in South Carolina soybean include Araneae, *Nabis* spp. (Hemiptera: Nabidae) and *Geocoris* spp. (Hemiptera: Geocoridae) (Turnipseed 1972). Beneficial arthropods that attack lepidopterans have the potential to help to control pest populations in soybean. This study was undertaken to determine the role of green cloverworm as an alternate host for natural enemies of lepidopteran pests of soybean in South Carolina.

Materials and Methods

During a 2-yr study (1985 and 1986), seven pairs of soybean fields were planted on or near the Clemson University Edisto Research and Education Center (EREC), Blackville, South Carolina. The fields in each pair were planted on the same date less than 0.4 km apart in 96-cm rows by using conventional tillage practices. Herbicides and nematicides were applied according to guidelines (Clemson University Cooperative Extension Service 1986). Weekly sampling for lepidopterans was initiated when plants were in the V-1 stage of development (Fehr et al. 1971). A randomized complete block design was used with four blocks (pairs of fields) and two treatments in 1985 and three blocks and two treatments in 1986. One field (ca. 2 ha) of each pair was treated as needed with permethrin (0.0225 kg [AI]/ha) by using a high clearance sprayer to maintain green cloverworm populations near zero until the occurrence of subsequent lepidopterans; the other field of each pair was the untreated control. The planting and treatment dates are depicted in Table 1. Previous studies indicate that planting date may affect green cloverworm increase (McCutcheon & Turnipseed 1989). However, other lepidopteran populations were not affected by planting date in those studies, and major natural enemies occurred in soybean planted on all dates.

Weekly larval collections and population estimates of arthropods were taken by using a 1.2 m × 1.0 m ground cloth (Turnipseed 1974). Each field was divided

Table 1. Pairs of soybean fields at seven locations near Blackville, South Carolina.

Fields	Planting date	Treatment dates ^a
	<u>1985</u>	
Pair A ^b	6 May ^c	25 June, 22 July
Pair B ^b	12 June ^c	19 July
Pair C ^b	1 July ^c	2 August, 26 August
Pair D ^d	7 June	25 June, 19 July
	<u>1986</u>	
Pair A ^b	14 June	21 July, 19 August
Pair B ^b	14 June	21 July, 19 August
Pair C ^b	14 June	19 August

^aOne field of each pair was treated at 0.0225 kg [AI]/ha with permethrin.

^bBraxton - maturity group VII.

^cSweet corn (0.8 ha) was planted on 2 April between treated and untreated fields of each pair to trap corn earworm.

^dCentennial - maturity group VI.

into quadrants by placing flag markers halfway along the field in both directions (i.e., along the rows and across the rows). Two sampling sites were near the center of each quadrant and two were near the center of the field. A total of 10 single-row beats (1 m each) was taken weekly. If necessary, up to 10 additional single-row beats were taken to collect 80 larvae of each species. The following predatory taxa were monitored: *Geocoris* spp., *Nabis* spp., *Notoxus* sp. (Coleoptera: Anthicidae), *Orius insidiosus* Say (Hemiptera: Anthocoridae), *Chrysopa* sp. (Neuroptera: Chrysopidae), Formicidae, and Araneae. Lepidopteran larvae (green cloverworm, soybean looper, corn earworm, and velvetbean caterpillar) were separated according to species and counted as they were collected. Larvae of corn earworm, because of their cannibalistic behavior, were placed singly in 30-ml plastic cups with a modified pinto bean-wheat germ diet (Greene et al. 1976) at the collection site. Green cloverworm, soybean looper, and velvetbean caterpillar were transported to the laboratory in 0.5-liter ice cream cartons containing soybean foliage from the individual collection areas. All larvae were categorized as small (first and second instars), medium (third and fourth instars), or large (fifth instar or older) and placed singly in cups with modified pinto bean-wheat germ diet. Green cloverworm larvae were placed individually in 9-cm plastic Petri dishes containing moistened filter paper and fresh foliage that had been washed with a 1% solution of NaClO to eliminate fungal conidia from leaves. The leaves were rinsed with distilled water.

Larvae were maintained at $26 \pm 2^{\circ}\text{C}$, $60 \pm 5\%$ RH, and a photoperiod of 14:10 (L:D) h. Larvae were checked every 2 d, and dates of parasitoid emergence, pupation, and death from disease or unknown causes were

recorded. Pupae were held 2 wk for possible emergence of parasitoids. Adult parasitoids were identified by the senior author.

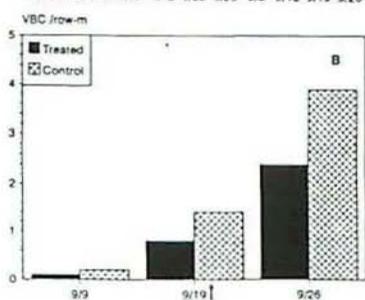
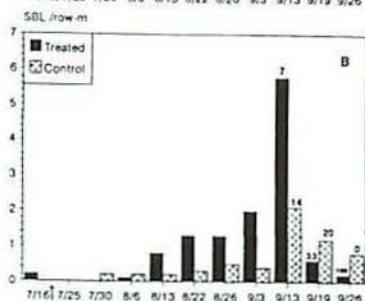
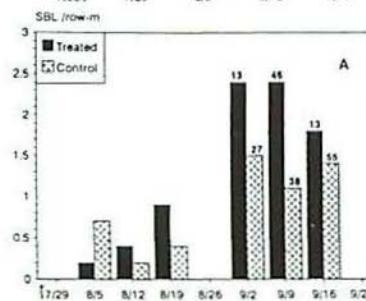
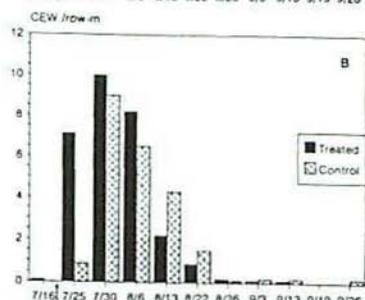
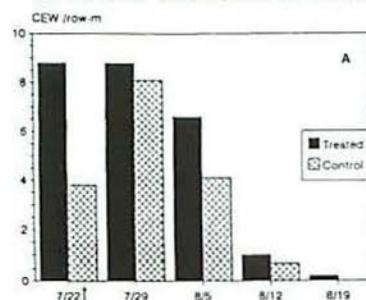
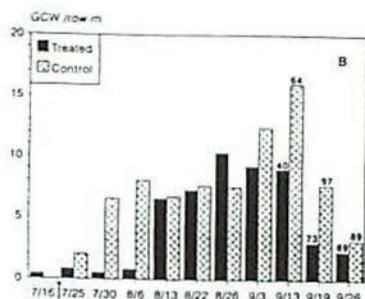
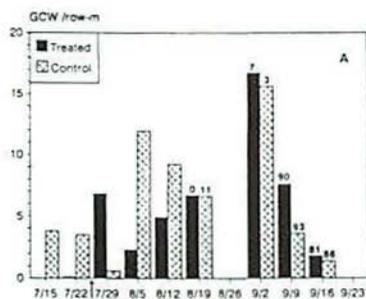
Parasitism and disease percentages were based on the total number of larvae collected minus the number of larvae that died of unknown causes. Only first through fourth instars were used in calculations of *C. marginiventris* parasitism because this parasitoid attacks small larvae and emerges from larvae before they are 12 d old (Kunnalaca & Mueller 1979). Percentages of parasitism, after being arcsine \sqrt{Y} transformed, were subjected to two-way analysis of variance (ANOVA). Densities of lepidopterans and predators were compared between treated and untreated fields of each pair (replicate) by analysis of variance (SAS Institute 1985).

Results and Discussion

Population density of lepidopterans. Population levels of lepidopterans at four sites sampled in 1985 showed significantly more green cloverworm larvae in untreated control fields than in the treated fields by the first week of August 1985 ($F = 13.36$; $df = 1, 3$; $P < 0.05$). No differences were detected across all sites in population density of subsequently occurring lepidopterans. Corn earworms did not reach economic threshold levels (18 caterpillars per meter before pod set) in either treated or untreated soybean (Clemson University Cooperative Extension Service 1986). Soybean looper populations remained far below 18 larvae per meter, the density at which significant foliage loss occurs. Velvetbean caterpillar populations also remained below the 18 large caterpillars per meter, which is their economic injury level. Differences in corn earworm and soybean looper population density (treated vs. untreated) were detected. On 22 and 25 July at sites A, B, and D (Figs. 1A, B, D), there were more corn earworms in treated soybean than in untreated controls ($F = 87.11$; $df = 1, 2$; $P = 0.068$). There were significantly more soybean loopers in treated soybean pairs A, B, and C than in the untreated soybean during early September ($F = 33.23$; $df = 1, 2$; $P < 0.05$) (Figs. 1A, B, C).

By mid-August in 1986, significantly more green cloverworms were detected in untreated control than in treated fields (Fig. 2) ($F = 40.12$; $df = 1, 2$; $P < 0.05$). The difference in populations lasted through early September when density exceeded 15 larvae per meter of row. Soybean loopers peaked during mid to late September. Generally, soybean looper populations increased after green cloverworm populations decreased. Velvetbean caterpillars exceeded economic threshold levels in Pair C on 1 October (Fig. 2C). Corn earworm populations remained below one per meter of row throughout the season in pairs A and B. Populations of corn earworm exceeded nine per meter of row in pair C with no differences in treated and untreated fields (Fig. 2C).

Incidence of fungal pathogens. The most prevalent pathogen of lepidopteran larvae in soybean fields was *N. rileyi*, which was more common in green cloverworm than in the other species (Fig. 1). The pathogen was detected from mid-August through September in green cloverworm larvae. There was an epizootic of *N. rileyi* in green cloverworm in treated and untreated 1985 pairs A, B, and C with no significant differences in percentage of infection in larvae from treated and untreated fields ($P > 0.05$). The pathogen was detected



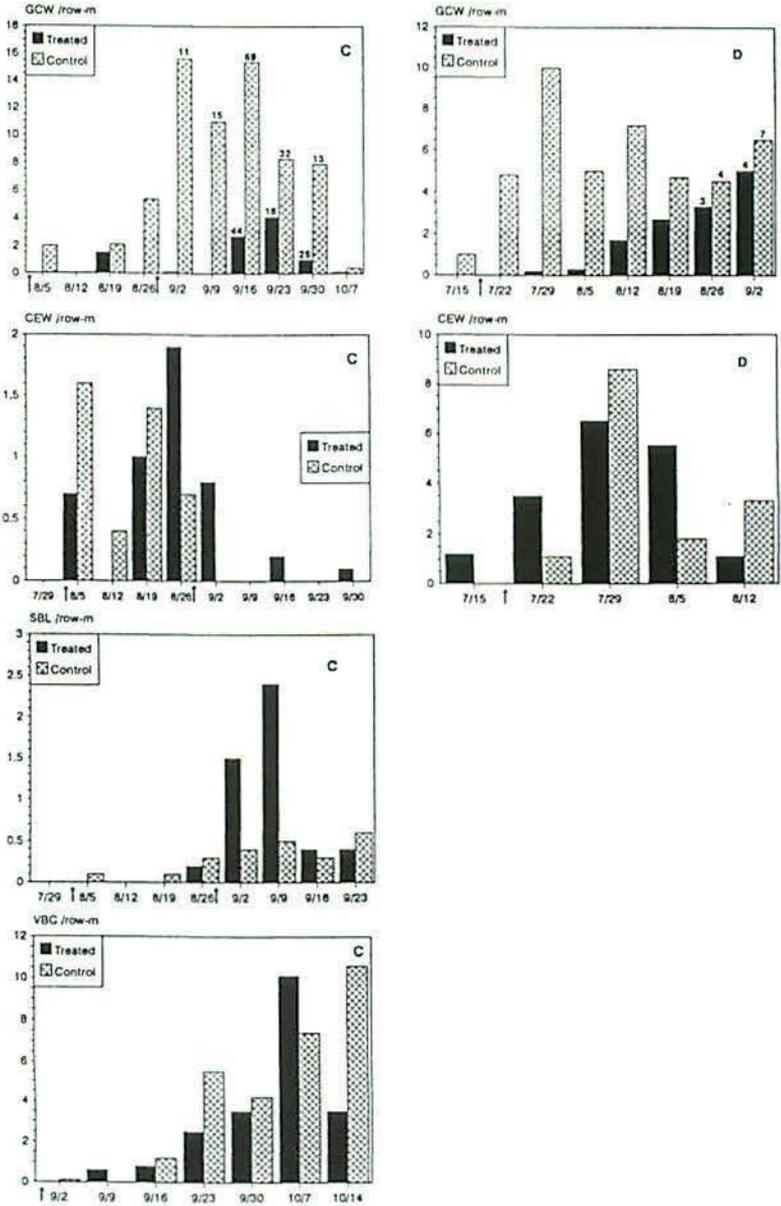
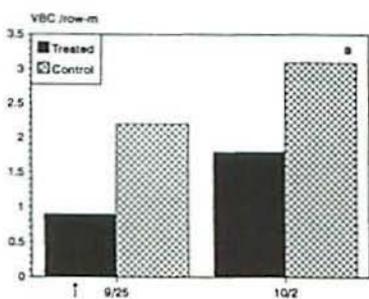
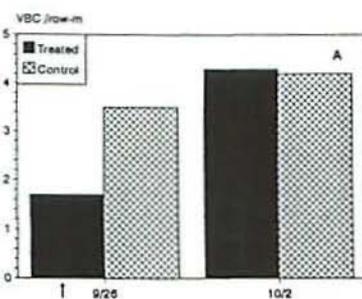
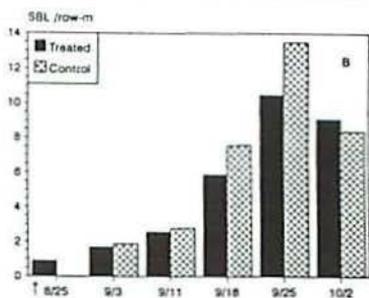
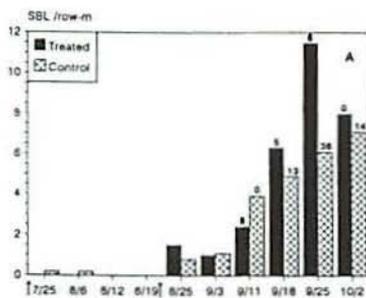
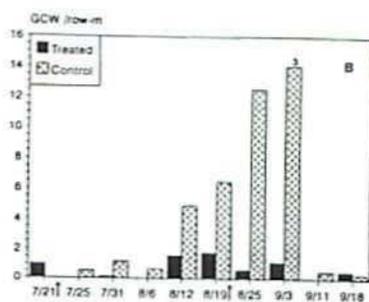
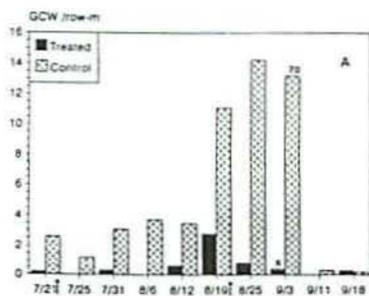


Fig. 1. Population density and incidence (%) of *N. rileyi* (denoted above each bar) for first through fourth instar green cloverworm (GCW), soybean looper (SBL), corn earworm (CEW), and velvetbean caterpillar (VBC) in soybean treated with 0.0225 kg [AI]/ha permethrin (denoted by arrow) and soybean that were untreated, (Blackville, South Carolina, 1985). Field pairs are denoted by upper case letters A, B, C, or D in upper right corner.



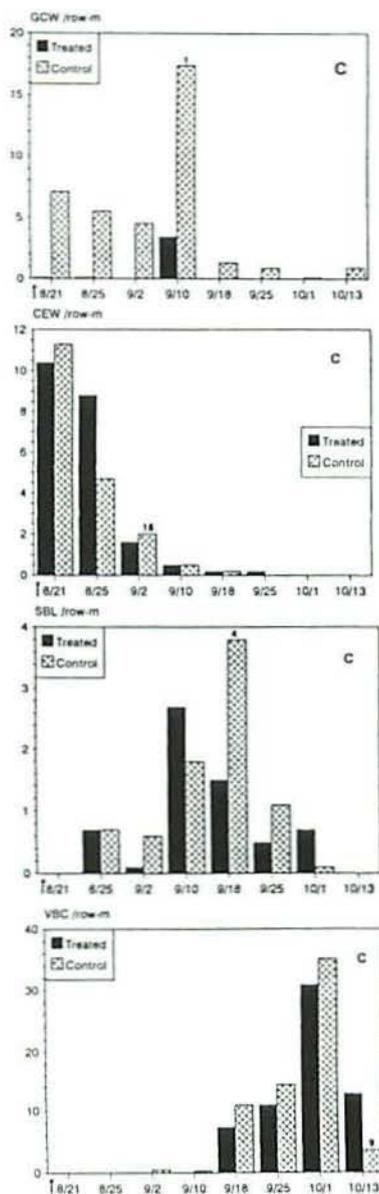


Fig. 2. Population density and incidence (%) of *N. rileyi* (denoted above each bar) for first through fourth instar green cloverworm (GCW), soybean looper (SBL), corn earworm (CEW), and velvetbean caterpillar (VBC) in soybean treated with 0.0225 kg [AI]/ha permethrin (denoted by arrow) and soybean that were untreated (Blackville, South Carolina, 1986). Field pairs are denoted by upper case letters A, B, or C in upper right corner.

on each sampling date in September in soybean loopers collected from treated and untreated soybean pairs A and B (Figs. 1A, B). Populations of soybean loopers were low and no differences in incidence of disease were detected. Pathogen incidence in total collections during the season was 80% in green cloverworm and 40% in soybean looper. *Nomuraea rileyi* was not detected in corn earworm or velvetbean caterpillar at any of these study sites. Data from other collections in 1985 showed that 13% of corn earworm and up to 20% of velvetbean caterpillars were infected with *N. rileyi* (G.S.M., unpublished data).

Only field pair A in 1986 exhibited high incidence of *N. rileyi* in green cloverworm populations (Fig. 2A). Its incidence in the untreated field was 70% in small and medium green cloverworm on 3 September during peak populations, which extended from mid-August to early September. Green cloverworm larval populations in the treated field remained low after the 19 August application of permethrin. Incidence of *N. rileyi* reached 36% in peak populations of soybean looper collected from the untreated field and 6% in the treated field during late September. *Nomuraea rileyi* increased in soybean looper in the absence of green cloverworm during September. Incidence of *N. rileyi* was higher in soybean looper from the untreated controls than in treated fields ($F = 19.24$; $df = 1, 2$; $P < 0.05$). These data support our hypothesis that *N. rileyi* can be enhanced later in the season subsequent to its infection of green cloverworm. It appears that this enhancement of *N. rileyi* can occur when green cloverworms are not available at the same time as the soybean looper. Puttler et al. (1976) reported that green cloverworms are more susceptible (48.2% mortality) than soybean looper (7.4% mortality) to *N. rileyi*. Therefore, the high incidence of *N. rileyi* in green cloverworm early in the season and the absence of green cloverworm as a host when soybean loopers occurred resulted in high incidence of *N. rileyi* in soybean looper. These data are consistent with reports by Ignoffo et al. (1976) where seasonal increase was more rapid in soybean when conidia were introduced.

Another entomopathogenic fungus *Entomophthora* sp. (Entomophthorales) was detected in green cloverworm. It occurred in 5% of green cloverworms on 18 September 1986 in the untreated field of pair A.

Parasitoids. Species of parasitoids reared from green cloverworm are listed in Table 2. Parasitism by *C. marginiventris*, the most abundant parasitoid, was more prevalent in green cloverworm and soybean looper than in the other lepidopterans, which were seldom parasitized during 1985. Parasitism in green cloverworm before soybean looper in the untreated field did not enhance the prevalence of *C. marginiventris* later in the season. The parasitoid was active in soybean looper populations, regardless of the host density or time of occurrence. Although population density and number of larvae collected were relatively high in green cloverworm compared with soybean looper in pairs A, B, and C (Fig. 1), the parasitoid was common in both lepidopterans (Table 3). The season-long incidence of parasitism indicated more frequent parasitism in soybean looper. Daigle et al. (1988) reported that soybean looper collected in the same fields are parasitized by *C. marginiventris* more frequently than green cloverworm in Louisiana.

Likewise, *C. marginiventris* was the most abundant parasitoid in 1986. It was prevalent in green cloverworm during August and early September and in

Table 2. Parasitoid species reared from green cloverworm larvae collected from soybean. Blackville, South Carolina (1985–1986).

Family	Species
Braconidae	<i>Cotesia marginiventris</i> (Cresson)
	<i>Protomicroplitis facetosa</i> (Weed)
Ichneumonidae	<i>Campoletis flavicincta</i> (Ashmead)
	<i>Mesochorus discitergus</i> (Say) ^a
	<i>Venturia nigriscapus</i> (Viereck)
Phoridae	<i>Dohrniphora cornuta</i> (Bigot)

^aHyperparasitoid of *C. marginiventris*.

Table 3. Season-long percent parasitism by *C. marginiventris* in treated (early-season control of *P. scabra*) and untreated soybean ('Braxton'- Maturity Group VII). Blackville, South Carolina (1985).

Field	n	Untreated			Treated ^a			
		<i>Plathypena scabra</i>	n	<i>Pseudoplusia includens</i>	n	<i>Plathypena scabra</i>	<i>Pseudoplusia includens</i>	
A	166	15.7	12	33.0	62	11.3	6	50.0
B	146	8.9	4	25.0	83	14.5	15	20.0
C	130	24.6	12	41.7	13	53.8	8	75.0

^aPermethrin at 0.0225 kg [AI]/ha.

soybean looper from mid-September to early October. Parasitism by *C. marginiventris* was prevalent in soybean looper reaching 89%, 82%, and 85% in pairs A, B, and C, respectively, late in the season during mid to late September. In green cloverworms, parasitism by *C. marginiventris* reached only 9%, 10%, and 18% in pairs A, B, and C, respectively, from mid-August to early September. The higher incidence in soybean looper could be a result of the later peak in host population when natural enemies from various ecosystems have had longer to propagate and disseminate. In addition, the only lepidopteran other than soybean looper that occurred during mid-September to early October was velvetbean caterpillar, which is seldom parasitized by *C. marginiventris* (McCUTCHEON & TURNIPSEED 1981).

Removal of green cloverworm early in the season did not affect the occurrence of *C. marginiventris* later in the season, which was active in both treated and untreated fields and in high- and low-density populations. In fact, its highest incidence during the study was near 90% in soybean looper during mid to late September 1986, when green cloverworm populations remained unusually low through mid-August and peaked in early September. Record-high temperatures were recorded during the summer of 1986, and the heat was possibly a factor in suppressing green cloverworm populations early in the season.

Predaceous arthropods. Predaceous arthropods varied in relative abundance between treated and untreated fields throughout the study. During 1985, the most abundant predators were geocorids and spiders, which comprised 26% and 27%, respectively, of the total predator population in treated fields. In the untreated fields, geocorids and spiders comprised 40% and 41%, respectively, of the total predator population. Populations of predaceous arthropods in treated fields increased but were not significantly higher ($P > 0.05$) following high populations of neonate corn earworm larvae that developed in treated fields. The predators were mostly geocorids, nabids, and spiders that feed on small larvae. Predator-prey relationships often develop in this manner. According to Croft (1990), in considering colonization of a herbivorous pest and its natural enemy in a given habitat over time, generalist predators usually only colonize a crop system after prey population densities have reached a threshold level sufficient to provide adequate nutrition. Peak predator populations were similar during late August in both treated and untreated fields (Fig. 3).

In 1986, predator populations were not influenced by the control of green cloverworms with permethrin (Fig. 4). Of the major predators, geocorids constituted 20% and 24%, spiders 19% and 22%, and anthicids 22% and 28% of the total predaceous arthropod population in treated and untreated fields, respectively. Populations peaked (11 predaceous arthropods per row-m) during early September.

With or without the early presence of green cloverworm in soybean ecosystems in South Carolina, major natural enemies that are generalists will play an important role in regulating pest lepidopteran populations. Although green cloverworm generally occurs before potentially damaging populations of lepidopterans during the soybean growing season in South Carolina, this species also occurs in a later generation concurrently with other lepidopterans.

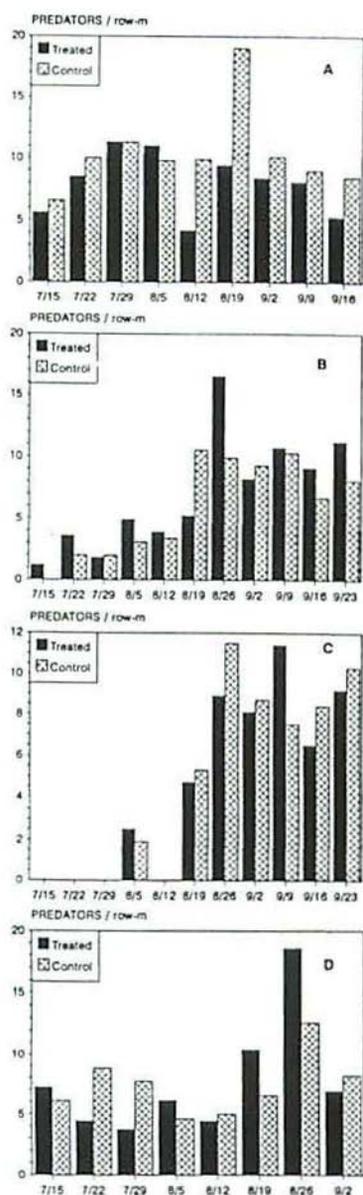


Fig. 3. Population density of predaceous arthropods in soybean treated with 0.0225 kg [AI]/ha permethrin and soybean that were untreated, (Blackville, South Carolina, 1985). Field pairs are denoted by upper case letters A, B, C, or D in upper right corner.

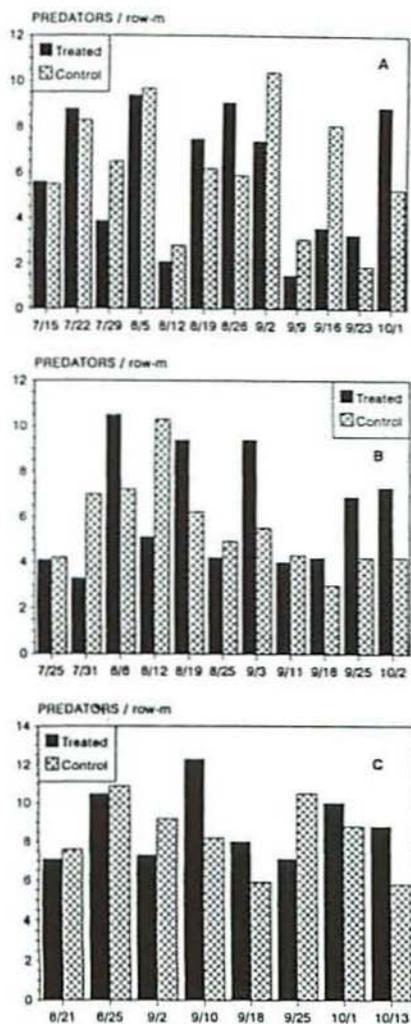


Fig. 4. Population density of predaceous arthropods in soybean treated with 0.0225 kg [AI]/ha permethrin and soybean that were untreated. Pairs A and B (Williston) and Pair C (Blackville), (South Carolina, 1986). Field pairs are denoted by upper case letters A, B, or C in upper right corner.

When this is the case, as in 1985 in the present study, acceptance of the null hypothesis is expected: no difference between permethrin-treated and untreated soybean as related to the occurrence and abundance of natural enemies of corn earworm, soybean looper, and velvetbean caterpillar. When green cloverworms did not occur later in the season concurrently with other lepidopterans, *N. rileyi* had higher incidence in soybean loopers in the untreated field where green cloverworm populations were allowed to increase. Caution should be taken in making conclusive statements because lepidopterans in only one pair of fields were infected with the pathogen during 1986. The occurrence and incidence of diseases are unpredictable because certain physical conditions, such as high humidity, are necessary for dissemination and propagation (Bell 1974). Additional studies may determine factors that are important in predicting the efficacy of pathogens.

The parasitoid *C. marginiventris* was in high incidence in both treated and untreated fields. Parasitism in green cloverworm as a host before soybean looper, which occurs in sporadic populations, did not appear to enhance the prevalence of *C. marginiventris* in soybean loopers. As observed in this study, *C. marginiventris* is maintained in both high- and low-density green cloverworm populations, and when soybean looper occurs, it begins to use a high percentage of the soybean looper population as hosts. Green cloverworm is important in maintaining the parasitoid in soybean ecosystems, but *C. marginiventris* is not totally dependent upon the prior occurrence of green cloverworm in the same field. In Florida, during the winter of 1972-1973, green cloverworm was the most important alternate host for *C. marginiventris* that attacked soybean looper and other lepidopterans (Martin et al. 1981). This parasitoid is a generalist, using hosts on many other crops. Its mobility and diversity lessen total dependence upon green cloverworm populations in a particular soybean field for effectiveness against soybean loopers. Soybean, as well as vetch, clover, and alfalfa in surrounding areas, can serve as reservoirs for the parasitoids (McCutcheon et al. 1995).

Fortunately, growers in South Carolina have not needed to control green cloverworm on any plant hosts with insecticide applications. However, relying solely on green cloverworm populations to serve as a reservoir for natural enemies of lepidopteran pests of soybean is not recommended based on our data. Various plant and other insect hosts also serve as reservoirs for natural enemies. Future control tactics against early-season pests, such as insects, weeds, and plant diseases, should include strategies to conserve natural enemies.

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Effect of *Beauveria bassiana* on Migratory Grasshoppers (Orthoptera: Acrididae) and Nontarget Yellow Mealworms (Coleoptera: Tenebrionidae) in Spraytower Bioassays¹

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ABSTRACT A grasshopper-derived strain of *Beauveria bassiana* (Balsamo) Vuillemin (Hyphomycetes: Moniliales) was tested on fourth-instar migratory grasshoppers, *Melanoplus sanguinipes* (F.), and adult yellow mealworms, *Tenebrio molitor* L., in spraytower bioassays. Spray treatments included air (control), 0.09 ml of oil, and 0.09 ml of oil containing 2.64×10^9 *B. bassiana* conidia per milliliter of oil. At 10 d, mortality of grasshoppers treated with *B. bassiana* was 72.5% and was significantly ($F = 15.26$, $P = 0.0001$) higher than mortality of yellow mealworms sprayed with conidia, and mortality of both species receiving air and oil treatments. Results suggest that fourth-instar migratory grasshoppers are more susceptible to fungal infection by grasshopper-derived *B. bassiana* than adult yellow mealworms in spraytower bioassays.

KEY WORDS *Beauveria bassiana*, Hyphomycetes, Moniliales, *Melanoplus sanguinipes*, Orthoptera, Acrididae, *Tenebrio molitor*, Coleoptera, Tenebrionidae, conidia

Grasshoppers are important pests on western rangeland in North America where outbreaks can dramatically reduce agricultural production (Mason & Erlandson 1994, Mann et al. 1986). Methods of grasshopper control have been predominately limited to insecticidal sprays. Unfortunately, applications of organophosphate insecticides have resulted in large, immediate decreases in certain nontarget arthropods (Pfadt et al. 1985, Quinn et al. 1991).

Beauveria bassiana (Balsamo) Vuillemin is a deuteromycete fungus (Hyphomycetes: Moniliales) that is pathogenic to grasshoppers (Inglis et al. 1995) and

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many other types of insects (Champlin et al. 1981, Anderson et al. 1989, Knudsen et al. 1990, Quintela et al. 1990, James & Lighthart 1994). However, there may be strain-specific differences in fungal virulence toward a single species of insect (Khachatourians 1992, Kosir et al. 1991). In addition, susceptibility to fungal infection varies with insect species depending on the efficiency of the hosts' defense response (Bidochka & Khachatourians 1987).

The infective form of the fungus is the conidia, which can be mass produced and aerially applied over large tracts of land. Laboratory studies using *B. bassiana* have been conducted on migratory grasshoppers, *Melanoplus sanguinipes* (F.) (Khachatourians 1992, Marcandier & Khachatourians 1987, Moore & Erlandson 1988), lesser mealworms, *Alphitobius diaperinus* (Panzer) (Steinkraus et al. 1991), and alfalfa leafcutting bees, *Megachile rotundata* (F.) (Goerzen et al. 1990), but treatments were applied directly to test insects by dipping, topical application, or injection. Laboratory studies using simulated aerial treatments are needed because results may differ from studies in which conidia are directly applied to insects. The purpose of this research was to evaluate the specificity of a grasshopper-derived strain of *B. bassiana* in spraytower bioassays. Migratory grasshoppers and yellow mealworms, *Tenebrio molitor* L., were used in the simulated aerial application studies (i.e., spraytower bioassays) for determination of *B. bassiana* strain specificity.

Materials and Methods

Fungal conidia (batch # 921114GHA) and an oil carrier solution were supplied by Mycotech (Butte, Montana). The oil carrier was an inert paraffin that is registered with the Environmental Protection Agency as an insecticide carrier (Johnson et al. 1991). Aerial application of *B. bassiana* was simulated in the laboratory with the use of a spraytower. A spraytower is an airbrush (Model H#, Paasche Airbrush Co., Harwood Heights, Illinois) connected to an air pump. The airbrush was mounted 1.83 m above the floor in a 9.75-m² room. Air pressure was provided by an air pump at 1.76 kg/cm² (25 psi), and treatments were injected into an airstream with a 1-ml syringe. Several practice tests were conducted using the oil solution and oil-sensitive paper (TeeJet[®] Spraying Systems Co., Wheaton, Illinois) to achieve a spray pattern equivalent to a field application rate of 1×10^{13} conidia/3.785 liter/0.405 ha (1×10^{13} conidia/gal/acre). Airbrush nozzle settings were adjusted accordingly and then maintained for the duration of the experiments.

Migratory grasshoppers were reared and maintained at South Dakota State University according to recommendations provided by Henry (1985). Fourth-instar migratory grasshoppers were obtained from laboratory cultures for evaluation in spraytower bioassays. Once treated, grasshoppers were placed individually in 60-ml clear plastic specimen cups. Rye clippings were provided as a food source. Food was added when necessary, and grasshopper feces was removed.

Yellow mealworm larvae were purchased from Ward's Natural Science Est., Inc. (Rochester, New York) and were raised to adults in wheat bran with apple. Following treatment, beetles were placed individually in 60-ml clear plastic specimen cups. Wheat bran (10% of container volume), fresh apple (1.5-cm³ slice), and paper towel (4 cm²) were added to each container. The paper towel

allowed overturned beetles to right themselves.

Prior to each spray event, clean newsprint was placed on the floor below the spraytower in the spray room. Test insects were immobilized in groups of 10 by cooling to 1.7°C. Thirty individuals were randomly arranged on the newsprint near the center of the spraytower, sprayed with air for 15 s, and kept as controls; 30 individuals were sprayed with 0.09 ml of the oil carrier; and 30 individuals were sprayed with 0.09 ml of oil containing 2.64×10^9 conidia per milliliter of oil. Oil-sensitive cards were placed adjacent to the insects (one card per 10 test insects) to confirm reception of treatment. Once treated, insects were placed in containers in the holding room. The airbrush was disinfected with bleach after each *B. bassiana* treatment. When not in use, the spray room was disinfected by using a germicidal light (General Electric 30 watt germicidal G30T8). Temperature and humidity of the holding room was maintained at 27°–29°C and 40%–50%, respectively. Insects were provided with food, and mortality was checked every 24 h, for 10 d.

The experimental design was a randomized complete block with repeated measures. Each treatment was replicated four times with a total of 360 individuals of each species used in three treatments. The procedures for the three treatments within a replication were conducted on the same day, but replications of the tests occurred on different days between 20 April 1993 and 4 July 1994. Data were analyzed by using the PROC MIXED procedure (Littell et al. 1996).

Results and Discussion

More than 50% of grasshoppers sprayed with *B. bassiana* expired by 7 d. At 10 d, mortality of grasshoppers treated with *B. bassiana* was 72.5% and was significantly ($F = 15.26$, $P = 0.0001$) higher than mortality of yellow mealworms sprayed with conidia, and mortality of both species receiving air and oil treatments. Mortality of grasshoppers treated with air and the oil carrier at 10 d was only 16.7% and 22.5%, respectively (Fig. 1). At 10 d, mortality of yellow mealworms was 20.8%, 14.2%, and 14.2% in *B. bassiana*, air, and oil treatments, respectively (Fig. 2). One or 2 d prior to death, infected grasshoppers usually became sluggish and feeding diminished. Many of the dead grasshoppers (in *B. bassiana* treatments) exhibited a dark red coloration, which is generally a symptom of grasshopper/*B. bassiana* infection (Marcandier & Khachatourians 1987).

Marcandier & Khachatourians (1987) observed 90%–100% mortality of grasshoppers, dipped in a distilled water suspension containing 2×10^7 conidia per milliliter, at 14-d posttreatment. Although mortality in our spraytower studies was lower, it may be more representative of that observed under standard field application procedures.

Results from spraytower bioassays suggest that migratory grasshoppers are more susceptible to fungal infection by grasshopper-derived *B. bassiana* than adult yellow mealworms. Steinkraus et al. (1991) used *B. bassiana* derived from wild house flies, *Musca domestica* L., and directly contacted larval and adult lesser mealworms with starch dust or aqueous suspensions containing conidia. Steinkraus et al. (1991) found that lesser mealworms were susceptible

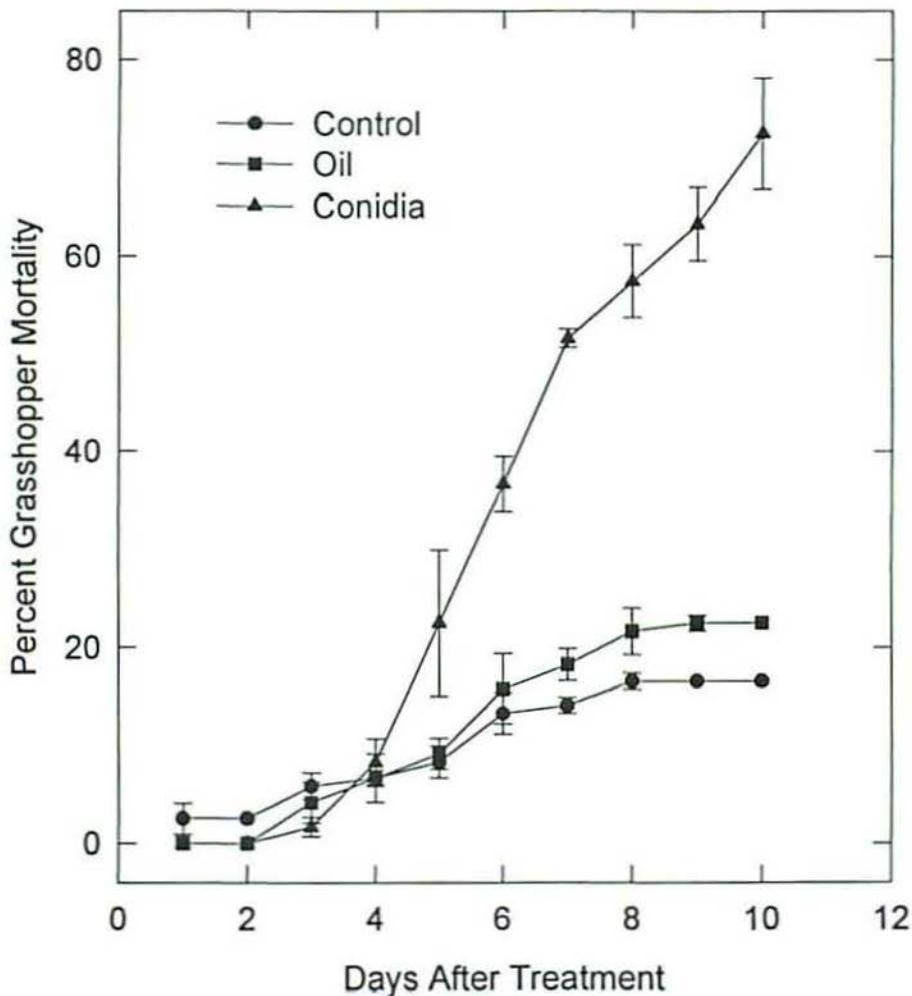


Fig. 1. Percent cumulative mortality of migratory grasshoppers sprayed with air, 0.09 ml of oil, and 0.09 ml of oil containing 2.64×10^9 *Beauveria bassiana* conidia per milliliter of oil.

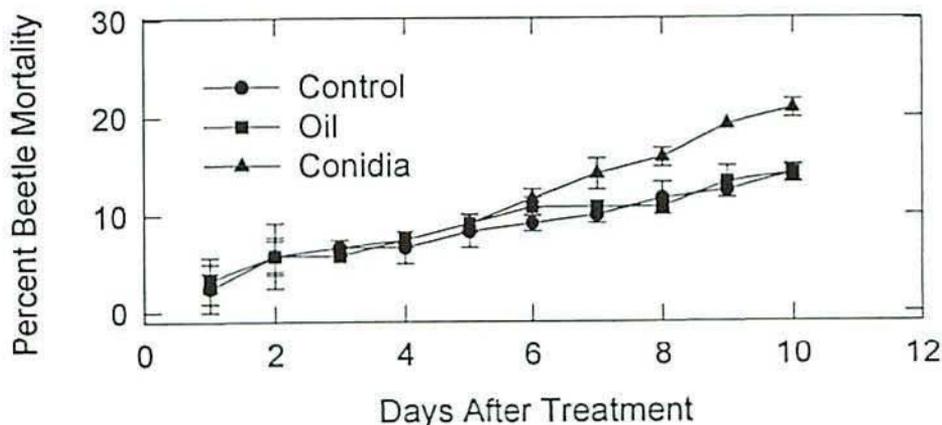


Fig. 2. Percent cumulative mortality of adult yellow mealworms sprayed with air, 0.09 ml of oil, and 0.09 ml of oil containing 2.64×10^9 *Beauveria bassiana* conidia per milliliter of oil.

to infection, but they used a different strain of *B. bassiana*. Tolerance of adult yellow mealworms to spray treatments may have been due to the isolate of *B. bassiana* used (Khachatourians 1992) or to the method of application. Direct contact of insects with conidia suspensions may result in a higher number of conidia attaching to cuticle than what might be observed in spraytower studies or in the field.

Grasshopper control methods must significantly reduce densities within a short time to prevent oviposition and extensive damage to vegetation. If applications can be effective and fast-acting in the field, *B. bassiana* may be a desirable alternative to chemical sprays. Results suggest that the tolerance observed in our studies may contribute to *B. bassiana* being viewed as a safe tool in rangeland grasshopper control, especially in ecologically sensitive areas. However, additional research should evaluate the impact on nontarget rangeland insect species. The levels of grasshopper mortality exhibited in our studies are sufficient to justify further field trials using *B. bassiana* for grasshopper control.

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Predation on Wandering Larvae and Pupae of Caribbean Fruit Fly (Diptera: Tephritidae) in Guava and Carambola Grove Soils¹

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ABSTRACT Laboratory-reared Caribbean fruit fly (*Anastrepha suspensa* [Loew]) larvae in the wandering period of the last instar were released singly onto the soil surface in guava (*Psidium guajava* L.) and carambola (*Averrhoa carambola* L.) groves in Florida. Crawling, burrowing, and interactions with predators on the soil surface before burrowing were observed. Four days after release, pupae were excavated from the soil and returned to the laboratory for rearing. Depth of pupation in all soils ranged from 0-27 mm. Four species of ants were observed attacking wandering larvae. Adult emergence of pupae recovered from all groves ranged from 0%-98%. Wireworm larvae, *Conoderus* sp., were observed eating pupae in the field. In the laboratory, the earwig *Euborellia annulipes* (Lucas) ate wandering larvae and pupae.

KEY WORDS Tephritidae, *Anastrepha suspensa*, predation

Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae), is a pest of export quarantine significance in guava, *Psidium guajava* L., and carambola, *Averrhoa carambola* L., fruit grown commercially in Florida. Wandering larvae leave the fruit and enter the soil to pupate (Bateman 1972). There are no preharvest management methods for wandering larvae and pupae in the soil in carambola and guava groves. The natural causes and rates of mortality of immature stages of Caribbean fruit fly in groves have not been determined. Bateman (1972) noted that wandering larvae were very vulnerable to predation during the period when they leave the fruit and search for a pupation site in the soil. Hennessey (1994) observed wandering Caribbean fruit fly larvae taking up to 6 h to burrow into highly compacted loam or marl soil in the laboratory. Ants have been noted as predators of immature stages of the apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera:

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Tephritidae) in Ontario (Allen & Hagley 1990); the Mexican fruit fly (*Anastrepha ludens* [Loew]) (Diptera: Tephritidae) in Texas and Mexico (Thomas 1993, 1995); the oriental fruit fly (*Bactrocera dorsalis* [Hendel]) in Hawaii (Marucci 1955, Newell & Haramoto 1968) and Malaysia (Serit & Tan 1990); and the Mediterranean fruit fly (*Ceratitis capitata* [Wiedemann]) in Hawaii (Wong et al. 1984) and Guatemala (Eskafi & Kolbe 1990). Newell & Haramoto (1968) remarked that ant and earwig predation were probably insignificant as major population control factors of fruit flies in guavas in Hawaii. Marucci (1955) noted that two species of earwigs were predators of *B. dorsalis* larvae in Hawaii. Hoffman (1987) indicated that *Euborellia annulipes* (Lucas) (Dermaptera: Carcinophoridae), a wingless earwig with worldwide distribution, was a scavenger and predator. Koppenhofer (1995) observed that the species was a predator of *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) larvae in the field in Kenya.

Soil insecticide treatments applied as a drench are accepted by the United States Animal and Plant Health Inspection Service to eradicate immature stages of oriental (Stefan 1991) and Mediterranean fruit flies (Smith 1993) under host fruit trees in quarantined areas. Insecticidal drench treatments are being investigated by the author for management of Caribbean fruit fly immature stages in guava and carambola groves in Florida. Knowledge of the nontarget predators that may be affected, the rate of natural background mortality of immature stages in the soil, and the depth at which larvae pupate in field soil is essential in evaluating efficacy of new soil drench treatments for Caribbean fruit fly. Duncan & McCoy (1996) studied depth of immature citrus root weevils (*Diaprepes abbreviatus* [L.]) (Coleoptera: Curculionidae) in soil to determine if applied entomopathogenic nematode treatments would be effective. They found nematodes did not persist above 3 cm in soil after 7 d. Hennessey (1994) found depth of pupation of the Caribbean fruit fly in the laboratory to vary with moisture and compaction levels, but it was above 3 cm for the three soil types tested.

The present investigations were conducted to determine depths of pupation of laboratory-reared Caribbean fruit fly larvae released into guava and carambola groves, identify predators, and provide estimates of rates of mortality due to predation. As part of these investigations, laboratory experiments also were conducted to confirm whether *E. annulipes*, an earwig commonly found in groves and suspected of being predaceous on fruit flies, would eat wandering fruit fly larvae and pupae.

Materials and Methods

Caribbean fruit flies were reared on an agar medium at the United States Department of Agriculture, Agricultural Research Service (USDA, ARS) station in Miami, Florida (Hennessey 1994). Timing of experiments coincided with the Florida harvest seasons for guava and carambola.

Two commercial guava groves in Homestead, Dade County, Florida, on different soil types, were used for the study. Heaviest guava production in the Sardinia grove was in the summer and fall of 1995. Heaviest guava production in the Brooks grove was in the spring and summer of 1996. The Sardinia grove

was under bedded cultivation on Biscayne gravelly marl and four replicates were conducted in September and October 1995. The Brooks grove was on Krome very gravelly loam, was not bedded, and four replicates were conducted in March, May, and July 1996. Both groves were treated for adult fruit flies with insecticide applied aerially by the grower every 10 d.

The two carambola groves were located on the Miami ARS station. They had a summer and a winter crop in 1995–1996. Both were on Krome very gravelly loam. One grove was a mature 15-yr-old stand and the other a young 3-yr-old stand. Both groves were sampled while they had mature fruit. Eight replicates were conducted in the mature stand in August, September, October, and February 1996. Two replicates were conducted in the young stand in January and February 1996. Neither grove was treated with insecticides.

Soil temperature, soil moisture, and air temperature were noted at the time of release. Soil moisture was measured with a Kelway Tester Model HB-2 (Kel Instruments, Wyckoff, New Jersey), and soil temperature was measured at a depth of 3 cm with a bimetal soil thermometer. Minimum temperature and rainfall over the test period were measured at the site. Soil types were determined by using the soil maps of Noble et al. (1996).

Field mortality and pupation depth. On the day of an experiment, approximately 200, 7-d-old wandering larvae that were leaving the agar medium to pupate were collected from rearing trays, held in a ventilated plastic cup in an insulated cooler (21°–26°C), and transported to the field. An excess was brought to the field to account for loss because of mortality and pupation during transit to the field. Within 1 h of collection, 40 larvae were dropped singly by hand from a height of 0.5 m (to simulate dropping out of a fruit on a tree) onto the soil surface in the grove. Larvae were dropped approximately 1 m apart under the leaf canopy near previously placed marker flags. The spot where each larva was dropped was marked immediately with a painted wooden toothpick to facilitate observation. Observations on crawling, burrowing, and predation were noted for a period of up to 30 min after larval release (all surviving larvae burrowed within 30 min). Larvae that were not released were held in aggregate in moist vermiculite as controls in the laboratory (26°–28°C, 85%–95% RH, photoperiod 14:10 [L:D] h) to determine percentage adult emergence.

Four days after release the pupae were excavated, placed individually in Petri dishes with damp vermiculite, and held for adult emergence in the laboratory alongside controls under the conditions described above. The depth at which pupae were recovered was noted. The 4-d period was used because all individuals were expected to have pupated within that time and any parasitoids emerge before adult eclosion.

Fruit fly predators observed. Representative predators were collected at sites during observation periods and submitted to the Florida Department of Agriculture and Consumer Services (FDACS), Gainesville, Florida, for identification. Voucher specimens are maintained at FDACS and the Miami ARS station.

Acceptance of flies as prey by earwigs. *Euborellia annulipes* were frequently observed under fallen fruits in carambola groves. Adult and large nymphs were collected on 3 July 1995 from the soil surface under fruits on the

ground at the Miami ARS station. The earwigs were maintained singly in the laboratory in 15-cm Petri dishes with dry dog food, water in a vial with a cotton plug, and several pieces of blotter paper for shelter. They were maintained and bioassayed under the environmental conditions described above. On 21 July, each of 55 earwigs was offered a single, laboratory-reared, mature Caribbean fruit fly larva in a no-choice test started within 1 h of removal of their regular food. The wandering larvae were collected from agar medium as described above. Observations on predation were made within the first 10 min and after 3 d. The larvae pupated within the 3-d period. The 3-d observation was made to determine if earwigs were interested in or capable of eating larvae and resultant pupae over a protracted period of time. After testing, the earwigs were maintained on their regular diet. On 7 August 1995, the same earwigs were used in a feeding test with with 3-d-old fruit fly pupae.

Results and Discussion

Field mortality and pupation depth. Sixty-seven of 160 (42%) wandering larvae released in the Sardinia guava grove were recovered as pupae (Table 1). The greatest pupation depth for all four test dates was 16 ± 7 mm (mean \pm SD, $n = 9$) on 26 October (Table 1). The range for all dates was 0–27 mm. Loss and predation accounted for the difference between the number released and recovered. For three of the four dates tested, adult emergence from recovered pupae was much lower than for control pupae (Table 1). Biological control agents such as pathogens or nematodes may have accounted for some of this difference.

Fifty-three of 160 (33%) wandering larvae released in the Brooks guava grove were recovered as pupae (Table 2), slightly fewer than for the Sardinia grove. The greatest pupation depth for the four test dates was 17 ± 1 mm (mean \pm SD, $n = 15$) on 5 July (Table 2). Depth ranged from 0–27 mm over all dates. Mean depth and range were not notably different from those of the Sardinia grove. This similarity may be explained, in part, by the finding of Hennessey (1994) that pupation depth did not differ for soil type but differed for compaction. Soils in both guava groves were loosely compacted under the canopy.

The low numbers of pupae recovered and the low percentage of emerged adults in the mature carambola grove were likely the result of predation (Table 3). Of 320 larvae released on eight dates, 79% (252) were killed by ants (Table 3). The greatest pupation depth for the eight test dates was 10 ± 1 mm (mean \pm SD, $n = 2$) (Table 3). The larvae may have been injured by ants, partially explaining the shallow (relative to the guava groves) pupation depth. Soil moisture and temperature conditions over the sampling period were similar to those recorded for the Brooks guava grove (Table 4).

The low number of pupae recovered in the young carambola grove was probably attributable, in part, to predation by the ants on the pupae beneath the soil. The greatest depth over all dates was 20 ± 1 mm (mean \pm 1 mm, $n = 5$) recorded for 9 February, and the range for both dates was 10–20 mm (Table 5). The environmental conditions (Table 4) were similar to those recorded for the mature carambola grove.

Table 1. Mortality of larvae and pupae of the Caribbean fruit fly in marl soil of the Sardinia guava grove at Homestead, Florida, in 1995.

Date	No. of larvae preyed upon out of 40	No. of pupae recovered	Mean depth (mm) pupae recovered ^a	Adult emergence (%) of recovered pupae (control)
22 Sept.	2 ^b	15	15 ± 9	57 (94)
28 Sept.	0	22	8 ± 5	77 (75)
6 Oct.	0	21	9 ± 7	57 (96)
26 Oct.	2 ^b	9	16 ± 7	58 (78)

^aValues are means ± SD.^b(Hymenoptera: Formicidae) *Solenopsis invicta* Buren.**Table 2. Mortality of Caribbean fruit fly larvae and pupae in loam soil of the Brooks guava grove at Homestead, Florida, in 1996.**

Date	No. of larvae preyed upon out of 40	No. of pupae recovered	Mean depth (mm) pupae recovered ^a	Adult emergence (%) of recovered pupae (control)
15 March	2 ^b	15	14 ± 6	67 (92)
22 March	2 ^c	22	13 ± 6	68 (98)
17 May	4 ^b	11	12 ± 6	58 (91)
5 July	0	5	17 ± 1	50 (90)

^aValues are means ± SD.^b*Solenopsis invicta*.^c(Hymenoptera: Formicidae) *Paratrechina parvula*.

Table 3. Mortality of larvae and pupae of the Caribbean fruit fly in loam soil of the mature carambola grove at Miami, Florida, in 1995–1996.

Date	No. of larvae preyed upon out of 40	No. of pupae recovered	Mean depth (mm) pupae recovered ^a	Adult emergence (%) of recovered pupae (control)
25 Aug. 95	20 ^b	0	—	—
31 Aug. 95	35 ^b	0	—	—
8 Sept. 95	28 ^b	0	—	—
14 Sept. 95	39 ^b	0	—	—
12 Oct. 95	39 ^b	0	—	— (96)
20 Oct. 95	40 ^b	0	—	— (94)
16 Feb. 96	17 ^b	1	6 ± 0	0 (60)
29 Feb. 96	34 ^b	2	10 ± 1	0 (60)

^aValues are means ± SD.

^b(Hymenoptera: Formicidae) *Leptothorax* sp. near *pergandei*.

Fruit fly predators observed. Four observations of predation, all by *Solenopsis invicta* Buren (Hymenoptera: Formicidae), were noted for 160 (3%) larvae released on four dates in the Sardinia guava grove (Table 1). This low predation rate may have been attributable to several factors. *Solenopsis invicta* was observed infrequently in the field because grove sanitation was good, and there was little fruit on the ground. Periodic insecticide spraying also may have affected the ants. The marl soil was very friable, with low compaction under the canopy. It also was wet, and the temperatures warm, on all sampling dates (Table 4), which allowed the wandering larvae to burrow rapidly and escape predation on the soil surface.

Two species, *S. invicta* and *Paratrechina parvula* (Mayr) (Hymenoptera: Formicidae), were observed to prey on larvae in the Brooks guava grove (Table 2). Another formicid, *Odontomachus brunneus* (Wheeler), was observed to encounter wandering larvae on several occasions but did not accept them as prey. The explanation for the 5% (8 of 160) predation rate (Table 2) may be the same as that for the Sardinia grove. The soil in the Brooks grove differed from the Sardinia grove in that it was a loam and moisture was lower (Table 4), but it was similar in that it had low compaction under the canopy and good sanitation. There was one observed instance of below-ground predation. One pupa from the 15 March release date was recovered at a depth of 11 mm and was being eaten by a wireworm larva *Conoderus* sp. (Coleoptera: Elateridae).

Table 4. Environmental conditions during Caribbean fruit fly soil experiments.

Fruit type	Grove	Date	Soil temp. (°C)	Soil moisture (%)	Minimum	
					air temp. (°C)	Rainfall (mm)
Guava	Sardinia	22 Sept. 95	25	100	23	10
		28 Sept. 95	28	100	22	10
		6 Oct. 95	24	100	26	48
		26 Oct. 95	23	100	19	5
Guava	Brooks	15 March 96	21	20	24	12
		22 March 96	12	35	7	0
		17 May 96	23	24	20	78
		5 July 96	27	18	23	35
Carambola	Mature	25 Aug. 95	—	50	—	—
		31 Aug. 95	29	40	24	18
		8 Sept. 95	27	40	22	60
		14 Sept. 95	31	20	26	0
		12 Oct. 95	26	25	23	59
		20 Oct. 95	27	75	22	98
		16 Feb. 95	18	8	2	3
29 Feb. 96	22	15	18	12		
Carambola	Young	25 Jan. 96	27	20	17	45
		9 Feb. 96	23	45	7	0

Table 5. Mortality of larvae and pupae of the Caribbean fruit fly in loam soil of the young carambola grove at Miami, Florida, in 1996.

Date	No. of larvae preyed upon out of 40	No. of pupae recovered	Mean depth (mm) pupae recovered ^a	Adult emergence (%) of recovered pupae (control)
25 Jan.	18 ^b	4	10 ± 1	25 (93)
9 Feb.	17 ^b	5	20 ± 1	60 (96)

^aValues are means ± SD.

^b*Monomorium* sp. near *viridum* Brown and *Solenopsis invicta* (Hymenoptera: Formicidae).

Conditions in the mature carambola grove differed from those in the guava groves in that there was no insecticide used and fallen fruit was on the ground. The fallen fruit harbored colonies of the ant *Leptothorax* sp. near *pergandei* Emery (Hymenoptera: Formicidae) (Table 3). Foragers of this ant were abundant on all sampling dates as was reflected in the numbers of larvae preyed upon (Table 3), which were high relative to those in the guava groves (Tables 1, 2). *Euborellia annulipes* were frequently observed under fallen fruits but were not observed to attack larvae in the field.

The young carambola grove differed from the mature grove in that the canopy was smaller and there were relatively fewer fruits on the ground. The species responsible for the 44% (35 of 80) predation rate over the two dates were a combination of the ants *Monomorium* sp. near *viride* Brown and *S. invicta* (Table 5). As for the mature grove, earwigs frequently were observed under fallen fruits.

Acceptance of flies as prey by earwigs. Because *E. annulipes* has been recorded as a soil insect predator, it was suspected, during these investigations, of being predaceous on wandering larvae and on pupae. The feeding bioassay indicated that 24% (13 of 55) of larvae were eaten within 10 min and 64% (35 of 55) within 3 d. The earwigs consumed 13% (7 of 55) of offered pupae within 10 min and 29% (16 of 55) within 3 d. Upon encountering a larva or pupa, an earwig was observed to seize and hold it with its cerci and then feed on it. It was concluded that large nymphs and adults of *E. annulipes* were capable of preying on fruit fly larvae and pupae.

Overall in the present study, wandering larvae burrowed into the soil within 30 min on all experimental occasions, probably because soils under the canopy were loosely compacted in all groves. The pupation depths over all groves and dates ranged from 0–27 mm for the 720 larvae released. Four species of ants were observed to prey on larvae before burrowing, and there was one instance of a wireworm feeding on a pupa below the soil surface during the study.

Twelve of the 320 (4%) larvae released in guava groves and 287 of 400 (72%) larvae released in carambola groves were preyed upon. *Solenopsis invicta* was found preying on larvae in both carambola and guava groves. Ants were observed to kill up to 100% (20 October) of the released larvae in a carambola grove and up to 10% (17 May) in a guava grove. The higher occurrence of ant predation in carambolas may have been attributable to the much higher presence of fruit on the ground, which supported large populations of ants. Ants are a considerable mortality factor for wandering larvae in carambola groves where fruit accumulates on the ground. The low emergence rate of pupae, relative to controls, for most dates and for all groves, indicated that unidentified factors such as pathogens or nematodes also contributed to the overall mortality observed. Parasitoids were not observed to be a mortality factor in the present study. Although earwigs were not observed feeding on larvae in the field, they are capable of killing and eating larvae and pupae based on laboratory bioassays, and probably contribute to mortality in the field.

Concerning Caribbean fruit fly management in groves, drench treatments should penetrate to, and be effective at, 27 mm in marl and loam soils to have an impact on wandering larvae and pupae in groves. Nontarget effects of drench treatments on predatory ants should be taken into account in determining their efficacy.

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Dispersal of the Egg Parasitoid *Trissolcus basalis* (Hymenoptera: Scelionidae) in Tomato^{1, 2}

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ABSTRACT Three experiments were conducted in Charleston, South Carolina, during 1993 and 1994 to study the dispersal of *Trissolcus basalis* (Wollaston), an egg parasitoid of the southern green stink bug, *Nezara viridula* (L.). Dispersal was determined by placing stink bug egg masses in tomato fields, releasing *T. basalis* at the center of each field, collecting the egg masses, and holding them to determine parasitism. *Trissolcus basalis* located egg masses within each field with no preference for direction or distance from the release point. Release of about 2,000 *T. basalis* adults in a 50 × 50 m field resulted in 90% parasitism of egg masses with 52.8 to 97.0% average parasitism of eggs in all directions. About 50% of the egg masses were parasitized when 369 *T. basalis* were released in a 50 × 50 m field and when 480 parasitoids were released in a 50 × 100 m field.

KEY WORDS Hymenoptera, Scelionidae, *Trissolcus basalis*, *Nezara viridula*, parasitism, dispersal, tomato

The scelionid wasp, *Trissolcus basalis* (Wollaston), is the most important and widely distributed parasitoid species that attacks the eggs of the southern green stink bug, *Nezara viridula* (L.) (Jones 1988). A single *T. basalis* emerges from each egg and it prefers southern green stink bug eggs, but also attacks those of *Thyanta custator accerra* McAtee, *Thyanta pallidovirens* Stal, *Euschistus conspersus* Uhler, *E. servus*, *Euschistus* spp., and *A. marginatum* (Palisot de Beauvois) (Hoffmann et al. 1991; W. A. Jones, USDA-ARS, personal communication). According to Clarke (1990), the synonymous names of *T. basalis* used by Australian biological control workers are:

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Microphanurus megacephalus (Priesner), *Telenomus megacephalus* (Ashmead), *Microphanurus basalis* (Nixon), and *Asolcus basalis* (Delucchi). Nishida (1966) first referred to this wasp as *Telenomus basalis* Wollaston.

Several workers reported that *T. basalis* females respond to both the eggs and adults of southern green stink bugs and to volatiles from extracts of their eggs (Sales et al. 1978, Sales 1979, Sales et al. 1980, Sales 1985, Bin & Vinson 1985, Bin et al. 1988, Mattiacci et al. 1993). Using an olfactometer, Bin et al. (1988) showed that *T. basalis* females are attracted to males and females of the southern green stink bug. Mattiacci et al. (1993) showed that this parasitoid is attracted to an area containing southern green stink bug adults, and observed that *T. basalis* females examined and probed glass beads coated with an acetone extract of the metathoracic gland from males or females. Mattiacci et al. (1993) discovered that the unsaturated aldehyde (*E*)-2-decenal, which is present in the defensive metathoracic gland secretions of southern green stink bugs, attracts *T. basalis* females. They also found that there are no (*E*)-2-decenal isomers in extracts of eggs and concluded that (*E*)-2-decenal appears to act as a long-range kairomone, serving as a cue for orientation of *T. basalis* to southern green stink bug populations. *Trissolcus basalis* females possess remarkable ability to orient and find southern green stink bug eggs, and the threshold for the stimulation of the female parasitoids' orientation is five eggs (Sales 1979). A strong attraction by the parasitoid to southern green stink bug eggs also was observed by Bin et al. (1988). Bin & Vinson (1985) demonstrated that a kairomone present in a glycoprotein used to glue the eggs to the substrate is important in oviposition behavior by *T. basalis*. They found that oviposition stimuli are both chemical and tactile, and that a spherical substrate and an aqueous solution of the sticky substance from ovarian southern green stink bug eggs are required to induce oviposition by *T. basalis*. Mattiacci et al. (1993) provided evidence that the adhesive that holds the eggs together in an egg mass is a host-recognition factor and showed that it elicits strong ovipositor-probing response by *T. basalis*. Sales et al. (1978, 1980) and Sales (1985) observed that a kairomone isolated from southern green stink bug eggs plays a role in attracting *T. basalis* females. This kairomone induces kinesis, chemotaxis, antennal palpation, searching, and reinforcement in female *T. basalis*.

Several introductions of *T. basalis* have been made in "hotspot" locations in Australia and in other countries to control southern green stink bug (Jones 1988, Clarke 1990). However, within-field spread of this parasitoid has not been studied, and the number of *T. basalis* necessary to control southern green stink bug populations has largely been postulated through post-release surveys of *T. basalis* parasitism (Hoffmann et al. 1991).

This study was conducted to obtain information on how *T. basalis* disperses within tomato fields after release from a central point. In addition, we hoped to gain insights about the number of *T. basalis* needed to provide southern green stink bug control.

Materials and Methods

Three experiments were conducted to study the within-field dispersal of *T. basalis* in tomato. One-month-old tomato plants were used, and no insecticides were applied. The tomato plants were transplanted and parasite releases were made just prior to blooming. Young plants were used to aid in reducing the possibility of high rates of parasitism by indigenous *T. basalis*, assuming that when few natural hosts are available, lower parasitism rates could be expected.

Dispersal was determined by placing southern green stink bug egg masses in tomato fields, releasing *T. basalis* at the center of each field, and collecting the egg masses for holding in the laboratory to determine parasitism.

Egg masses obtained from colony-reared stink bugs were stored in a freezer at -75°C until ready for use. Egg masses with 60 or more eggs were glued (Elmer's Glue-All, Borden, Inc., Ohio) to small rectangular strips of cardboard. Empty egg chorions were excluded from the counts. One week prior to *T. basalis* release, benchmark parasitism was determined by placing freezer-stored southern green stink bug egg masses within each field at 4-m intervals equidistant from a designated central release point towards the north, northeast, east, southeast, south, southwest, west, and northwest directions. Egg masses were placed in each field by stapling cardboard strips with egg masses to the undersides of tomato leaves. After 1 wk, egg masses were collected, and each was placed into individual labeled test tubes and covered with fine nylon mesh. The egg masses were held inside a rearing room at $24 \pm 5^{\circ}\text{C}$ and $60 \pm 5\%$ RH with a photoperiod of 14:10 (L:D) h to allow parasitoids to emerge.

On the day when egg masses for benchmark parasitism were collected, freezer-stored southern green stink bug egg masses were again placed in each field, following the same pattern. Parasitized eggs with *T. basalis* expected to emerge within 24 h were then placed at a central release point in each field by gluing the eggs inside a plastic container (17 cm high, 15 cm diameter) that was fixed, bottom up, on top of a wooden stake. Food was provided to newly emerged *T. basalis* adults by attaching cotton balls soaked in 80% honey solution along the inner rim of the container. The base of the wooden stake was coated with Tree Tanglefoot (The Tanglefoot Co., Michigan) to prevent crawling predators from attacking the eggs. After 1 wk, southern green stink bug egg masses were collected from each field and held inside a rearing room pending parasitoid emergence. Numbers of parasitoids released into each field were determined by counting the exit holes made by emerging *T. basalis*. Wind velocity, temperature, and relative humidity were recorded.

Experiment I. The first experiment was conducted in a 50×50 m field at Clemson University's Coastal Research and Education Center, Charleston, South Carolina, in October 1993. Plants of tomato cultivar 'NC5G' were grown in bush culture and planted in beds (25 cm high, 100 cm wide) without plastic mulch. Plants were spaced 61 cm apart with a 2-m distance between rows. Two thousand parasitized southern green stink bug eggs were placed at the center of the field, and 40 freezer-stored southern green stink bug egg masses were attached to plants at 4-m intervals away from the release point.

Experiment II. The second experiment was carried out in a field with the same dimensions as in Experiment I at the United States Vegetable Laboratory, USDA-ARS, Charleston, South Carolina, in May 1994. Plants of tomato cultivar 'NC5G' were planted in beds with no plastic mulch. One thousand parasitized southern green stink bug eggs were placed at the release point as soon as 40 freezer-stored southern green stink bug egg masses were placed at 4-m intervals along eight directions in the field away from the central release point.

Experiment III. The third experiment was conducted in a commercial tomato field measuring 50 × 100 m on Wadmalaw Island, Charleston, South Carolina, in May 1994. Tomatoes ('Sunny' variety) were grown in stake culture and planted in beds (25 cm high, 100 cm wide) with plastic mulch. Plants were transplanted 46 cm apart with about a 2-m distance between rows. Two thousand parasitized southern green stink bug eggs were introduced into the field at the central release point on the same day that 72 freezer-stored southern green stink bug egg masses were placed at 4-m intervals along eight directions from the release point (11 egg masses along the length of the field, 10 along the diagonal, and five along the width).

Data were analyzed using analysis of variance (ANOVA) and means were separated with the least significant difference (LSD) test at $P < 0.05$.

Results

Experiment I. None of the egg masses that were placed within the 0.25-ha tomato field before parasitoid release were parasitized. Almost 100% of *T. basalis* emerged from parasitized southern green stink bug eggs placed at the release site. Justo (1994) showed that the male:female ratio was ca. 50:50; thus, about 969 females were available to disperse and find egg masses. Parasitism by *T. basalis* on southern green stink bug egg masses placed equidistantly from the central release point within the field is shown in Fig. 1. Out of 40 egg masses placed in the field after release of *T. basalis*, 35 were parasitized, two were taken by predators, and three were not attacked. The total egg mass parasitism was 87.5% (Table 1). Average parasitism by *T. basalis* of southern green stink bug egg masses in all directions ranged from 52.8 to 97.0% (Fig. 2), and the incidence of parasitism at different intervals away from the release site is shown in Fig. 3. There was no significant difference ($P < 0.05$) between percentage parasitism in any direction except along the north where parasitism dropped to ca. 53%. There was some indication that parasitism was declining (65%) at 16 m away from the release site, but it increased again to 78% at 20 m. No significant differences in percentage parasitism of egg masses at any of the sites away from the central release point were detected ($P < 0.5$). Almost all eggs in each mass were parasitized.

Experiment II. The natural levels of parasitoids in the field was very low because no parasitoids emerged from egg masses placed in the 0.25-ha field before parasitoids were released. Only 369 *T. basalis* adults (36.9%) emerged from parasitized eggs placed at the release site. The pattern of parasitism on southern green stink bug egg masses in different directions after *T. basalis* release is shown in Fig. 4. Out of 40 egg masses, 10 were parasitized, 21 were

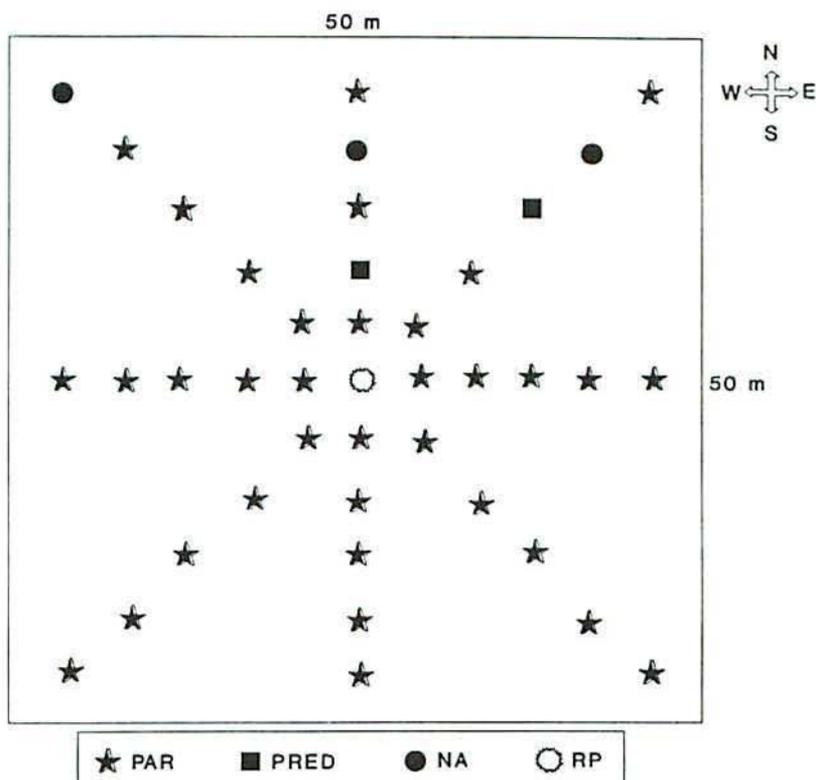


Fig. 1. Parasitism (PAR) by *Trissolcus basalis* on egg masses of *Nezara viridula* placed equidistantly from the central release point (RP) within a tomato field at the Coastal Research and Education Center, Charleston, South Carolina, 7–14 October 1993. Predation (PRED) on egg masses and those not attacked (NA) also are shown.

eaten by predators, and nine were not attacked. Thus, the combination of parasitoids and predators accounted for 77.5% of the mortality of southern green stink bug egg masses, and *T. basalis* parasitized 52.6% of the egg masses that were not taken by predators (Table 1).

Experiment III. No parasitoids were recorded for benchmark parasitism. Only 480 (24%) *T. basalis* emerged from egg masses at the release point. One week after release of *T. basalis*, 32 southern green stink bug egg masses were parasitized, eight were attacked by predators, and 32 escaped attacks out of 72 egg masses placed within the field. Parasitism in different directions on southern green stink bug egg masses placed in the field is shown in Fig. 5. *Trissolcus basalis* parasitized 50% of the egg masses that were not taken by predators (Table 1).

Table 1. Parasitism and predation on egg masses of the southern green stink bug (SGSB), *Nezara viridula* (L.), placed within tomato fields after release of the scelionid egg parasitoid *Trissolcus basalis* in 1993 and 1994 (Charleston, South Carolina).

Expt. no. ^a	Tomato culture	Field dimension (m)	<i>T. basalis</i> released (no.)	SGSB egg masses exposed	Egg masses eaten		Egg masses parasitized	
					(no.)	(%)	(no.)	(%) ^b
I	Bush	50 × 50	1,938	40	2	4.9	35	87.5
II	Bush	50 × 50	369	40	21	52.5	10	52.6
III	Stake	50 × 100	480	72	8	11.1	32	50.0

^aI and II, 'NC5G' cultivar planted in beds without plastic mulch; III = 'Sunny' variety with plastic mulch.

^bEgg masses eaten by predators were not included in the calculation of percentage egg mass parasitism.

Discussion

The poor emergence of *T. basalis* from parasitized southern green stink bug eggs placed at the release point for Experiments II and III may have been due to the heat generated inside the plastic container where these parasitoid embryos were kept pending emergence. Conversely, almost 100% *T. basalis* emerged during Experiment I, possibly due to the cooler weather in October 1993.

The high number of southern green stink bug egg masses attacked by predators in Experiment II may have been due to the semidwarf characteristic of 'NC5G' tomato and to the absence of plastic mulch that may have made it easier for crawling predators to search for southern green stink bug egg masses. In contrast, the low predation in 'NC5G' tomato in Experiment I may have been due to the cold weather slowing down predatory activity at the site. The low predation in 'Sunny' tomato is consistent with the findings of Justo (1994) and Shepard et al. (1994) who reported that predation on southern green stink bug eggs in mulched, staked tomato was low in South Carolina.

There were no indications that wind velocity, temperature, or relative humidity influenced the dispersal of *T. basalis* during the time these three experiments were conducted, although lower parasitism occurred along the northerly direction. Parasitism by *T. basalis* on southern green stink bug egg masses placed in tomato fields indicated that this wasp was highly efficient in locating egg masses. Data from Experiment I show about 2,000 *T. basalis* were sufficient to provide adequate biocontrol in a 50 × 50 m tomato field. The average percentage egg parasitism obtained from Experiment I is similar to levels recorded by Su & Tseng (1984) after the introduction of *T. basalis* in

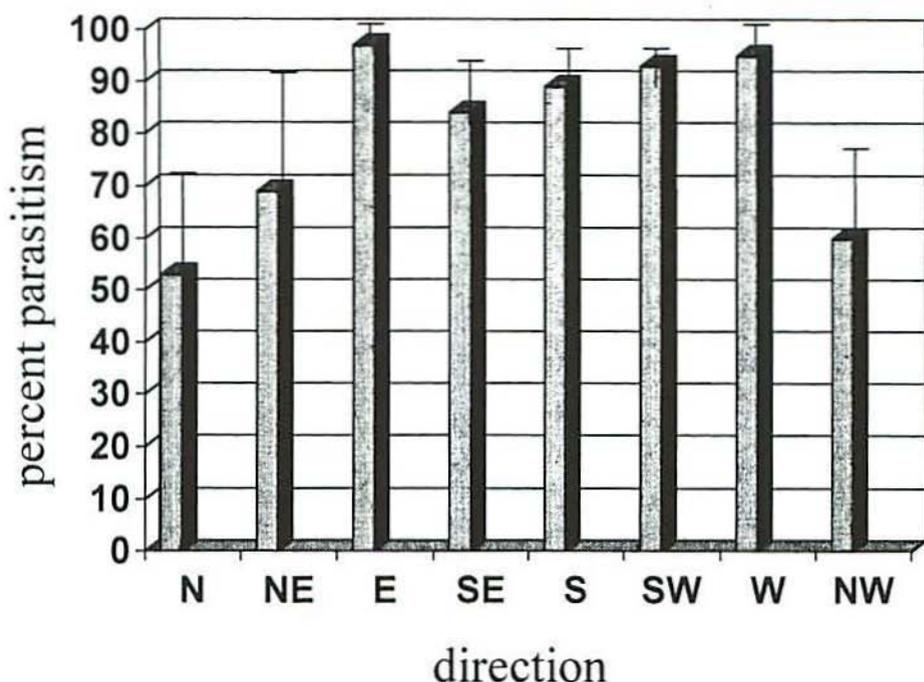


Fig. 2. Average parasitism by *Trissolcus basalis* on eggs of *Nezara viridula* placed equidistantly from the central release point (RP) within a tomato field along the N, NE, E, SE, S, SW, W, and NW directions, respectively. Coastal Research and Education Center, Charleston, South Carolina, 7–14 October 1993.

Taiwan to control southern green stink bug. Also, post-release surveys of parasitism by *T. basalis* (Hoffmann et al. 1991) in California were about the same as in the above studies. Hoffmann et al. (1991) found 79.9% parasitism by *T. basalis* of all southern green stink bug eggs and 87.2% parasitism of the eggs per egg mass on egg masses placed in the field and in naturally occurring egg masses.

Our data suggest that augmentative and inundative releases of *T. basalis* may be made anywhere in fields of similar dimensions we used because of the high efficiency of this parasitoid in locating southern green stink bug egg masses. This observation is similar to the findings of Sales (1979) who reported that *T. basalis* females possess remarkable ability to orient and find southern green stink bug eggs. Using a stochastic model that simulates interactions of *T. basalis* with southern green stink bug, Powell et al. (1983) reported that a parasitoid:host ratio of 1:60, with two releases of *T. basalis*, should provide satisfactory control.

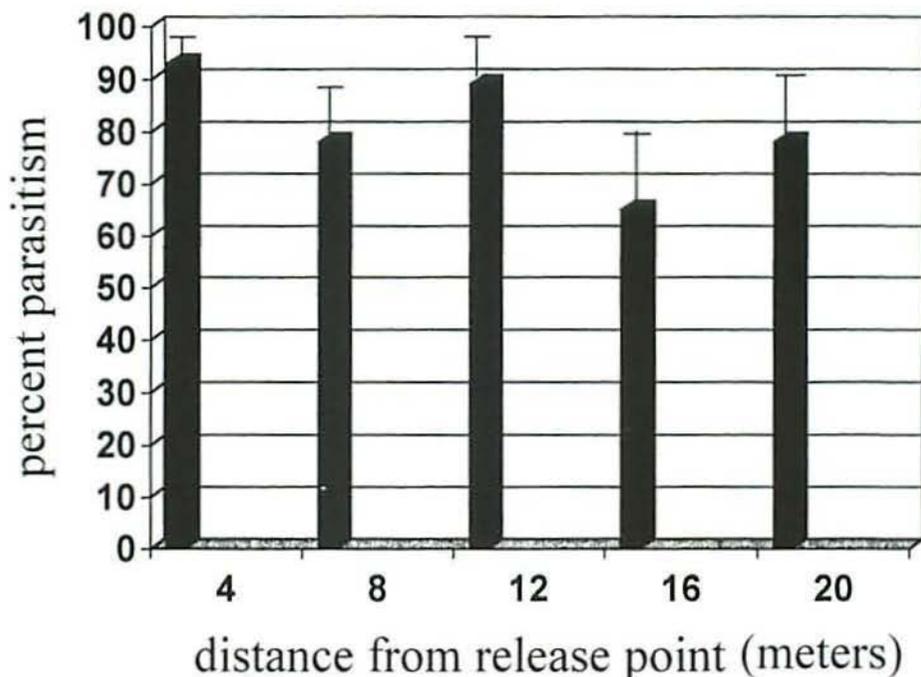


Fig. 3. Average parasitism by *Trissolcus basalis* on eggs of *Nezara viridula* placed at 4-m intervals from the central release point (RP) within a tomato field. Coastal Research and Education Center, Charleston, South Carolina, 7–14 October 1993.

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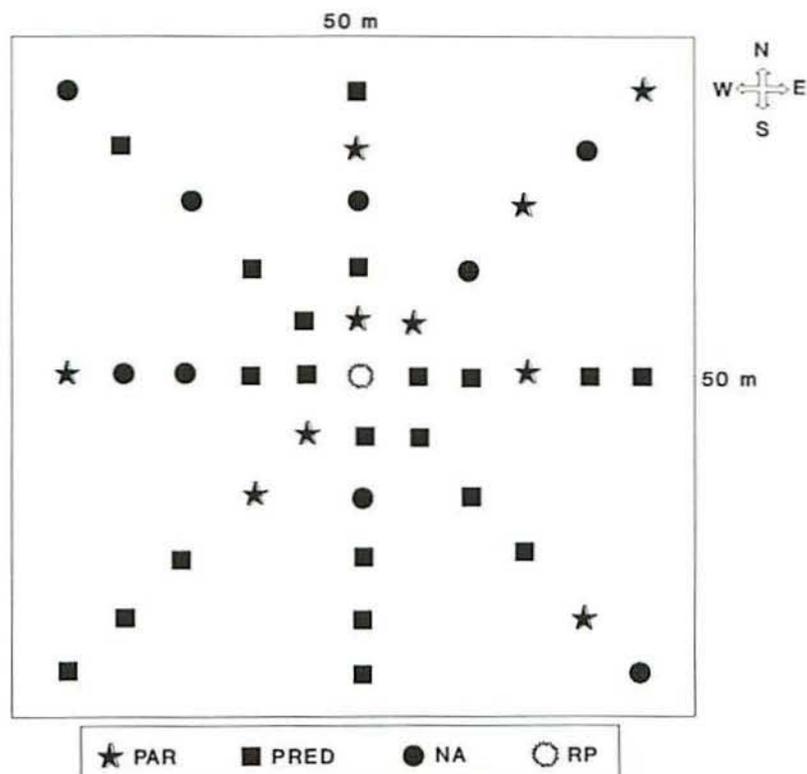


Fig. 4. Parasitism (PAR) by *Trissolcus basalis* on egg masses of *Nezara viridula* placed equidistantly from the central release point (RP) within a tomato field at the USDA-ARS, Charleston, South Carolina, 24–31 May 1994. Predation (PRED) on egg masses and those not attacked (NA) also are shown.

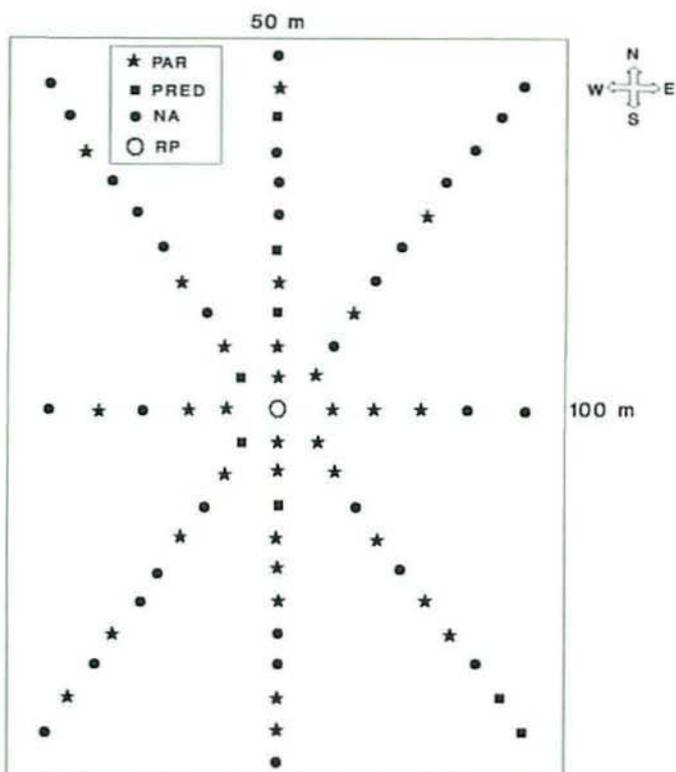


Fig. 5. Parasitism (PAR) by *Trissolcus basalis* on egg masses of *Nezara viridula* placed equidistantly from the central release point (RP) within a commercial tomato field on Wadmalaw Island, Charleston, South Carolina, 13–20 May 1994. Predation (PRED) on egg masses and those not attacked (NA) also are shown.

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Predation on Fall Armyworm (Lepidoptera: Noctuidae) in Sweet Sorghum¹

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ABSTRACT Predatory arthropod fauna was investigated to determine both composition and potential for controlling fall armyworm (*Spodoptera frugiperda* [J. E. Smith]) populations in sweet sorghum, *Sorghum bicolor* (L.) Moench. The fauna was comprised predominantly of red imported fire ants, *Solenopsis invicta* Buren. Predation was responsible for at least a 3-fold reduction in *S. frugiperda* infestation levels on whorl-stage sweet sorghum. Whorl-feeding behavior by *S. frugiperda* in plots with suppressed predator populations resulted in 10 to 15% plant defoliation. Post-whorl phenological stages of sweet sorghum were not attacked by subsequent generations of *S. frugiperda* larvae. Although significant differences were not detected, there was a 6.4% trend toward a reduction in total sugar yield in plots with higher *S. frugiperda* levels in 1985. However, a 72.5% reduction in *S. frugiperda* infestations likely prevented a repeat of this trend in 1986. Thus, early-season *S. frugiperda* damage to whorl-stage sweet sorghum was not found to cause significant economic losses in either year. Natural control by *S. invicta* was shown to be a major regulating factor in *S. frugiperda* population dynamics in sweet sorghum.

KEY WORDS Lepidoptera, *Spodoptera frugiperda*, Formicidae, *Solenopsis invicta*, predation

Sweet sorghum, *Sorghum bicolor* (L.) Moench, is one of several crops used for making fuel ethanol. It also has been cultivated on very limited hectareage in the southeastern United States for making sorghum syrup (Freeman et al. 1973). Increased sweet sorghum production in the early 1980s was undertaken to enhance domestic energy resources. At the time of these studies it had limited production, although it has been shown to have a promising future as an alternative energy resource (Nathan 1979).

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Insect pest management of sweet sorghum in Louisiana has focused on the sugarcane borer, *Diatraea saccharalis* (F.) (Reagan & Flynn 1986, Fuller et al. 1988). The red imported fire ant, *Solenopsis invicta* Buren, is the major predator in controlling *D. saccharalis* in sweet sorghum in Louisiana (Fuller & Reagan 1988). Other lepidopterous pests, including the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), have been known to attack this crop. Severe crop destruction by this pest was found during the seedling stages in sorghum grown in Georgia (Luginbill 1928). Pest density levels associated with this damage caused by *S. frugiperda* were not stated, and the necessity for treating *S. frugiperda* infestations in whorl-stage sweet sorghum remains undetermined. Thus, our study was undertaken to determine whether damage caused by *S. frugiperda* from early-season infestations is of economic importance in the production of sweet sorghum and to assess the role of predation in regulating *S. frugiperda* populations.

Materials and Methods

Sweet sorghum cultivar 'Wray' was planted in 0.1-ha plots (ca. 64 m × 64 m; rows spaced 0.9 m apart) in 1985 and 1986 on the St. Gabriel Research Station and the Gay Sugarcane Plantation sites located in Iberville Parish, Louisiana. Plant stands ranged from five to eight plants per meter of row. Plots were arranged in a randomized complete block design with four replications. Treatments consisted of sorghum plots receiving an early season (post-planting) application of chlordane at 1.1 kg (AI)/ha to eliminate *S. invicta* populations and untreated (natural) controls. Therefore, the relative abundance of *S. invicta* was used to investigate the role it plays in regulating *S. frugiperda* population levels. Following plot establishment, *S. invicta* presence was monitored using peanut oil-soaked index cards (7.6 cm × 12.7 cm) placed on the soil surface for 1 h within each plot according to methods of Ali & Reagan (1986). Detection prompted retreatment of the suppression plots, however, only one additional chlordane application was needed to suppress predator populations. Beginning at early whorl stage, 15 plants per plot were dissected to determine the number of *S. frugiperda* larvae per plant. Sampling continued weekly throughout the growing season. Two pitfall traps (Greenslade 1964) were placed within each plot to monitor relative abundance of soil-surface-dwelling arthropods on a weekly basis. All plots were checked for size and number of active *S. invicta* colonies in mounds as described by Markin et al. (1973) at harvest in 1985. Mound counts were not taken in 1986 due to severe postharvest lodging that precluded accurate assessments. Voucher specimens were deposited in the Louisiana State Arthropod Museum, Department of Entomology, Louisiana State University, Baton Rouge, Louisiana.

Sugar yield data were obtained by randomly selecting two 25-stalk bundles from a 22.9-m section of row per plot. These selected rows received applications of monocrotophos at 0.85 kg (AI)/ha to remove late-season *D. saccharalis* infestations and limited damage to *S. frugiperda* (only other observed pest). Sugar yield samples were processed using the press method of Tanimoto (1967). Total sugar (all fermentable sugars) was determined using techniques described by Ricaud & Arceneaux (1984). Data were

subjected to the GLM procedure (SAS Institute 1985), and differences between means were identified using the F statistic.

Results

Significantly ($P < 0.05$) fewer *S. invicta* were captured in pitfall traps in suppression treatment plots compared with plots without chlordane (97 vs. 1,017 in 1985; 192 vs. 1,120 in 1986). Similarly, no foraging *S. invicta* were found in foliar samples from suppression plots. The mean number of active (ant-occupied) *S. invicta* mounds found was 12.8 and 0.8 per plot in natural and suppressed areas, respectively. Also, field-margin mound colonies (those within 10 m of plot perimeters; usually in grassy breaks or along field edge) averaged 2 and 0.5 mounds per plot for these treatments, respectively. Colonies of *S. invicta* were randomly distributed throughout the study area. However, 86% of the colonies were located within field plots, and only 14% were found in the field margins. Small colonies were more prevalent (57.6%) when compared with the numbers of medium (13.5%) and large (28.9%) mounds.

Leaves of whorl-stage sweet sorghum in predator suppression plots were ragged in appearance due to *S. frugiperda* feeding. All *S. frugiperda* instars were found and their maximum density throughout the season reached a level of 12 larvae per plant. However, visual estimates of most plants showed 10 to 15% foliar injury in association with less than two larvae per plant. Plots with suppressed predator populations contained a 3-fold higher number of *S. frugiperda* larvae as compared with plots with natural predator populations during both years (Table 1). After whorl stage, *S. frugiperda* larvae were rarely observed on sweet sorghum. Those discovered following whorl-stage sweet sorghum were late instars feeding in the ligular area of leaves. Small distinctive feeding indentations (ca. 1 cm diam. and 3 mm deep) into stalk tissue also were observed. This second type of plant injury was found in <1% of harvested stalks.

Although *S. frugiperda* infestations were significantly ($P < 0.05$) higher in plots with suppressed arthropod predator populations, yield of total sugar (all fermentable plant sugars) did not reflect significant differences in relation to the variation in pest densities (Table 1). However, total sugar yield was reduced by 6.4% in plots with higher *S. frugiperda* levels in 1985. This trend for lower yield was not repeated in 1986 when field populations of *S. frugiperda* were substantially reduced (72.5%).

Pitfall data from the untreated plots included several soil-surface-dwelling predators (Carabidae, Araneae, Dermaptera, Staphylinidae, Cicindelidae, and other Formicidae); however, *S. invicta* represented 55.9 and 68.8% of total predator counts in 1985 and 1986, respectively. The next most abundant predator complex included Araneae (Lycosidae, 64.4%; Clubionidae, 13.3%; Linyphidae, 8.9%; Nesticidae, 5.6%; Theridiidae, 3.9%; and all other spiders, 3.9%) that comprised 19.7 and 16% for both years, respectively. The remaining predatory taxa represented <5% of total predators with the exception of the Cicindelidae, 16.7 and 10.5% in 1985 and 1986, respectively.

Table 1. Fall armyworm infestations and sugar yield observed in sweet sorghum plots at Iberville Parish, Louisiana, during 1985 and 1986.

Year	Predator populations	Sugar yield ^a (kg/ha)	<i>S. frugiperda</i> (larva/plant)
1985	Natural	12,482a	0.50b
	Suppressed ^b	11,687a	1.65a
1986	Natural	9,989a	0.11b
	Suppressed	10,742a	0.48a

^aMeans within columns and year followed by the same letter are not significantly different ($P > 0.05$) using the *F* statistic.

^bSuppression of arthropod predators achieved using soil-surface applied liquid chlordane at 1.1 kg (AI)/ha.

Discussion

A 3-fold reduction in *S. frugiperda* populations in untreated plots was likely due to soil-surface-dwelling predators (*S. invicta*, Araneae, Cicindelidae). The predominance and potential predatory importance of *S. invicta* in this group closely parallels the findings for *D. saccharalis* control in Louisiana sweet sorghum fields (Fuller & Reagan 1988, Reagan 1986). Removal of predators from early whorl-stage sweet sorghum did not significantly impact yield in either year of this study. However, natural populations of *S. frugiperda* may not have been sufficient to cause yield reduction. Nevertheless, predatory arthropod taxa were responsible for a 3- and 4-fold reduction of *S. frugiperda*. There currently is no evidence that predator effectiveness would have been diminished, if prey were available at higher density levels. Thus, the value of *S. invicta* and other predators can not be overlooked. Management considerations for sweet sorghum should include those options that safeguard and protect the natural enemies of *S. frugiperda* and other pests.

Our study further suggests that when leaf-feeding damage of approximately 15% occurs in whorl-stage sweet sorghum, insecticidal treatment should be avoided in deferment to the crop's natural ability to compensate for early-season damage. Additionally, reductions in early-season pesticide applications could result in greater natural control of not only *S. frugiperda* but also of other important pests such as *D. saccharalis* (Fuller & Reagan 1988).

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Fecundity and Longevity of the Yellowmargined Leaf Beetle (Coleoptera: Chrysomelidae) on Crucifers¹

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ABSTRACT Fecundity and longevity of adult yellowmargined leaf beetle, *Microtheca ochroloma* Stål, reared on cabbage, *Brassica oleracea* var *capitata* L.; collard, *B. oleracea* var *acephala* L.; mustard, *B. juncea* Cosson; turnip, *B. rapa* L.; and radish, *Raphanus sativus* L., were quantified in a laboratory experiment. Number of eggs per female (mean \pm SE) was significantly higher on turnip (490.7 ± 116.0) than on collard (198.9 ± 28.9) but did not differ significantly among cabbage (271.3 ± 39.1), mustard (424.9 ± 46.4), and radish (440.1 ± 50.1). Daily oviposition was significantly higher on turnip (5.9 ± 0.7) and mustard (5.9 ± 0.4) than on collard (3.3 ± 0.5), but was similar on cabbage (4.0 ± 0.5) and radish (4.6 ± 0.6). Beetles lived significantly longer on radish (109.2 ± 5.9 d) than on cabbage (64.7 ± 5.7 d), collard (63.9 ± 5.7 d), mustard (75.9 ± 6.1 d), or turnip (61.8 ± 5.9 d). The sex of the beetle did not significantly affect longevity.

KEY WORDS *Microtheca ochroloma*, Coleoptera, Chrysomelidae, Cruciferae, longevity, fecundity, oviposition

The yellowmargined leaf beetle, *Microtheca ochroloma* Stål, is a cool season pest of crucifer crops in the southeastern United States (Chamberlin & Tippins 1948, Rohwer et al. 1953, Oliver & Chapin 1983). This beetle, native to South America, was accidentally introduced into the United States in the 1940s (Chamberlin & Tippins 1948). Field populations of the beetle have been recorded from locations in Alabama, Florida, Louisiana, Mississippi and Texas, on cabbage, *Brassica oleracea* var *capitata* L.; collard, *B. oleracea* var *acephala* L.; mustard, *B. juncea* Cosson; turnip, *B. rapa* L.; radish, *Raphanus sativus* L.; and watercress, *Nasturtium officinale* R. Brown (Chamberlin & Tippins 1948, Spink 1959, Woodruff 1974, Balsbaugh 1978, Oliver & Chapin 1983).

There is little information on the biology of this beetle despite its wide distribution in the southeastern United States and its potential for damage to crucifer crops. Reported herein are the results of laboratory studies on the fecundity and longevity of adult *M. ochroloma* on cabbage, collard, mustard, radish, and turnip.

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Materials and Methods

To study fecundity and longevity of adult beetles on the five crucifer host plants, a source of insecticide and disease-free foliage was established by raising the plants in a greenhouse. The cultivars 'Early Round Dutch' cabbage, 'Georgia' collard, 'Florida Broadleaf' mustard, 'Scarlet Globe' radish, and 'Purple Top White Globe' turnip were used. Plants were raised on a Jiffy-mix[®] medium (Jiffy Products of America, Inc., Batavia, Illinois) in Jiffypots[®] (about 6.4 cm²)(Jiffy Products of America, Inc., Batavia, Illinois), and fertilized on alternate days by using Miracle-Gro[®] (Stern's Miracle-Gro Products, Inc., Port Washington, New York), a 20:20:20 water-soluble NPK fertilizer mixture. New plantings were made every 10 d to ensure that young foliage (3 to 5 wk) was continuously available to feed the beetles.

To begin the study, about 500 eggs were collected from a laboratory culture that had been maintained for >11 generations on mustard foliage. These eggs were divided into five groups, and each group was randomly assigned to a host plant. Beetles were reared on the leaves of assigned host plants from the first through the second generation in filter-paper-lined Petri dishes (100 mm × 15 mm) in a growth chamber maintained at 20°C and a photoperiod of 14:10 (L:D) h. As soon as the second generation adults eclosed, 20 1-d-old beetles of each sex were randomly selected by host-plant treatment and transferred in mixed-sex pairs to covered dishes. The dishes were arranged by host plant in plastic storage containers (10 cm × 15 cm × 30 cm) in a Hotpack[®] incubator (Hotpack Co., Philadelphia, Pennsylvania). Pairs were supplied with a leaf disc (15 cm²) of the appropriate plant every 48 h, and the eggs laid by each female were carefully collected and counted. Morphometric data (length and weight) were recorded for each adult beetle specimen. Fecundity defined as the total number of eggs laid per female was determined on each host. Longevity was designated as the number of days from adult emergence until death and was recorded for each host. Hatchability of the eggs also was determined for five randomly selected females by host plant. Daily ovipositional rate was calculated as the fecundity of a female divided by its longevity.

Statistics. Host-plant effects on fecundity, daily oviposition, and adult longevity were analyzed with analysis of variance, and included sex as well as host-plant effects on longevity as appropriate for a completely randomized design (PROC ANOVA, SAS Institute 1990). Fecundity was correlated with longevity, and both parameters also were correlated to the morphometric data by host-plant treatment (PROC CANCORR, SAS Institute 1990).

Results and Discussion

There was a significant effect of host plant on fecundity ($F = 3.90$, $df = 4$, $P = 0.0057$), daily oviposition ($F = 4.55$, $df = 4$, $P = 0.0021$), and adult longevity ($F = 11.40$, $df = 4$, $P = 0.0001$) of *M. ochroloma*. Number of eggs per female was significantly higher on turnip than on collard but did not otherwise vary significantly with host plant (Table 1). Fecundity ranged from a high of 1,497 eggs on turnip to a low of 10 on collard. Significant differences also were found in daily oviposition by host plant treatment, with beetles maintained on turnip

Table 1. Fecundity, adult longevity, and daily oviposition of the yellowmargined leaf beetle ($n = 20$) on cabbage, collard, mustard, radish, and turnip.

Host plant	Total fecundity (Mean \pm SE)	Longevity (d) (Mean \pm SE)	Daily oviposition (Mean \pm SE)
Cabbage	271.3 \pm 39.1ab	69.3 \pm 5.8a	4.0 \pm 0.5ab
Collard	198.9 \pm 28.9b	67.9 \pm 8.2ab	3.3 \pm 0.5b
Mustard	424.9 \pm 46.4ab	74.9 \pm 7.6a	5.9 \pm 0.4a
Radish	440.1 \pm 50.1ab	105.3 \pm 7.5b	4.6 \pm 0.6ab
Turnip	490.7 \pm 116.0a	67.8 \pm 9.9a	5.9 \pm 0.7a

Means within columns followed by the same letter(s) are not significantly different ($P = 0.05$); Tukey test, SAS Institute 1990.

and mustard laying significantly more eggs per day (5.9 ± 0.7 and 5.9 ± 0.4 , respectively), than those maintained on collard (3.3 ± 0.5) (Table 1). There was no significant difference in daily oviposition on cabbage (4.0 ± 0.5) and radish (4.6 ± 0.6) (Table 1). Eclosion was consistently high at 98.7% (952) on cabbage, 99.1% (934) on collard, 99.0% (1,823) on mustard, 99.1% (2,722) on radish, and 99.0% (2,424) on turnip. Beetles lived significantly longer on radish than on the other four host plants tested (Table 1). Longevity was not affected by sex; no significant main effect ($F = 0.53$, $df = 1$, $P = 0.4677$) or interaction with host plant ($F = 0.51$, $df = 4$, $P = 0.7301$) was detected. Longevities ranged from a high of 186 d on radish to a low of 16 d on collard.

In general, female beetles were significantly heavier and longer than males (Table 2), but size did not significantly affect fecundity and longevity ($P < 0.05$). However, correlations were found between fecundity and longevity on all hosts except radish (Table 3).

Fecundity and longevity of *M. ochroloma* on cabbage, collard, mustard, and radish had not been previously reported. This research demonstrated that the beetle can survive and lay viable eggs when fed cabbage, collard, mustard, and radish. Oliver & Chapin (1983) had earlier reported similar information for the beetle on turnip. However, the beetles maintained on turnip in our study, on average, laid more eggs and lived longer (490 eggs and 68 d) than the beetles raised by Oliver & Chapin (1983) (83 eggs and 43 d). This might be due to the fact that our beetles were fed relatively young foliage obtained from insecticide- and disease-free plants raised in the greenhouse. The beetles in Oliver & Chapin (1983) were fed foliage obtained from the field.

Information on the fecundity and longevity of *M. ochroloma* can be used as a quantitative measure of the suitability of each of the five crucifer plants as a

Table 2. Morphometric data of a laboratory population of the yellowmargined leaf beetle ($n = 20$) maintained on cabbage, collard, mustard, radish, and turnip.

Host plant ^a	Sex ^b	Length (mm) (Mean \pm SE)	Weight (mg) (Mean \pm SE)
Cabbage	Female	5.9 \pm 0.1	11.9 \pm 0.4
	Male	4.9 \pm 0.1	7.5 \pm 0.3
Collard	Female	5.9 \pm 0.1	11.6 \pm 0.5
	Male	5.0 \pm 0.1	7.4 \pm 0.3
Mustard	Female	5.8 \pm 0.1	11.6 \pm 0.3
	Male	4.8 \pm 0.1	7.4 \pm 0.2
Radish	Female	5.8 \pm 0.1	9.8 \pm 0.5
	Male	5.1 \pm 0.1	6.7 \pm 0.4
Turnip	Female	5.9 \pm 0.1	10.1 \pm 0.3
	Male	4.9 \pm 0.1	6.9 \pm 0.3

^aNo significant effect of host plant on length and weight ($P = 0.05$; Tukey test, SAS Institute 1990).

^bFemales were significantly heavier and longer than males ($P = 0.05$; Tukey test, SAS Institute 1990).

host for the beetle. This information also could be used to assess the potential size of beetle population on field plantings of these crops. Based on fecundity data, it is apparent that mustard, turnip, radish, and cabbage could be regarded as suitable hosts and be expected to support a relatively higher population of the beetle than collard. Thus, field plantings of the former plants could incur more damage than the latter crop. Although field densities of the beetle have never been quantified on any of these plants, many workers have reported field observations of unusually high numbers of the beetle on turnip and mustard relative to the other plants (Haeussler 1951, Rohwer et al. 1953, Anonymous 1976, Oliver & Chapin 1983).

The relatively high fecundity of beetles maintained on cabbage was not expected because Chamberlin & Tippins (1948) reported that a field population of the beetle did not feed on cabbage in Theodore, Alabama, where turnip and cabbage plants were in close proximity. They suggested that this beetle does not feed on cabbage if there are choices of highly preferred plants, for example, turnip or mustard. The fact that beetles fed cabbage, a less preferred plant, laid as many eggs as beetles fed turnip and mustard, the more preferred plants, suggests that it is necessary to investigate the factors responsible for the apparent lack of preference for cabbage.

Table 3. Correlation coefficients for the relationships of fecundity to length, weight, and adult longevity of a laboratory population ($n = 20$) of the yellowmargined leaf beetle maintained on cabbage, collard, mustard, radish, and turnip.

Host plant	Parameter	Correlation coefficient	$P > F$
Cabbage	Length	0.4484	0.0474
	Weight	0.5539	0.0113
	Longevity	0.5794	0.0074
Collard	Length	0.1723	0.4676
	Weight	0.1895	0.4236
	Longevity	0.1262	0.5959
Mustard	Length	-0.0224	0.9274
	Weight	-0.3745	0.1142
	Longevity	0.7176	0.0005
Radish	Length	0.0488	0.8429
	Weight	0.1250	0.6101
	Longevity	-0.1330	0.5863
Turnip	Length	-0.1353	0.5809
	Weight	-0.2345	0.3339
	Longevity	0.9444	0.0001

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Action Thresholds for Controlling *Phyllonorycter crataegella* (Lepidoptera: Gracillariidae) Leafminers Based on Pre-bloom Captures of Adults on Visual Traps¹

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ABSTRACT We compared the relationship of pre-bloom captures of adult *Phyllonorycter crataegella* (Clemens) leafminers on sticky red rectangle traps stapled in vertical position at knee height to south sides of tree trunks with densities of first-generation larvae on foliage in 32 commercial apple orchard blocks that received no pesticide treatment against the first generation. Regression analysis revealed a highly significant linear relationship between cumulative adult trap captures from the silver tip to the tight cluster stage of bud development and densities of first-generation larvae ($r^2 = 0.645$). The same was true for cumulative adult trap captures from the silver tip to the full pink stage of bud development ($r^2 = 0.838$). In neither case was there a significant quadratic relationship between these variables. Action thresholds of 7 and 14 first-generation larvae per 100 leaves translated into action thresholds of a cumulative of 4 and 8 adult trap captures, respectively, from silver tip to tight cluster and a cumulative of 9 and 21 adult trap captures, respectively, from silver tip to full pink. We describe advantages offered by sticky red rectangles attached to tree trunks compared with other approaches used to monitor *P. crataegella* populations prior to the appearance of visually detectable mines.

KEY WORDS *Phyllonorycter*, leafminers, visual traps, action thresholds

The apple blotch leafminer, *Phyllonorycter crataegella* (Clemens) (Lepidoptera: Gracillariidae), and the spotted tentiform leafminer, *Phyllonorycter blancardella* (F.), are important pests of apple foliage in commercial orchards of northeastern North America. Indeed, in a recent survey completed by more than 75% of Massachusetts tree fruit growers, *Phyllonorycter* leafminers were ranked fourth in importance among all arthropod pests of apple in terms of concern for potential crop damage (Prokopy et al. 1996a). In northeastern North America, *Phyllonorycter* leafminers usually complete three generations per year. Damage is believed to be greatest by second-generation

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larvae and can result in early ripening and premature drop of fruit, reduced fruit size, and reduction in fruit set the following year (Reissig et al. 1982). Parasitoids of *Phyllonorycter* larvae may in some orchards provide considerable population suppression of miners (reviewed in Maier 1994), but most commercial growers rely on pesticides for control.

Several approaches exist for monitoring the abundance of *Phyllonorycter* leafminer populations to aid in determining the need to apply pesticide (reviewed in Coli & Leahy 1994). These include monitoring adults by using sex pheromone traps (Trimble 1986, Vincent et al. 1986) or visual traps (Coli et al. 1985, Green & Prokopy 1986), monitoring eggs deposited on the foliage (Nyrop et al. 1990, Agnello et al. 1991), and monitoring larvae feeding on foliage (Reissig et al. 1982, Agnello et al. 1991, Schmaedick & Nyrop 1993).

Although pheromone traps may provide accurate information on initial emergence and peak activity periods of the adults, thus far it has not been possible to establish a good relationship between captures of adults in pheromone traps and subsequent larval densities (Nyrop et al. 1990). A sequential sampling plan involving pre-bloom monitoring of first-generation eggs has proven reliable for predicting subsequent larval densities of *P. blancardella* in many New York orchards (Nyrop et al. 1990). However, from our experience in Massachusetts, and from information gained from several other states (Coli & Leahy 1994), comparatively few growers engage in monitoring eggs on account of the considerable training and experience necessary for identification of eggs, which are minute in size (ca. 0.3 mm), about the same color (whitish green) as the undersides of leaves on which they are laid, and sometimes masked by the hairiness of leaves. Survey results indicate that examining leaves for evidence of mines made by larvae is the most frequently used method of determining leafminer abundance (Coli & Leahy 1994). This approach is the method of choice for justifying post-petal-fall application of pesticides such as methomyl against first- or second-generation larvae. But it is not an effective approach for pre-bloom application of pesticides such as oxamyl, pyrethroids, or endosulfan, or for petal-fall application of pesticides such as imidacloprid or abamectin, whose principal effects are on adults, eggs, or sap-feeding larvae. In these cases, pesticide application must be made before the majority of individuals in a population is detectable as sap-feeding miners (annually in Massachusetts, for example, fewer than 10% of sap-feeding *P. crataegella* miners are detectable by petal fall, with 90% or more not detectable until a week or more after petal fall). Use of visual traps to monitor moth abundance is a promising approach to determining need for application of those pesticides aimed at first-generation adults, eggs, or sap-feeding larvae or whose labels restrict use to application no later than petal fall.

Among the various types of visual traps evaluated for capturing *P. crataegella* adults, sticky-coated red rectangles have proven more effective than sticky-coated rectangles of other colors or shades (Green & Prokopy 1986). When Coli et al. (1985) compared first-generation larval abundance with pre-bloom captures of *P. crataegella* adults on sticky red rectangles hung in horizontal position at a height of 1.5 m in the interior canopy of trees in 32 commercial apple orchard blocks, they obtained a highly significant positive

relationship. Subsequently, Green & Prokopy (1986) found that red rectangles placed 0.5 m above ground next to the tree trunk captured nearly six times more *P. crataegella* adults than traps hung anywhere in the tree canopy.

Here, in 32 commercial apple orchard blocks that received no pesticide treatment against first-generation *P. crataegella* populations, we compared the relationship of pre-bloom captures of adults on sticky red rectangles attached in vertical position to tree trunks at 0.5 m above ground with densities of first-generation larval populations.

Materials and Methods

We stapled one Tangletrap[®]-coated 20 cm × 30 cm vertically oriented plastic red rectangle (Gempler's, Inc., Box 270, Mt. Horeb, Wisconsin) at 0.5 m above ground to the southern face of the trunk of each of five apple trees in each of 32 commercial orchard blocks. Each block was 2–4 ha. Other characteristics of the blocks are described in Prokopy et al. (1996b). One trap was positioned ca. 5 m inward from each corner of each block and one near the center. All traps were emplaced at the silver tip stage of fruit bud development (before adult emergence) and remained in place through the full pink stage (Anonymous 1996). Traps were examined at both the tight cluster and full pink stages of development, with all *Phyllonorycter* moths counted and removed at each examination.

The 32 blocks studied here during 1991 to 1994 received no pesticide against first-generation *Phyllonorycter* adults, eggs, or larvae and were populated predominantly (80% or more of collected individuals) by *P. crataegella* (voucher specimens deposited in Fernald Hall at the University of Massachusetts in Amherst). We sampled 10 interior fruit cluster leaves (third to sixth oldest leaves) (Leahy & Prokopy 1992) on each of 20 trees per block in late June, when all first-generation larvae had completed development and were readily detectable by evidence of stippled tentiform-shaped mines (total = 200 leaves per block). Sampled trees were chosen in an X pattern across the block.

For statistical analyses, we pooled all trap capture and mine count data for each block. We then subjected all data to linear and quadratic regression analysis by using Microsoft EXCEL 5.0.

An action threshold for New York has been suggested as 200 second-generation *Phyllonorycter* mines per 100 leaves (Reissig et al. 1982). In Massachusetts and other parts of New England, orchard trees are frequently under stress from summer drought and foliar damage from calcium chloride sprays (applied to enhance fruit firmness during storage). These two factors, coupled with the loss of daminozide as a chemical for preventing premature ripening and drop, have prompted us to establish a lower provisional action threshold of 100 second-generation mines per 100 leaves for non-McIntosh cultivars and 50 second-generation mines per 100 leaves for McIntosh. McIntosh is the dominant cultivar in New England and is much more susceptible to premature ripening and drop as a consequence of leafminer feeding, drought, and calcium chloride injury than are other principal cultivars (Anonymous 1996). Data gathered in 1981 and 1982 from 18 Massachusetts commercial orchard blocks that received no pesticide against first- or second-generation *P. crataegella*

leafminers revealed an average 7.4-fold level of population increase from first- to second-generation larvae (Coli et al. 1985). Data gathered by us from 1991–1994 in 26 of the 32 blocks studied here that did not receive pesticide against second-generation leafminers revealed likewise an average 7.4-fold level of population increase from first- to second- generation larvae. Hence, we have adopted provisional action thresholds of 14 first-generation larval mines per 100 leaves for non-McIntosh cultivars ($100 \div 7.4$) and 7 first-generation larval mines per 100 leaves for McIntosh ($50 \div 7.4$).

Results

Regression analysis revealed a highly significant linear relationship between cumulative captures of *P. crataegella* adults from the silver tip to the tight cluster stage of bud development and densities of first-generation mines ($r^2 = 0.645$, $P = 0.0001$, Fig. 1). The same was true for cumulative adult trap captures from silver tip to the full pink bud stage and densities of first-generation mines ($r^2 = 0.838$, $P = 0.0001$, Fig. 2). In neither case was there a significant quadratic relationship between trap captures and mine densities. Inspection of the linear regression lines shows that action thresholds of 7 and 14 first-generation mines per 100 leaves translate into action thresholds of a cumulative of 4 and 8 adult trap captures, respectively, from silver tip to tight cluster and a cumulative of 9 and 21 adult trap captures, respectively, from silver tip to full pink. By extrapolation, an action threshold of 28 first-generation mines per 100 leaves (equivalent to the New York standard) translates into a cumulative of 17 and 42 adult trap captures from silver-tip to tight cluster and full pink, respectively.

Discussion

Our findings suggest that the level of pre-bloom captures of *P. crataegella* leafminer adults on sticky red rectangles stapled at knee height to south sides of apple tree trunks could be a useful predictor of growers need to take action or not to take action against this pest. Unfortunately, our study turned out to be less robust than we had planned. In addition to traps placed in the 32 blocks reported here, we placed traps in another 64 blocks. Growers judged 48 of these 64 blocks to be in need of treatment to prevent establishment of potentially damaging leafminers populations and hence applied recommended pesticides (Anonymous 1996) against first-generation leafminers, denying the possibility of relating trap captures to larval densities. Exclusion of data from these blocks meant that only blocks with comparatively low or moderate leafminer populations were useable for analysis. Leafminer populations in the other 16 blocks consisted substantially (20% or more) of *P. blancardella* and were excluded from consideration.

What advantage do sticky red rectangles stapled in vertical position to tree trunks offer over sticky red rectangles hung in horizontal position (sticky side up) in tree canopies? First, adult captures on horizontal canopy traps, though being significant predictors of *P. crataegella* larval density ($r^2 = 0.567$ for captures from silver tip to full pink) (Coli et al. 1985), are not as strong

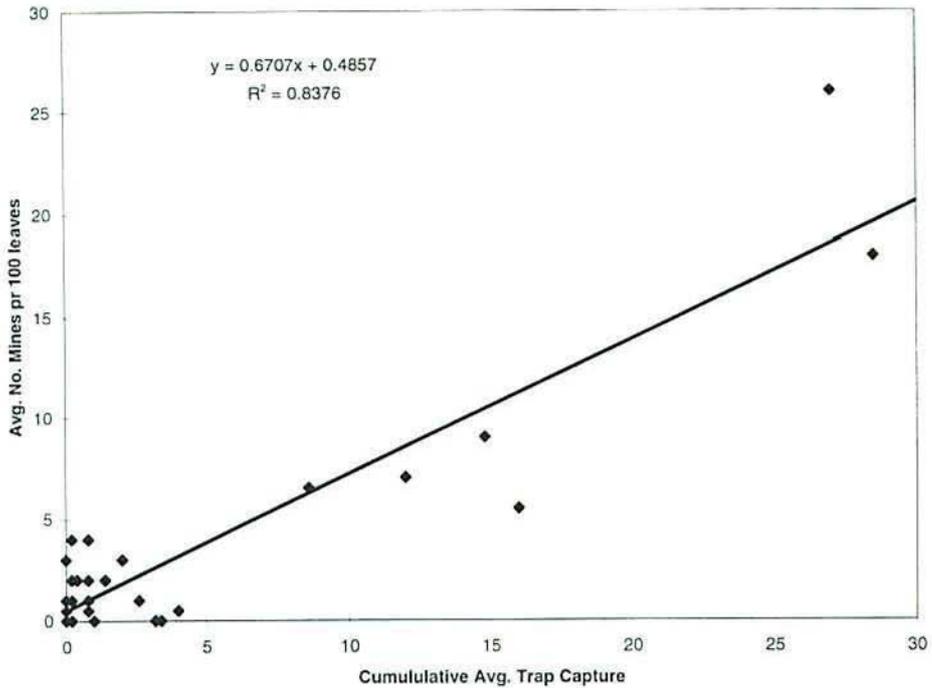


Fig. 1. Linear regression curve describing the relationship between adult *P. crataegella* trap captures from silver tip to tight cluster stages of apple and bud development and density of first-generation larvae. Of the 32 sets of data comprising this linear regression, only 19 data points appear here. The remaining 13 data points are identical with some of the 19 that appear.

predictors as are adult captures on vertical trunk traps ($r^2 = 0.838$ for the same time period) (Fig. 2), possibly because vertical trunk traps yield considerably greater capture levels (Green & Prokopy 1986). Second, in our experience and that of private consultants and several growers who have used red rectangles in both positions, it is much easier to identify *Phyllonorycter* adults on vertical than horizontal traps. One reason is that adults on horizontal traps are much more prone to lose wing scales during rainfall. Excessive loss of wing scales causes difficulty in species identification, especially for an inexperienced person. Hanging red rectangles vertically in tree canopies offers no advantage in this regard because they are far less effective in capturing *P. crataegella* adults than are horizontal red rectangles in tree canopies (Green & Prokopy 1986).

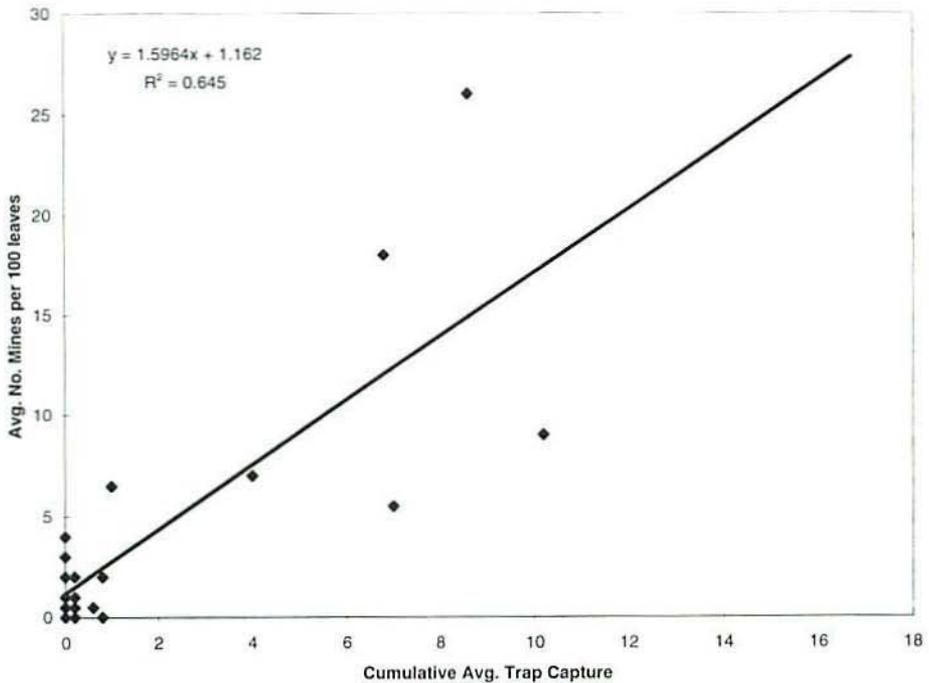


Fig. 2. Linear regression curve describing the relationship between *P. crataegella* adult trap captures from silver tip to full pink stages of apple bud development and density of first-generation larvae. Of the 32 sets of data comprising this linear regression, only 26 data points appear here. The remaining 6 data points are identical with some of the 26 that appear.

In a survey of 34 tree fruit extension personnel in North America (Coli & Leahy 1994) and in a survey of more than 75% of Massachusetts tree fruit growers (Prokopy et al. 1996a), 30%–40% of the respondents indicated that they use sticky red rectangle traps for monitoring *Phyllonorycter* leafminer abundance. Results reported here offer a firm prospect for making correct control decisions for *P. crataegella* based on levels of pre-bloom adult captures on such traps. Choice of using captures up to tight cluster or up to full pink will depend on whether pesticide will be applied at tight cluster, pink, or petal fall. In future studies, we plan on determining the degree to which captures of *P. blancardella* adults on sticky red rectangle traps on tree trunks are useful for predicting tentiform mine densities in orchards dominated by that species and on determining optimal numbers of traps per hectare or per orchard block needed to provide an accurate estimate of *Phyllonorycter* adult population density.

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Phenology and Dispersal of *Harpalus rufipes* DeGeer (Coleoptera: Carabidae) in Agroecosystems in Maine¹

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ABSTRACT The phenology and movement of adult *Harpalus rufipes* DeGeer (Coleoptera: Carabidae) was studied in Presque Isle and Stillwater, Maine during 1989-1996. Samples collected from pitfall and light traps indicated *H. rufipes* is the dominant ground beetle species (up to 78.4% of the total Carabidae) in Maine potato agroecosystems. This species overwinters as both adults and larvae. Overwintered adults became active in early May and densities peaked by the end of June. Overwintered larvae started pupating in mid-July; the resulting summer adult population peaked by mid-August. Seasonal adult population catches in light traps coincided with the two peak pitfall trap catches in most years. Most of the light trap catches (97%) represented sexually immature adults suggesting that long-distance dispersal by flight occurs before oviposition. Mean tethered flight durations for females and males were 188.5 ± 242.6 (mean \pm SD) and 100.4 ± 84.4 min, respectively. The mean dispersal distances per day by ovipositing females after mark and release was 9.8 ± 5.2 m in a potato field and 11.2 ± 7.0 m in an oat field. The mean dispersal distance per day by males was 6.5 ± 6.1 m in a potato field and 5.7 ± 4.7 m in an oat field. This suggests that adult *H. rufipes* are very active on the soil surface and have potential to numerically respond to localized weed seed densities, which is an important characteristic of a weed seed predator.

KEY WORDS Coleoptera, Carabidae, *Harpalus rufipes*, phenology, potato, flight

There are over 400 species of ground beetles (Coleoptera: Carabidae) in Maine (Anonymous 1983). One species, *Harpalus rufipes* DeGeer, was inadvertently introduced into North America from Europe shortly before 1937 (Dunn 1981). The first documented report of this species in Maine was from the town of Topsfield in 1966. At that time *H. rufipes* was abundant; therefore, we suspect that it must have been present

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in this area for several years prior to the report. By 1978, *H. rufipes* had not been reported west of New Hampshire and Quebec and south of Maine (Anonymous 1983). *Harpalus rufipes* now is found in Newfoundland, Quebec, Prince Edward Island, Nova Scotia, and New Brunswick in Canada; and in Maine, New Hampshire, Vermont, Rhode Island, and Connecticut in the United States (Zhang et al. 1994).

Little is known about the ecology of this species in the northeastern United States and in eastern Canada. The adults are dull black, elongated oval, and 1.27–1.59 cm long with reddish legs and may be found throughout the year in Maine (Zhang 1993). Although active during the day if disturbed, the beetles are most active at night. Upon maturity each female lays small white, inconspicuous eggs in the soil that hatch within 10–14 d. The first two larval instars are active and can be captured in pitfall traps (Hartke 1996). In Europe, pupation of the overwintered larvae occurs within the soil beginning in early July, and a new generation of beetles (usually lighter in color, often tan) begins to appear in the middle of July (Briggs 1965, Luff 1980). Both larvae and adults overwinter. The beetles feed primarily on seeds of various grains and weeds that they cache in burrows or beneath plant residue (Zhang 1993).

Growing concern over the environmental impacts of intensive herbicide use has led to increased interest in cultural and biological methods of weed management (Altieri & Liebman 1988).

The objective of this paper is to describe the phenology and dispersal ability of adult *H. rufipes*, a potentially important weed seed predator in Maine potato agroecosystems.

Materials and Methods

Seasonal incidence. Seasonal incidence of *H. rufipes* adults was recorded in Stillwater, Maine, during 1989 and 1990 and in Presque Isle, Maine during 1989–1991. Adult beetles were sampled for a duration of 7 d with pitfall traps (0.5-liter plastic containers) containing antifreeze and water in a 1:1 ratio. Forty-eight pitfall traps were randomly set out three times during 15 June to 24 July in 1989 and nine times during 11 June to 15 August in 1990 in a 0.1-ha potato field at the University of Maine's Roger's Farm, Stillwater, Maine. In Presque Isle at the University of Maine's Aroostook Farm, 10 pitfall traps were set out 11 times during 21 June to 5 September in 1989; 12 traps were set out seven times during 19 July to 30 August in 1990, and 32 traps were uniformly set out 19 times during 26 April to 12 September in 1991 in a 0.6-ha potato field. No insecticides were applied in either of the potato fields. Five traps were randomly set out in each of 48 barley and 48 potato plots (0.08 ha) in a large ecosystem project (Alford et al. 1996) in late June and mid-August in 1991 and 1992 for 7 d. All traps were protected from rain with 20 cm × 20 cm aluminum rain covers. Adult *H. rufipes* and all other adult ground beetles were counted, identified (Lindroth 1969), sexed, and recorded.

Mating behavior and egg laying. Observations on the reproductive behavior of adults caught from dry pitfall traps (without antifreeze or water) during July and August 1992 were made in the laboratory. Mature adults were separated by sex and held in isolation 1 to 2 d before allowing the sexes to mix

for mating behavior observations. Male/female pairs were placed in 9-cm petri dishes with moist filter paper and supplied with 30 barnyard grass (*Echinochloa crusgalli* [L.] seeds each day. Mating duration was recorded with a stop watch. Ninety-two female *H. rufipes* were dissected during 1991 beginning 23 June to determine the phenology of egg maturation. Egg laying by females was studied by direct observation. Numerical estimates of fecundity were obtained by recording oviposition by 136 females collected from dry pitfall traps in potato and barley fields during 11 July to 15 August 1994 and 173 females during 9 July to 16 August 1995. Beetles were sexed and placed within covered 473-ml plastic deli containers (oviposition chambers) filled with moist sand. Each oviposition chamber contained one adult female, monitored daily and fed lambsquarters (*Chenopodium album* L.), with a photoperiod of 16:8 (L:D) h at a constant temperature of 18°C.

Flight duration. Forty-one adults collected during 8–13 June and 20–23 July 1992 from dry pitfall traps and a light trap were tethered with Duro® superglue (Lactite Corp., Rocky Hill, Connecticut) on the pronotum, to the head of an insect pin. The insect pin was then attached to a suspended styrofoam block (ca. 1 cm³) connected to a 50-cm thread allowing free flight in any direction. Tethered flight tests were conducted under laboratory conditions (ca. 20°C) after 1800 h. Observations were conducted under a red torch light. Flight durations were measured and recorded by direct observation. Tethered adults were disconnected after 4 h if they did not fly. Females were dissected after flight test experiments to determine ovarian development by checking for mature eggs. Flight characteristics such as flight duration and frequency are presented as mean ± standard deviation.

Flight phenology. A black light-trap was used to capture flying *H. rufipes* during 1991–1996 at the University of Maine's Aroostook Farm. The light trap was turned on at dusk and off at dawn. It was checked every 2 d starting 1 June throughout the potato growing season until 10 October in 1991 and until 1 September in 1992–1996. Adults caught in the light trap were collected at dawn, identified, and sexed. In 1991 trapped females were dissected for determination of ovarian development (Zhang 1993). By using hourly temperature data collected at the University of Maine's Aroostook Farm (weather station ca. 10 m from light trap), we investigated the capability of various temperature indices to predict days of first light-trap catch and peak light-trap catch of *H. rufipes* adults between 1991 and 1996. The measures we used were as follows: day of the year, accumulated degree days from 1 April of each year using the base threshold temperatures of 0°–16°C at 2°C intervals; the average temperature on the day of first catch or peak catch; number of days where the minimum daily temperature was greater than 0°, 5°, and 10°C from 1 April to date of catch; number of days where the average daily temperature was greater than 0°, 5°, 10°, 16°, and 20°C from 1 April to date of catch; and the mean of the minimum temperature from the 5 d before first trap catch and peak trap catch. For the 6-yr period, the mean and standard error were calculated for each of the measures and the standard error:mean ratio was used as an index of precision to evaluate the best predictor.

Walking dispersal ability. Two mark-recapture experiments of adult *H. rufipes* were conducted to determine adult walking dispersal ability during July

and August 1991 at the University of Maine's Aroostook Farm. Adults were collected with dry pitfall traps 1–4 d before experiments. Adults were marked with Testors® red and white paint (The Testor Corp., Rockford, Illinois) on the pronotum. The first experiment was conducted in a potato field (0.6-ha). Ten females and 97 males captured from dry pitfall traps were released with red paint, and 10 females and 90 males were released with white paint on 10 July 1991 at two release centers surrounded by 57 concentrically spaced pitfall traps in five rings. The second experiment involved releasing 48 females and 298 males marked with red paint on 8 August 1991 in the center of a 2.5-ha oat field. Four, 8, 8, 12, 16, and 12 dry pitfall traps were set up at an equal distance along circumferences with radii of 3.0, 6.1, 9.1, 12.2, 15.2, and 30.5 m, respectively. In both experiments pitfall traps were visited daily for 2 wk after the initial release and adults were examined for marks and then released back to the field. Dispersal ability is presented as mean \pm standard deviation. Dispersal distance due to beetle sex and crop habitat were compared with a two-way ANOVA (Gagnon et al. 1989).

Weed seed rain. Ten enamel trays ($32 \times 48 \text{ cm}^2$) covered with 3.1-mm metal screening (to exclude adult beetles and other seed predators) were randomly placed between the rows of a 0.06-ha potato plot to catch seed rain of weeds common in potato production in northern Maine during 21 May–31 August 1993. Seed trays were checked on a weekly basis and brought back to the laboratory for species identification (Hartke 1996).

Results and Discussion

Seasonal incidence. *Harpalus rufipes* is the dominant ground beetle in potato-grain systems in Aroostook Farm. Most (49.7%–78.4%) of the carabids caught in pitfall traps in June and August, 1991–1992 were *H. rufipes* (Fig. 1). The next most dominant ground beetle was *Pterostichus melanarius* DeGeer, which made up 0.3%–25.6% of the total carabid community (Fig. 1). There were no significant differences in the relative abundance (proportion *H. rufipes* relative to total carabid community) of adult *H. rufipes* between barley and potato fields ($F = 0.323$; $df = 1, 196$; $P = 0.571$; $F = 2.804$; $df = 1, 158$; $P = 0.096$, 1991 and 1992, respectively). However, there were significant differences in the number of adult *H. rufipes* trapped in potato fields compared with barley fields. Barley contained significantly more adults than potatoes ($F = 101.00$; $df = 1, 26$; $P < 0.001$; $F = 16.91$, $df = 1, 26$; $P = 0.003$, 1991 and 1992, respectively; 1991 mean adults/trap/day in potato = 0.54 ± 0.08 , in barley = 1.75 ± 0.23 ; 1992 mean adults/trap/day in potato = 0.23 ± 0.02 , in barley = 0.49 ± 0.09).

Adult *H. rufipes* became active in April and May in Maine (Figs. 2, 3). This is similar to its phenology in Europe (Briggs 1961). Patterns of adult population incidence were similar during 1989–1991 in Presque Isle (Figs. 2, 3) and during 1989–1990 in Stillwater (Fig. 2). The first peaks appeared in the end of June. All beetles caught during the first peaks were darkly pigmented and so were overwintered beetles (Zhang 1993). Newly emerged adults (light tan) were observed between mid-July and the end of August. These summer beetles take 6–17 d to darken and contribute to the second adult peak in mid to late August (Zhang 1993). The percentage of newly emerged adults compared

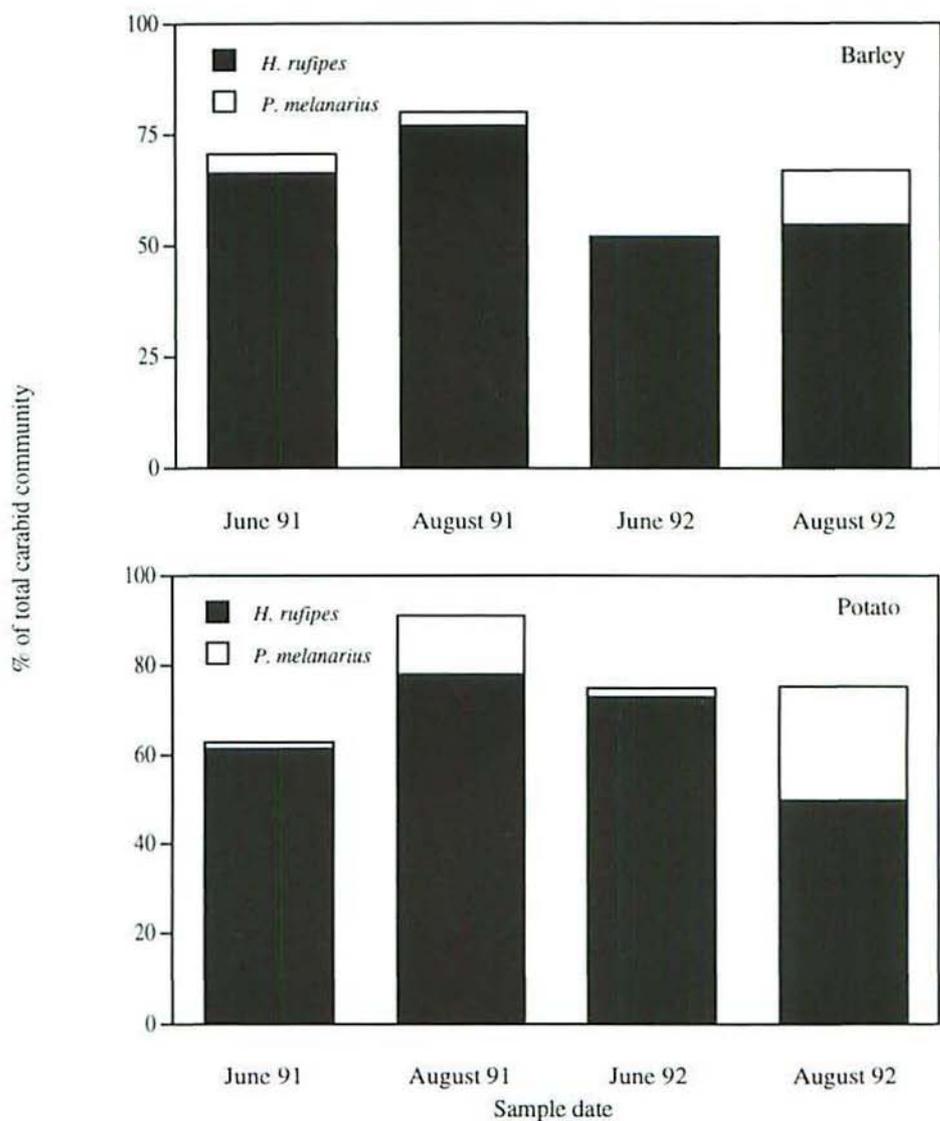


Fig. 1. Relative abundance of *H. rufipes* and *P. melanarius* in carabid communities caught in pitfall traps in a potato field at Aroostook Farm, Maine.

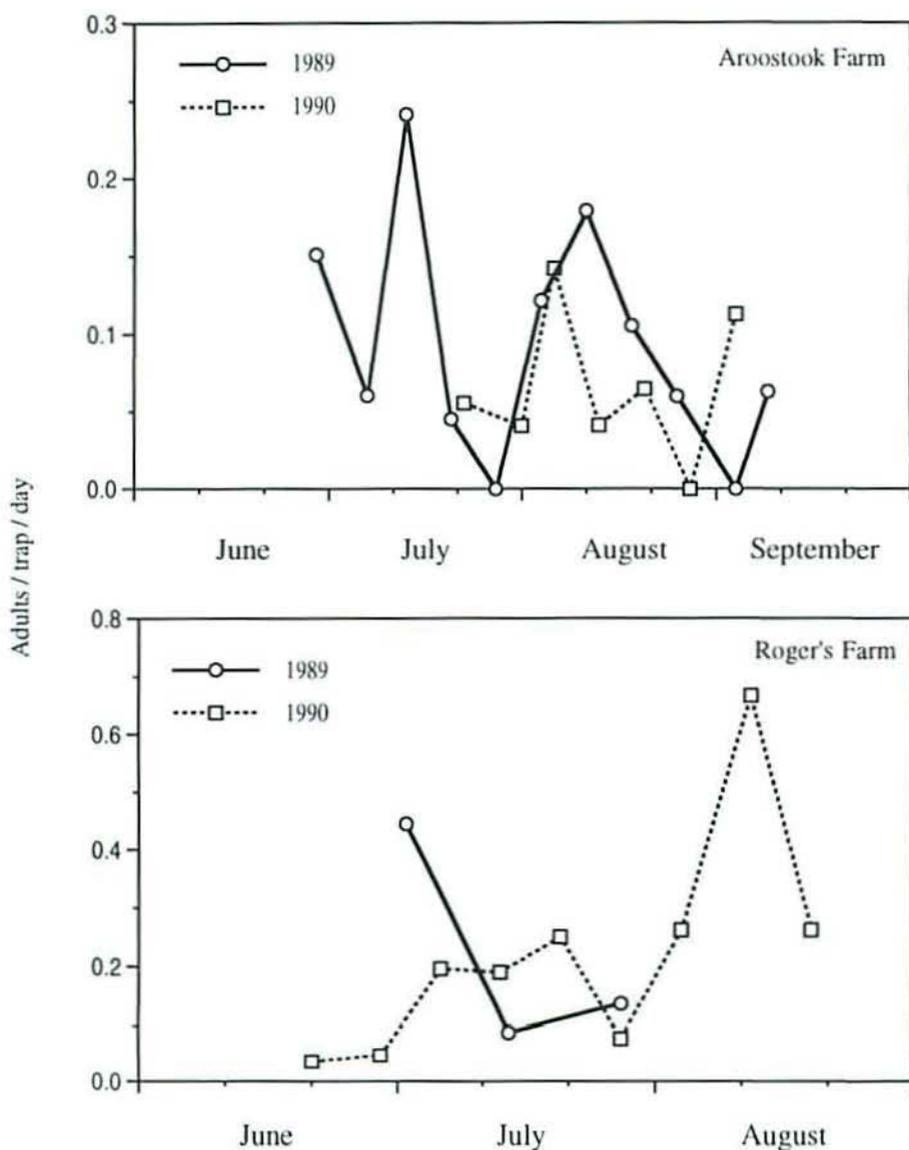


Fig. 2. Seasonal pitfall trap catches of adult *H. rufipes* in Aroostook Farm and Roger's Farm, Maine, in 1989 and 1990.

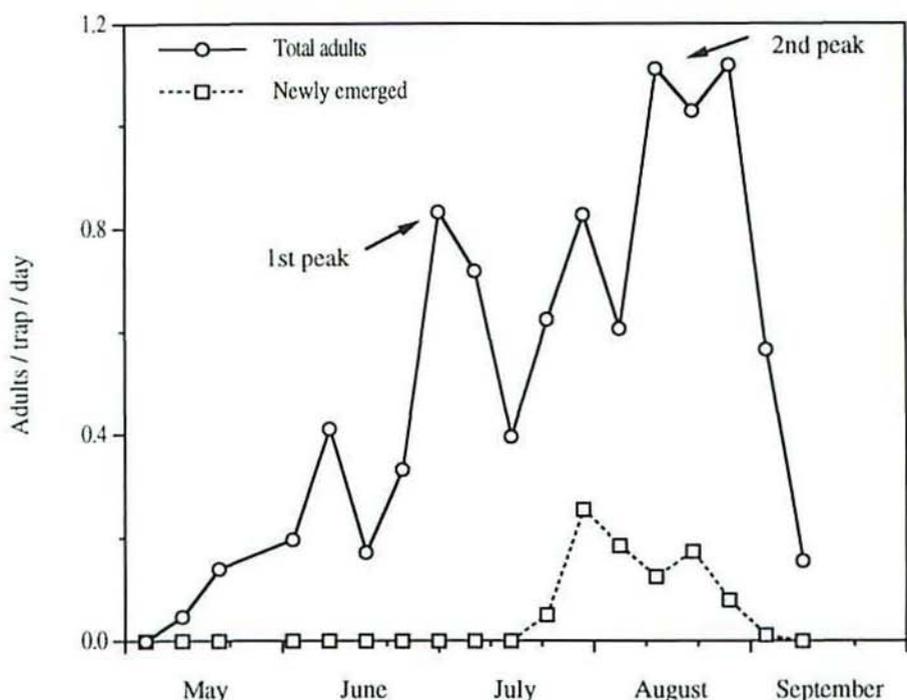


Fig. 3. Seasonal pitfall trap catches of adult *H. rufipes* at Aroostook Farm in 1991.

with total adults in 1991 ranged from 1% (21 August–27 August) to 30.8% (25 July–30 July) (Fig. 3). Similarly, newly emerged beetles begin to appear in the middle of July in Europe (Briggs 1965).

Female sex ratio (females:total) is low early in the season (first generation) and increases to >50% by September (Fig. 4). In Europe, Luff (1980) also found that males predominate in trap catches early in the season. Many more beetles (both males and females) were trapped in dry pitfall traps than in traps containing antifreeze. Female sex ratio was much lower in dry pitfall traps (seasonal average adult per day = 91.71 ± 62.89) than in traps containing antifreeze (seasonal average adult per day = 32.81 ± 8.64) (Fig. 4, $t = 6.196$; $df = 1, 4$; $P < 0.001$). This difference could be due to live females attracting males to the dry pitfall traps. However, Thiele (1977) suggests that aggregation of ground beetles is not governed by sex pheromones, based on investigations of aggregation behavior by *Brachinus* spp. (Wautier 1971) and *Pterostichus adstrictus* Eschscholtz and *P. pensylvanicus* Leconte (Goulet 1974).

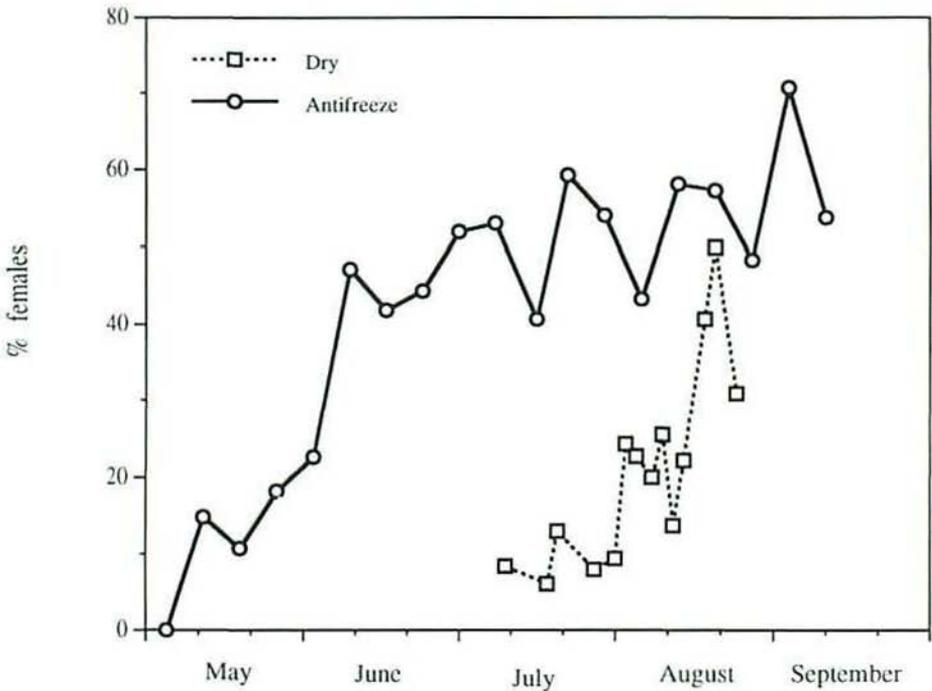


Fig. 4. Percentage of females *H. rufipes* caught in pitfall traps in 1991 at Aroostook Farm. Antifreeze: pitfall trap with 1:1 antifreeze:water solution (32.81 ± 8.64 adults/d); Dry: no antifreeze or water (91.71 ± 62.89 adults/d).

Mating behavior and egg laying. A high copulation rate between field-collected females and males was observed in the laboratory, especially after 1–2 d isolation. Males find females immediately after they are put together. Almost all females are mated when the sex ratio is male biased. The mating duration (mean \pm SD) observed for 18 couples during 5–8 August 1991 was 6.1 ± 2.4 min (range 2–10 min). No interference behavior between adults was observed during mating when 20 adults were in a group. Human activity and disturbance of mating pairs had little effect on mating behavior. Mating was observed to take place during both the day and night in the laboratory.

Overwintered females do not carry mature eggs. Females develop eggs in June, but peak egg maturity is not reached until late July (Fig. 5). The percentage of females with mature eggs increases to ca. 50% before summer adult emergence. After emergence of new adults, ovarian maturity increased to

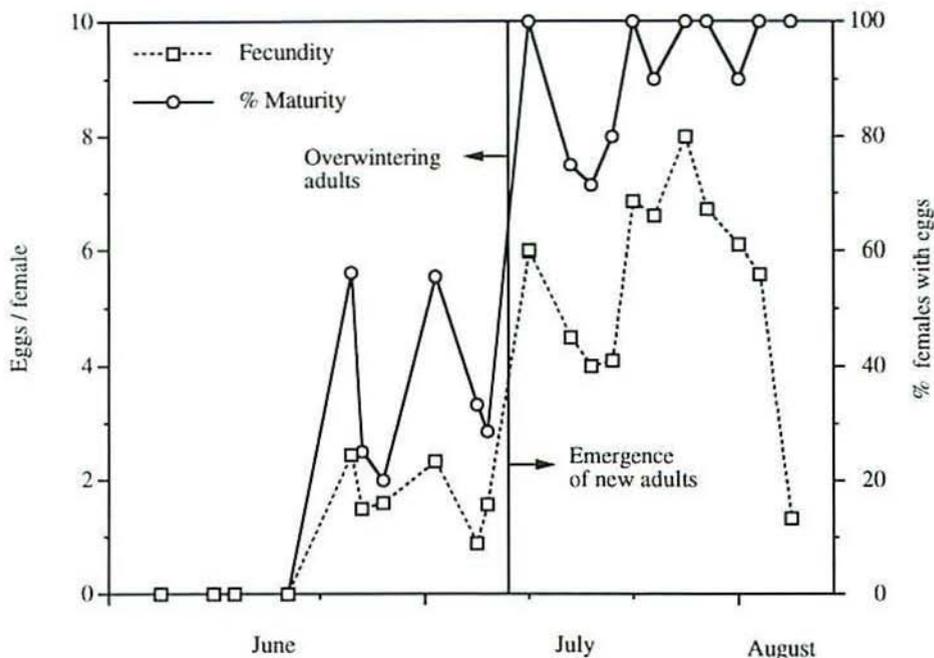


Fig. 5. Ovarian maturity and potential fecundity of *H. rufipes* females in 1991 at Aroostook Farm. Maturity: percentage females ($n = 92$) with mature eggs, fecundity: egg numbers per dissected female.

100% (Fig. 5). Mature eggs ready for oviposition first appear in late June but fecundity peaks in early July and quickly declines by mid-August (Fig. 5). Females have 12 ovarioles, six per ovary. There were 5.3 eggs per female on average (range 1–12, $n = 92$ females). During 30 July to 8 August 1992, only one female was observed laying eggs under laboratory conditions. On the first day, seven eggs were laid and from the second day until the last day one egg per day was laid. A total of 15 eggs was laid. In 1994, within moist oviposition chambers with 16:8 (L:D) h and 18°C, a total of 310 eggs was laid by 136 females during the summer (June–August), resulting in a mean of 2.3 ± 0.3 eggs per female with a maximum of 13 eggs per female. In 1995 a total of 471 eggs was laid by 173 females during the summer, resulting in a mean of 2.7 ± 0.2 eggs per female and a maximum of 11 eggs per female. More than 90% of the eggs laid during 1994 and 1995 hatched. There are few estimates of fecundity reported in the literature because researchers have had a difficult time getting *H. rufipes* to lay eggs in the laboratory (Luff 1980, 1982), although Briggs (1965) measured a high level of fecundity with an average of 84 eggs laid by 19 females.

Flight duration. Potential flight duration can be measured by tethered flight (Dingle 1972). Twenty-one percent (9 out of 41) of the beetles tethered flew under laboratory conditions (Table 1). The mean tethered flight duration of all beetles was 119.9 ± 199.2 min (188.5 ± 242.6 and 100.4 ± 84.4 min for females and males, respectively). Adults flew several times during the test period. The mean numbers of flights were 3.5 ± 0.7 and 3.1 ± 2.3 for females and males, respectively. The longest single flight durations were 170 and 159 min for female and male beetles, respectively. The longest cumulative flight durations (total flight duration for an individual for all flights during one night) were 360 min and 272 min by female and male beetles, respectively. These data suggest that *H. rufipes* can disperse long distances by flight. This is corroborated by the rapid spread of *H. rufipes* in the northeastern United States since its introduction on Prince Edward Island, Canada, in the 1930s (Zhang et al. 1994).

Flight phenology. We found that *H. rufipes* can be periodically caught in black light traps indicating that they will disperse by flight (Fig. 6). Briggs (1965) also reported catching *H. rufipes* adults in light traps in Europe. Ninety-seven percent of the females (70 out of 72) that we caught during 1991 and 1992 in the black light trap did not have matured eggs. This suggests that most *H. rufipes* fly before eggs are mature, a type of prereproductive dispersal with an oogenesis flight syndrome (Johnson 1969). The highest catch we recorded was 167 adults on 29 June 1991. Initial light trap catch in 5 of 6 yr was at the mid through end of June. In 1993 initial trap catch occurred in July.

Table 1. Percentage of adult *H. rufipes* flying in the laboratory in 1992.

Date	No. of beetles tethered	No. of beetles that flew	Flight percentage
9 June	5	0	0
12 June	3	0	0
13 June	12	7	58.3
22 July	12	0	0
23 July	9	2	22.2

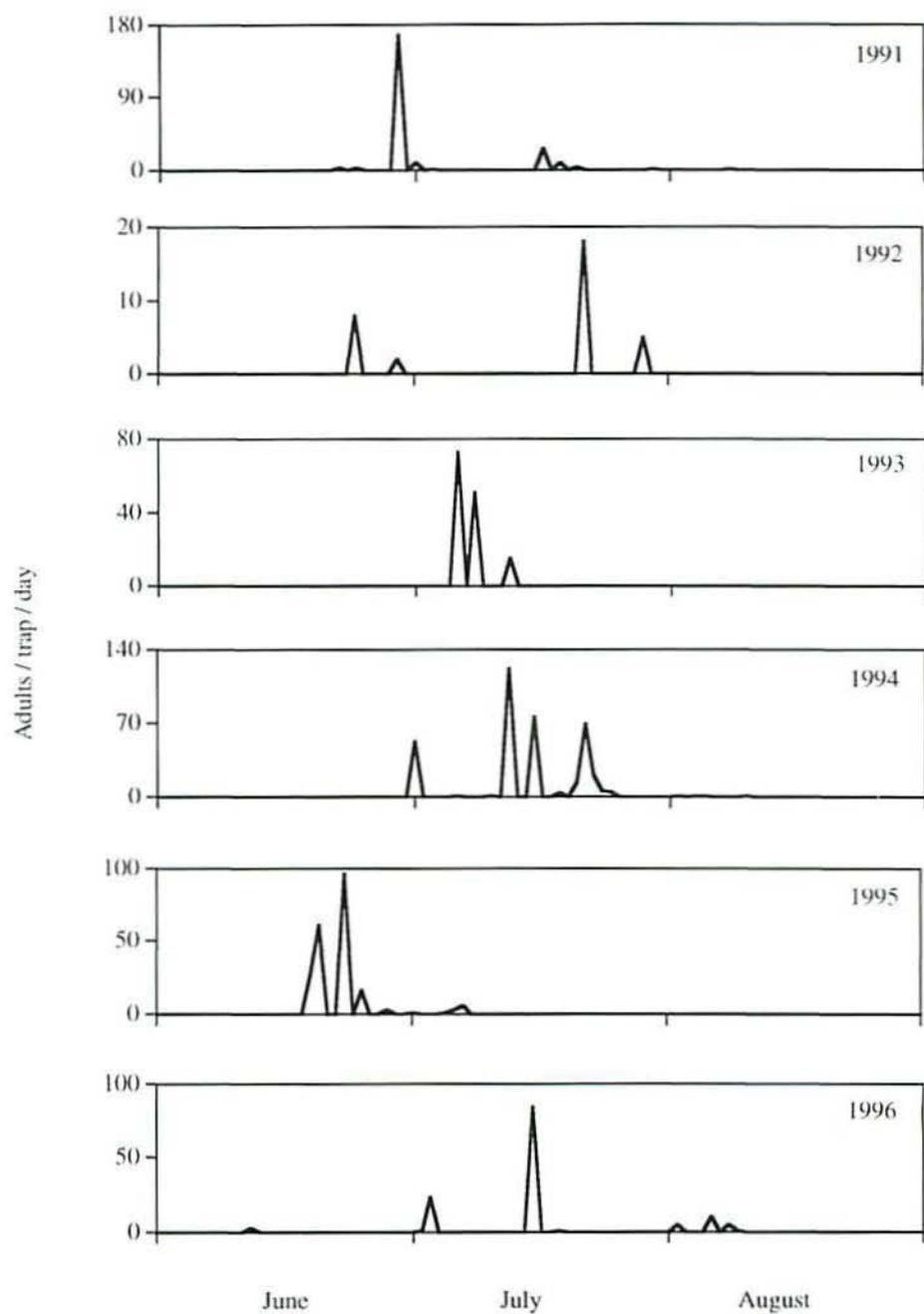


Fig. 6. Black light trap catches of adult *H. rufipes* at Aroostook Farm in 1991–1996.

The overall pattern of peak catch varies during June and July. In 1993 (July) and 1995 (June) only one major flight of beetles was observed. In 1991, 1992, 1994, and 1996 there were two flight periods. In 1991 the largest flight occurred at the end of June and a second occurred in mid-July. In 1992 and 1994 a similar pattern was seen as in 1991 with a flight in the end of June and a larger flight in mid to late July. August flight was observed only in 1996 (Fig. 6). In 1991, the first flight peak occurred at the same time as the first peak catch in the pitfall traps in late June. We hypothesize this first peak to be the overwintering generation. The second small peak appeared when newly emerging adults were caught in pitfall traps in mid-July 1991. The second light trap catch peak, therefore, probably represents the summer generation of *H. rufipes* in 1991, 1992, 1994, and 1996 (Figs. 5, 6).

Of the measures we evaluated for predicting date of first light-trap capture and peak trap capture, day of year was the best predictor. The standard error to mean ratio for day of year was 0.02 and 0.02 for first trap catch and peak trap catch, respectively. The mean day of year for first trap catch over the 6-yr period was day 175 with a range of 163–187. The mean day of year for peak trap catch was 189 with a range of 174–202. The next-best predictor for first trap catch was accumulated degree days with a base threshold of 0°C (degree day = 914 degree days, se:mean ratio = 0.05). The second best predictor for peak trap catch was the number of days with an average temperature greater than 0°C (number of days = 83.8, se:mean ratio = 0.04). The average minimum air temperature during 5 d before the first catch was $12.6 \pm 2.6^\circ\text{C}$. Van Huizen (1979) found that the "take-off" temperature threshold for carabids (*Harpalus* spp. not included) was 16°C. However, in the case of *H. rufipes*, we found that the average number of days before initial flight with maximum air temperature above 16°C was 48.9 ± 5.3 d.

Walking dispersal ability. Adult *H. rufipes* appear to disperse mostly on the soil surface at night, as exhibited by most carabids (Thiele & Weber 1968). Two red marked females, seven red marked males, and two white marked males were recaptured within 10 d after release of a total of 20 females and 187 males in the potato field. Three females and 29 males were caught after release of a total of 47 females and 298 males in the oat field. All five females were recaptured within 24 h after release. The longest dispersal distance by a female on the first day after release was 15.2 m (in the oat field). The mean dispersal distance of females in 1 d was 9.8 ± 5.2 m in the potato field and 11.2 ± 7.0 m in the oat field (Table 2). Twenty-eight males were recaptured on the first day after release. The mean male dispersal distance on the first day, 7.5 ± 5.4 m in the potato field and 6.3 ± 4.7 m in the oat field, was not significantly different from the distance moved by females ($F = 1.678$; $df = 1, 29$; $P = 0.205$). There also was no significant difference in the 1-d dispersal ability of both female and male *H. rufipes* between the oat and potato fields ($F = 0.002$; $df = 1, 29$; $P = 0.966$).

Only males were recaptured beyond the second day after release. Marked males were caught up to 10 d after release in the potato field and 7 d after release in the oat field. The mean dispersal distance per day by males of all marked males caught after release was 6.5 ± 6.1 m in the potato field and 5.7 ± 4.7 m in the oat field ($F = 0.186$; $df = 1, 36$; $P = 0.669$). The longest total

Table 2. Dispersal distance of marked *H. rufipes* in a potato and oat field, 1991.

	Potato		Oats	
	Male	Female	Male	Female
After 1 d	7.5 ± 5.4 ^a	9.8 ± 5.2	6.3 ± 4.7	11.2 ± 7.0
After 2 wk	21.7 ± 23.9	^b	7.1 ± 6.6	-
Distance per day for experimental period	6.5 ± 6.1		5.7 ± 4.7	

^aMean ± SD (distance in meters).^bNo. females recaptured after 1 d.

dispersal distance of males in the potato field was 63.5 m over 4 d, and the longest dispersal distance in the oat field was 30.5 m over 7 d. Male *H. rufipes* dispersal in the potato field was greater than in the oat field, possibly due to the rows channeling the beetles in the potato field. The mean total dispersal distance by all recaptured marked males over 2 wk was 21.7 ± 23.9 m in the potato field compared with 7.1 ± 6.3 m in the oat field ($F = 9.229$, $df = 1, 36$; $P = 0.004$). Wallin & Ekblom (1988) measured adult *H. rufipes* movement on the soil surface to be 7.3 ± 1.5 m over night, where Lys & Nentwig (1991) found the average movement distance over a night was 14.2 ± 2.7 m. The difference in the reported dispersal distances may be related to vegetation, topography, or weather conditions at these sites.

Harpalus rufipes has become the dominant ground beetle in Maine agroecosystems in the past 50–60 yr since its introduction from Europe. Understanding the biology of *H. rufipes* is important for the evaluation of this potential weed biocontrol agent. We have found that adult *H. rufipes* are very active, dispersing by flight early in the season and moving on the soil surface throughout the season. Adult flight in June and July leads to dispersal of the adult population from overwintering habitats such as field edges where there are more weed seeds available and less soil disturbance. Colonizing spring and early summer adults invade crop land by flight before oviposition. Subsequent reproduction leads to an increase in the beetle population in agricultural fields and can potentially result in increased seed predation (Zhang 1993). Their activity on the soil surface during the season in an annual cropping system is very important for this predator in maximizing its numerical response to patchily distributed weed populations.

The temporal synchronization of predator and prey incidence also is an important characteristic for an effective biological control agent. We have

found that in the Maine potato agroecosystem, the overwintered adults become active in early May, and the adult population initially peaks at the end of June but continues to increase until late in the season when weed seeds are being produced and shed (Fig 7). This synchronization of weed seed production and the occurrence of a mobile adult beetle population results in a situation where seed predation should be maximized. Weed seeds tend to be highly visible on the soil surface in the fall, thereby increasing the likelihood of numerical response due to beetle predation and oviposition in areas of high weed-seed density. The above-mentioned characteristics of *H. rufipes*, in addition to it being polyphagous on weed seeds (Chiverton 1987, Hamon et al. 1990, Sunderland et al. 1987, Zhang 1993) and having a relatively stable population abundance not greatly affected by agricultural production practices (Zhang 1993), suggests that *H. rufipes* may play a role in the population dynamics of weeds in Maine potato production.

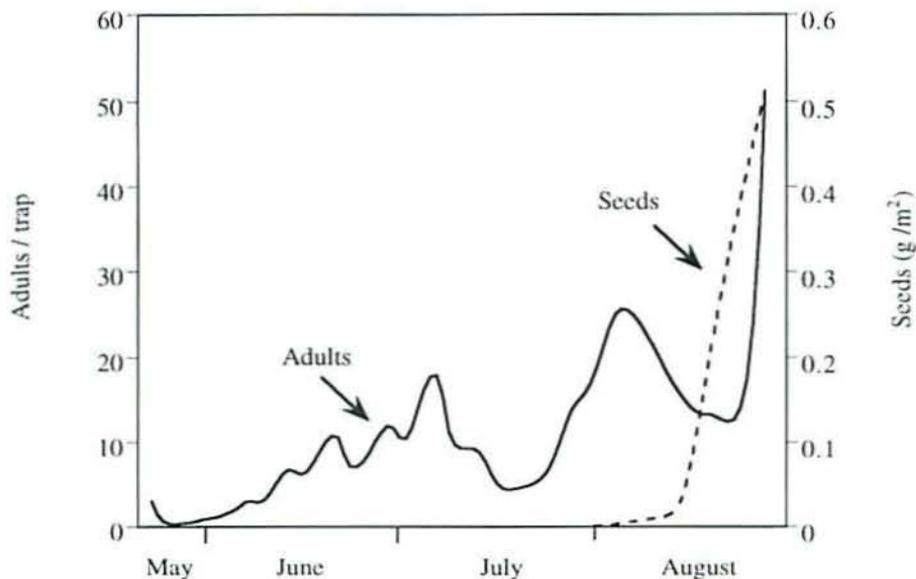


Fig. 7. The temporal dynamics of adult *H. rufipes* measured as mean catch from pitfall traps and weed seed released onto the ground in a potato field, Presque Isle, Maine, in 1993.

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Effect of Marking Aphids With Fluorescent Powders on Virus Vectoring Activities^{1, 2}

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ABSTRACT Studies were conducted to determine whether marking aphids with fluorescent powders would affect aphid processes and activities that could influence their role in the epidemiology of virus diseases. Aphids were dusted with fluorescent powders while they infested source plants. The powders readily adhered to the exoskeltons of four aphid species: *Myzus persicae* Sulzer (green peach aphid), *Aphis gossypii* Glover (cotton aphid), *Aphis fabae* Scopoli (bean aphid), and *Aphis craccivora* Koch (cowpea aphid). The dusted aphids then were easily identified at a later date by their fluorescent glow under ultraviolet light. The powders had no perceptible effect on plants or on the dispersal behavior of the green peach aphid. In flight chambers, about the same percentage of labeled and unlabeled green peach aphid *alatae* took flight, and their preference for white, yellow, or gray at landing was not affected as compared with unmarked aphids from the same population. Marking green peach aphid *apterae* did not affect aphid fecundity, longevity, movement to and among plants, or capacity to acquire and transmit potato leafroll virus. The powders were rinsed from plants after 3 h of sprinkler irrigation but not from aphids. We conclude that marking aphids with fluorescent powders could be a suitable method to trace dispersal patterns of both alate and apterous aphids and could be useful as a tool for elucidating the epidemiology and control of aphidborne virus diseases.

KEY WORDS *Myzus persicae*, *Aphis gossypii*, *Aphis fabae*, *Aphis craccivora*, potato leafroll virus, fluorescent powder, epidemiology, labeling, fecundity, color preference

Development of effective virus disease control measures for a crop often requires an in-depth understanding of the ecology of the virus in the area where the particular crop is grown (Matthews 1991). The means by which the virus spreads to and within the crop is usually the dominant factor in viral disease epidemiology (Matthews 1991). In the case of viruses dependent on aphid transmission, virus spread is largely dependent on the behavior of the alatoid

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and apterous vectors. When monitoring aphids as vectors of plant viruses, researchers have generally quantified various aspects of vector abundance (Hanafi et al. 1989, Raccah & Irwin 1988) with little or no certain knowledge regarding their sources. However, insect activities associated with vector migration, selection of preferred hosts, and movement and distribution to and within crops and overwintering hosts are very important in the development of virus disease epidemics (Raccah 1983). An ability to trace the movements of vectors could help elucidate these complex activities.

An example of the importance of identifying the sources of vectors and virus can be cited from efforts to control beet curly top disease in the western United States. Vast, uncultivated areas of the desert, both in California (Cook 1933) from 1931–present and Idaho (Douglass et al. 1955) in 1950 and 1953, were treated with insecticide in an effort to control beet curly top virus disease by killing the leafhopper vector on its overwintering hosts. Although the incidence of beet curly top disease in crops may have been reduced somewhat (Bennett 1971), after the treatments began, it was not possible to determine whether the treatments actually caused the reductions in disease or if the areas treated were the sources of vectors or virus that caused the disease.

Researchers have used numerous methods to trace the movement and dispersal of insects and to identify the sources of insect vectors of virus disease (Kring 1972), including ratio of male to female leafhoppers, determination of body fat content, and spotting of insects with colored lacquers (Lawson et al. 1951). These and other methods have generally been time consuming and expensive, and they often risk alteration of insect processes and behavior that could influence major aspects of insect ecology and virus disease epidemiology (Southwood 1978). The use of topically applied fluorescent powders to mark insects for later identification under ultraviolet light appears to have overcome most of these problems (Zukel 1945). This technique has been applied to larger insects such as bees (Smith & Townsend 1951, Mayer & Lunden 1991), beetles (Naranjo 1990, Cook & Hain 1992), bugs (Stern & Mueller 1968, Khattat & Stewart 1974), and moths (Kipp & Lonergan 1992). It also has been used to mark mosquitoes (Zukel 1945, Brust 1980), black flies (Doddall et al. 1992), leafhoppers (Purcell & Suslow 1982), and whiteflies (Cohen et al. 1988, Byrne et al. 1996). Cook & Hain (1992) reported that marking with fluorescent powders decreased the life span but not the dispersal activities of bark beetles. However, several researchers have found that the powders did not influence the survival, behavior, or recapture of other insect species (Purcell & Suslow 1982, Naranjo 1990, Doddall et al. 1992, Kipp & Lonergan 1992). A major advantage to using fluorescent powders is that insects from different sources may be distinguished by using different colors for labeling.

If they are suitable for marking aphid populations, fluorescent powders would offer new possibilities for describing the epidemiology and control of aphidborne virus diseases. We are particularly interested in developing strategies to control potato leafroll virus (PLRV), a virus that causes a costly disease of potatoes worldwide (Bacon et al. 1976). PLRV may be transmitted in a persistent manner by about 10 species of aphids, but the green peach aphid (*Myzus persicae* Sulzer) is the most efficient vector and the predominant vector of PLRV worldwide (Kennedy et al. 1962). Aphids acquire the ability to

transmit PLRV only by feeding on infected plants (Day 1955). They retain the ability to transmit for life, but the virus is not passed transovarially to offspring produced either viviparously or oviparously.

In the Columbia Basin of Washington and Oregon, our chief area of interest, *M. persicae* overwinters in the egg stage on peach trees (*Prunus persicae* L.) (Powell & Mandor 1976). A small spring flight from peach trees distributes the aphids without PLRV (Thomas et al. 1992) to herbaceous winter annual and early spring hosts. A summer flight begins when spring hosts start to mature and senesce and this distributes aphids and PLRV (Thomas 1983) to summer hosts. A fall flight from summer hosts begins in September. It distributes aphids and virus to fall-planted crops (Thomas et al. 1993) and returns aphids to the overwintering peach host.

Before this technology can be used to elucidate the activities of aphids associated with virus disease epidemiology, however, it must be established that marking aphids with fluorescent powders does not affect aphid activities that may influence the epidemiology of virus diseases (Southwood 1978).

The objective of this study was to determine the effect of marking aphids with fluorescent powders on their longevity, fecundity, flight and walking activity, color preference, and capacity to acquire and transmit PLRV.

Materials and Methods

Green peach aphid cultures were kept on *Datura stramonium* L. and potato (*Solanum tuberosum* L. cv. Russet Burbank) plants. *Aphis gossypii* Glover, *A. fabae* Scopoli, and *A. craccivora* Koch were collected from field plants (cotton, potato, and alfalfa, respectively). Plants were grown in an insectproof greenhouse and, unless otherwise specified, the experiments were conducted in temperature-controlled chambers maintained at 20°–22° C, with a 16:8 (L:D) h photoperiod and ca. 2,000 lux illumination.

Fluorescent powders. The fluorescent powders used in this study were Yellow Potomac, Saturn Yellow, Chartreuse 320, Fire Orange, Blaze Orange, Neon Red, Corona Magenta, Sunset Orange, Signal Green, Blue Horizon, and Blue Columbia. They were obtained from Day Glow, Cleveland, Ohio, and Swada, Ltd., London. All were fluorescent under ultraviolet (UV) light in relative darkness. We used a high intensity UV long wave UV-366 nm lamp (Mineralight & Blacore-Ray Lamps, San Gabriel, California).

Adhesion of powders to aphids. Aphids and plants were marked with fluorescent powders by gently dusting the powders on aphid-infested plants by using a powder insufflator. Intensity of coverage was not critical, but sufficient powder was applied so that each aphid was marked with at least 10 grains of powder. Alate and apterous aphids were then transferred with a small brush and used in various experiments as described below.

The adhesion of fluorescent powders to aphid exoskeletons was tested on *M. persicae*, *A. gossypii*, *A. fabae*, and *A. craccivora*. All other tests were performed using only *M. persicae*.

To determine whether fluorescent dyes adhered to the tarsi of aphids that walked across a treated surface, a circular path (5 cm wide and 30-cm inside diameter) was constructed with Fire Orange-dusted paper on a flat surface.

Twelve *D. stramonium* plants, 7 cm tall in 8-cm square pots, were placed around the outside circumference. Fifty aphids were released at the center of the circle in a petri dish. Aphids that reached the plants were required to walk across the dusted path. All aphids on the plants after 24 h were examined under UV light.

Effects on plants. To determine whether fluorescent powders caused damage to treated plants, 10 plants of each of 12 plant species belonging to seven families were dusted with 11 different powder colors and kept in a greenhouse. After 5 wk, treated and control plants were compared with regard to size, color, and general appearance. Several plant species were tested against all the fluorescent powders: Amaranthaceae (*Gomphrena globosa* L.); Chenopodiaceae (*Beta vulgaris* L., *Chenopodium quinoa* Willd.); Asteraceae (*Helianthus annuus* L., *Zinnia elegans* Jacq.); Cucurbitaceae (*Cucumis sativus* L.); Brassicaceae (*Capsella bursa-pastoris* L., *Raphanus sativus* L.); Fabaceae (*Vigna sinensis* [Torner] Savi); and Solanaceae (*Datura stramonium* L., *Physalis floridana* Rydb., *Solanum tuberosum* L.)

Effects on aphid longevity. The effect of dusting aphids with fluorescent powders on aphid longevity was determined. Fifty apterae labeled with each of three fluorescent powders (Fire Orange, Signal Green, and Blue Horizon) were individually transferred into each of five leaf cages. Cages were round, plastic (Plexiglas™) cylinders 3 cm diameter by 2 cm tall, one end of which was covered with a removable nylon screen cap; the other end was open. Fifty nonlabeled, control apterae from the same colony were transferred to five leaf cages. The cages were divided into five sets, each set containing one cage treated with each of the three powders and one with unmarked control aphids. Each set was placed on a different *D. stramonium* plant. Plants were the same age, selected for uniformity, approximately 40 cm tall, and multibranching with a foliage canopy about 25 cm in diameter. Each cage was clamped with the open end against the undersurface of a single leaf. Leaves used for the cages were the same age and size—the first fully expanded leaf below a growing point. At 1, 3, 5, 7, and 9 d after marking aphids, one set of cages was removed, with the leaves still clamped to cages. The cages with leaves still attached were taken to the laboratory and removed from the cages. Progeny were separated from original aphids, and original aphids remaining alive were counted. This experiment was repeated three times. Analysis of covariance was done on aphid mortality data by using the SAS GLM procedure (Anonymous 1992). Mean separation for mortality data was performed using the least significant differences test.

Effects on aphid flight and color preference. The effects of fluorescent powders on aphid flight and color preference were assayed in an aphid flight chamber similar to the one described by Kring (1966). Three wooden cylinders 60 cm in diameter were stacked on top of each other producing a chamber 100 cm tall. Fluorescent (cool white) lamps at the top provided 1,100 lux light intensity, and an exhaust fan fitted over a net-covered hole at the top provided air movement to cool the chamber and to stimulate flight. The inside of the flight chamber was painted white. Yellow, white, and gray plastic cards (20 × 20 cm) were attached on the side walls of the middle cylinder 42 cm above the base. The cards were painted with the transparent sticky substance called

Rimifoot (Jewnin and Joffe, Rishon LeZion, Israel). Fluorescent powder-dusted and nondusted control alatae were simultaneously released from leaf cages placed in a petri dish at the bottom of the chamber, and the numbers trapped on the different colored sticky cards were counted the next day. This experiment was repeated four times. The position of the colored cards was changed in each replication. The temperature in the flight chamber was maintained at 22°–24°C. Analysis of variance was done on aphid flight data by using the SAS GLM procedure (Anonymous 1992). Mean separation for color preferences was performed using the protected least significant differences test.

Effects on aphid acquisition and transmission of PLRV. To assay the effect of marking aphids with fluorescent powders on their capacity to acquire and transmit PLRV, noninfective aphids from colonies on *D. stramonium* plants were dusted with Fire Orange powder. Dusted leaves containing aphids were detached and cut into segments approximately 2 cm². The segments were placed into leaf cages, as described above, and the cages were clamped to leaves of the same age on an infected *D. stramonium* plant. Untreated control aphids from the same colony were transferred to leaves of the same infected *D. stramonium* plant in the same manner. After a 3-d acquisition period, 50 dusted and 50 control aphids were transferred, one per plant, to virus-free *D. stramonium* seedlings. The aphids were killed by fumigation with nicotine sulfate after plants were exposed for 48 h. Plants were then transferred to an insectproof greenhouse, and PLRV presence was assayed by ELISA (Kaniewski & Thomas 1988) after 3 wk. Data were analyzed using a *t*-test (Anonymous 1992).

Effects on aphid fecundity. To assay the effect of marking aphids with fluorescent powders on fecundity of *M. persicae* apterae, one nondusted (control) aphid was transferred from an aphid source leaf to a cotyledon on each of 10 *D. stramonium* seedlings. The aphid source leaf was then dusted with Fire Orange powder, and one dusted aphid was transferred to a cotyledon on each of 10 additional *D. stramonium* seedlings. The source leaf was from a *M. persicae* colony on *D. stramonium*. The seedlings were covered with cylindrical plastic cages (8 cm diameter x 14 cm high with nylon mesh tops), and numbers of nymphs were counted 1, 3, and 6 d after aphids were placed on the seedlings. This experiment was replicated three times in an insectary with a 16:8 (L:D) h photoperiod and 2,000 lux at 18°–20° C. Analysis of covariance was done on aphid fecundity data by using the SAS GLM procedure (Anonymous 1992). A *t*-test was performed to compare treatment effects (Anonymous 1992).

Effects on aphid dispersal. Dispersal of marked versus nonmarked apterae from a neutral point to and among plants was assessed in a growth chamber. Fifty Fire Orange-dusted apterae were placed in a 5.5-cm diameter petri dish. Fifty nondusted aphids from the same aphid colony were introduced into a second petri dish. The petri dishes were placed side by side with the top rims at soil level at the center of a row of young, Russet Burbank potato plants growing in a flat. Six plants with foliage about 4 cm in diameter were spaced 14 cm apart on each side of the petri dishes. The number of dusted and nondusted aphids on each plant was counted after 24 h. This experiment was replicated three times. Analysis of covariance was done on the distances aphids moved using the SAS GLM procedure (Anonymous 1992).

Results and Discussion

Fluorescent powders and their adhesion to aphids. All fluorescent dusts adhered instantly to both alatae and apterae of the four aphid species tested. Aphids marked with all of the powders were easily observed by their strong, distinctive fluorescence under UV light in darkness. A single grain of fluorescent powder was sufficient to mark an aphid, and aphids marked with 10 or more grains were visually detectable from a distance of 8–15 m. Although the fluorescence of all the powders tested was easily observed, and all of the powders were judged satisfactory for marking aphids, brightness of fluorescence varied among the different powders. Fire Orange clearly appeared brightest. Yellow Potomac and Blue Columbia were the least bright. More important than brightness was an ability to distinguish readily between colors when more than one color was used to mark different groups of aphids. Different shades of the same color were not readily distinguishable without known references that could be used for comparison. Groups of colors that were not instantly distinguishable were Fire Orange, Blaze Orange, and Sunset Orange; Yellow Potomac, Saturn Yellow, and Chartreuse 320; and Blue Columbia and Blue Horizon. Potato leaves have a low level of natural fluorescence, mainly in the reddish-brown range, which interfered somewhat with observation of aphids marked with Corona Magenta and Neon Red. The colors we selected as best for work on potato leaves were Fire Orange, Blue Horizon, and Signal Green.

The powders seemed to adhere more strongly to the aphids than to plants. After 3 h of overhead irrigation, most of the plants were rinsed clean of fluorescent powder but green peach aphids still glowed under the UV lamp. This glow on the aphids persisted for at least 8 d. Untreated aphids that walked across a surface that had been dusted with fluorescent powder were easily identified because the powder adhered to their tarsi. Under UV light, the tarsi appeared as six bright, fluorescent spots.

Effects on plants: The powders caused no visually perceptible effects on plants for at least 5 wk after treatment. Groups of plants that had been treated were visually indistinguishable from untreated control groups.

Effects on aphid longevity. Marking green peach aphids with fluorescent powders did not affect their mortality rate (Table 1). Mortality increased significantly with time over 9 d ($F = 8.84$; $df = 1, 53$; $P < 0.004$) but marking with fluorescent powders did not affect mortality ($F = 1.01$; $df = 3, 53$; $P < 0.3978$). The largest difference in mortality rates occurred between aphids treated with Fire Orange and Blue Horizon on day 5, but this difference was not significant.

Effects on aphid flight and color preference. Aphids trapped on the colored sticky cards that were used to test the effects of fluorescent powders on the tendency of aphids to take flight and on their attraction to different colors were dispersed randomly across the surfaces of the cards. This is evidence that they flew to the cards. Had they walked from the release site to the cards, the aphids would have been trapped on the edges of the cards.

There was no effect of marking green peach alatae with the Fire Orange fluorescent powders on their tendency to take flight ($F = 0.05$; $df = 1, 18$; $P > 0.823$)

Table 1. Effect of marking *Myzus persicae* with fluorescent powders on mortality rate of apterae.

Interval ^b (days)	Aphids alive at intervals after marking ^a			
	Control	Signal Green	Fire Orange	Blue Horizon
1	35a	31a	35a	32a
3	26a	25a	27a	24a
5	24a	25a	26a	21a
7	23a	24a	26a	21a
9	25a	24a	27a	25a

^aMean number remaining alive per 50 alatae marked in three replications.

^bA different group of 50 aphids was used in each treatment at each interval.

At each interval, means followed by the same letter are not significantly different ($P > 0.10$).

(Table 2). The same percentage of treated (31%) and untreated (32%) alatae released in a flight chamber were trapped on the sticky cards.

No differences were found between dye-treated and untreated green peach alatae in landing response on different colored cards ($F = 0.17$; $df = 2, 18$; $P > 0.8466$) (Table 2). Both labeled and unlabeled flying alatae exhibited typical (Kring 1972, Marco 1986) color preference ($F = 57.3$; $df = 2, 8$; $P < 0.0001$); they preferred yellow (Kring 1972) and were reluctant to land on white (Marco 1986) (Table 1).

Effects on aphid acquisition and transmission of PLRV. In four experiments to determine the effect of marking aphids with fluorescent powders on their capacity to acquire and transmit PLRV, the percentages of acquisition and transmission of PLRV was 47 versus 64, 57 versus 52, 70 versus 38, and 65 versus 77%, respectively, for control versus marked apterae. Acquisition and transmission of PLRV was the same ($t = 0.31$, 6 df , $P > 0.77$) for control and Fire Orange-marked apterae.

Effects on fecundity. Marking green peach apterae with Fire Orange did not affect the percentage of aphids that were reproductive (Fig. 1A) ($t = 0.14$; $df = 16$; $P > 0.89$) or the rate of accumulation of offspring (Fig. 1B) ($F = 0.02$; $df = 1, 179$; $P > 0.89$).

Table 2. Effect of fluorescent dye marking of *Myzus persicae* on alatae flight response and attraction to sticky traps of different colors.

Exp	No. of alatae attracted to colored traps ^a						Flight response	
	Yellow		White		Gray		% trapped	
	Label	No label	Label	No label	Label	No label	Label	No label
1	13	14	1	0	5	3	38	34
2	12	10	0	2	4	5	32	34
3	9	12	3	1	2	4	28	34
4	7	8	2	2	4	3	26	26

^aNumber per 50 alatae attracted to sticky colored cards (20 cm × 20 cm).

Effects on aphid dispersal. The spread of apterous aphids to and among plants was not affected by treatment with fluorescent dye (Fig. 2). Significantly more aphids were found on plants closest to the source of aphids ($F = 77$; $df = 1, 25$ df ; $P < 0.0001$), but marking aphids with fluorescent dye did not affect either the number of apterae that moved or the distance they moved from plant-to-plant ($F < 0.001$; $df = 1, 25$; $P > 1.0$).

Marking green peach aphids with fluorescent powders had no measurable effect on any of several fundamental aphid activities and processes; the data suggest that movement and dispersal behavior of aphids obtained by marking aphids with the powders may be valid.

We did not test the effect of marking aphids on timing and orientation of aphid flight patterns over large geographical regions. The report by Brust (1980) that mosquitoes marked with fluorescent powders could be captured and identified up to 33 d after their release and at distances up to 11 km from the release site lends plausibility to the concept that fluorescent powders could be used to trace aphid flights. The strong fluorescence of marked individuals and the distinctive colors that are available provide an effective method for finding and identifying marked individuals. By dusting different aphid source areas with different colors, it may be possible to determine the importance of various overwintering sources of virus.

Our work illustrated that the movement of apterae over short distances can be followed by labeling with fluorescent powders. Thus, the technology may provide a tool for studying the plant-to-plant movement activities of apterous aphids in selecting hosts and spreading virus. Work is underway to study plant-to-plant spread of PLRV by green peach apterae in susceptible and resistant potato germplasm (Marco & Thomas 1992).

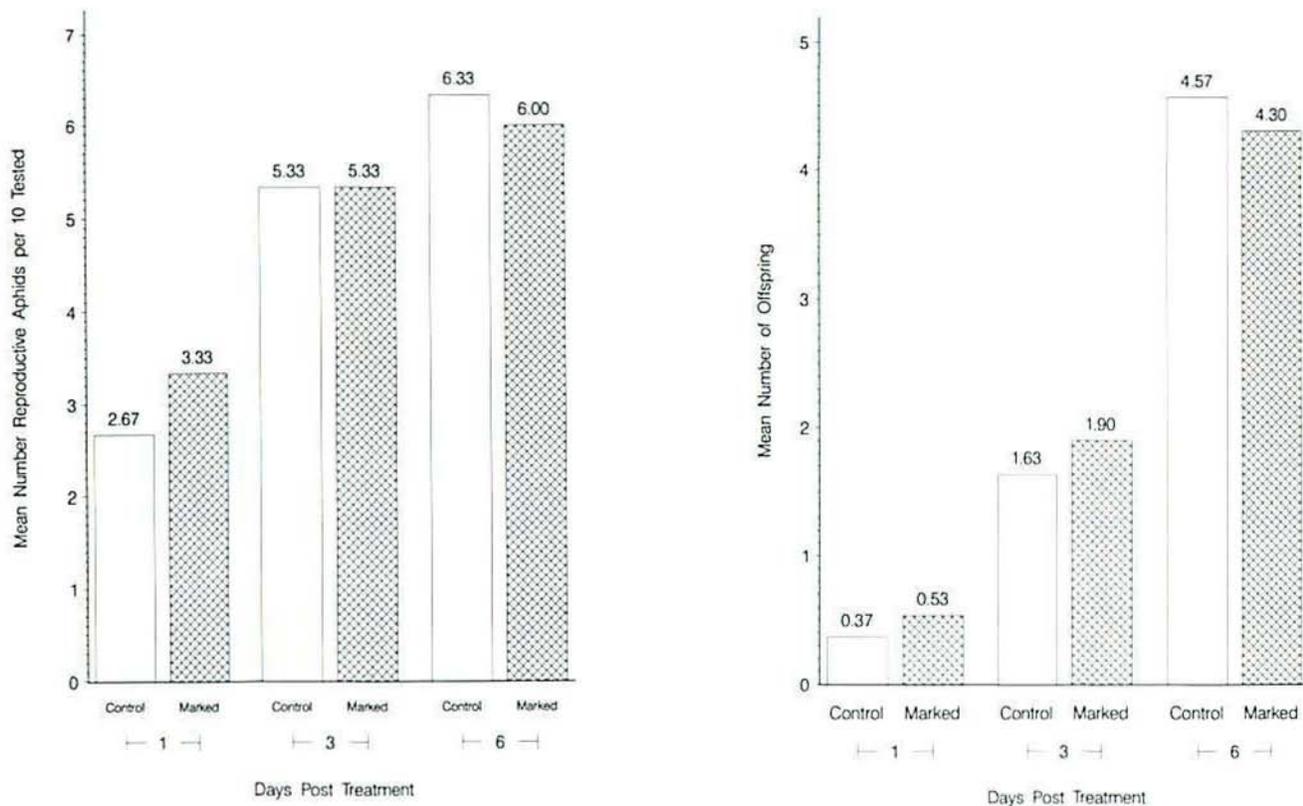


Fig. 1. Effect of fluorescent powder marking on fecundity of *M. persicae* apterae. (Left) Mean number of apterae that were reproductive among 10 treated and 10 nontreated apterae at 1, 3, and 6 d after treatment in each of three replications. (Right) Mean number of offspring propagated by each of 10 treated and 10 nontreated apterae at 1, 3, and 6 d after treatment in each of three replications.

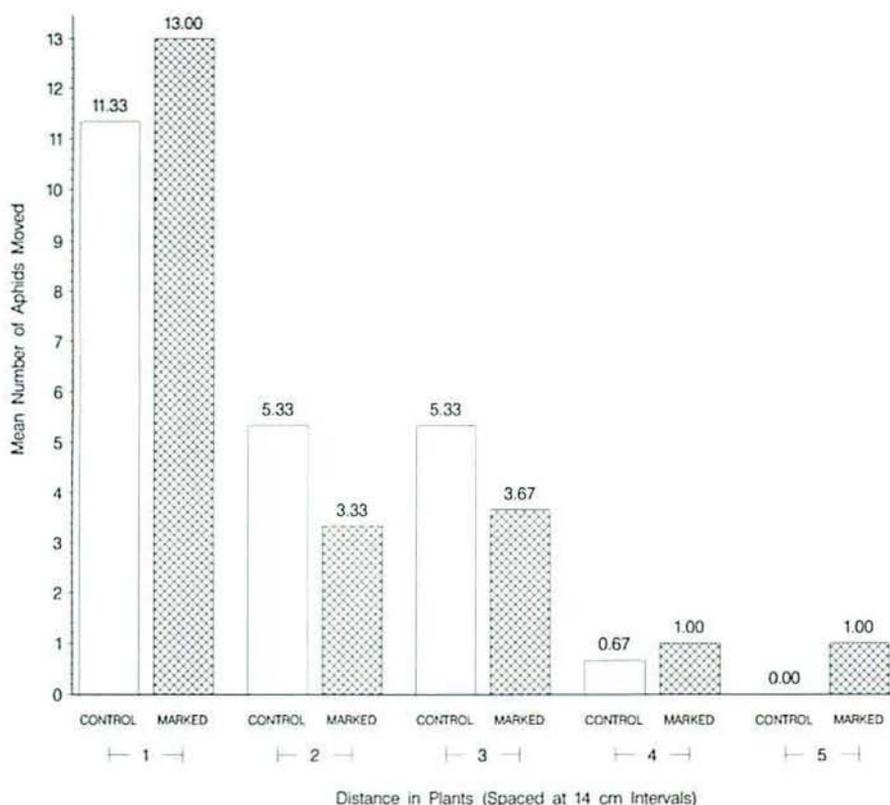


Fig. 2. Effect of fluorescent dye marking of *M. persicae* on movement of apterae from a petri dish to young Russet Burbank potato plants spaced 14 cm apart on each side of the aphid source. Aphids were counted 24 h after release. Data are the means of three independent experiments performed in a control chamber at 24°–27°C, a photoperiod of 16:8 (L:D) h, and light intensity of 1,700 lux.

The bulk of this work concentrated on the green peach aphid. Our only evidence that fluorescent powders could be used to study movement activities of other aphids is that the powders adhered to exoskeletons of three other aphid species. Because the powders have been used successfully on several insect species and on green peach aphid, they could probably be used for other aphid species.

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Control of Lone Star Ticks on Cattle with Ivermectin^{1, 2}

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ABSTRACT Pastured cattle were treated twice weekly with ivermectin either at 200 µg/kg oral or 40 µg/kg by injection. The numbers of lone star ticks, *Amblyomma americanum* (L.), on the treated and the untreated groups within a single herd were observed for 4 wk. There was no significant ($P > 0.05$) difference in the number of unengorged, small (4-6 mm), and medium (6-8 mm) female lone star ticks on the untreated control compared with those treated orally. However, cattle receiving the oral treatment had significantly ($P < 0.05$) fewer large (8 mm) females than the untreated cattle. Significantly ($P < 0.05$) fewer small, medium, and large female ticks were found on the injected cattle compared with the untreated controls. Interestingly, there were significantly ($P < 0.05$) more unengorged females on the animals treated by injection than on those treated orally or left untreated. Animals treated orally had 4-6 ppb ivermectin in their blood serum whereas those treated by injection had 13-15 ppb.

KEY WORDS *Amblyomma americanum*, lone star ticks, ivermectin, systemic acaricide, tick control, cattle

An extensive literature deals with the discovery, development, and activity of ivermectin (Campbell 1989). Its unique mode of action and broad spectrum of activity against arthropod pests of livestock (Drummond 1985), as well as agricultural crops (Lasota & Dybas 1991), establishes ivermectin as an important member of a new class of pesticides. Minimum effective daily oral and subcutaneous dosages against several species of ticks have been determined (Drummond et al. 1981, Lancaster et al. 1982, Nolan et al. 1981). Because ivermectin is active against ticks at low dosages, the potential exists for the development of novel delivery systems for administering the drug. With most long-lasting, controlled-release delivery systems such as implants (Miller et al. 1983, Boyce et al. 1992) or ruminal boluses (Miller et al. 1979, 1986; Soll

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²Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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et al. 1990; Taylor & Kenny 1990), the objective is usually to establish uniform daily dosing. However, with simpler systems, such as medicated mineral or protein blocks where consumption is by choice, the dosing regime may be cyclic or even sporadic. Nolan et al. (1985) found that injections of ivermectin at 200 µg/kg at 3-d intervals controlled *Boophilus microplus* (Canestrini). Miller & Oehler (1996) determined that when cattle were held on pasture, twice weekly treatments at 3–4 d intervals produced ≈5 ppb ivermectin in serum for those treated orally at 200 µg/kg and ≈15 ppb for those treated subcutaneously at 40 µg/kg. Another interesting observation in their study was that for the same oral dosage, cattle held in stanchions had twice the level of ivermectin in serum as cattle held on pasture. However, when cattle were treated by injection, no differences in serum levels were found for cattle held in stanchion or on pasture.

Miller et al. (1989) found that ≥35 µg/kg ivermectin administered as a daily oral treatment to Spanish goats, *Capra hircus* (L.), or to white-tailed deer, *Odocoileus virginianus* (Zimmerman), was highly effective against lone star ticks, *Amblyomma americanum* (L.), feeding on these animals. They suggested that an ivermectin-medicated bait could be used for control of lone star ticks in recreational areas or for control of *Ixodes scapularis* (Say) in areas with Lyme disease. The success of such an approach requires an understanding of the efficacy against these ticks when consumption may not be daily.

The present study was conducted to determine whether an oral treatment at 200 µg/kg or a subcutaneous injection at 40 µg/kg of ivermectin provided to cattle twice weekly could provide control of lone star ticks under pasture conditions. The primary focus of the study was to assess minimal efficacious levels as an aid to the development of delivery systems and strategies to control ticks on cattle and white-tailed deer, rather than to determine the actual tick control achieved. Although Miller & Oehler (1996) determined ivermectin levels in serum resulting from these identical treatments, they did not assess resultant impact on ticks.

Materials and Methods

A herd of 20 Hereford cows held on an 800-acre ranch with a history of heavy lone star tick populations was selected for the trial. Prior to the determination of tick numbers, three groups of five mature cows each were selected and randomly assigned for treatment. The remaining five cows, although ranging with the herd, were not used in the study. The animals were gathered twice weekly at 3–4 d intervals and moved through a chute for treatment. At each gathering, one group was treated orally with Ivomec Paste® (Merck & Co., New Jersey) at the rate of 200 µg/kg, one group was treated by subcutaneous injection with Ivomec Injectable® (Merck & Co., New Jersey) at 40 µg/kg, and the third group was the untreated control. Because scales were not available on the ranch, a beef cattle “weighing tape” was used to estimate the weights of each treated animal by measuring the girth of the animal at the withers.

On the first gathering of each week, the number of lone star ticks on the left side of each treated and control animal was counted. The number of males, unengorged females (diam <4 mm), small engorging females (4 mm ≤

diam <6 mm), medium engorging females (6 mm \leq diameter <8 mm), and large engorging females (diam \geq 8 mm) was recorded at 0, 7, 14, 21, and 28-d posttreatment. A small, clear, plastic scale with 4-, 6-, and 8-mm diameter holes was used as an aid in estimation of size of engorging ticks. In addition, the cattle were bled from the jugular vein at 0, 11, and 18-d posttreatment to obtain samples of blood for analysis of ivermectin concentration by using HPLC (Oehler & Miller 1989). Two samples were collected from each animal in 13-ml SST Vacutainers®.

An analysis of variance (ANOVA) was conducted for each category of tick to determine if there was an effect due to the treatment group, time posttreatment, and treatment by time interaction. When any of these variables was significant ($P \leq 0.05$), Fisher's least significant differences test (LSD) was used to compare multiple means (SAS 1994).

Results and Discussion

The mean numbers of ticks of each category—males, unengorged females, and small, medium, and large engorging females—for each time period are presented in Table 1. As a result of the complete randomization process prior to treatment, the control group had greater numbers of unengorged females but fewer partially engorged females than the two treated groups. Analyses of variance were conducted using only the posttreatment data to avoid the obscuring effect of the pretreatment counts particularly in the medium and large female categories. There was a significant overall treatment effect ($P < 0.05$) on each category of female ticks, but no significant effect ($P > 0.05$) due to time posttreatment or treatment group by time interaction was found. For male ticks, there was no significant effect due to treatment at the 5% level but a significant effect at the 10% level. Because posttreatment time and treatment by time interaction were not significant, the data were pooled across time and another analysis of variance was conducted for each tick category. A *t*-test was then used for separation of the overall means for the treatments (Fisher's Protected Least Significant Difference, SAS 1994). There was no significant difference ($P > 0.05$) in the numbers of unengorged, small, and medium female ticks when the untreated controls and the oral 200 $\mu\text{g}/\text{kg}$ ivermectin treatment were compared. However, the oral treatment resulted in significantly ($P < 0.05$) fewer large engorging females than on the control cattle. Significantly ($P < 0.05$) fewer small, medium, and large females were on the injected cattle compared with the controls. The number of small and medium females also was significantly less ($P < 0.05$) on the injected animals than on those treated orally; however, the number of large females on those treated orally and those injected was not significantly different. Interestingly, there was a significantly ($P < 0.05$) larger number of unengorged females on the animals receiving the injection than on those treated orally or left untreated. One explanation for this observation may be that because ivermectin interferes with tick feeding, the unengorged females were attaching and remaining in this stage for a longer time. The number of males on cattle treated orally or with the injection was significantly ($P < 0.05$) less than that on the untreated controls but not significantly different from each other.

Table 1. Numbers of lone star ticks on cattle treated with ivermectin twice weekly either with oral paste at 200 µg/kg or subcutaneous injection at 40 µg/kg.

	Number of ticks on days posttreatment				
	0	7	14	21	28
	Males				
Control	34.2	31.8	25.0	31.2	19.8
Oral	25.8	19.4	22.4	13.8	17.0
Injection	34.8	24.4	22.0	13.4	14.2
	LSD (5% Level) = 8.1				
	Unengorged females				
Control	33.2	23.0	19.2	17.6	20.2
Oral	30.8	18.0	19.8	15.6	21.2
Injection	30.2	36.4	30.2	39.6	29.4
	LSD (5% Level) = 7.0				
	Females (4 mm ≤ diameter <6 mm)				
Control	4.2	10.2	8.6	9.2	5.4
Oral	6.0	5.4	13.6	13.0	10.0
Injection	6.2	5.4	8.4	1.4	1.4
	LSD (5% Level) = 3.3				
	Females (6 mm ≤ diameter <8 mm)				
Control	0.8	2.8	1.8	1.4	2.0
Oral	1.6	1.8	1.0	1.4	2.6
Injection	1.6	0.2	0.4	0.2	0.0
	LSD (5% Level) = 0.8				
	Females (diameter ≥ 8 mm)				
Control	0.2	1.6	2.6	1.0	1.0
Oral	2.8	0.6	0.8	0.8	0.6
Injection	2.2	0.0	0.0	0.0	0.0
	LSD (5% Level) = 0.7				

Ivermectin was found in the serum of treated cattle in both posttreatment collections. On day 11 posttreatment, animals receiving the oral treatment had 4.3 ± 0.3 (mean \pm SE) ppb ivermectin in their blood serum and those treated by subcutaneous injection had 13.3 ± 3.2 ppb ivermectin. The blood collected on day 18 posttreatment showed 6.0 ± 0.7 ppb ivermectin for those treated orally and 14.6 ± 1.7 ppb ivermectin for those treated by injection. These serum concentration levels are in close agreement with that previously reported by Miller & Oehler (1996) for cattle treated in this same manner under pasture conditions.

Within 7 d after initiation of the treatments, we began to notice effects on the ticks. The ticks on the untreated controls appeared normal with a dark brown coloration and a plump shape. Those on cattle receiving the oral treatment were discolored to a mottled, lighter brown but otherwise were normal in appearance. The ticks on the cattle that were injected with ivermectin were discolored and had a wrinkled appearance, which was particularly noticeable in ticks that had started to engorge.

The results of this trial have important implications in the use of ivermectin for control of ticks on both cattle and white-tailed deer. The results indicate that although an oral treatment of 200 $\mu\text{g}/\text{kg}$ at 3–4 d intervals (twice a week) can impact ticks feeding on cattle, it appears that the 4–6 ppb ivermectin in serum resulting from the oral treatment would be inadequate for an acceptable level of tick control. In this study, each animal received a target dose of 200 $\mu\text{g}/\text{kg}$ oral treatment at 3–4 d intervals. However, in a free-choice treatment using medicated mineral or bait, normal variation in consumption between animals or within animals between feeding time would be expected to produce wider variations in ivermectin serum levels and, therefore, produce less dependable control. To compensate for these natural variations in consumption, it appears that levels of oral treatment would have to approach 200 $\mu\text{g}/\text{kg}/\text{d}$ for dependable control of lone star ticks. On the basis of these results, Pound et al. (1996) found in a subsequent study that the feeding of ivermectin-medicated corn to provide a dosage of 200 $\mu\text{g}/\text{kg}/\text{d}$ to white-tailed deer resulted in 20–30 ppb in the serum and was adequate for the control of free-living populations of lone star ticks.

Injections of 40 $\mu\text{g}/\text{kg}$ at 3–4 d intervals and the resultant 13–15 ppb ivermectin in serum was adequate to prevent completion of engorgement and, thus, should be sufficient to control lone star females feeding on pastured cattle or white-tailed deer. Therefore, an implant or other form of sustained-release injectable, which could produce these levels of ivermectin in serum, should be efficacious under field conditions.

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A Two-Year Grower Survey of Thrips and *Tospovirus* Incidence and Management in Maine Greenhouses^{1, 2}

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ABSTRACT Two mail surveys determined the presence and importance of pest thrips species and *Tospovirus* in Maine greenhouses for growing years 1993 and 1994. Respondents were licensed growers with at least 93 m² (1,000 ft²) of growing area. The study's objectives were to develop a grower demographic profile; determine the incidence of pest thrips species with specific focus on *Frankliniella occidentalis* Pergande and two thrips-vectored tospoviruses, tomato spotted wilt and impatiens necrotic spot; and identify current thrips management strategies. The surveys indicate that greenhouse growers in Maine are seasonal, experienced, and retail oriented; their growing areas average less than 929 m² (10,000 ft²); they produce a diverse crop mix; and they choose to import production stock as much as propagate it themselves. Approximately one-third of the surveyed growers detected thrips in both years. The severity of thrips and tospoviruses has increased in Maine greenhouses over the past 10 yr. Larger, year-round greenhouses are more likely to have infestations of thrips and higher virus incidence. The majority of surveyed growers employed an integrated pest management strategy. Ninety percent of growers used insecticides to control thrips. Less than 6% of growers used natural enemies to manage thrips. However, 64% of growers responded that future research in pest management should focus on biological control.

KEY WORDS Thysanoptera, Thripidae, *Frankliniella occidentalis*, western flower thrips, *Tospovirus*, impatiens necrotic spot virus, tomato spotted wilt virus, pest management, greenhouse, mail survey

Frankliniella occidentalis Pergande (Thysanoptera: Thripidae) western flower thrips, is an important pest of field and fruit crops in the western and southern United States and Canada and in many parts of Asia, South America, and Europe (Palmer et al. 1989, Oetting et al. 1993). It is considered the major pest species of greenhouse floriculture crops throughout the United States and

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Canada (Parrella 1995b). *Frankliniella occidentalis* transmits tomato spotted wilt virus (TSWV) and impatiens necrotic spot virus (INSV), two potentially fatal tospoviruses infecting a broad range of wild and cultivated plants (Sether & DeAngelis 1992, German et al. 1992). In the last decade, *F. occidentalis* has dispersed rapidly throughout the world in shipments of infested plant material and has become a major pest in greenhouse production systems (Brødsgaard 1989, Vierbergen 1995).

Along with *F. occidentalis*' ability to vector tospoviruses, other factors contribute to its pest status and economic importance in greenhouses. All active life stages feed on vegetative and reproductive tissue; it demonstrates thigmotaxis, resulting in its inhabiting protected flower and leaf spaces and potentially avoiding contact with insecticides; and it has developed resistance to most insecticides used for its control (Robb et al. 1988, Robb & Parrella 1995).

Frankliniella occidentalis is the predominant thrips species found in Maine greenhouses, notably during the spring cropping season (Folsom 1995). Smith & Lopes (1991) identified *F. occidentalis* as the predominant thrips species in Massachusetts greenhouses. Other phytophagous thrips can be found in Maine greenhouses at various times during the year; however, *F. occidentalis* appears to have displaced other pest species in number and economic importance (R. Folsom, MDAFRR, personal communication).

Frankliniella occidentalis and its associated tospoviruses are relatively recent problems for Maine greenhouse growers. *Frankliniella occidentalis* was first detected by Maine Department of Agriculture, Food and Rural Resources (MDAFRR) horticulturists in 1981 on African violets, *Saintpaulia ionantha* H. Wendland, which were marketed in supermarket chain stores (R. Folsom, MDAFRR, personal communication). In 1982 and 1983, *F. occidentalis* incidence increased dramatically and infestations occurred on many ornamental crops. *Tospovirus* was positively identified in Maine for the first time in 1990. INSV is currently the predominant thrips-vectoring virus detected in ornamental crops in Maine greenhouses (B. Watt, University of Maine Cooperative Extension, personal communication).

Considerable research worldwide currently focuses on integrated pest management (IPM) of *F. occidentalis* (Parrella 1995a, b). One goal of greenhouse IPM research is to design practical control strategies for growers. Successful application of an IPM strategy requires information about growers' perceptions and knowledge of thrips and tospoviruses, and about the current status of thrips management.

Surveys can be vital tools to determine the importance of an issue and the means for solving a problem (Warwick & Lininger 1975). They can contribute to an understanding of grower practices, attitudes, and concerns, and provide an assessment of the current status of the greenhouse industry. The objectives of this study were to develop a demographic profile of Maine greenhouse growers, determine the incidence of thrips and TSWV/INSV in two successive growing seasons, and identify current thrips management approaches.

Materials and Methods

Survey procedures. During 1993, MDAFRR reported a total of 1,348 licensed producers and dealers of nursery stock in Maine, with a total greenhouse production and retail area of nearly 232,000 m² (2.5 million ft²) (MDAFRR 1993). "Nursery stock" includes both woody and herbaceous plants grown for sale. Of these businesses, 496 produced greenhouse crops for retail or wholesale markets. The survey sample of 372 greenhouses included all businesses with production areas of at least 93 m² (1,000 ft²).

As a general guideline for implementing the mail survey, the "total design method" described by Dillman (1978) was used. A pretest survey was mailed in November 1993 to eight licensed greenhouse growers, six University of Maine agriculture specialists, and two MDAFRR horticulturists. The pretest packet included a cover letter; the survey; an information sheet on *F. occidentalis* identification, basic biology, and management options; and a postage-paid return envelope. The pretest was used to identify construction flaws in the survey and to solicit suggestions about the format and content.

The revised survey questionnaire was mailed to 372 growers in December 1993, requesting information about the 1993 crop year. A cover letter emphasized the importance of the study to the Maine greenhouse industry and promised confidentiality to respondents. A follow-up survey was sent after 3 wk to nonrespondents, with a revised cover letter. An additional follow-up survey was sent 4 wk later to nonrespondents. Of the respondents, only the growers who were in business in 1993 were included in the survey analysis. This reduced the sample population to 353. The response rate for the 1993 survey was 248 growers (70.3%).

A similar mailing procedure requested information about the 1994 growing season. The 248 growers who responded to the 1993 survey were sent a 1994 survey. The first survey was mailed in December 1994 and follow-up surveys were mailed similarly as in 1993. Only those respondents who were in business in 1994 were included in the survey analysis. The response rate was 178 growers of a corrected sample of 244 (73%).

Nonresponse error is defined as "nonrespondents who are different from respondents in a way that pertains to the study focus" (Salant & Dillman 1994). Response rate is the most frequently used criterion measuring nonresponse error. Response rates between 60% and 75% for mail surveys are desired (Dillman 1978). Our response rates, greater than 70% in both years, were within this range. However, this single criterion may not accurately assess nonresponse error (Salant & Dillman 1994). Two other measures were considered. First, because greenhouse size was correlated with thrips incidence in both years' surveys, we compared greenhouse size of nonrespondents with that of respondents. There was no difference between the groups ($P > 0.05$). Second, greenhouse crops are grown during specific periods of the year, based on their market potential. This market potential for crops is quite uniform across geographic areas. Bedding plants are sold in May and June, whereas poinsettias are sold in December. This similarity may logically be considered to apply to pest complexes throughout the geographic region surveyed. Again, no difference was found between respondents and nonrespondents. We concluded

from the three criteria (high response rate, thrips by greenhouse size interaction, and time and space similarity) that there was low nonresponse error; i.e., nonrespondents were similar to respondents, and therefore, the study results represent the Maine commercial greenhouse industry.

Grower profile. Demographic indicators used to describe Maine greenhouse growers included size of greenhouse, marketing strategy, years in operation, crops grown and their relative economic value, methods of crop accession and production, and months of the year the business was in operation. Some data, such as crop value, propagation method, greenhouse size, years in operation, and months of operation were regrouped into ordered categories to better describe the results. A seasonal greenhouse was designated as having operated 10 mo or less per year and a year-round greenhouse as having operated more than 10 mo per year. Marketing strategy was expressed in terms of how growers sold their crops—retail, wholesale, or a combination of the two. Crop data were described as both the mean percentage of the crop types produced (a profile of the average grower's crop mix) and as the percentage of all growers producing specific crops. Growers were asked to describe the percentage of plants obtained or propagated in various ways. Data were described as both the mean percentage of the source of production stock or propagation method used and as the percentage of all growers using each source or propagation method. Questions pertaining to greenhouse size and years in operation were asked in the 1993 survey only.

Thrips and TSWV/INSV. Greenhouse growers were not asked to identify the thrips they reported. However, thrips species other than *F. occidentalis* were rarely found by state inspectors in Maine greenhouses in the years this survey was conducted (R. Folsom, personal communication). Therefore, we assume that *F. occidentalis* was the predominant species of thrips detected by growers for this survey.

In the 1993 survey, growers were asked whether they had ever detected thrips, in what year they first detected them, each year they found them, infestation pressure in 1993 compared with 1992, and on what crops and specific plants thrips caused damage. In 1994, growers were asked whether they found thrips in 1994, infestation pressure in 1994 compared with 1993, and on what crops and specific plants thrips caused damage. In the 1993 survey, growers were asked whether they had ever detected TSWV, in what year they first detected it, if it was positively identified by an expert, what plant species were infected, and if they discarded any of the crop because of virus. In the 1994 survey, the same questions were asked about TSWV/INSV incidence for the 1994 season only (with the exception of the question "first year detected"). INSV was included in 1994 because prior to that time, INSV in Maine had been identified as TSWV (impatiens strain). Only growers who found thrips in 1993 were considered for analysis of thrips-virus interactions related to 1993 demographics and comparison with 1994 growers reporting thrips or TSWV/INSV. Only growers who found thrips in either 1993 or 1994 answered additional survey questions related to thrips and virus incidence.

Thrips management. Growers were asked to report the management tactics they used to control thrips and whether the measures were effective. If growers applied insecticides, the data were categorized by the percentage of

growers who used each compound listed and by the three insecticides each grower relied on most frequently to control thrips. Because growers use different combinations of insecticides, responses to the question pertaining to pesticide reliance were weighted, assigning the highest value to the insecticide each grower used most frequently. The transformed data were summed for each compound. These weighted sums were divided by the number of growers using each compound, giving a rating index from 0 to 3. The closer the rating was to 3, the more the insecticide was relied on, or on average, used most frequently. Growers also estimated the effectiveness of their insecticide applications.

Growers were asked if they had ever used natural enemies to control thrips. Those who reported thrips incidence but did not use biological control as an IPM tactic were asked why they did not consider this control option. Growers were asked where they obtained information about pest management and in what areas future IPM research should focus. Respondents wrote general comments regarding thrips management at the end of the questionnaire and a brief summary of these comments is included in the discussion.

Statistical analysis. Responses to questions were coded by a variable name and given a number corresponding to the response. A master codebook was developed to record data used in the survey (Salant & Dillman 1994). Data were analyzed in SYSTAT 5.2 (Wilkinson 1989). Frequency distributions were computed for all questions in both years' surveys. Where questions about grower demographics were the same in both years' surveys, means were pooled if no differences were found between 1993 and 1994. The statistical difference between two means was computed by a *t*-test at a significance level of $P \leq 0.05$. Both years' results were included in graphs and tables for other categorical data. Interactions between categorical variables and frequency distributions were analyzed with contingency tables and the Pearson chi-square statistic at a significance level of $P \leq 0.05$. For analysis purposes, tables with sparse cell frequency ($n < 5$) were reduced in dimension to assess interaction significance by using chi-square (Sokol & Rholf 1981).

Results and Discussion

Grower profile. No differences in grower responses to demographic questions were found between 1993 and 1994 ($P > 0.05$). Means for grower profile data were pooled for both years.

Most greenhouses in Maine (61.7%) are smaller than 465 m² (5,000 ft²) and 82% are smaller than 929 m² (10,000 ft²) (Fig. 1A). The largest grower had a greenhouse area of 7,060 m² (76,000 ft²) and only one other grower's greenhouse area exceeded 4,645 m² (50,000 ft²). Nearly half of all growers (47.3%) have been in business more than 10 yr and 80.8% at least 5 yr (Fig. 1B). Less than 3% responded that 1993 was their first year in business.

At least 92% of all Maine greenhouses operated from March through June and nearly 100% operated in April and May, indicating the prevalence of spring greenhouses (Fig. 1C). The majority of greenhouses (71%) were seasonal (operating 10 or fewer mo per year). Most growers either sold their entire crop in the retail market (34.5%) or sold more than 50% of their crop in the retail market (49.2%), whereas 4% were wholesale growers only (Fig. 1D).

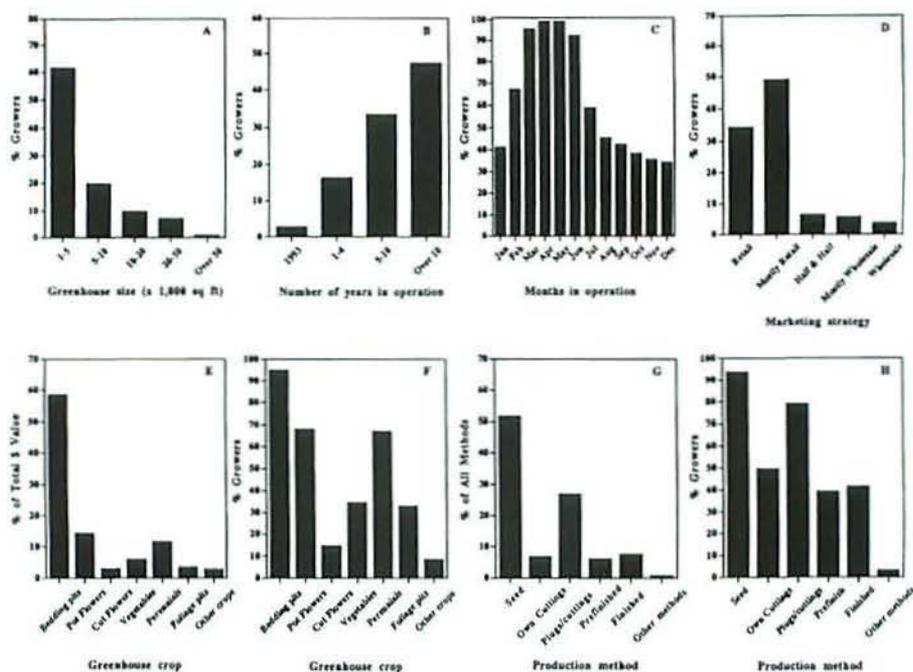


Fig. 1. (A) Greenhouse size expressed as the percentage of growers in each size category ($n = 248$). (B) Number of years greenhouse business has been in operation expressed as the percentage of growers in each category ($n = 239$). (C) The months of the year growers are in operation expressed as the percentage of growers in each month, averaged over 1993–1994 ($n = 171$). (D) Marketing strategy expressed as the percentage of growers marketing their crops in different ways, averaged over 1993–1994 ($n = 178$). (E) Mean values of specific crops expressed as percentages of the total value of all crops grown averaged over 1993–1994 ($n = 178$). (F) Mean percentage of growers producing any amount of each crop averaged over 1993–1994 ($n = 178$). (G) Mean percentage of production stock each grower obtained or propagated averaged over 1993–1994 ($n = 178$). (H) Mean percentage of growers propagating production stock with various methods averaged over 1993–1994 ($n = 178$).

Bedding plants were the major crop; nearly 4 times more value was in bedding plants than in potted flowering plants or perennials (Fig. 1E). Bedding plants also were grown by the most growers; 95% of all growers produced bedding plants whereas two-thirds produced potted flowering plants and perennials and one-third produced vegetables and foliage plants (Fig. 1F). The data indicate that although the greatest dollar value in the total crop mix was in bedding plants, growers produced a diverse crop mix.

Seed was the primary propagation method, but most growers supplemented seed propagation with other methods (Fig. 1G). Crops propagated from seed made up 52.3% of total production stock and 26.4% were purchased as plugs or cuttings from other growers. Less than 10% of production stock was obtained by the other methods. Growers used multiple sources of production stock; at least 40% of all growers relied on all propagation methods (Fig. 1H). Only 6.5% of growers propagated all their production stock themselves, but 61.3% self-propagated at least 50% of their production stock.

Year-round growers were more diversified than seasonal growers in their marketing approach. More seasonal growers (42.5%) than year-round growers (17.9%) relied solely on the retail market and 85.3% of seasonal growers and 78.9% of year-round growers sold at least 50% of their product in the retail market. Only 2.7% of seasonal growers and 6.8% of year-round growers sold wholesale only ($\chi^2 = 14.827$; $df = 4$; $P = 0.0051$). Most of growers in business more than 10 yr were year-round (62%) and 13% of year-round growers had been in business less than 5 yr ($\chi^2 = 9.468$; $df = 3$; $P = 0.0237$). Smaller operations (less than 465 m² [5,000 ft²]) were seasonal (69%). The majority of greenhouses over 929 m² (10,000 ft²) were year-round (56%) ($\chi^2 = 47.1911$; $df = 4$; $P = 0.0001$). Of growers with more than 50% of their total crop value in bedding plants, 64% were seasonal. Eleven percent of seasonal growers had a bedding plant value of less than 25% of their total crop value. Forty-eight percent of year-round growers had bedding plant values between one-quarter and one-half of their total crop value ($\chi^2 = 17.521$; $df = 3$; $P = 0.0006$). Of growers with more than 25% of their total crop value in potted flowering plants, 12% were seasonal ($\chi^2 = 15.596$; $df = 3$; $P = 0.0014$). In comparing perennial, cut flower, vegetable, or foliage plant crop values with months of greenhouse operation, no trend was seen ($P > 0.05$).

In summary, an average Maine greenhouse grower is seasonal, experienced, and retail oriented, has a growing area of less than 929 m² (10,000 ft²), produces a diverse crop mix emphasizing bedding plants, and imports some production stock in addition to producing it in-house.

According to 1993 and 1994 data on floriculture crops, greenhouses in Connecticut, Maryland, and Massachusetts averaged more than 1,858 m² (20,000 ft²) and had the majority of their sales in bedding plants and potted flowering crops (USDA 1995). Therefore, the Maine greenhouse industry is comprised of growers similar to those in the other three states, except that Maine's greenhouse size is, on average, half as large. The industry in Maine also contributes less to the state's total agricultural cash receipts. The greenhouse/nursery industry accounted for 20% of all agricultural cash receipts in New England in 1993, whereas greenhouse and nursery industry cash receipts in 1993 totaled 4% of the total agricultural cash receipts in Maine (USDA 1994).

Thrips and TSWV/INSV. Of the 248 respondents in 1993, 34% reported thrips in their greenhouses in 1993 or earlier, 55.6% had never detected thrips, and 10.4% were not sure if they had ever found thrips (Fig. 2A). Considering 1993 only, 26.7% reported finding thrips in that year. In 1994, 31% of growers reported thrips, 62% didn't find thrips, and 7% weren't sure.

Although *F. occidentalis* has infested Maine greenhouse crops for about 14 yr, 3.6% of all growers reported finding them prior to 1988 and 9.3% reported finding them for the first time in 1992 (Fig. 2B). Additional growers detected thrips for the first time in each year until 1993, when first-time thrips detection fell by more than half. There was a steady rise in the percentage of growers finding thrips from 1988 through 1993 (Fig. 2B). Similar thrips incidence has been documented in other New England states (Blessington et al. 1992, Wick et al. 1994).

Frankliniella occidentalis has been found infesting the flowers of every major plant family (Robb & Parrella 1995). In our 2-yr survey, growers reported finding thrips on 51 different taxa, belonging to 42 genera in 26 families (Table 1). Thrips were especially troublesome on Balsaminaceae, Asteraceae, Geraniaceae, Gesneriaceae, Solanaceae, and Begoniaceae species in Maine greenhouses, but many greenhouse crop families are susceptible.

Potted flowering plants (68.7%, 1993; 65.1%, 1994) and bedding plants (54.8%, 1993; 63.5%, 1994) were listed most often as the crops infested with thrips (Table 2). Because many growers produced potted baskets consisting of stock also sold as bedding plants, some misrepresentation may have occurred. Single impatiens was listed most often as the plant infested with thrips (22.1%, 1993; 26.4%, 1994) (Table 3). Three types of impatiens were reported, and when combined, they comprised 46.1% and 49.5% of all plants listed in 1993 and 1994 respectively.

Tospovirus incidence has increased steadily in Maine greenhouses since 1988. At least 8 species of thrips, including *F. occidentalis*, transmit tospoviruses (German et al. 1992). At least 60 plant families and 550 plant species, including both monocots and dicots, are known to be hosts (Sether & DeAngelis 1992). Some plant species or cultivars remain asymptomatic and are marketable. Others show varying symptoms of the disease and may be unmarketable. Economic losses may be considerable and to prevent virus infection, exclusion and control of infected thrips are important management tactics (Parrella 1995a). Growers who purchase any of their crop from wholesale production sources incur an additional risk of acquiring the virus by importing either infected thrips or infected plants (Zitter et al. 1989).

Of the 84 growers who detected thrips in 1993 or before, 26 (31%) also reported TSWV/INSV-infected plants (Fig. 3A). Of the 55 growers reporting thrips in 1994, 17 (30.9%) reported virus-infected plants. When all years are averaged, 50.8% of growers found no virus and 17% were not sure if they had ever found TSWV/INSV. No virus was reported by the surveyed growers to have occurred prior to 1988 (Fig. 3B). The number of growers who reported the virus for the first time steadily increased (with a small drop in 1991) until 1992, when nine cases (34.6% of the total) were reported for the first time. A 65% drop in the rate of virus spread occurred in 1993, matching a similar decline noted with first-time thrips incidence (Fig. 2B).

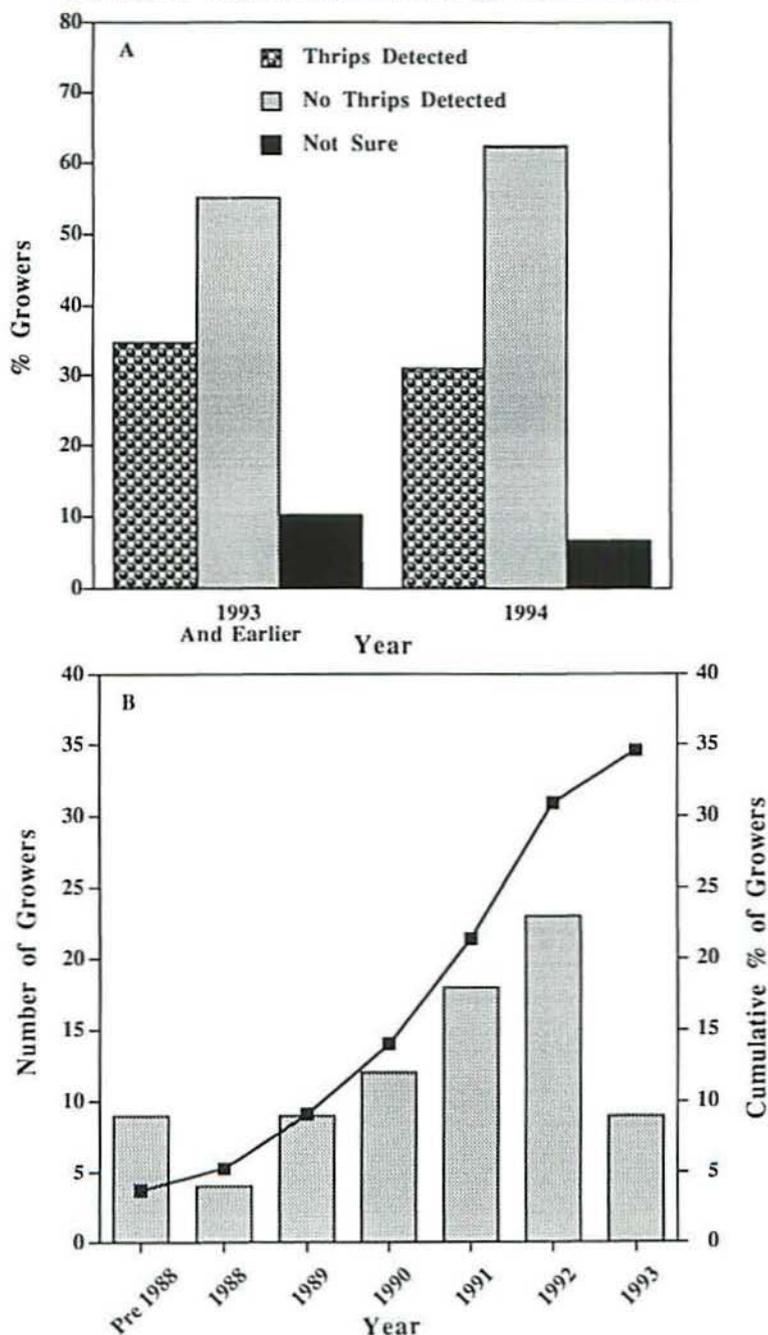


Fig. 2. (A) Percentage of growers reporting thrips in 1993 and earlier ($n = 248$) and in 1994 ($n = 178$). (B) Number of growers reporting the first year thrips was found (left Y axis: bar graph); and the cumulative percentage of growers reporting thrips in each year (right Y axis: line graph) ($n = 248$).

Table 1. Taxa infested with thrips and/or infected with tomato spotted wilt virus/impatiens necrotic spot virus reported by Maine greenhouse growers in 1993 ($n = 84$) and 1994 ($n = 55$).

Family	Genus	Common name	Cultivar
^a Asteraceae	<i>Dendranthema</i>	chrysanthemum	
^c Asteraceae	<i>Gerbera</i>	gerbera	
^a Asteraceae	<i>Dahlia</i>	dahlia	
^c Asteraceae	<i>Brachycome</i>	Swan River daisy	
^c Asteraceae	<i>Tagetes</i>	marigold	
^c Asteraceae	<i>Ageratum</i>	floss flower	
^a Asteraceae	<i>Senecio</i>	cineraria	
^c Asteraceae	<i>Felicia</i>	felicia daisy	
^c Solanaceae	<i>Solanum</i>	eggplant	
^c Solanaceae	<i>Petunia</i>	petunia	
^c Solanaceae	<i>Petunia</i>	double petunia	
^a Solanaceae	<i>Lycopersicon</i>	tomato	
^c Solanaceae	<i>Capsicum</i>	pepper	
^a Balsaminaceae	<i>Impatiens</i>	single impatiens	
^a Balsaminaceae	<i>Impatiens</i>	Accent impatiens	'Accent'
^a Balsaminaceae	<i>Impatiens</i>	Blitz impatiens	'Blitz'
^a Balsaminaceae	<i>Impatiens</i>	double impatiens	
^a Balsaminaceae	<i>Impatiens</i>	New Guinea impatiens	
^a Gesneriaceae	<i>Sinningia</i>	gloxinia	
^c Gesneriaceae	<i>Saintpaulia</i>	African violet	
^c Gesneriaceae	<i>Streptocarpus</i>	cape primrose	
^c Lamiaceae	<i>Salvia</i>	annual salvia	
^c Lamiaceae	<i>Salvia</i>	perennial salvia	
^a Lamiaceae	<i>Plectranthus</i>	Swedish ivy	
^a Geraniaceae	<i>Pelargonium</i>	ivy geranium	
^c Geraniaceae	<i>Pelargonium</i>	zonal geranium	
^c Verbenaceae	<i>Verbena</i>	verbena	
^a Verbenaceae	<i>Lantana</i>	lemon verbena	
^c Euphorbiaceae	<i>Euphorbia</i>	poinsettia	
^c Euphorbiaceae	<i>Euphorbia</i>	Freedom poinsettia	'Freedom'
^c Saxifragaceae	<i>Tolmeia</i>	piggy-back plant	
^b Saxifragaceae	<i>Saxifraga</i>	strawberry begonia	
^c Primulaceae	<i>Primula</i>	primrose	
^c Primulaceae	<i>Cyclamen</i>	cyclamen	
^c Begoniaceae	<i>Begonia</i>	begonia	
^b Begoniaceae	<i>Begonia</i>	Rieger hybrids	
^c Iridaceae	<i>Gladiolus</i>	gladiolus	
^c Fabaceae	<i>Lupinus</i>	lupine	
^c Onagraceae	<i>Fuchsia</i>	fuchsia	
^c Oxalidaceae	<i>Oxalis</i>	oxalis	
^c Amaranthaceae	<i>Celosia</i>	celosia	
^c Violaceae	<i>Viola</i>	pansy	
^c Lobeliaceae	<i>Lobelia</i>	lobelia	
^c Cucurbitaceae	<i>Cucumis</i>	cucumber	
^c Piperaceae	<i>Peperomia</i>	peperomia	
^c Scrophulariaceae	<i>Antirrhinum</i>	snapdragon	
^c Rosaceae	<i>Fragaria</i>	strawberry	
^c Caryophyllaceae	<i>Dianthus</i>	carnation	
^c Apocynaceae	<i>Mandevilla</i>	mandevilla	
^c Asclepiadaceae	<i>Hoya</i>	wax vine	
^c Araceae	<i>Aglaonema</i>	Chinese evergreen	

^a Plants with thrips and TSWV/INSV.

^b Plants with TSWV/INSV only.

^c Plants with thrips only.

Table 2. Percentage of Maine greenhouse growers in 1993 ($n = 84$) and 1994 ($n = 55$) listing crops on which thrips were found.

Crop	% Growers	
	1993	1994
Potted flowering plants	68.7	65.1
Bedding plants	54.8	63.5
Perennials	9.2	8.9
Foliage plants	13.8	7.3
Cut flowers	6.2	8.1
Vegetables	2	3.2

Table 3. Percentage of Maine greenhouse growers in 1993 ($n = 84$) and 1994 ($n = 55$) listing plants on which thrips were most often found.

Crop	% Growers	
	1993	1994
Single impatiens	22.1	26.4
Double impatiens	13.4	11.9
New Guinea impatiens	10.6	11.2
All impatiens	46.1	49.5
<i>Sinningia</i>	14.8	12.8
<i>Dendranthema</i>	13.5	8.9
<i>Saintpaulia</i>	9.6	7.1
<i>Tagetes</i>	8	6
<i>Pelargonium</i> (zonal)	8	7
<i>Pelargonium</i> (ivy-leaf)	4	13
<i>Begonia</i>	6	9

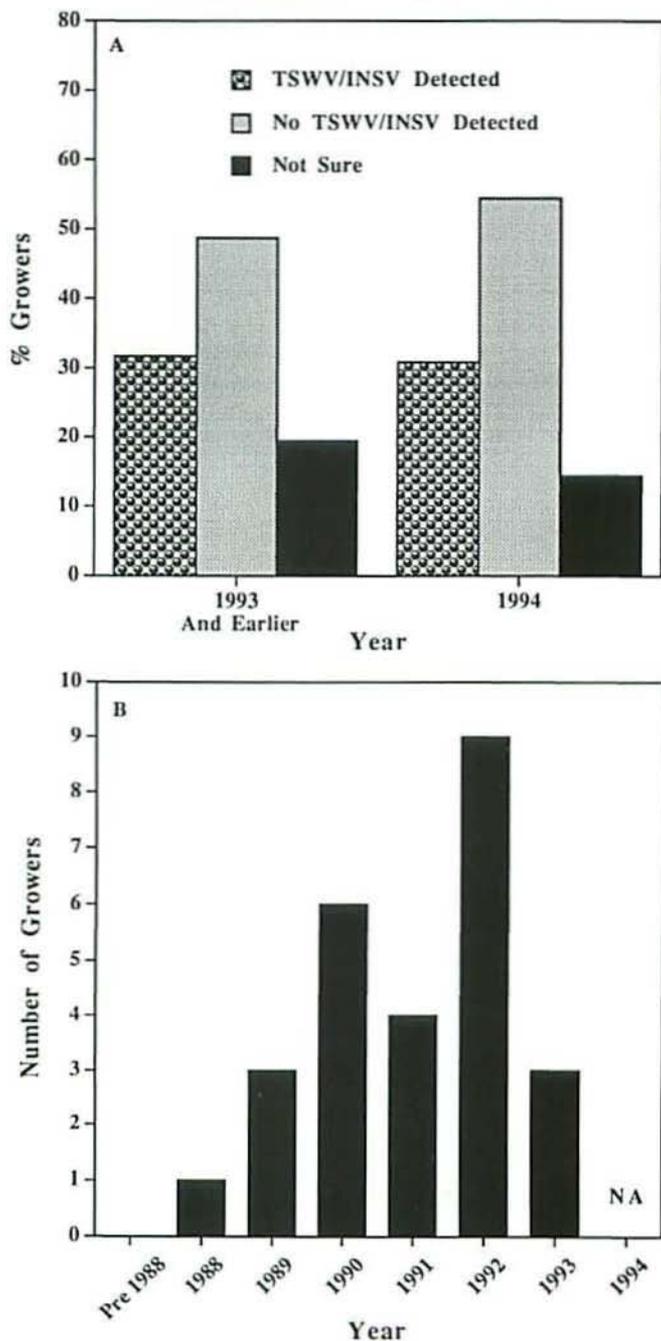


Fig. 3. (A) Percentage of growers reporting TSWV/INSV in 1993 or earlier ($n = 84$) and in 1994 ($n = 55$). (B) Number of growers reporting the year the virus was detected for the first time ($n = 84$); NA, question not asked.

The species composition of virus-infected plants (Table 4) was similar to thrips-susceptible plants (Table 3), with impatiens reported most often. Nearly 88% of growers reporting virus in both survey years discarded some portion of their infected crop, indicating the economic importance of the disease.

In 1993, 19 growers (73%) had TSWV/INSV positively identified by an expert. In 1994, nine cases (52.9%) were positively identified. The high percentage of growers (18%) who were uncertain whether their crop was infected with virus reflects the difficulty growers have recognizing disease symptoms. There are a number of possible reasons for this: symptoms can be distinct for different plant species; damage may not be detectable early enough in the crop's growth cycle to prevent disease spread; and symptoms may easily be confused with other biotic or abiotic problems (Zitter et al. 1989). Presently, plant inspectors in Maine suspect that TSWV/INSV goes undetected or misdiagnosed by many more greenhouse growers than are accurately assessing and reporting it (E. A. Gibbs, MDAFRR, personal communication).

Among growers with greenhouses larger than 929 m² (10,000 ft²), 66% reported thrips, whereas 19% of growers with greenhouses smaller than 465 m² (5,000 ft²) reported thrips ($\chi^2 = 24.821$; df = 4; $P = 0.0001$). The same trend occurred with virus incidence among growers reporting thrips; 71% of growers detected virus in greenhouses exceeding 929 m² (10,000 ft²), compared with 14% in greenhouses less than 465 m² (5,000 ft²) ($\chi^2 = 8.986$; df = 3; $P = 0.0295$). More year-round growers (62%) detected thrips than seasonal growers ($\chi^2 = 33.427$; df = 2; $P = 0.0001$).

We speculate that growers who propagated all of their own plant stock from seed or from stock cuttings had less thrips and virus pressure because no plant material was shipped in from outside sources. Twelve growers propagated 100% of their plants in 1993 and 14 propagated all their stock in 1994. Of these growers, none reported thrips in 1993 and two reported thrips in 1994. Of the two in 1994, neither reported the virus. Producing all plant stock in-house appears to have helped reduce thrips and virus. However, these data did not differ from growers who obtained all their stock from outside production sources. Eight of 11 growers who purchased all their plant material from outside sources in 1993 and 8 of 10 growers in 1994 also reported finding no thrips ($\chi^2 = 4.665$; df = 2; $P = .0971$). Two growers in each year also reported virus incidence.

We conclude that there is a serious thrips and *Tospovirus* problem in Maine greenhouses. One-third of all growers detected thrips and another 10% were not certain. One-third of this group also detected INSV and 15%–20% were not certain. Considering crop damage and virus potential, the chance for economic loss is substantial. Based on the number of growers who were not sure whether they had thrips or virus, an accurate assessment of insect and disease presence in the greenhouse during the growing season should be a priority for Maine growers. This requires accurate thrips monitoring and virus symptom identification.

Thrips management. IPM appears to be practiced by many Maine greenhouse growers. Both years' data reflect a broad application of management tactics (Table 5). Thirty percent of all growers used at least seven of the 10 tactics. More growers (90%) used insecticides to control thrips than

Table 4. Percentage of Maine greenhouse growers in 1993 ($n = 26$) and 1994 ($n = 17$) listing plants on which TSWV/INSV was most often found.

Plant	% Growers	
	1993	1994
Double impatiens	15.4	12.3
Single impatiens	11.6	10.2
New Guinea impatiens	5.2	3.3
All impatiens	31.3	25.9
<i>Sinningia</i>	6.6	3.6
<i>Plectranthus</i>	4.0	1.4
<i>Begonia</i>	3.2	4.0
<i>Lycopersicon</i>	3.0	2.2
<i>Dendranthema</i>	2.2	2.6
<i>Pelargonium</i> (ivy-leaf)	2.1	2.9
<i>Cineraria</i>	2.1	2.9

any other control option. More growers used Orthene (acephate) than any other insecticide (73%, 1993; 61.2%, 1994) (Table 6). Orthene was also relied on, or on average, used more frequently by the growers who applied it (rated value, 2.5) (Table 6). Avid (abamectin), resmethrin, and Talstar (bifenthrin) were applied in both years by at least 30% of growers. As a class, pyrethroids (bifenthrin, resmethrin, and fluvalinate) were used by the most growers, but with the exception of fluvalinate (rated value, 2.75 in 1994), were not necessarily the compounds relied on most often by individual growers to control thrips. Dursban (chlorpyrifos) and Fulex (nicotine) were used most frequently by growers who applied them (rated value, 3). However, these compounds were applied by less than 10% of growers. The so-called biorational insecticides, such as Margosan-O and Azatin (azadirachtin), horticultural oil, and insecticidal soap, were applied by fewer than 30% of growers. Azadirachtin had a high reliance rating in 1993 (2.7), whereas oil and soap had ratings of about 2. There was no correlation between the percentage of growers using a particular insecticide and their reliance on the insecticide ($P > 0.05$).

Only 15% of growers indicated that they achieved 100% control with the insecticides they applied. Forty-seven percent said that at least 75% efficacy was achieved and 23% didn't know what the results of the pesticide applications were. Growers indicated in both years that removing weeds and applying insecticides were the two most effective management tactics (Table 5). However, many growers commented that insecticides are becoming less and

Table 5. Percentage of Maine greenhouse growers using various pest management tactics for thrips control in 1993 ($n = 84$) and 1994 ($n = 55$); and percentage of growers rating tactics as being effective or ineffective in 1993 ($n = 84$) and 1994 ($n = 55$).

Tactic	% Growers					
	1993			1994		
	Used	Effective	Ineffective	Used	Effective	Ineffective
Apply insecticides	90.5	56.0	10.7	89.1	63.6	11.0
Remove weeds	75.0	51.2	9.5	81.8	67.3	7.3
Fallow greenhouses	63.1	48.8	3.6	50.9	30.9	9.1
Rotate insecticides	59.5	39.3	8.3	69.1	50.9	5.5
Purchase clean stock	52.3	32.1	14.3	43.6	29.1	12.7
Monitor thrips	50.0	33.3	7.1	56.4	40.0	5.5
Quarantine crops	31.0	19.0	4.8	27.3	20.0	5.5
Screen vents	6.0	4.8	1.2	11.0	7.3	3.7
Sanitize benches	3.9	1.2	2.7	4.0	1.2	2.8
Discard infested plants	3.8	3.8	0	3.6	3.0	0.4
Propagate in-house	3.6	2.4	0	9.1	7.3	1.8
Use natural enemies	2.4	1.2	1.2	5.5	1.8	3.7
Other	2.9	2.9	0	1.8	0	0

less effective. Eleven percent of growers indicated that insecticides were ineffective.

Insecticide resistance is a serious and widespread problem in managing *F. occidentalis* (Brødsgaard 1989, Robb et al. 1995). It is an important reason why *F. occidentalis* has achieved global importance. Alternating chemical classes may slow resistance in a susceptible population. Many growers (59.5%, 1993; 69.1%, 1994) used chemical class rotation (Table 5) when applying insecticides, presumably to reduce selective resistance of thrips to the chemicals applied. However, some growers (24.3%, 1993; 19.5%, 1994) who indicated that chemical rotation was an effective tactic, also said they applied only one insecticide class, indicating a lack of incorporation of this tactic into their overall management strategy.

Growers felt they were achieving success with a variety of management tactics (Table 5). It is noteworthy that 14.3% of growers in 1993 who purchased production stock from outside sources claiming pest-free stock found this tactic unreliable. This contributes to at least part of the thrips and virus problem for Maine growers. However, nearly all growers who purchased 100% of their production stock from outside sources also reported no thrips. This anomaly is notable; however, any thrips control strategies these particular growers used

Table 6. Insecticides Maine greenhouse growers used to control thrips in 1993 ($n = 84$) and 1994 ($n = 55$); and rated values showing, on average, how frequently each insecticide was used by growers who applied it.

Insecticide ^{a,b}	% Growers ^c		Rated value ^d	
	1993	1994	1993	1994
Orthene (acephate)	73	61.2	2.45	2.5
Talstar (bifenthrin)	37.8	38.8	2	1.85
Avid (abamectin)	35.1	40.8	2.35	2.3
Resmethrin (resmethrin)	35.1	30.6	2	1.75
Mavrik (fluvalinate)	32.4	18.4	2.2	2.75
Malathion (malathion)	23	24.5	2.35	2.25
Enstar (kinoprene)	—	26.5	—	1.75
Knox-Out (diazinon)	20.3	28.6	2	2
Insecticidal soap (potassium salts)	20.3	22.4	2.12	1.75
*Oxamyl (vydate)	20.3	16.3	2.12	2.5
Margosan-O/Azatin (azadirachtin)	18.9	28.6	2.7	2.5
Other insecticide	13.5	16.3	2.25	2.75
Sunspray (horticultural oil)	8.1	10.2	2.12	1.8
Dursban (chlorpyrifos)	8.1	4.3	3	3
*Fulex (nicotine)	6.8	4.3	3	3
Dithio (sulfotepp)	5.4	10.2	2	2
*Thiodan (endosulfan >10%)	5.4	4	1.75	1
Dycarb (bendiocarb)	4.1	0	0	0
Dibrom (naled)	0	2	0	0
Decathlon (cyfluthrin)	0	0	0	0

^aRegistered insecticides for thrips in Maine greenhouses; * denotes restricted use insecticides.

^bEnstar was not included in 1993 survey.

^cPercentage of all growers using various insecticides in 1993 and 1994 to control thrips.

^dThe average value of grower reliance on each insecticide if the insecticide was used to control thrips in either 1993 or 1994; 0, least reliance; 3, most reliance.

would necessarily affect thrips incidence.

Monitoring for pest presence and weed removal are promoted as useful practices in greenhouse pest management (Parrella 1995a). Thrips monitoring was used by 50% of growers in 1993 and 56.4% in 1994 (Table 5). We speculate that monitoring thrips populations may have been more prevalent for year-round growers. No differences were observed between seasonal and year-round growers ($\chi^2 = 5.2617$; $df = 3$; $P = .0819$ in 1993 and $\chi^2 = 4.1198$; $df = 3$; $P = .1021$ in 1994). Weed removal was used by 75% of growers in 1993 and 81.8% in 1994.

Closing all or some of the greenhouses in a range, either in the heat of summer or cold of winter, can reduce or eradicate a thrips population. Seasonal

growers have more opportunity to fallow greenhouses. In Maine, about 74% of surveyed growers were seasonal and of these 62% in 1993 and 57% in 1994 reported using this tactic to manage thrips. Both seasonal growers (84%) and year-round growers (70%) found fallowing greenhouses to be effective in 1993 ($\chi^2 = 1.781$; $df = 2$; $P = 0.410$), but more seasonal growers (88%) than year-round growers (25%) in 1994 found that fallowing greenhouses was effective ($\chi^2 = 12.467$; $df = 2$; $P = 0.002$).

Two growers in 1993 and three growers in 1994 released biological control agents of thrips. They used a predatory mite, *Neoseiulus (Amblyseius) cucumeris* Oudemans, and *Encarsia formosa* Gahan, which is a whitefly parasite and is not effective against thrips. All the growers indicated that the natural enemies they used did not provide adequate control, either because a pesticide was used prior to release, possibly killing the biological control agent, or they didn't release enough of the agents. The two most frequent reasons why growers reporting thrips incidence did not try biological controls were limited knowledge about their use (57%) and adequate control was obtained with insecticides (46%). Nearly 20% responded that it would be too risky and 10% indicated that it would be too costly.

Most growers use multiple sources to obtain information about pest management (Table 7). The most frequently listed sources were grower publications (81.1%, 1993; 64.1%, 1994) and University of Maine Cooperative Extension (67.2%, 1993; 58.9%, 1994). At least 30% used all listed information sources. There was a high grower response in both years for future pest management research to focus on biological control, the use of resistant crop cultivars, and cultural management (Table 8). These results appear to be a request for pest management alternatives to conventional reliance on pesticides. We also were interested if growers who found thrips in either year chose a particular method of information gathering or requested a particular research focus. There were no differences in information seeking or research focus between growers who reported thrips compared with those who did not report finding them ($P > 0.05$).

About 15% of respondents in both years added comments about pest management, particularly thrips control, at the end of the survey. Two themes characterized the comments. First, western flower thrips has become the most challenging pest problem many growers have had to confront in their years in the greenhouse business. Difficulty in accurately assessing the levels of infestations, the suddenness of thrips outbreaks, and the ineffectiveness of insecticides were mentioned frequently. Second, growers were not sure where, how, and when to detect thrips. Thrips were confused with other more "long-standing" greenhouse pests, such as aphids and whiteflies. Finally, some growers warned that if "you don't think you have western flower thrips, look a little closer."

Status of Thrips and INSV in 1995 and 1996. The status of *F. occidentalis* and INSV in Maine greenhouses in 1995 and 1996 was considered to be very serious (R. Folsom, MDAFRR, personal communication). Many cases of thrips were encountered during greenhouse inspections, especially on impatiens and seed-propagated tuberous begonia. The virus was considered more prevalent in 1995 and 1996 than in the years reported in this survey, and

Table 7. Percentage of Maine greenhouse growers in 1993 ($n = 248$) and 1994 ($n = 178$) using various pest management information sources.

Source	Growers using information source (%)	
	1993	1994
Grower publications	81.1	64.1
University of Maine Cooperative Extension	67.2	58.9
Reference books	64.9	59.4
Other growers	62.6	41.2
Maine Department of Agriculture	55.5	41.2
Trade shows or grower meetings	42.6	39.4
Sales representatives	36.1	30.2
Other source	6.7	2.9

Table 8. Percentage of Maine greenhouse growers in 1993 ($n = 248$) and 1994 ($n = 178$) requesting that pest management research be focused in specific areas.

Research area	Growers requesting research (%)	
	1993	1994
Biological control	63.8	61.3
Crop resistance	62.5	59.4
Cultural management	43.8	37.4
Pesticide application	33.0	29.7
Pesticide resistance	25.0	29.0
Biotechnology	17.9	16.8
Other area	2.7	4.5

some growers' entire impatiens and begonia crops were lost to the virus due to carrying over infected thrips to the new spring crop or importing infected plantlets. ELISA test results confirmed that the prevalent virus in Maine greenhouses is INSV (B. Watt, University of Maine Cooperative Extension, personal communication). Entomologists recommend that growers spot test plugs and cuttings arriving in the spring to identify the virus early, as well as control thrips during the entire growing season.

This survey provides a glimpse at the greenhouse industry in Maine over a 2-yr period, and how growers manage one of the most damaging pests of greenhouse crops. Although the Maine greenhouse industry is small relative to other eastern states, there are many individual growers. The need to provide greenhouse growers with information about their industry and to inform them about pest identification, biology, and management is evident from this study. Updates on pest status and effective pest management options are required to supplement growers' knowledge about the management of *F. occidentalis* and INSV.

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Program Symposium
1996 Entomological Society of America Annual Meeting
Louisville, Kentucky

Biorational Approaches to Urban Pest Management

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Introduction to the Symposium Proceedings

Faith M. Oi and Nancy C. Hinkle

Chemicals are typically used as the first line of defense against insects in the urban environment because of their ease of use and ready accessibility. As evidence of the prevalence of pesticide use in or around the home, a 1976–1977 Epidemiologic Pesticide Study Center of Colorado State University survey found that 9 of 10 residents interviewed reported using pesticides in their house, garden, or yard. “Over three times as many householders use pesticides **in** (emphasis added) their houses as in their yards. Such widespread use of pesticides in the home environment is undoubtedly a significant source of exposure of the general population to pesticides” (NRC 1980).

Improper use of lawn care chemicals can contribute significantly to non-point source pollutant loadings (<http://www.epa.gov/OWOW/MMGI/Chapter4/ch4-6.html>), and to contamination of indoor home environments when pesticides are tracked in on shoes (Nishioka et al. 1996). Workers using typical homeowner equipment also received a measurable dose of pesticide when applying diazinon granules for Japanese beetle, *Popillia japonica* Newman, control (Weisskopf et al. 1988), and golfers are regularly exposed to pesticides on the golf course, although the risks could not be quantified (Bogert et al. 1994). Pesticide residues also may be transported indoors by pets or other items that move from the yard into the house. In summary, people may be exposed to pesticides in their homes, yards, or public areas (e.g., parks, restaurants, playgrounds, etc.).

However, left uncontrolled, these urban pests cause human and environmental risks. Wood-destroying organisms and lawn and ornamental pests can cause significant economic loss to property. Termites alone cost consumers more than \$1 billion per year for control and damage repair (Su & Scheffrahn 1990). Pests such as cockroaches, fleas, and fire ants can pose significant medical problems. Cockroaches can cause severe allergies, fleas can transmit tapeworms that can infest humans, and fire ant stings cause painful, itching pustules. In addition, allergic responses to

the sting can cause death from respiratory failure. Unlike agricultural systems where there may be a certain number of pests that are acceptable in crops, the threshold for pests in the urban environment is usually zero.

The alternative to using chemicals alone as the primary method of control is an integrated pest management (IPM) approach. IPM is not just "nonchemical" control: it is an active decision-making process that includes monitoring to determine the pest identity and determining if there really is a problem and its severity.

Urban IPM is bringing to bear all the appropriate methods to achieve pest suppression. In urban environments, methods include physical modifications to structures, cultural controls that are mainly related to sanitation, legislative methods that control who can do certain types of pest control, and chemical control. IPM requires knowledge of the pest biology and behavior to make the environment less attractive to the pest.

One IPM method that has received little attention in the area of urban pest management is biological control and the emerging use of biorationals. Like most terminology, "biorational" has evolved. In early works, the term "biorational" was used to describe "pheromones, insect growth regulators, and hormone antagonists" (Djerassi et al. 1974). For purposes of this Symposium, we have used the term "biorational" to include classical biological control agents; biologically based or naturally occurring active ingredients such as entomopathogenic fungi, bacteria, and viruses or their by-products; soaps and oils; and nonchemical approaches, in addition to the Djerassi definition. Admittedly, the lines between "hard" chemicals such as the organophosphates and pyrethroids and "soft" chemicals such as the insect growth regulators will blur with the new chemistries being developed.

The intent in developing this Symposium was to explore the potential for using biorationals in the urban environment and to answer the question: "Do biorationals have a place in urban pest management?" To answer this question, there are several characteristics of an urban environment that must be defined. First, a pest control operator's most difficult hurdle in any pest problem is dealing with people's fears. Pest control professionals find themselves dealing with a wide variety of people with a range of tolerances for pests and pesticides. They must deal with people who are highly entomophobic and people who are highly chemophobic. Often, people can be highly entomophobic and highly chemophobic and still demand pest control with a tolerance of zero insects. Most people lie somewhere in between.

Second, a pest control operator must deal with people's pest control "wants," but not necessarily "needs." Many people will accept the concept of biological control in agriculture in an effort to reduce pesticide use on their food. Biological control assumes that some portion of the pest population will be left to maintain the beneficial population. However, is there such a thing as an acceptable number of termites in a house? A pest control operator can help ensure that customer expectations are in line with what he or she is able to deliver by explaining the methods to be used. Sometimes the pest control operator will end up in court, regardless of efforts to explain the attempts made at pest control.

The third characteristic of doing pest management in the urban environment is the courtroom, when a client's fears are realized or needs not met. Lawsuits

can be filed for reasons ranging from actual, severe structural damage because of negligence on the part of the pest control operator, damage to structures in spite of the pest control operator's best efforts, chemical contamination of structures so they are not habitable (real and perceived), physical reactions due to chemicals (real or perceived), and punitive lawsuits for "mental distress" due to treatments correctly or incorrectly performed.

Urban pest management is driven by customer demand and legal restraints, much more so than in agricultural pest management. So why use biorationals in urban environments? One reason is that customers say that they want alternatives. Urban pest control methods are driven by consumer demand. In 1995, over 45% of residential and over 50% of commercial and industrial customers inquired about IPM techniques that included the use of biorationals (Guyette 1995). Additionally, the use of biorationals will reduce chemical exposure and environmental pesticide load; biological control systems, especially in landscape, may be self-sustaining, and the use of biorationals may minimize visits by the pest control operator and lead to less expense for the homeowner and pest control company.

Each of the nine symposium papers addresses three questions: (1) What are the characteristics in an urban environment that would support a biological/alternative control program for a pest/area? (2) What are the characteristics in an urban environment that would hinder a biological/alternative control program for a pest/area? and (3) How might efficacy be monitored so that pest control advisors could make future recommendations?

The authors would like to promote continued discussions of the following topics as they relate to biorational approaches to urban pest management: (1) Who will pay for the cost of research and development of biorationals? (2) How will biorationals be registered and regulated? (3) What is the potential incompatibility with other IPM components?

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Biocontrol Options for Urban Pest Ants¹

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ABSTRACT Several factors have led to increasing interest in biological control of urban ants but practical applications are limited. Some aspects of the urban environment that make it difficult for biological control to be adopted include: (1) a low pest threshold and tolerance for insects; (2) the lack of proven biocontrol agents; (3) the interactions between indoor and outdoor ant populations; and (4) the inability to formulate biocontrol agents into products suitable for the urban market. Parasites, predators, and pathogens are considered as agents in biological control programs and each group may be advantageous for certain situations. However, pathogens have the highest potential for use in urban environments because the microorganisms can be delivered in acceptable formulations for use in the urban environment. Pathogens offer a wide range of characteristics that make them suitable for biopesticide formulation and use in biological control programs. Parasitic organisms also may be useful in classical biological control of outdoor ants. Predators are not likely to be useful as introduced biocontrol agents, although they may be important in natural control of ants.

KEY WORDS Ants, Formicidae, urban pests, parasites, predators, pathogens, biological control, biopesticides, microbial control

In the urban environment, a number of ant species are known to invade homes and other human structures (Table 1) (Thompson 1990). These pest ants cause problems that vary from simple annoyance to the transmission of diseases and deaths from allergic reactions to stings (Adams 1986, Busvine 1980, Eichler 1990, Jemal & Hughes-Jones 1993). According to Whitmore et al. (1992), more than a third of the households in the United States are treated for ant problems. Ants other than fire ants and carpenter ants are major pests in 13% of the households in the United States. Fire ants are pests in 5.9% of households (Whitmore et al. 1992).

Ant control in agricultural and urban environments usually is accomplished with chemical pesticides. Biological control of ants has received relatively little attention.

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Table 1. Principal urban pest ant species in the United States.

Scientific Name	Common Name
<i>Camponotus</i> spp.	carpenter ant, wood ant
<i>Formica</i> spp.	black field ant
<i>Linepithema humile</i> Mayr (<i>Iridomyrmex humilis</i>) Mayr	Argentine ant
<i>Monomorium pharaonis</i> L. <i>Monomorium minimum</i> Buckley	Pharaoh ant little black ant
<i>Paratrechina longicornis</i> Emery <i>Paratrechina</i> spp.	crazy ant
<i>Pheidole megacephala</i> F.	big-headed ant
<i>Solenopsis invicta</i> Buren <i>Solenopsis xyloni</i> McCook <i>Solenopsis molesta</i> Say	red imported fire ant southern fire ant thief ant, sugar ant
<i>Tapinoma melanocephalum</i> F. <i>Tapinoma sessile</i> Say	blackheaded ant odorous house ant
<i>Tetramorium caespitum</i> L.	pavement ant

However, demand for biological control solutions to ant problems has been increasing in recent years. Three factors contributing to this increase are: (1) the desire for a decrease in chemical exposure; (2) the demand for alternative control tactics; and (3) the low efficacy of ant control products on the market.

Urban Environment and Biological Control

Several factors of the urban environment must be considered when evaluating biological control options (Table 2), many of which are related to humans or human perception of pests, their control, and possible biological control organisms.

Two of the most important factors to consider are the pest threshold and the tolerance for biocontrol agents. The threshold is the maximum number of pest insects that will be tolerated in the urban environment and this number is usually very low, especially indoors. Larger populations of pest ants can be tolerated outdoors, depending on the location and ant species present.

Table 2. Characteristics of the urban environment that influence the choice of biological control options for indoor and outdoor situations.

Characteristic	Indoor	Outdoor
Pest threshold	Low	Low (but higher than indoor)
Tolerance for biocontrol agent	Minimum	Some
Availability of biocontrol agents	Limited	Greater
Source of pest (nest)	Indoor or Outdoor	Outdoor
Structural damage	Possible	Not likely

Tolerance for insects, whether they are harmful, beneficial, or neutral, varies widely among humans. However, in urban environments, tolerance for the presence of insects is usually minimal. A "no-bugs-allowed" attitude is typical and can determine whether or not biocontrol organisms are used.

The lack of proven biological control agents for urban ants is the most important factor preventing large-scale use of this form of control. Although a few biocontrol agents have been tested outdoors, no serious attempts have been made to apply biocontrol indoors. Increased funding for research in this area is needed before commercial options become available to the public.

Another important factor to consider is the source of the pest. Indoor pest ants often originate from larger outdoor populations. Control of indoor ants can serve only as a quick remedy and will not solve the problem because reinfestations will occur. In these cases, source populations need to be controlled, or measures are necessary to prevent movement of ants from outdoors to inside structures. However, indoor ant nests may be established without connection to outside populations. These indoor ant nests may be difficult to locate, but application of biological control agents that reach the pest nest may be necessary to achieve the desired level of control.

Attractive baits are the preferred formulations for control of indoor and outdoor ants. Biological control agents that can be formulated or delivered in baits or bait stations will be more easily adopted by the public. Applications of biocontrol agents requiring more complex efforts by the applicator may require professional pest control operators.

Biological Control Options

Parasites, predators, and pathogens are used in biological control programs. Although competitors (and antagonists) can be important in the control of natural ant populations (Buren 1983, Stimac & Alves 1994), these organisms usually are not considered as agents in classical biological control programs

because of lack of specificity. Each of the biocontrol agents offers some advantages but certain ones may be better adapted to urban situations. However, with urban ant pests, some concerns with the use of the different agents must be addressed. Possible problems may be related to the nature of the biocontrol agent and others are related to human perceptions and possible restrictions of their use. By understanding possible problems, biocontrol agents with greater chances of successful acceptance by the public can be selected.

Parasites and Parasitoids

Little is known about parasites and parasitoids of ants and examples cited in the literature provide a mixture of beneficial and harmful effects in relation to human interests. For example, the straw itch mite, *Pyemotes tritici* LaGreze-Fossat & Montange, a parasite of the red imported fire ant, *Solenopsis invicta* Buren (Bruce & LeCato 1980), causes itching and other problems to humans and domestic animals. Other parasites, such as parasitic ants (Silveira-Guido et al. 1973) may have undesirable characteristics such as a wide host range that would prevent their use in urban biocontrol programs.

Advantages to using parasites and parasitoids. Natural occurrences of parasites and parasitoids serve as indicators that these organisms can be effective in classical biological control programs. Parasites and parasitoids are capable of searching for the host and can be released away from the ant pest population. Once established in the environment, control of the host can be continuous, requiring no more introductions.

Specificity is a very important advantage and most hymenopteran and dipteran parasites and parasitoids restrict their attack to a single species or a group of related species. This characteristic allows for use of these parasitic organisms without effects on nontarget ant populations. However, specificity also can be disadvantageous in urban situations where multiple ant species may occur, thus necessitating use of multiple control agents or tactics.

Possible problems with the use of parasites and parasitoids. Parasites and parasitoids often have complex ecological relationships with their hosts and other elements of the environment. This complexity complicates their use in urban environments. For many parasitic insects, the host is the substrate for the larval stage but the adult depends on nectar or other energy sources. A supply of food, resting places, and other resources may or may not be available in the urban landscape. A complex life cycle also complicates the production of large numbers of parasitic insects for augmentative releases.

The most commonly considered parasitoids are in the Diptera and Hymenoptera but other groups also may be important (Wojcik 1989). Because these are flying insects, their presence in households and other urban structures may not be desirable, despite any beneficial effects. Deliberate release of flying insects in houses may not be accepted by most of the public. Individuals with lower acceptance of insects or with mild to severe entomophobia may attempt to control beneficial insects as though they were pests. Also, behavioral changes in parasitized ants may cause them to become more apparent, increasing their level of annoyance. Furthermore, many parasites are unlikely to be able to survive or reproduce in indoor environments.

Because parasites and parasitoids of urban pests evolved in natural environments, the effect of the urban environment on their search behavior also must be considered. Parasitic insects use different cues in their search for hosts (Doutt et al. 1976). These cues can be associated with host characteristics (odor, shape, excrements) or with the host habitat (nest, damage to food substrate). In the urban environment, many of these cues may not be easily available for the parasitic organisms. Construction materials may alter visual and chemical cues, and search behavior of parasitic insects may be radically modified in urban settings. Parasitic species may not adapt to indoor urban environments.

Containment of parasitic insects also may be a problem in outdoor urban environments. Because ant control in the urban environment is not done on an areawide basis (as with mosquito control), control agents must be contained to small areas within owners' property boundaries. This creates the problem of containment when the released parasites move away from the treated property with consequent dilution of their effects on the pest ants at the release sites.

A serious problem with parasitic insects is the lag time between release of the biocontrol agent and the reduction of the pest population to acceptable levels. Parasitic insects may require a long period, sometimes years, to control the pest population. In the urban environment, property owners are not willing to wait long for their pest problems to be resolved.

The lack of specific candidates is perhaps the most serious problem preventing the use of parasitic organisms for biocontrol of ants. Few species have been identified attacking urban pest ants and detailed studies of these organisms have not been conducted in most cases. However, for the red imported fire ant, a serious search for biocontrol organisms has revealed some parasitic organisms (Jouvenaz et al. 1981, Jouvenaz 1983). Similar efforts for other pest ants may provide useful candidates for classical biocontrol agents for outdoors.

Types of parasites. Many types of parasites are known to affect ant colonies (Table 3). Social parasites are ants that take advantage of the colonies of other ant species for their survival and reproduction. Some live in permanent association with pest ants but may not serve as biocontrol agents because they do not reduce host populations significantly. Although some ants use host ant colonies temporarily and limit populations of pest ants, use of these social parasites as biocontrol agents is probably an undesirable solution because they do not eliminate pest ant colonies.

Ectoparasites are organisms that attach to the exterior of the ants and derive their nutrients from the ant host or host food. The presence of the parasite causes weakening of the host and may lead to host death and colony decline. Mites are the most common ectoparasitic organisms, although many mites found on ants have a phoretic or mutualistic relationship with the host (Hölldobler & Wilson 1990).

Endoparasites complete their development inside the body cavity of ants. Several species of flies and wasps have been identified parasitizing ants (Williams & Whitcomb 1974, Williams et al. 1973, Waller & Moser 1990, Feener 1981, Disney 1980). Several fly species are being considered for introduction in the United States as biocontrol agents for fire ants (Porter et al. 1995). Although these parasitoids can cause reduction of ant populations in the

Table 3. Types of parasites associated with ants.**Social parasites**

Temporary parasitism

Slave-making (dulosis)

Permanent parasitism (inquilinism)

Solenopsis (Labauchena) daguerrei (Santschi)**Ectoparasites**Straw itch mite (*Pyemotes tritici* LaGreze-Fossat & Montagne)**Endoparasites**Phorid flies (*Pseudacteum* supp.)Eucharitidae wasps (*Orasema* spp.)**Myrmecophiles**

field, their effects on ant populations in urban situations will depend on the suitability of urban microclimates for the parasitoids.

Many insects and other arthropods are known to occur in ant nests and are referred to as myrmecophiles (Hölldobler & Wilson 1990). Among these organisms, many potential parasites may occur. The biology of these myrmecophiles has not been studied in detail so the nature of their relationships with the ant host is not well understood. Some of these myrmecophiles are known to have detrimental effects on ant colonies (Wojcik 1975, Hölldobler & Wilson 1990), and detailed studies may reveal the presence of potential biocontrol agents.

Predators

Predators are multicellular organisms, usually insects or other arthropods, that consume insects as the principle part of their diet. The use of predators such as lady beetles and lacewings is well established for control of garden and agricultural pests. Predators of ants, especially newly mated queens, are reported in the scientific literature (Whitcomb et al. 1973, Nichols & Sites 1991), but few are specialized predators of ants. Although predation may be very important in reducing numbers of newly mated queens during establishment of new colonies, the high reproductive capacity of ant species compensates for mortality of reproductives.

Predators are usually much larger than their prey and therefore larger than the parasitic insects. Ant predators vary from small insects (other ants and beetles) to larger animals such as spiders, birds, and mammals. The sizes of these organisms make them undesirable for use within houses and other buildings.

Because of factors already mentioned above in relation to parasitic insects, problems with containment, searching behavior, and lag time also must be considered for predators. Other advantages and disadvantages of predators usually coincide with those cited for parasites and parasitoids. However, the biggest problem with predators is that they are usually less specific and their use in biocontrol programs must be considered in relation to possible effects on other beneficial organisms. For example, introduction of generalist ant species as suggested by Buren (1983) could be dangerous because it would be virtually impossible to guarantee that the introduced species would not become pest problems themselves. Although the use of native generalist predators as augmentative biological control agents may be possible, their deliberate use in urban environments is not likely. As with parasitic insects, problems with nuisance and entomophobia may prevent widespread use of predators for ant control in urban environments, especially indoors.

Pathogens

Pathogens are microorganisms that cause diseases in ants. The pathogens used in biological control do not cause harm to humans, pets, and other higher animals, except possibly allergic reactions. These pathogens are increasingly being considered for control of agricultural and urban pests, including ants (Jouvenaz 1986). Great variability exists in the effects of pathogens for insect populations. Some cause chronic diseases not leading to immediate mortality but weaken the individual and the ant colony. Other pathogens cause acute infections and mortality of insects within a few days. Both types of pathogens have been proposed as microbial control agents for different pest populations but limited studies have been conducted with urban pest ants.

Laboratory and field tests have demonstrated the potential of fungal pathogens for control of fire ants (Broome 1974, Stimac et al. 1987, Pereira et al. 1993, Oi et al. 1994). *Beauveria bassiana* (Balsamo) Vuillemin also has been tested as the active ingredient in attractive baits against several urban pest ants, including fire ants, carpenter ants, and Pharaoh ants (Pereira & Stimac, unpublished data). Results demonstrated that this fungus can be as effective as commercial chemical baits against some ant species. Other fungal species and other types of pathogens could be developed.

Advantages to using pathogens. Because the different groups of pathogens have different characteristics, advantages of pathogens may vary from one group to the other. Many pathogens have been identified as candidate biological control agents (Table 4). These offer a wide choice of characteristics, some of which may be more adequate for urban environments than others.

In contrast with the parasites and predators, pathogens cannot move actively and can be contained relatively easily, preventing problems with interference in human activities. Because many pathogens can be applied as spores that infect the hosts, no other substrate is needed for nutrition and growth. Also, the biology and ecology of many pathogens are relatively simple and they are faster acting than parasites and parasitoids. Quick-acting, nonspecific pathogens are the principal organisms being considered as microbial control agents for ants. However, greater specificity of other

Table 4. Pathogens and other microorganisms associated with ants.

Pathogen	Host	References
Bacteria		
1) Nonpathological associations		
Bacteria	Attine ants	Scheld et al. 1971
Bacteria	<i>Atta laevigata</i>	Tauk & Serzedello 1975
2) Pathogens isolated from ants		
<i>Bacillus finitimus</i>	<i>Solenopsis</i> spp.	Jouvenaz et al. 1980
<i>Serratia</i> spp.	<i>Solenopsis</i> spp.	Miller & Brown 1983
<i>Enterobacter</i> spp.	<i>Solenopsis</i> spp.	Miller & Brown 1983
<i>Pseudomonas aeruginosa</i>	<i>Solenopsis</i> spp.	Miller & Brown 1983
<i>P. chloroaphis</i>	<i>S. invicta</i>	Jouvenaz 1990
3) Pathogens of other insects		
<i>B. thuringiensis</i>	<i>S. invicta</i>	Mycogen ^a
<i>B. sphaericus</i>	<i>S. invicta</i>	Jouvenaz 1990
<i>B. larvae</i>	<i>S. invicta</i>	Jouvenaz 1990
<i>B. pulvificiens</i>	<i>S. invicta</i>	Jouvenaz 1990
Protozoa		
<i>Thelohania solenopsae</i>	<i>Solenopsis</i> spp.	Knell et al. 1977
<i>Mattesia geminatae</i>	<i>Solenopsis</i> spp.	Jouvenaz & Anthony 1979
<i>Vairimorpha invictae</i>	<i>Solenopsis</i> spp.	Jouvenaz & Ellis 1986
<i>V. "undeeni"</i>	<i>S. geminata</i>	Jouvenaz 1986
<i>Burenella dimorpha</i>	<i>Solenopsis</i> spp.	Jouvenaz & Hazard 1978
<i>Nosema</i> sp.	<i>S. invicta</i>	Jouvenaz 1990
Viruses		
Virus (?)	<i>Solenopsis</i> spp.	Avery et al. 1977
Iridescent (?) virus	<i>Formica lugubris</i>	Bailey 1973
Nematodes		
10 nematode species (+ several unidentified specimens) in 20 genera and 58 species of ants		Poinar 1975, Bedding 1984
Mermithid	<i>S. geminata</i>	Mitchell & Jouvenaz 1985
Mermithid	<i>S. richteri</i>	Jouvenaz & Wojcik 1990
<i>Tetradonema solenopsis</i>	<i>Solenopsis</i> spp.	Nickle & Jouvenaz 1987
<i>Aphelenchoides composticola</i>	<i>Acromyrmex octospinosus</i>	Kermarrec et al. 1986
<i>Caenorhabditis dolichura</i>	<i>Iridomyrmex humilis</i>	Markin & McCoy 1968
<i>Rabbiun paradoxus</i>	<i>Camponotus castaneus</i>	Bedding 1984
<i>Rhabditis</i> spp.	<i>Ac. octospinosus</i>	Kermarrec et al. 1986
<i>Steinernema carpocapsae</i>	Several	Kermarrec et al. 1986 Laumond et al. 1979
Fungi		
1) Ectoparasites		
<i>Rickia wasmanii</i>	<i>Myrmica</i> spp.	Espadaler & Suñer 1989
<i>Aegeritella</i> spp.	Several	Espadaler & Wisniewski 1987 Espadaler & Roig 1993 Espadaler & Suñer 1989 Balazy et al. 1986 Balazy et al. 1990 Blachwell et al. 1980
<i>Termitariopsis cavernosa</i>		

Table 4. Continued.

Pathogen	Host	References
2) Endoparasites		
<i>Akanthomyces gracilis</i>	Several	Evans 1974
<i>Alternaria</i> sp.	<i>Formica rufa</i>	Marikovskiy 1962
<i>Aphanocladium album</i>	<i>Anoplolepis longipes</i>	Humber 1992
<i>Aspergillus</i> spp.	Several	Sikorowski et al. 1973
<i>Beauveria</i> spp.	Several	Stimac et al. 1987 Alves et al. 1988 Kermarrec & Decharme 1982
<i>Conidiobolus</i> sp.	<i>S. invicta</i>	Sanchez-Pena & Thorvilson 1992
<i>Cordyceps</i> spp.	Several	Evans & Samson 1982, 1984 Evans 1974, 1982 Hywel-Jones 1996
<i>Desmidiospora myrmecophila</i>	<i>Camponotus</i> spp.	Evans & Samson 1984
<i>Erynia</i> spp. (<i>Pandora</i> spp., <i>Entomophthora</i> spp.)	Several	Balazy & Sokolowski 1977 Loos-Frank & Zimmermann 1976 Turian & Wuest 1969 Evans 1989 Kermarrec & Mauleon 1975
<i>Gliocladium</i> spp.	<i>Macromischoides inermis</i>	Evans 1974
<i>Hirsutella</i> spp. (<i>Cordyceps</i>)	Several	Evans & Samson 1982
<i>Hymenostilbe</i> spp. (<i>Cordyceps</i>)	Several	Evens 1974 Evans & Samson 1984
<i>Metarhizium anisopliae</i>	<i>S. invicta</i>	Allen & Buren 1974 Stimac et al. 1987 Alves et al. 1988 Kermarrec & Decharme 1982
<i>Myrmecomycetes annellisae</i>	<i>Solenopsis</i> spp.	Sanchez-Peña & Thorvilson 1992
<i>Myrmecinosporidium durum</i>	Several	Jouvenaz & Kimbrough 1991
<i>Paecilomyces</i> spp.	Several	Sanchez-Peña et al. 1993 Stimac et al. 1987 Alves et al. 1988 Kermarrec & Decharme 1982
<i>Pseudogibellula formicarum</i>	Several	Evans 1974
<i>Sporothrix</i> spp.	Several	Evans 1974
<i>Stilbella burmensis</i>	Several	Evans 1989 Evans & Samson 1984
<i>Stilbum</i> spp.	Several	Evans 1974
<i>Tarichium</i> sp.	<i>Tetramorium caespitum</i>	Marikovskiy 1962
<i>Tilachlidium</i> sp.	Several	Evans 1974
<i>Torrubiella</i> spp.	<i>Paltothyreus tarsatus</i>	Evans 1974
<i>Verticillium nodulosum</i>	Several	Evans 1974

^aAnnouncement by this company that it had a bacterial isolate effective against fire ants.

pathogens makes them potentially very useful for field application with little or no threat to nontarget species.

Possible problems with the use of pathogens. A certain degree of microbiophobia is prevalent among humans, and the addition of microbes to what is perceived to be a sterile environment in households may cause concern. Educational programs may be necessary to emphasize the safety of microbial agents for control of urban pests. Also, some fungi considered for control of ants can cause undesirable allergic reactions in sensitive individuals when excessive exposure occurs. This must be addressed during the product development phase and during the registration process. The fungal pathogen *Metarhizium anisopliae* (Metschnikoff) Sorokin received approval for use in traps for cockroach control in the household (Stix 1994). During the process of registration by the United States Environmental Protection Agency, possible health effects on humans were addressed and resolved. Similar concerns can be expected to be raised with other pathogens proposed as control agents for ants but resolutions of these concerns should not prevent use of these pathogens in the urban environment.

Certain characteristics of the pathogens, such as humidity requirements, determine the need for environmental conditions under which these organisms can survive and function. Conditions such as temperature and humidity can affect the performance of the pathogens after application. Also, these conditions may be important during storage of the products formulated with pathogens, affecting their shelf life. Requirements for specific environmental conditions in storage and after application may be a hindrance for the commercialization and adoption of some microbial control agents. Characteristics of the formulations containing ant pathogens also affect dose and delivery of the pathogens to the targets. Baits, used for formulation of the fungus *B. bassiana* for indoor and outdoor ant control, must be prepared so as to provide good survival conditions for the fungus and be attractive to different ant species. Other formulations also must address similar concerns in relation to pathogen survival and delivery.

Several factors affect the presence and efficacy of pathogens in populations of social insects. The most important factors are related to: (1) the genetics of the social insect populations (Sherman et al. 1988), (2) social behavior (Oi & Pereira 1993), and (3) the nest environment. Genetic variability affects the susceptibility of ants to diseases. Ant social behaviors, such as nest hygiene, brood care, and grooming can eliminate pathogenic infective units or assist with the spread of disease organisms within and between ant nests. The nest environment provides pathogen exposure to high host densities, favorable temperatures and humidities, and protection from harmful UV radiation. However, secretions produced by ants and ant symbionts present in the nest environment can be detrimental to microorganisms (Jouvenaz et al. 1972, Schildknecht & Koob 1971, Storey et al. 1991). Thus, selected biocontrol agents must be adapted for maximum survival and development under conditions in ant nests.

Another important factor in determining the susceptibility of ants to pathogens is the structure of the digestive tract, and the type of diets ingested by the different developmental stages. Adult fire ants have complex filtering

structures that prevent ingestion of solid particles. This filtering can prevent the ingestion of pathogens that are transmitted *per os*, but larval stages can ingest solid particles and be infected with larger pathogens.

Types of Pathogens

Bacteria. Very little is known about bacterial diseases of ants and their occurrence is not even mentioned in comprehensive works about ants such as Hölldobler & Wilson (1990). However, in other Hymenoptera such as honey bees, bacteria can be important population control agents.

Bacteria are generally found as larval pathogens and this may account for the lack of information on possible bacterial diseases of ants. Because most of the observations and collections concentrate on adults, the importance of bacteria infecting larval stages may not be recognized. The lack of specific candidates is the major obstacle to the use of bacteria for control of ants. As microbial control agents, bacteria are advantageous due to ease of production and the consequent low cost of material with biological activity. Also, because bacteria usually infect through the digestive system, these pathogens can be incorporated into attractive baits for ants without filtering capabilities.

Reports of bacteria associated with ants can be divided into three classes: (1) nonpathogenic associations with ecological adaptation of the bacteria to the nest environment, (2) true pathogens found infecting ants, and (3) pathogens of other insects tested against ants (Table 4). The first group may serve as carriers or vectors for toxins or other control agents into the nest. Bacterial pathogens of other insects have been tested without much success (Jouvenaz et al. 1980, Miller & Brown 1983, Jouvenaz 1990). Although bacteria isolated from non-ant hosts can cause a low level of mortality, the bacterial pathogens isolated directly from ants hold the highest potential for use in microbial control. Further survey studies are needed to identify other bacterial pathogens.

Protozoa. Because of their abundance and large size, protozoa are relatively easy to find in adults and brood of *Solenopsis* species but these pathogens have not been identified from other ant species. Some protozoa are important in the natural regulation of ant populations (Briano et al. 1995) and have been considered for classical biological control of fire ants (Patterson 1994).

Protozoan pathogens are generally highly specific and, therefore, are good candidates for introduction in natural areas. However, their complex biology, including the requirement for an intermediate or alternate host in some cases, can complicate the use of protozoa in control programs. Also, because protozoa require a living host, production of large quantities of the pathogens would be difficult.

Like bacteria, protozoa infect insects *per os* and therefore could be added to attractive baits. However, the large size of spores makes these pathogens more prone to filtering in the buccal cavity of ant adults. Some protozoan pathogens also can be transferred transovarially and infection of nest queens could be important in the spread of protozoans in ant populations.

Viruses. Very little is known about viruses in ants, and only two reports on virus-like particles from ants have been published (Avery et al. 1977, Bailey

1973). Because of their minute size, viruses are probably overlooked even when high mortality is observed in ant colonies. The lack of candidates prevents studies on possible use of viruses for control of ants, although several viruses are known from other Hymenoptera (Bailey 1973).

Viruses can infect both *per os* and by transovarial transmission and therefore these pathogens could be easily disseminated in ant populations. However, difficulties in the production, because of the living host requirement, may prevent the use of viruses for ant control.

Nematodes. Nematodes are commonly reported from ants (Poinar 1975, Bedding 1984), perhaps due to their large size and obvious deformations to the host body. Although nematodes can move on their own and actively seek hosts as parasites and predators do, they may not move fast enough to infect ants. Although baits may facilitate application of nematodes for ant control, problems can arise if the nematodes escape from the attractive baits and miss the opportunity to encounter hosts.

Some entomopathogenic nematode species are produced in large-capacity fermenters at relatively low cost. However, most nematode species are produced in living hosts, a very expensive and complex system. Besides production problems, use of nematodes also may be inefficient due to ant behavior. Because of their large size, nematodes can be readily recognized and removed by the ants during grooming. This recognition also may trigger ant behavioral changes that lead to nest movement or other activities limiting efficacy of the nematodes (Drees et al. 1992).

Except for *Steinernema* spp., no other nematode is used extensively as a commercial biopesticide. Although several nematodes species have been described to infect ants, most of these species would probably have no potential as a commercial product or classical biological control agent.

Fungi. Fungi are the most frequently reported pathogens in ant populations (Table 4) and the fungus *B. bassiana* has been described infecting a 25-million-yr-old ant in amber (Poinar & Thomas 1984). This high frequency of reports does not necessarily mean that these are the most important natural pathogens (Oi & Pereira 1993). Other reasons may account for recognition of fungi as pathogens of ants, such as large and peculiar fungal structures and changes in ant behavior. Infected ants may change their foraging time (Poinar et al. 1989) or move into areas normally not visited (Marikovskiy 1962, Evans 1982).

Because of the many examples of natural fungal epizootics in ant populations (Evans 1974, 1982, 1989; Evans & Samson 1982, 1984), fungi are probably excellent candidates for biological control of ants. Natural epizootics demonstrate that these pathogens can spread through nests affecting large numbers of individuals. This characteristic, as well as ease of production, may justify emphasis on fungi for control of ants.

Unlike other pathogens, fungal infection occurs mainly through the host cuticle and fungal spores must contact the exterior of the insect body to cause the disease. This requirement for cuticular penetration is disadvantageous when attractive baits are used. Baits that are ingested without much contact with the exterior of the ant body are not effective. Formulations used with most fungi must promote contact between fungal spores and the ant cuticle.

This contact can be accomplished with dry, powdery baits that are attractive but difficult to transport by the ant, causing an increase in the contact between the pathogens and the ant body with consequent infection.

Among the ant-infecting fungi, some can be classified as ectoparasitic; that is, their entire life cycle occurs on the external surface of the ant cuticle. These species usually cause minimum interference with ant biology and most likely will never be important as biocontrol agents. Other fungi penetrate the insect hemocoel and are classified as endoparasites. Some cause benign infections but other species kill the ant host. Among the endoparasites are various fungi that are currently used or being considered for control of insects. These species (e.g., *B. bassiana* and *M. anisopliae*) and possibly others have great potential for development into commercial biological formicides (Stimac et al. 1987, Stimac & Alves 1994, Alves et al. 1988, Pereira et al. 1993, Oi et al. 1994).

Conclusions and Future Research Needs

Several options are available for biological control of some urban pest ant species such as fire ants, carpenter ants, and Pharaoh ants. Many other organisms await identification as potential candidates and much research is needed. However, due to the characteristics of the urban environment and the control measure requirements dictated by the public, research on pathogens as biocontrol agents is more likely to produce practical results. Pathogens can be applied both indoors and outdoors with little interference with the public. Thus, the presence of the biocontrol agent will not be an annoyance to users.

Some pathogens have been formulated as biopesticides to be commercialized through traditional marketing structures used for chemical pesticides. Pathogens could be incorporated into different formulations, including bait stations, normally used in households. Other possible formulations can be restricted for application by pest control operators. This will facilitate the adoption of these agents for control of ants, as it can serve the different levels of the market. Also, application by pest control operators allows use of pathogens that require special handling before or during application.

Parasitic organisms may be useful for control of ants in outdoor situations. Most of this use would be as classical biological control agents with introduction of the parasite or parasitoid for areawide control of source populations. This control strategy may facilitate control of introduced pests such as the red imported fire ant that moved into the United States without natural enemies (Porter et al. 1997). Other introduced outdoor ant pest species in different regions of the globe could be controlled in a similar fashion.

Biological control of larval stages is an important mortality factor in populations of insects. Ants spend a great deal of energy in brood tending so there may be effective agents that could reduce larval populations and reproductive potential of ant colonies. Few examples of biocontrol agents attacking ant larval stages are known. Studies are needed to identify pathogens and other biocontrol agents attacking the larvae and pupae of different ant species.

Because social behavior is important in the survival and success of ant colonies, research also is needed on the possible interactions between social

behavior and the effects of biocontrol agents. This information also can be helpful in the design of control strategies, and ant behavior may serve as a tool in the efficient delivery of pathogens and parasites.

As new biocontrol agents are identified and developed into products, and the demand for their use is intensified, biological control will become an integral part of ant control programs. The quality of human life in urban environments will increase with the use of biological control against urban ants and other insect pests.

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Alternative Control Strategies for Ants Around Homes¹

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ABSTRACT Recent research in urban ant control provides some new opportunities for developing alternative pest management strategies. Current control of urban pest ants relies heavily on broadcast spraying of residual insecticides. However, recent findings suggest that a combination of ant repellents, baits, and exclusion techniques may be equally effective and offer a safer alternative. Low-toxicity pesticides and nonchemical methods would significantly reduce the risk of exposure to pesticides, as well as serve as a model for ant control based on an environmentally safe approach.

KEY WORDS Ant repellents, ant baits, IGRs, boric acid, nonchemical pest control

These are challenging times for research and development of innovative techniques in the management of household pests, particularly with regard to strategies that reduce risk of pesticide exposure. A trend toward the use of low-toxicity pest control continues into the 21st century. Consequently, we are looking at more judicious use of pesticides in the future through precise, target-specific treatments. In this spirit of low-toxicity pest control, we address some breakthroughs in ant control research that may offer alternative strategies for controlling ants around homes.

In a recent national survey in the United States, ants were considered by homeowners to be a more serious household pest than cockroaches (Whitmore et al. 1992). To control ants, many pest control operators rely heavily on insecticide sprays (Klotz et al. 1995). For example, barrier sprays or perimeter treatments have been and are currently a common method of ant control (Haack & Granovsky 1990). As a result, as much as 10 gallons of insecticide solution is sometimes applied per 1,000 ft² around a structure to prevent the entry of ants. Such a strategy does not eliminate or suppress ants but is intended to form a barrier to prevent access into a structure. Achieving this end is difficult because any small gaps or breaks in the barrier are potential passageways. In addition, perimeter sprays are usually broad-spectrum

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insecticides that kill many of the beneficial insects present around homes and may result in secondary pest outbreaks (Smith et al. 1996).

In contrast to the traditional approach of using sprays, there is a resurgence in the use of baits for controlling ants. Baits are fairly target specific and use less insecticide. In comparison with chemical barriers, baits offer distinct advantages. First, very little insecticide is required, and the toxicant can be contained within a bait station. Consequently, baits are safer for the environment than a chemical barrier. Second, baits eliminate the necessity of finding the nest, usually a labor-intensive procedure because of the cryptic nesting habits of many pest ants. And third, baits capitalize on the social behavior of ants, whereby scout ants recruit nestmates to a newly discovered bait, and these recruited ants return to a nest to share the bait with the rest of the colony.

The objective of this paper is to summarize our research results on barrier treatments, bait development, and nonchemical techniques in light of their potential use as alternative strategies for ant control. First, we address a possible alternative to broad-spectrum insecticides—using ant repellents formulated for barrier or perimeter sprays. Second, we describe our research on low-toxicity bait toxicants for ant control. And third, we briefly discuss some nonchemical methods for ant control, such as exclusion. Although labor-intensive, we believe these nonchemical techniques could be as effective as chemical control.

Ant repellents. Chemical signals dominate the communication systems of social insects such as ants (Wilson 1971). For example, the Argentine ant, *Linepithema humile* (Mayr) (= *Iridomyrmex humilis*), produces a trail pheromone from the sternal gland (Wilson & Pavan 1959). Over evolutionary time, ants, other insects, and plants have developed chemical methods of defending themselves against ant attack (Hölldobler & Wilson 1990). We may be able to exploit these same chemical defenses in management of pest ants. Progress in the use of repellents in pest ant management has gone farthest in agriculture, where research has focused primarily on plant protection (Shorey et al. 1992, 1993, 1996). For example, repellents applied in a band around the trunks of citrus trees can prevent Argentine ants from tending honeydew-producing homopterans. Candidate chemicals are placed on cotton twine or rubber tubing and then wrapped around tree trunks; the number of ants crossing this potential barrier is counted to evaluate a compound's repellency (Shorey et al. 1992). In Table 1 the efficacy of the repellent farnesol with the carrier Stickem is evaluated against a Lorsban (chlorpyrifos) spray. Farnesol-Stickem remains effective as a barrier for 5 mo. Other carriers for repellents have been investigated, but the best so far has been Stickem Special (Seabright, Emeryville, California), which shows some repellency of its own (Table 1) (Shorey et al., unpublished data).

To identify potential repellents, food sources may be set out in a laboratory foraging arena and surrounded by each chemical compound to be evaluated as barriers to foraging ants (Shorey et al. 1996). In this type of assay, farnesol provides an effective barrier against Argentine ants.

Repellents may have a place in urban settings for management of ants in orchard or shade trees around homes, and in sensitive areas such as hospitals,

Table 1. Mean number^a (95% confidence interval) of Argentine ants per minute climbing lemon trees in Ventura County, California (1994).

Treatment ^b	April	May	July	Sept.
Fransol-Stickem band	0	0	1 (0-3)	0
Stickem-only band	1 (0-3)	6 (2-14)	5 (2-12)	2 (0-8)
Lorsban-boom (broadcast spray)	1 (0-3)	1 (0-3)	8 (2-22)	4 (1-13)
Lorsban-back pack (trunk + 2-m radius on soil surface spray)	0	0	1 (0-2)	6 (1-21)
Untreated	21 (7-57)	23 (11-45)	5 (2-12)	59 (52-67)

^aOriginal data were transformed to $\ln(x+1)$ for analysis and determination of 95% confidence intervals. Numbers presented here are transformed back to the original scale.

^bSix replicates, 25 trees/plot (=0.10 ha each). Each tree observed for 2 min at each sampling date, counting numbers of ants proceeding both up and down. Data from Shorey, H. H., R. G. Gerber & P. A. Phillips, unpublished.

animal rearing facilities, computer equipment, vending machines, and food processing and storage facilities. If repellents could be formulated in sprays for perimeter treatments, they would be an ideal way to pestproof homes prone to ant infestation. Or, used in concert with baits, repellents might alleviate the immediate problem of ants entering a house while allowing time for slower-acting baits to take effect on the outside.

Low-toxicity baits—Insect growth regulators (IGRs) and boric acid

A bait consists of four components (Cherrett & Lewis 1974): an attractant, usually a food or pheromone that enhances the probability that the bait will be accepted and readily picked up (Peregrine 1973); a palatable carrier that gives the physical structure or matrix to the bait; a toxicant that should be nonrepellent and delayed in action, effective over at least a 10-fold dosage range (Stringer et al. 1964); and other materials added for reasons of formulation, such as emulsifiers or antimicrobial agents. Each of these components must be developed and tested in combination for efficacy.

This manuscript focuses on the toxicant component of a bait and discusses low-toxicity alternatives for controlling ants by examining two compounds that have potential in this respect.

Insect growth regulators. Juvenile hormone analogs (juvenoids) and chitin synthesis inhibitors have low mammalian toxicity (Staal 1975) and have been used effectively in ant control. Their current use is focused primarily on control of the red imported fire ant, *Solenopsis invicta* Buren. The use of IGRs

should be expanded to include other urban pest ants, such as has been done with methoprene against Pharaoh ants, *Monomorium pharaonis* (L.) (Edwards 1986). Methoprene was available for commercial use for Pharaoh ant control but is no longer marketed.

Contrary to popular belief, IGRs are just as effective as metabolic inhibitors (Williams 1994). In field tests with fire ants, the juvenoids fenoxycarb and pyriproxyfen and the chitin synthesis inhibitor teflubenzuron were compared with the metabolic inhibitors hydramethylnon and sulfluramid, and the reduction in the population index after 6–10 wk was similar for all five compounds (Table 2).

More recent work in the laboratory has shown similar success with IGRs for control of Pharaoh ants (Williams & Vail 1993, Vail & Williams 1995). Large colonies were exposed to 0.5% fenoxycarb, methoprene, or pyriproxyfen. In comparison with the control, all of these IGRs had a significant impact on worker numbers. As in the field studies with fire ants, after 6 wk the colonies exposed to fenoxycarb and pyriproxyfen showed significant reduction in worker numbers. Methoprene took a little longer to show effects.

In field tests with concentrations of pyriproxyfen (0.25%, 0.5%, and 1.0%) at housing complexes infested with Pharaoh ants, significant reductions in foraging ants were attained (Vail et al. 1996). All concentrations reduced the foraging population almost to zero after 12 wk. In fact, the 0.25% pyriproxyfen treatment reduced foraging population 85.5% by 2.5 wk after the second treatment.

Insect growth regulators affect various stages in an ant's life cycle (Banks 1990). In the case of pyriproxyfen against Pharaoh ants, it interferes with larval and pupal development, the reproductive physiology of the queen, and has a possible toxic effect on adult ants (Vail & Williams 1995).

Insect growth regulators could be important tools in an urban pest management strategy: used first in a baiting program to penetrate the entire colony and then followed by faster-acting insecticides or baits to reduce the immediate problem of foraging ants (Vail et al. 1996).

David H. Oi (Auburn University, personal communication) discovered in laboratory tests that metabolic inhibitors were not spread as widely into a population as IGRs. In controlling a Pharaoh ant colony with multiple nests, a bait with a metabolic inhibitor usually affects only the nests closest to the bait, whereas a bait with an IGR eventually eliminated all of the nests.

Boric acid. Boric acid is another bait toxicant that, at low doses, has low mammalian toxicity (Quarles 1992) and exhibits delayed action (Klotz & Moss 1996). Boric acid has been used in ant baits for over 100 yr (Quarles 1993, Riley 1889, Rust 1986), but our research indicates that it has probably been misused (Klotz et al. 1997). For example, doses of $\geq 5\%$ boric acid are common in currently used ant baits. Our research has shown that at low doses ($\leq 1\%$), boric acid shows delayed toxicity (Table 3) whereas higher doses ($\geq 5\%$) are faster acting but not as readily consumed (Fig. 1). Here is a classic case of "less is better" in pest control.

Baits shift the emphasis in pest control from a treatment strategy based on applying gallons of pesticide solutions to make an impervious barrier to a strategy that exploits the social behavior of the insect to effect control.

Table 2. Field efficacy of bait toxicants against *Solenopsis invicta*.

Chemical ^a	Mode of action ^b	Active ingredient (g/ha)	% Reduction in population index after weeks indicated ^c	
			6-10	>20
Fenoxycarb	IGR	6.2-25.1	94	92
Hydramethylnon	MI	4.2-10.4	86	79
Pyriproxyfen	IGR	5.3-24.5	83	87
Sulfuramid	MI	6.7-10.1	93	79
Teflubenzuron	IGR	0.051-0.2	77	- ^d

^aAll baits were formulated in a soybean oil-pregel defatted corn grit mixture containing the active ingredient.

^bMI, metabolic inhibitor; IGR, insect growth regulator.

^cSee Lofgren & Williams (1982) for an explanation of the method of determining the population index. Percentages are means of the population index.

^dData not recorded for this period. Data from D. F. Williams (1994).

However, a control program for household pests should not rely on only one strategy such as baits but also should be supplemented with nonchemical techniques.

An effective nonchemical technique for ant control is pest-proofing or exclusion, i.e., finding out where the ants are getting into a structure and then sealing that point of entry. In their natural habitat, foraging ants prefer to follow preexisting edges and other structural features in the environment to and from their nest (Klotz & Reid 1992). This natural behavior predisposes ants to travel along the structural guidelines that are provided in the urban environment, such as wires, pipes, and conduits. Therefore, an effective strategy for preventing ant entry should include pest-proofing the points where utility lines enter a structure.

Guideline orientation also has important implications for chemical control strategies. Insecticides should be applied along guidelines to optimize their efficacy as well as to reduce the amount used. Dust is an excellent formulation for this purpose. Ants readily pick up dusts that are lightly applied to their trailways. Ideally, dusts should be applied during construction when there is easy access to wall voids. They also can be applied for remedial treatment by using a power duster (Hansen & Akre 1993). In this technique, all of the switch plates within a structure are removed and dust is then injected behind the switch boxes with a power duster to spread both laterally and vertically in the wall voids. This procedure thoroughly treats all the utility lines in the void space that the ants could use as runways.

Table 3. The LT_{50} s of *Camponotus floridanus* (Buckley) workers fed boric acid bait.

Conc. molar (%)	LT_{50} (95% CL) days	Slope \pm SE	No. of ants	Chi square	<i>P</i>
0.02 (0.13)	9.7 (8.1–13.3)	2.87 (\pm 0.47)	110	7.3	0.06
0.04 (0.25)	4.5 (4.3–4.8)	5.26 (\pm 0.38)	110	9.7	0.08
0.06 (0.38)	5.1 (4.8–5.5)	3.90 (\pm 0.32)	110	7.5	0.19
0.08 (0.50)	4.2 (3.9–4.5)	3.80 (\pm 0.28)	110	10.8	0.06
0.10 (0.63)	3.1 (2.8–3.3)	3.54 (\pm 0.24)	110	8.3	0.08
0.15 (0.94)	3.3 (3.0–3.5)	3.62 (\pm 0.30)	70	7.1	0.21
0.20 (1.25)	2.8 (2.5–3.0)	2.71 (\pm 0.20)	110	7.5	0.18
0.25 (1.56)	2.5 (2.3–2.7)	3.62 (\pm 0.27)	80	3.2	0.67
0.30 (1.88)	3.1 (2.8–3.4)	2.67 (\pm 0.22)	90	0.7	0.98
0.35 (2.19)	2.4 (2.1–2.6)	3.21 (\pm 0.25)	80	10.0	0.08
0.50 (3.13)	1.5 (1.2–1.7)	2.83 (\pm 0.31)	60	2.4	0.67

Data from Klotz & Moss (1996).

Future development of alternative strategies for household ant control depends on the commitment of both industry and academia to support research on innovative techniques that would significantly reduce the risk of exposure to pesticides and offer an environmentally safe alternative for ant control.

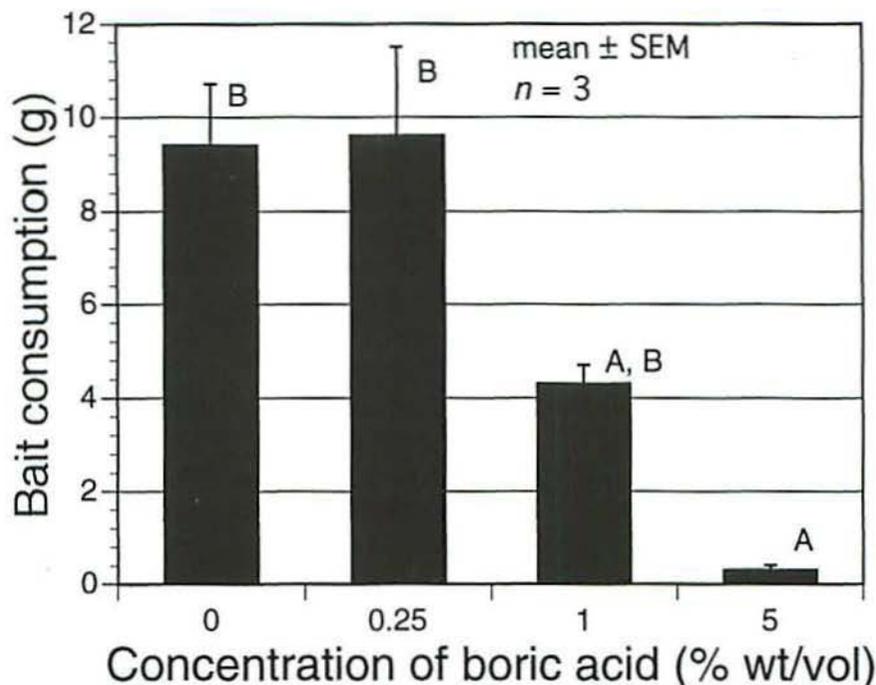


Fig. 1. Consumption of boric acid in 10% sucrose-water solutions by *S. invicta* colonies in 24 h. Consumption was significantly different by ANOVA ($F = 14.7$; $df = 3, 8$; $P = 0.0013$) for the various concentrations of boric acid. Means followed by the same letter are not significantly different ($P \leq 0.05$; Scheffe's F test). Data from Klotz et al. (1997).

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Biological Suppression of Synanthropic Cockroaches¹

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ABSTRACT Synanthropic cockroaches are those that live in close association with human beings and their abodes. A review of published research on the biological control of synanthropic cockroaches revealed a wealth of laboratory-generated information on the biology, ecology, and efficacy of several cockroach natural enemies; however, few field studies were conducted to evaluate these enemies. Pathogens, particularly fungi, appear to be the most promising group for the biological control of German cockroaches, *Blattella germanica* (L.). The most promising natural enemies for the biological control of peridomestic cockroaches appear to be the oothecal parasitoids and some pathogens. Searches abroad for classical biological control agents of cockroaches are highly recommended. Due to the harshness, ecological instability, and physical impediments associated with the indoor and outdoor environments where cockroaches are found, releases of biological control agents will necessarily be periodic and inundative. Conservation of existing natural biological controls can be achieved by switching from residual sprays to baits whenever possible. A multitude of questions regarding issues associated with the biological control of cockroaches are presented for discussion.

KEY WORDS Biological control, urban pest management, cockroach, parasitoid, pathogen, predator, natural enemy, Blattellidae, Blattidae

It has been estimated that nearly all insect species have a complex of parasites, predators, and pathogens that attack and destroy them. Many natural enemies are extremely host specific and attack only certain species or genera. Research and development on the biological control of urban pests is lacking when compared with biological control efforts of agricultural pests. Biological control efforts for cockroaches are more advanced than for any of the other urban pests considered in this symposium. Unfortunately, efforts to develop biological control programs for cockroaches have met with limited success.

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Literature Review

The following cursory review is meant to highlight trends in the biological control literature of cockroaches. The literature is broken into two sections: (1) natural enemies of German cockroaches (i.e., domestic pests) and (2) natural enemies of peridomestic cockroaches (i.e., the Blattidae) plus the brownbanded cockroach, *Supella longipalpa* (F.). This split was because of the dominance of oothecal parasitoids of peridomestic and brownbanded cockroaches. Peridomestic cockroaches are those that live and breed around and often inside structures, but which are not obligate domiciliary pests. Peridomestic cockroaches include many species in the *Periplaneta*, *Blatta*, *Eurycotis*, and *Parcoblatta*. Within each of the two divisions, the literature was further categorized as to whether the article involved some aspect of the enemy's biology, whether the natural enemy was used in some type of applied study, or whether the article was taxonomic, a survey, or a review article. In general, there is a wealth of laboratory-generated data on the biology and ecology, life cycle, and natural history of a multitude of promising natural enemies of synanthropic cockroaches, but just a few studies where biological control organisms have been deployed and evaluated under field situations.

German Cockroach Natural Enemies

Currently, the German cockroach natural-enemy complex consists of a few invertebrate (insects and other arthropods) and vertebrate predators, but more of the enemies are pathogens and nematodes (Table 1). Pathogens appear to be the most promising group for the biological control of German cockroaches. Due to the small size of the natural-enemy complex of German cockroaches, several nonspecific natural enemies (e.g., *Steinernema carpocapsae* [Weiser], *Beauveria bassiana* [Balsamo], and *Metarhizium anisopliae* [Metsch.]) are currently being investigated for use as biological control agents against the German cockroach (Koehler et al. 1992; Appel et al. 1993; Andis 1994; Appel & Benson 1994; Miller 1994; Kaakeh et al. 1996, 1997).

Pathogens. The bulk of our current knowledge of fungal pathogens of German cockroaches comes from a series of articles by Archbold et al. (1986; 1987a, b) and Appel et al. (1987). These studies detailed a lethal fungal infection from laboratory colonies of German cockroaches. Cadavers were dark and flaccid, and infected colonies had a putrid odor. Infected cockroaches were characterized by shortened, curled antennae; uneven wings; and emulsified fat bodies. Reproduction by infected females also was significantly affected. Disease progression was over a 20-d period with complete mortality after 30 d.

Roth & Willis (1960) reported several fungal pathogens that demonstrated considerable pathogenicity to the German cockroach. For example, *Cordyceps blattae* Petch was collected twice from a *Blattella* sp. at Hakgala, Sri Lanka, and the effect was described as "A slight covering of brown mycelium overran the insect and fastened it to the underside of a living leaf." *Herpomyces ectobiae* Thaxter was recorded from Massachusetts, Minnesota, Burma (Tenasserim), Argentina (Buenos Aires), France, Chile, and the Philippines; infection occurred in various locations on the German cockroach, but definitive pathogenicity was

Table 1. Literature summary of German cockroach natural enemies.

Area of research	No. of articles found	
	Pathogens	Nematodes
Biology	12	15
Applied	0	2
Taxonomy	0	0
Surveys	0	0
Reviews	1	2

not established. *Herpomyces* are highly host specific, but many species in this genera apparently cause no adverse effects to their cockroach hosts. No studies were found where fungal pathogens were field tested for efficacy.

There are several protozoans (Haplosporidia, Microsporidia) that seem to cause some adverse effects when present in cockroach fat body and Malpighian tubules, but most are not known to be harmful to their hosts. They have been recovered from German cockroaches in New York City, France, England, Germany, and India. The taxonomy of this group of protozoans is unsettled. The reader is referred to Roth & Willis (1960) (pages 185-186), Daoust (1981), and Strand & Brooks (1977) for taxonomic purposes, including complete synonyms and some biological notes. The basic life cycle of many of these microsporidia natural enemies is unknown. Haplosporidia and microsporidia from the Malpighian tubules of German cockroaches apparently encyst in the tubule wall and pass through the hindgut with the feces.

Tsai & Cahill (1970) reported, in addition to two nematode species, seven protozoans from German cockroaches collected in New York City. Nearly 83% of the city-collected cockroaches harbored *Neophridiophaga blattellae* (Crawley). There are no published reports of the field efficacy of microsporidia for German cockroach control.

Although Roth & Willis (1960), Daoust (1981), and Strand & Brooks (1977) reported a total of 29 bacterial species from German cockroaches, in only a few cases (*Serratia* sp. and *Bacillus* sp.) were cockroaches susceptible. Commercial preparations of *Bacillus* were effective in the biological control of German cockroaches on naval ships. Nymphs were more susceptible than adults, and mortality occurred 10 to 20 d after infection. The bacillus was transmitted as a result of cannibalism.

Nematodes. Research on generalist nematodes of the genus *Steinernema* dominate the recent nematode-related literature. Once again, the majority of

work on this group comes from the laboratory. Koehler et al. (1992), Appel et al. (1993), and Appel & Benson (1994) demonstrated that German cockroaches were susceptible to *S. carpocapsae* in Petri dish bioassays. Appel et al. (1993) and Manweiler et al. (1993) provided field evidence for the control of German cockroaches with *S. carpocapsae*. Exposure stations containing 2×10^6 nematodes significantly reduced German cockroach populations in infested apartments.

Roth & Willis (1960) reported several commensal thelastomatid nematode parasites, *Blattelicola blattelicola* Basir and *Blatticola blattae* (Graeffe), of German cockroaches. Tsai & Cahill (1970) discovered *B. blattae* in 96.2% of German cockroaches collected in New York City. In general, however, most of the 10 species of nematode listed in Roth & Willis (1960) are commensals and do not harm their German cockroach host.

Parasitoids and predators. The reproductive strategy of German cockroaches has excluded the hymenopterous oothecal parasitoids, one of the largest groups of cockroach natural enemies, from the natural-enemy fauna of German cockroaches. Blattid cockroach species bury or cover their egg case, possibly a protective mechanism against parasitism. German cockroaches protect the ootheca against parasitism by carrying it until just before hatch, thus protecting it from parasitoids. McKittrick (1964) hypothesized that, in addition to protection from desiccation, oothecal parasitoids have been a driving force in the variety of oviposition patterns in the Blattaria.

There have been several vertebrate (mammals) and invertebrate (spiders, mites) predators associated with German cockroaches, but very few insect predators. Several mite species have been shown to decrease survival and vigor of colonized German cockroaches. Often, females prematurely dropped their ootheca when infested with mites. The cockroach mite, *Pimeliaphilus cunliffei* Jack (Pterygosomidae), destroyed whole colonies of German cockroaches (Field et al. 1966). *Blattisocius tineivorus* (Oudemans), a member of the predaceous mite family Phytoseiidae, and a *Caloglyphus* species (Acaridae) also are known to cause decline of German cockroach colonies (Roth & Willis 1960). The spider *Steatoda grossa* (C. L. Koch) and crab spiders are known to feed on German cockroaches under controlled conditions (Ebeling 1978, Mallis 1990). *Dolichurus corniculus* (Spinola) (Ampulicidae) and *Rhipidius pectinicornis* Thunberg (Rhipiphoridae) are insect predators of the German cockroach (Roth & Willis 1960).

A determining factor in the success of biological control programs for German cockroaches will be homeowner acceptance of the agent, a factor directly related to its visibility. Most homeowners do not discriminate among insect species, and the presence of insect predators or parasites is likely to be viewed as unacceptable as the cockroach infestation itself. For example, Thoms & Robinson (1987) showed that residents often killed oothecal parasitoids.

Research on Generalist Natural Enemies of the German Cockroach

What little work has been done on the biological control of German cockroaches has been with nonspecific organisms pathogenic to a wide variety of pests. Several reasons underlie the biased use of nonspecific natural enemies

as opposed to specific ones. First, there has not been an effort to explore the German cockroach's native range for specific natural enemies. As a result, manufacturers and researchers are left with natural enemies currently available on a large scale. These are common nonspecific nematodes and pathogens such as *S. carpocapsae*, *B. bassiana*, and *M. anisopliae*. The work of Archbold et al. (1986; 1987a, b) and Appel et al. (1987) reflect the current situation. Their data are some of the best we have on fungal pathogens of German cockroaches, yet their project was initiated because the fungus was discovered by accident. It had infected the University of California, Riverside's, German cockroach colony and they initiated a pathological study that resulted in four refereed publications. These studies constitute the majority of our information on species-specific fungal pathogens of German cockroaches.

Another reason for the biased use of nonspecific natural enemies is that rearing procedures and conditions, and thus production costs, are generally less critical for nonspecific natural enemies. Although a species-specific natural enemy may be more effective at controlling a target pest, its specificity may actually increase costs of production due to elaborate rearing procedures. Nonspecific pathogens, such as *B. bassiana*, can be cultured on rice, and *S. carpocapsae* can be cultured in large vats containing bacteria and nutrients. Environmental conditions (e.g., pH, water quality, and temperature) for rearing and producing species-specific natural enemies are likely more critical and rigid. Also, conditions under that species-specific pathogens would be formulated might be critical to ensure pathogen viability while maintaining a competitive shelf life.

Peridomestic Cockroach Natural Enemies

In reviewing the peridomestic cockroach natural-enemy literature, similar trends to the German cockroach literature were evident. Thus, there is a wealth of literature on the biology and ecology of pathogens, nematodes, and parasitoids from a multitude of laboratory studies, but few in-depth studies using these enemies in the field (Table 2).

Pathogens. The pathogens infecting peridomestic cockroaches are evenly split between viruses and microsporidia. The bulk of the virus-related literature comes from several papers on a virus of smokybrown cockroaches, *Periplaneta fuliginosa* (Serville) (Suto et al. 1979, Yang et al. 1992, Hu et al. 1994). Several studies have been published on the biology of infection of blattid cockroach species by microsporidia (Strand & Brooks 1977, Daoust 1981). Unfortunately, the microsporidia are a poorly understood group. There are no field studies available involving either viruses or microsporidia.

Nematodes. Although a wealth of commensal nematodes was discovered during this literature search, they have not been included in this review, as they typically do not demonstrate pathogenicity toward their cockroach hosts. A majority of the work published to date involves laboratory biology, pathogenicity, and efficacy studies with generalist *Steinernema* spp. Koehler et al. (1992) demonstrated a correlation between habitat and susceptibility to *S. carpocapsae* among several peridomestic cockroach species, with American cockroaches, *Periplaneta americana* (L.), being significantly more resistant to

Table 2. Literature summary of peridomestic cockroach natural enemies.

Area of research	No. of articles found		
	Pathogens	Nematodes	Parasitoids
Biology	16	7	61
Applied	0	0	8
Taxonomy	2	0	6
Surveys	0	1	6
Reviews	1	1	4

infection than either smokybrown or Australian cockroaches, *Periplaneta australasiae* (F.). There were no studies involving the field efficacy of nematodes for peridomestic cockroach control.

Parasitoids. In a recent review of the hymenopterous natural enemies of cockroaches, LeBeck (1991) listed 20 species of parasitoids (Evaniiidae, Eulophidae, Pteromalidae, Eupelmidae, and Encyrtidae), and a few species of predators (Ampulicidae) of the Blattellidae. I found 61 articles on the laboratory biology, natural history, and ecology of oothecal parasitoids (Table 2), including several in which a parasitoid was used in inoculative or inundative releases (Slater et al. 1980; Coler et al. 1984; Hagenbuch et al. 1989; Pawson & Gold 1993; D. R. S., unpublished data). The work of Coler et al. (1984) and Slater et al. (1980) stand as our best examples of cockroach biological control. In 1984, Coler's release of *Comperia merceti* (Compere) in an insect rearing room to control brownbanded cockroaches caused a dramatic shift in age structure, leading eventually to a significant reduction in cockroach population. Recently, Hechmer & Van Driesche (1996) published an update on Coler's initial parasitoid release. The original release has remained, unaugmented, for some 10 yr since the last release, and 15 yr since the first release, and continues to maintain a significant level of brownbanded cockroach control.

Importations

Classical biological control, or importation, involves the exploration of a pest's native range for natural enemies that could be imported, quarantined, researched, and developed for biological control. The goal of classical biological control is simple: reacquaint the newly introduced pest with natural enemies that held its numbers at equilibrium in its native range.

Searches abroad for cockroach natural enemies will necessarily be multidisciplinary and likely involve the services of insect pathologists, hymenopterists, taxonomists, urban entomologists, and government officials, to name a few. Upon discovery, candidate material must be returned to the area of pest infestation, placed in quarantine, and observed to gather data on its biology, pathogenicity, environmental impact, and effect on nontarget organisms. Data gathering of this magnitude is costly and often requires years to accomplish.

There has not been a single concerted effort to search the native range of the German cockroach for natural enemies. Indeed, the history of classical approaches for the biological control of cockroaches is nonexistent. Are there opportunities? Roth's (1985) taxonomic revision of the *Blattella* provides convincing evidence that southern and southeastern Asia are the evolutionary origins of the German cockroach. Roth (1985) divides the 49 species of *Blattella* into six species-groups based on male morphology. In the Germanica group are six closely related species from locations throughout the southern and southeastern Asian mainland and from islands in the south Pacific. Only the German cockroach, *Blattella lituricollis* (Walker), and *Blattella asahinai* Mizukubo are found outside of this range.

Future searches for biological control agents of the German cockroach should be concentrated in southern and southeastern Asia. It is interesting to note that *B. asahinai* and *B. lituricollis* have been taken on Sri Lanka. As noted earlier, a fungal pathogen was collected twice at Hakgala, Sri Lanka, from a *Blattella* sp. in what appeared to be outdoor, field collections from wild German cockroaches (Petch 1924); *H. ectobiae* also was recorded from Burma (Tenasserim) and the Philippines from the German cockroach, but definitive pathogenicity was not established (see Roth & Willis 1960, page 135 for references). Recently, the fungal pathogen *Hymenostilbe ventricosa* sp. nov. was recovered from blattid cockroach species in Thailand (Hywel-Jones 1995). These records are highly suggestive that foreign exploration of southern and southeastern Asia might produce pathogens that are specific for cockroach pests.

Periodic Releases

The indoor and outdoor environments of cockroaches are heterogeneous and ecologically unstable (Flanders 1986). The indoor environment is chemically harsh, consisting of a plethora of household cleaners, pesticides, spices, soaps, and detergents. It is also structurally complex with numerous cracks, crevices, voids, and impediments to movement. In addition, there are many thermal and moisture microhabitats, and unnatural lighting and air movement (i.e., fans). The harsh ecological diversity of the indoor environment may adversely impact the performance of cockroach natural enemies by limiting dispersal, host location, and survival. Application of a natural enemy that evolved under different environmental conditions to the inhospitable environment of a home may not be successful.

The potential for biological control of cockroach pests in confined, nonhuman inhabited environments (e.g., treeholes, sewers, greenhouses, plumbing chases,

animal rearing facilities, and zoos) is higher than the potential for biological control indoors. These types of confined environments tend to be less heterogeneous and more stable ecologically than the indoor environment. Thus, parasitoids, for example, might be better able to search for and locate hosts because dispersal is reduced and survival is enhanced. As mentioned previously, Coler et al. (1984) controlled brownbanded cockroaches in a confined insect rearing facility, and Pawson & Gold (1993) and Hagenbuch et al. (1989) also had some success with parasitoid releases against American cockroaches in closed environments.

When parasitoids are released, they have a tendency to disperse. Lewis et al. (1972, 1975) enhanced parasitism of Lepidoptera eggs by wild and released *Trichogramma* in Petri dishes, greenhouses, and cotton fields by prerelease application of n-tricosane, a kairomone found in moth scales and used by the parasitoid as a host location cue. The kairomone prevented dispersal of parasitoids by stimulating host searching, resulting in higher rates of egg parasitism. The oothecal parasitoid *Aprostocetus hagenowii* (Ratzeburg) (Fig. 1) uses the hydrocarbon 6,9-heptacosadiene found on American cockroach oothecae as a host location kairomone (Suiter et al. 1996). The material is easily extracted from adult cockroach cadavers, oothecae, and frass, or is easily synthesized. This kairomone could be used to prevent parasitoid dispersal, and for monitoring parasitoid populations in the field before and after periodic releases.

There are other problems associated with releases of natural enemies. For instance, availability of artificial, mass rearing techniques for beneficial organisms is often a limiting factor in biological control programs. If the release of cockroach natural enemies is to be economically feasible, methods for their artificial, mass production must be developed. Dai et al. (1993) reported development of an artificial rearing technique for *A. hagenowii*.

Due to the environmental instability of the indoor and to a certain degree, outdoor environments, biological control programs for cockroaches will likely take the form of periodic, inundative releases. Periodic releases can be inoculative or inundative (Piper & Frankie 1978). Biological control by inoculation results from the progeny of released natural enemies and from subsequent generations. Inoculative releases seek long-term, slow reductions in the pest population. The objective of inundative biological control is to overwhelm the pest by releasing large numbers of natural enemies resulting in the immediate reduction of the pest population.

Conservation of Natural Enemies by Modifying Insecticide Use

Natural biological control is the baseline level of pest management achieved by naturally occurring parasites, predators, and pathogens. Disruption of natural controls must be avoided. Conservation of natural enemies is currently being performed by some pest control professionals without their knowledge. The use of target-specific toxic baits in locations where peridomestic cockroaches live conserves oothecal parasitoids. Fleet & Frankie (1975) showed that up to 80% of oothecae outdoors in Texas were parasitized by *A. hagenowii*. However, rates of oothecal parasitism were lower around structures treated



Fig. 1. The cockroach oothecal parasitoid *A. hagenowii* parasitizing an American cockroach ootheca. Photograph by D. R. S.

with a perimeter spray than structures where perimeter bands were not applied (Piper & Frankie 1978). Switching from sprays to baits in targeted areas will conserve natural enemies and allow them to maintain a high level of natural control. Hagenbuch et al. (1989) used this method to achieve control of American cockroaches in test kitchens. Lane M. Smith II (Auburn Univ., personal communication) has provided information on the ecologically disruptive effect of perimeter treatments on the ecology of the outdoor environment around structures.

Critical Questions

Before embarking on development of a biological control program for cockroaches, there are a number of questions to initially address. For instance,

Is there a need for the biological control of cockroaches, or are we trying to use agricultural models of biological control and force them into urban pest management? What is the driving force for development of biological control programs for cockroaches? Is it a reduction in insecticide use, the need to develop new markets, resistance management, or urban integrated pest management (IPM)? Furthermore, Who will pay for the development of biological control programs for cockroaches? Currently, cockroach research is not recognized by federal mechanisms that typically fund biological control research. Recent corporate experiences with biological control of cockroaches, the accompanying expenses involved in development of biological control organisms, and the risk of failure have made biological control unattractive from a manufacturers perspective at this time. From an operational viewpoint, Can biological control approaches compete with chemical approaches in effectiveness, cost of production (i.e., mass rearing), ease of application, and shelf life in the urban arena? Moreover, Do biological control approaches have to compete with chemical approaches in these areas?

These and other questions must be addressed before committing finite and dwindling resources. It is clear that classical approaches to the biological control of cockroaches are worthy of investigation. In addition, the philosophy of natural-enemy conservation fits nicely with the pest control industry's current push towards IPM-related pest control strategies.

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Nonchemical Approaches to Cockroach Control¹

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ABSTRACT Cockroaches require access to air, food, water, and a protected harborage for survival and reproduction. Access to food and water can be reduced by sanitation and proper construction practices but probably cannot be removed entirely. Harborages also can be modified or possibly eliminated from small areas if they can be identified. Harborage preferences are specific and influenced by harborage size and other abiotic factors, as well as by the presence of con- and heterospecifics. For example, all stages of the German cockroach, *Blattella germanica* (L.), were repelled from preferred areas by air velocities of 0.5–4.0 m/s. In an experiment with a cockroach-infested simulated kitchen cabinet, German cockroaches were moved from a preferred location with an air velocity of 4.75 m/s. The use of flowing air to repel cockroaches could be a useful noninsecticidal tool for German cockroach control. Harborage removal or modification by use of air could be an important new tactic in cockroach management systems.

KEY WORDS Dictyoptera, Blattidae, Blattellidae, *Blattella germanica*, *Periplaneta americana*, *Periplaneta fuliginosa*, German cockroach, American cockroach, smokybrown cockroach, repellency, air flow, choice box

Cockroaches are among the most important insect pests in and around the home. They contaminate food and food preparation areas with their saliva, feces, and body parts and may mechanically vector a number of disease-causing organisms (Roth & Willis 1960; Mullins & Cochran 1973a, b; Rivault et al. 1993; Kopanic et al. 1994). Probably as important, cockroaches and their secretions may be allergenic to sensitive people (Brenner et al. 1990, Kang 1990) and can induce psychological problems such as delusory parasitosis and delusory cleptoparasitosis (Roth & Willis 1957, Grace & Wood 1987, Brenner 1995).

Control of both domestic (primarily indoor) and peridomestic (primarily outdoor) cockroaches almost exclusively relies on the use of insecticides. Inorganic (e.g., boric acid), natural (e.g., pyrethrins), or synthetic (e.g., chlorpyrifos, deltamethrin) insecticides are used. Many different formulations are used, such as aerosols, baits,

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emulsifiable concentrates (EC), microencapsulates (ME), suspension concentrates (SC), and wettable powders (WP), and they are usually applied indoors to the cracks and crevices where cockroaches harbor (e.g., Rust 1986). Banded broadcast applications are used around the entire perimeter of homes for outdoor peridomestic cockroach control.

In this paper, nonchemical control generally refers to no direct application of chemicals of any type, including attractants, insecticides, kairomones, etc. Control methods should be based solely on the biology and physiology of the pest itself. Gold (1995) reviewed a number of nonchemical alternative strategies for German cockroach, *Blattella germanica* (L.), control, including light, temperature treatments, trapping, and various electronic pest control devices such as electromagnetic, vibration, and sonic and ultrasonic generators. In general, none of these strategies has been proven to reduce cockroach populations.

A newer German cockroach control strategy is the use of vacuuming, either alone or in conjunction with repellent sprays or heated air to physically remove cockroaches. Even though physical removal is an attractive nonchemical treatment, the analogous removal strategy of trapping-out has not been shown to be effective at reducing German cockroach populations (e.g., Barak et al. 1977).

Resource requirements for cockroaches. All cockroaches require air, food, water, and harborage for survival and reproduction. Cockroaches are opportunistic feeders and consume a variety of organic materials ranging from human foods to glue, pet feces, and other insects (e.g., Roth & Willis 1960). Especially indoors, there is typically an abundance of food available. Dirty dishes, garbage, and organic waste are all suitable foods. German cockroach population size, measured as trap catch or by visual inspection, has been correlated with poor sanitation in some studies (Wright 1979, Sherron et al. 1982, Schal 1988, Wright & Dupree 1988), but other studies have not shown such a relationship (Bennett 1978, Gold 1995). It is unlikely that the availability of food, particularly indoors, can be sufficiently reduced to significantly affect cockroach populations. Bertholf et al. (1987) removed food debris as part of a sanitation program but did not significantly reduce cockroach populations. Many researchers have conducted studies in low-income housing and could attest to the difficulty in removing sufficient amounts of cockroach food from the environment. Grease deposits that provide nutrients for cockroaches also may affect conventional insecticide efficacy by absorbing the lipophilic active ingredients, severely limiting their surface availability (e.g., Rust & Reiersen 1988).

Outdoors, food may be available periodically (flowers, ripe fruit). Smith & Appel (1996) demonstrated that lack of sufficient food can influence consumption of insecticides. House and landscape characteristics, including the presence of companion animals (presumably as a measure of pet food and feces), are well correlated with the relative abundance of smokybrown cockroaches, *Periplaneta fuliginosa* (Serville) (Smith et al. 1995).

Water is usually an abundant resource indoors. Locations such as sinks, toilets, condensation on pipes, and refrigerator drain pans all provide consistent sources of water. Many indoor areas infested with German cockroaches have

had poor maintenance with leaking plumbing. Outdoors, condensation (primarily in the evening), pools and ponds, rain, and water used to irrigate lawns and ornamental plantings offer almost unlimited access to water. Limiting the availability of water both indoors and out is difficult, if not impossible.

Harborages are abundant indoors (e.g., cracks and crevices, wall voids, and appliances) and also outdoors (e.g., tree holes, under mulch, cracks in masonry, out buildings). Even though the use of foams and other expanding materials could fill many potential harborage areas, it is unlikely that the monetary costs of such treatments could be justified. In addition, even if most void areas are made unsuitable as cockroach harborages, there are many void areas, such as in appliances and computers, that cannot be treated. Controlling access of peridomestic cockroaches into structures through the use of caulking (e.g., Thoms & Robinson 1987) and screening might reduce invasion rates but cannot reduce outdoor harborages. Because food, water, and harborage are abundant resources, which resource can be best modified to limit cockroach population growth? It may be possible to reduce availability or suitability of harborages as part of an integrated pest management (IPM) program.

Introduction to harborage selection. Harborages may be defined as daytime resting sites. Darkness, therefore, is the overwhelmingly important factor, but there are a number of abiotic and biotic factors that affect harborage selection or suitability (Fig. 1). Humidity, temperature, and their interaction (i.e., saturation deficit) affect harborage selection (Cornwall 1968). Both can be attractive or repellent and both can be affected by air flow. Harborage texture and size (see below) also can affect selection. Chemicals such as kairomones and sex pheromones affect harborage selection as do intra- and interspecific interactions. For example, the feces of even closely related *Blattella* spp. might be attractive, neutral, or repellent to nymphal German cockroaches (Rust & Appel 1985). Appel (1994) showed that American cockroaches, *Periplaneta americana* (L.), are significantly repelled from traps by smokybrown cockroaches but will enter traps containing conspecifics. Smokybrown cockroaches, however, readily enter traps containing con- or heterospecifics. A volatile kairomone, although unidentified, was assumed to mediate these responses. Part of these responses also may be due to volatile sex pheromones. Females of American, brownbanded (*Supella longipalpa* [F.]), German, and smokybrown cockroaches produce the pheromones to attract males. We must understand these types of inter- and intraspecific dynamics if we intend to understand harborage selection by cockroaches. Finally, traditional insecticides, repellents, and surface modifications such as silica gels and their interactions may affect harborage selection.

Harborage size selection. Berthold (1967), Berthold & Wilson (1967), and later Koehler et al. (1994) investigated the preferences of German cockroaches for various harborages widths. Younger (and presumably smaller) individuals preferred narrower harborages than older individuals. Similarly, early instars of American and smokybrown cockroaches preferred narrower harborages than older instars and adults (Appel & Smith 1996).

Potential outdoor harborage materials of American and smokybrown cockroaches, including juniper branches (*Juniperus horizontalis* L.), pine (*Pinus*

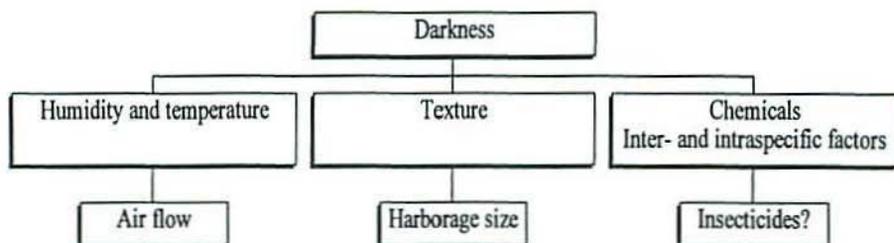


Fig. 1. Relationships among several harborage selection criteria used by cockroaches.

taeda L.) straw, soil, rocks, or grass (*Eremochloa ophiuroides* [Munro] Hack) thatch were studied by Appel & Smith (1996) in a series of laboratory experiments. None of the potential harborage materials was toxic to either species, and only dry soil was repellent to smokybrown cockroaches. American cockroaches preferred juniper branches more than other potential harborages when tested using mixed-stage groups. Smokybrown cockroaches also preferred juniper branches, but many also were found harboring under pine straw and rocks. Tested with single stages, small and medium American and smokybrown cockroach nymphs preferred pine straw rather than juniper indicating an interaction between cockroach stages and harborage selection. Even though relative humidity was significantly ($P = 0.05$) greater ($45.4 \pm 2.2\%$) under juniper, light intensity was lower (7.9 ± 3.9 lux) under pine straw. Smaller nymphs selected harborages with smaller interstitial spaces than larger nymphs; pine straw had the smallest interstices of any of the tested harborage material.

Harborage selection by cockroaches is mediated by the physical characteristics of the harborage and the presence of con- and heterospecifics. Because different cockroach species have different size ranges, only certain harborage dimensions are acceptable. Similarly, microclimate is an important factor in harborage selection and identification. Treatment strategies must, therefore, take into account the harborage preferences of the target species.

Development of a nonchemical repellent for German cockroaches. Ebeling et al. (1966) developed the 'choice box' for simultaneously evaluating the toxicity and repellency of insecticide deposits against cockroaches. Briefly, the choice box is a simple, square wooden box divided into two equal compartments that are connected by a small hole. Both compartments are covered by clear Plexiglas, but one compartment also is covered by an opaque sheet. Food and water are placed into the compartment covered with just clear Plexiglas (lighted) and a treatment is placed in the darkened compartment. The dark compartment simulates a harborage such as a void under a kitchen cabinet, where cockroaches would normally be found during the photophase. Cockroaches found alive and in the lighted side of the choice box are considered to be repelled from the dark side. Repellency in the choice box is, therefore,

relative to that of light. The lighted side simulates a counter top or floor with food and water readily available. Rauscher et al. (1985) investigated the effects of food, water, and insecticide placement on the distribution of German cockroaches in choice boxes. Their results generally agree with those of Ebeling et al. (1966), in that light was the major environmental factor affecting distribution. The Ebeling choice box, measures potential "control efficacy" in a similar choice situation as exists in an infested environment.

To test repellency of moving air, Oswalt et al. (1997a) developed a choice box (Fig. 2) composed of two parallel Plexiglas tubes, screening, and an electric fan. The tube that was connected to the fan was painted black and was exactly analogous to the 'darkened side' of an Ebeling choice box. Access holes were drilled and a clear tube was attached to part of the darkened tube. Food and water were placed into the clear 'lighted side' and a treatment, consisting of various air velocities was applied to the darkened side. Baffles were placed into the darkened tube to facilitate laminar air flow.

Perception of moving air. There are at least three body parts where cockroaches could potentially detect moving air: antennae, legs, and cerci. Volatile chemicals, temperature, and relative humidity are detected by the antennae (Cornwell 1968); however, the air movement escape system of the American cockroach is mediated by the cerci (Camhi 1984). Tarsi with movable setae, and various small mechanoreceptors on other body regions also may detect moving air. In a series of ablation and blocking experiments, antennae and/or cerci were removed; antennae were treated with butylmaleimide, blocking odor perception (Berger & Estes 1987); and tarsi were cauterized. Treated cockroaches were tested for repellency in electric choice boxes. Repelled cockroaches (those in the lighted compartment) perceived the moving air, whereas cockroaches found in the dark side of the electric choice box were assumed to be unable to perceive the moving air. In these tests, antennae were the only organ determined to be critical for the relevant detection of moving air.

Repellency of moving air. To summarize the results of Oswalt et al. (1997a), repellency of all German cockroach stages increased from 0% to about 100% between air velocities of 0.5 and 4 m/s; repellency increased linearly between these velocities. What is relevant about air velocities of around 4 m/s? This is about the velocity of air that comes out of forced air heating and air conditioning systems. Thus, air velocities that are repellent to cockroaches are actually found in homes today! The repellent properties of moving air against American and smokybrown cockroaches are currently being investigated.

A simulated kitchen cabinet, equipped with an adjustable fan (Fig. 3) was infested with German cockroaches to measure the effect of air movement on a cockroach population (Oswalt et al. 1997a). A repellent air velocity of 5 m/s was applied into a void at the top of the cabinet. Pegboard with 5-mm-diameter holes, covered with 0.25-mm² mesh "no-see-um" netting, was used as the bottom of the void, and as the bottom of the cabinet. Slits were cut into the pegboard so air could be directed at the inside walls of the cabinet to provide an even, downward flow. Clear Plexiglas was used as the cabinet door and was covered with black felt to prevent light from entering the cabinet. Approximately 200 mixed-stage German cockroaches were introduced into each of the cabinets and allowed to acclimate for 3 d. After 3 d, the position of all

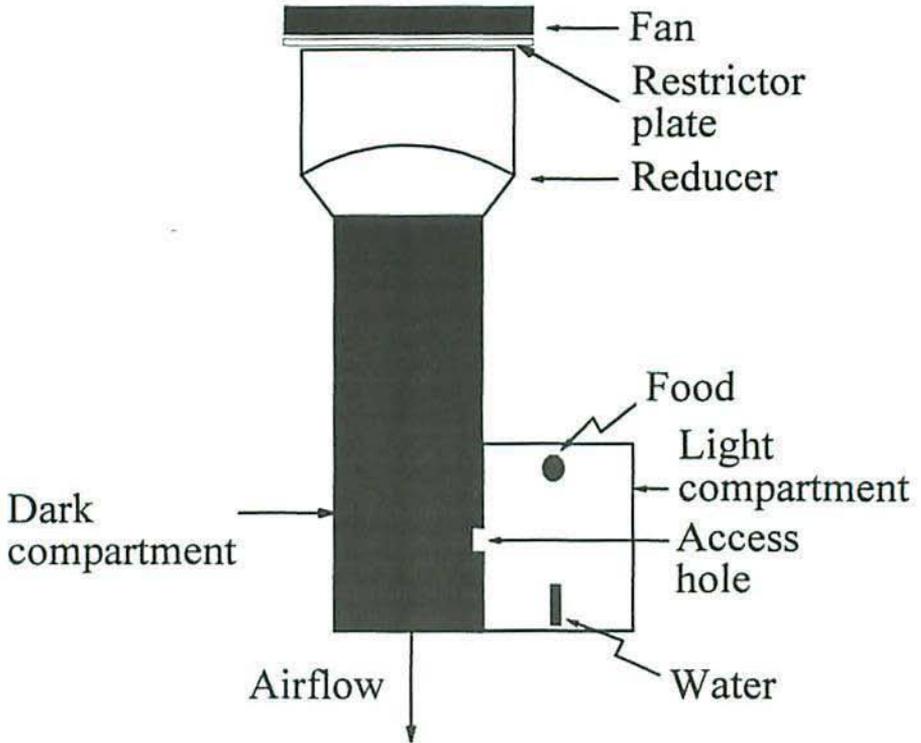


Fig. 2. Schematic diagram of electric choice box used to examine repellency of moving air (after Oswalt et al. 1997a).

cockroaches was recorded: top, middle, and bottom third of the cabinet. Then the air flow was applied. Another similarly prepared cabinet was used as an untreated (no air flow) control. After another 3 d, the position of all cockroaches was again recorded. Net movement, or the number on day 6 subtracted from its respective number on day 3, was calculated for each position in the cabinet.

Moving air significantly ($P < 0.001$) affected the distribution of cockroaches within the simulated kitchen cabinets. Net movement of cockroaches was significantly different in cabinets receiving the moving air treatment; cockroaches moved out of the top third ($t = 6.75$, $df = 23$, $P < 0.001$) and into the bottom third ($t = -4.08$, $df = 23$, $P < 0.001$). Therefore, German cockroaches can be repelled from preferred harborage by exposure to moving air. This approach might be useful as a tool in German cockroach management. If cockroaches could be driven and directed where we want them to go by directing air streams into normal harborage areas we would stress cockroach populations and facilitate conventional control strategies. Stress, such as low humidity, reduces cockroach reproductive rates and could make

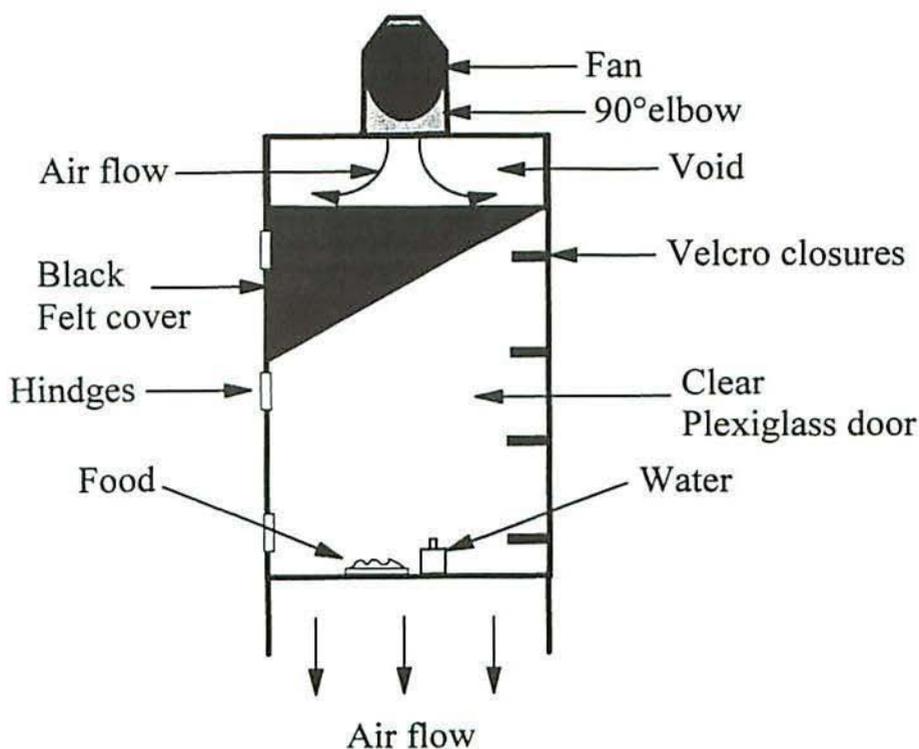


Fig. 3. Schematic diagram of simulated kitchen cabinet used to examine the effects of moving air on the distribution of German cockroach populations (after Oswald et al. 1997a).

them more sensitive to insecticides (Cornwell 1968). Desiccation caused by moving air rapidly killed German cockroaches (Oswald et al. 1997b). Cockroaches leaving normal preferred harborages could possibly be driven onto insecticide deposits or into closer proximity to baits.

Possibilities. A portion of bulk air flow from forced-air heating and cooling systems might be redirected into cockroach harborages in kitchens. One possibility would be to pressurize a wall void behind cabinets or appliances, tap the air from that void, and direct it into appropriate areas. Cabinets could be modified to maximize air flow and minimize humidity. For example, cabinet shelves could be constructed with slats rather than solid sheets to increase air flow similar to old-time basement coolers and dumb waiters. Raised vents could be used to connect cabinets with counters and increase air flow under counters.

Flowing air also might be used as an inspection tool to locate German cockroach harborages. Rather than use aerosol repellents, exposure to air of sufficient velocity may flush as well as chemical, but without a residue. The rapid flushing properties of moving air are being investigated.

Problems. Use of flowing air is not without several potential drawbacks. Just like a repellent insecticide, cockroach populations could be spread throughout a residence by application of repellent air flow. Thus, flowing air should only be used cautiously in areas with long established infestations. Increased air movement would probably increase the distribution or concentration of allergens, such a cockroach feces, shed skins, and setae, in living spaces. Removal of allergens by vacuuming of cockroach debris and chemical treatment of sites known to contain high levels of allergens should be carried out prior to adopting a moving air strategy.

Gel and paste baits that contain high levels of water will dry out faster when exposed to moving air because desiccation reduces palatability of baits to cockroaches (Appel 1992). Moisture-laden baits might have to be housed in impermeable stations to remain hydrated.

Conclusions. Relevant velocities of moving air are repellent to German cockroaches. Slight structural modifications could be used to facilitate air flow into cockroach harorage sites limiting infestation and possibly increasing the effectiveness of conventional insecticides.

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Biological Control Strategies for Suppression of Termites¹

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ABSTRACT Recent research and progress in developing biological control strategies for termite management are reviewed. Biological control involves the use or manipulation of parasites, predators, or pathogens. There is very little documentation of termite parasitism. Ants are the most important predators of termites, and the interactions of various ant species with termites have recently received a fair amount of research attention. Certain species of ants are effective in excluding termite foragers from localized wood resources, but they are quite limited in their ability to penetrate into subterranean termite galleries in the soil. The greatest potential for biological control of termites appears to lie with insect pathogens, or microbial control. Laboratory studies with insect-pathogenic fungi are particularly promising, although field efficacy data are lacking. The potential advantages of microbial control are such that further research is well justified. However, the technical difficulties that must be overcome are sufficiently daunting that we must temper our enthusiasm with cautious realism.

KEY WORDS Isoptera, *Reticulitermes*, *Heterotermes*, *Kaloterms*, *Coptotermes*, microbial control, entomopathogens

Biological control is generally perceived as both providing more permanent insect control and as having less potential for damage to the environment or to nontarget organisms than chemical pest control interventions, although the latter perception is not without controversy (Howarth 1991). With cryptic insects such as termites (Isoptera), detection prior to the occurrence of significant damage and the effective delivery of insecticides to kill the population or (with subterranean species) to block the path of entry into the threatened structure are particularly challenging. Typically, large quantities of persistent insecticide solutions have been applied to the soil to prevent subterranean termite attack, raising concerns about applicator safety, environmental contamination, and possible deleterious effects on nontarget animals.

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The use of biological control agents to hunt or to infect termites within their hidden galleries is appealing. The many social interactions within a subterranean termite colony and their maintenance of a dark, damp habitat also would seem to favor survival and distribution of pathogenic microorganisms that could be introduced or encouraged to flourish in microbial control strategies.

This paper represents a selective review of recent research and progress in developing biological control strategies for control of termites. It focuses on termites as pests of urban structures, although recent collaborative efforts between Kenya and Denmark addressing termites of agricultural importance in East and Central Africa (Danish Technological Institute 1992) also are discussed. Thus, this paper should be considered an update on the state of biological termite control, and the reader is referred to the literature citations, and particularly to the review by Logan et al. (1990), for discussions of earlier work in the field.

Parasites of Termites

Biological control may be achieved through the actions of parasites, predators, or pathogens. No insect parasites of termites have yet been reliably documented, although mites considered to be parasitic have been collected from termites (K.L. Strong & J.K. Grace, unpublished data), and phoretic mites are frequently noted by termite researchers (Phillipsen & Coppel 1977, Costa-Leonardo & Soares 1993). However, in laboratory studies with *Reticulitermes flavipes* (Kollar), inoculation of groups of termites with extremely large numbers of phoretic mites proved to have no discernable negative effects on termite feeding or survival (M.H. Zoberi & J.K. Grace, unpublished data).

Predators of Termites

Specialized predators of termites are rather limited in number, possibly because of the cryptic and protected habitats in which termites live. One of the more interesting of these predators, and one deserving of further attention by researchers, is the beroid larva *Lomamyia latipennis* Carpenter that lives within termite nests and was reported to prey upon termite workers by emitting a vapor-phase toxicant (Johnson & Hagen 1981).

More visible specialized predators of nesting and foraging termites include certain ponerine and myrmicine ant species and vertebrates such as aardwolves, aardvarks, and anteaters (Logan et al. 1990). It is extremely doubtful that any of these tropical ant species or large vertebrates could be used in control programs, although the thought of tethering an aardvark in the substructure space beneath an urban dwelling is intriguing.

Opportunistic predation on termites is quite common. During swarming periods, termite alates are readily fed upon by entomophagous arthropods (such as ants and spiders), birds, fish, lizards, geckos, toads, and mammals (including humans). Ants are the most obvious predators of foraging termites, and anecdotal observations of a decline in termite activity within structures associated with Argentine ant, *Linepithema humile* (= *Iridomyrmex humilis*)

(Mayr), infestations are common (c.f., Olkowski & Drlik 1994). In laboratory studies, Wells & Henderson (1993) observed that *Coptotermes formosanus* Shiraki groups with abnormally low soldier proportions were less likely to explore new areas, and that the relatively high proportion of soldiers normally found in colonies of this introduced termite species conferred greater protection from predation by the red imported fire ant, *Solenopsis invicta* Buren, than the smaller number of soldiers typical of native *Reticulitermes* spp. Holmgren colonies in North America. In similar studies with the bigheaded ant, *Pheidole megacephala* (F.), Cornelius & Grace (1997) found that the principal defensive role of termite soldiers was to temporarily prevent ants from invading galleries while the workers sealed breaks in the tunnels and built soil barriers to block further ant invasion.

Ant species commonly found in the urban environment in Hawaii, or collected in termite-infested wood and in traps at field sites, were recently evaluated at the University of Hawaii as potential biological control agents for the Formosan subterranean termite. Behavioral assays with solvent extracts from different ant species established that termite responses to the presence of ants were largely chemically mediated (Cornelius & Grace 1994a). Termites retreated from contact with the dolichoderine ant *Ochetellus glaber* (Mayr) (Cornelius & Grace 1994a, b) due to the repellence of its monoterpene anal gland secretion *cis,trans*- and *trans,cis*-dolichodial (Cornelius et al. 1995). This compound is toxic as well as repellent to termites and may prove to have some value as an insecticide model (Cornelius et al. 1995). However, the strong avoidance behavior exhibited by *C. formosanus* when contacting this ant species, and their subsequent rapid retreat and construction of soil barriers to prevent ants from following (Cornelius & Grace 1995) limit the potential of *O. glaber* as a biological control agent.

In contrast to the semiochemically mediated avoidance of *O. glaber* by termites, chemosensory cues associated with *P. megacephala* stimulate termites to rapidly attack this species (Cornelius & Grace 1994a, 1995, 1996). The bigheaded ant also is more invasive of termite galleries in the soil than *O. glaber*, and *C. formosanus* is forced to retreat further into the soil to construct defensive barriers and suffers greater losses from combat than with *O. glaber* (Cornelius & Grace 1995, 1996, 1997). In practical terms, *P. megacephala* can exclude termites from foraging in a particular location and is more invasive of termite galleries than *O. glaber*, but it also is prone to suffer greater combat casualties than *O. glaber* due to the termites' aggressive response. A more suitable model for a biological control agent is offered by the ant *Tetramorium simillimum* (F. Smith), which did not stimulate a visible response by *C. formosanus* in laboratory assays and was able to cause greater mortality among the unsuspecting termites than either *O. glaber* or *P. megacephala* (Cornelius & Grace 1994a, 1995). However, no interactions of this ant species with termites in Hawaii have yet been observed in the field.

Pathogens of Termites and Microbial Control

The greatest potential for application of biological control to suppression of termite populations appears to lie with pathogenic microorganisms. Certainly,

it is this area that has received the most significant research and regulatory (Grace 1994) attention. Nematodes have proven to be extremely virulent in confined conditions in the laboratory (Trudeau 1989) but appear to have a temporary impact on termite foraging activities in the field (Mix 1986, Epsky & Capinera 1988). Although nematode products are currently available in North America for application to the soil to control subterranean termites, the small amount of research published to date indicates that efficacy would be limited to the immediate area of application and quite temporary. Logan et al. (1990) suggested that nematodes might be most efficacious in direct application to small aboveground termite infestations within the branches of high-value crops such as tea bushes.

Only a small amount of research has been reported with either viruses or bacteria as potential termite control agents. Al Fazaury & Hassan (1988) successfully infected the drywood termite *Kaloterme flavicollis* F. with a nuclear polyhedrosis virus isolated from the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval), and recently reported the histopathology of the infection in some detail (Al Fazaury & Hassan 1993). Most bacterial research has concentrated on *Bacillus thuringiensis* Berliner (*Bt*), although termites are susceptible in nature to other bacterial infections, such as *Serratia marcescens* Bizio (Khan et al. 1977). *Bt* is generally considered more of a microbial insecticide than a self-sustaining biological control agent, and recent work by Grace & Ewart (1996) took that approach. These authors investigated the application of the delta-endotoxin of *Bt*, expressed by a recombinant leaf-colonizing bacterium *Pseudomonas fluorescens* (Trevisan) Migula, and then bio-encapsulated within that killed and fixed bacterium, against Formosan subterranean termites. Neither Lepidoptera nor Coleoptera-active endotoxins proved active against *C. formosanus*, but termites readily consumed large quantities of the genetically engineered bacterium, suggesting that other more termite-active toxins might be encapsulated in this fashion as baits (Grace & Ewart 1996).

Insect-pathogenic fungi have been the major foci of research on microbial control of termites. Such fungi are much less invasive than nematodes, causing fewer immediate physiological and behavioral changes in the insect and killing more slowly. Thus, these pathogens appear to have a greater potential for distribution through social contacts among colony members. The fairly constant temperatures and damp, dark conditions in subterranean termite galleries also favor fungal growth. Currently, collaborative efforts between researchers in Kenya and Denmark are addressing the potential for fungal control of termites in African agriculture (Danish Technological Institute 1992). Recent theses at the Royal Veterinary and Agricultural University, Copenhagen, have reported the isolation of *Cordycepioideus bisporus* Stifler from *Macrotermes subhyalinus* Rambur and laboratory evaluations of strains of *C. bisporus* and *Paecilomyces fumosoroseus* (Wize) Brown and Smith against *M. subhyalinus* (Ochiel 1995) and of the more well-known pathogenic fungi *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Balsamo) against *Macrotermes michaelsoni* (Sjöstedt) (Gitonga 1996). Gitonga (1966) also tested the use of fungus-inoculated sawdust and rice grains as termite baits near *M. michaelsoni* mounds, as well as inoculating mounds with dry conidia.

However, these treatments were much less effective than the termite mound treatments reported by Fernandes (1991) and colleagues in Brazil, or by Milner et al. (1996) in Australia. It may be necessary to deposit large quantities of conidia within the central portion of the nest to infect and kill the entire termite colony (Gitonga 1996).

In addition to reported success in treating termite mounds directly with conidia of *M. anisopliae*, Milner et al. (1996) also suggest the use of conidial sprays on wood to repel foraging termites. Although no efficacy data have yet been published, it is logical to expect that a similar effect and thus temporary protection of the treated wood would occur from application to termite-infested wood of a *M. anisopliae* conidial formulation recently announced for sale in North America, BioBlast™ Biological Termiticide (EcoScience, New Brunswick, New Jersey) (Quarles 1997). Repellent effects elicited by nonpathogenic fungi also may have some utility in termite control (Grace et al. 1992).

Both *M. anisopliae* and *B. bassiana* have been isolated from termites (Zoberi & Grace 1990b, Zoberi 1995) in North America and Hawaii, and the relative virulence of these and other strains has been established in laboratory screenings (Grace & Zoberi 1992, Jones et al. 1996, Wells et al. 1995). Researchers in Australia (Milner et al. 1996) and Japan (Suzuki 1991, 1996) also have screened large numbers of fungal isolates. It is entirely possible that additional pathogens will be found by more extensive examinations of the microflora and fauna associated with different termite species (Zoberi & Grace 1990a). However, although Rosengaus & Traniello (1993) have suggested that pathogens may have had profound effects on patterns of inbreeding and outbreeding in termites, there is still a distinct absence of documentation of naturally occurring epizootics among termite populations. There is also an absence of field efficacy data from microbial applications, with the exception of treatment of mounds with massive quantities of conidia. Suzuki (1996) attempted field applications of three pathogenic fungi (*M. anisopliae*, *B. bassiana*, and *P. fumosoroseus*) against *C. formosanus* but could not establish their efficacy.

Delate et al. (1995), Jones et al. (1996), and Grace (1995) have approached control of *C. formosanus* with pathogenic fungi from the standpoint of bait applications. Isolates of *M. anisopliae* and *B. bassiana* were identified that elicited slow mortality but were highly active at low spore concentrations with little variability in termite responses (Jones et al. 1996). Because of the difficulty of infecting a large proportion of a subterranean termite colony (which may contain several million individuals) with conidial "dusts," these researchers isolated a series of 12 cyclic peptides known as destruxins from *M. anisopliae* (Paš et al. 1981), including three novel compounds (Wahlman & Davidson 1993), and evaluated their potential as bait toxicants for *C. formosanus*. Although feeding on concentrations from 1,500 ppm to 3,300 ppm of destruxin A1 or destruxin E resulted in gradual and consistent termite mortality, subsequent choice tests established that both compounds were too repellent to be effective bait toxicants (Grace 1995). Of course, novel bioencapsulation methods (Grace & Ewart 1996) may prove useful in the future to overcome such repellence.

Living fungal cultures offer a distinct advantage in baiting systems by serving as a constant source of inoculum to termite foragers, thus potentially infecting a larger proportion of the colony than would be possible with dust or aerosol applications of conidia. Delate & Grace (1995) established in laboratory assays that *C. formosanus* foragers would investigate fungal cultures grown on agar-coated paper, leading to transfer of conidia and high mortality despite the repellence of isolated conidia and isolation behavior toward infected individuals (Zoberi & Grace 1990b). However, maintenance of a viable living culture of either *M. anisopliae* or *B. bassiana* within termite bait stations in the field is an extremely challenging proposition.

Potential for Biological Termite Control

Ants certainly act naturally to constrain termite foraging activities to some extent. However, the future of biological control interventions with termites clearly lies with pathogenic microorganisms and microbial control. From a technical standpoint, a mobile or readily distributed and possibly self-perpetuating control agent should mean more complete and less labor-intensive termite control. It must be noted, however, that pathogens generally have little or no mobility on their own, and that isolation behavior towards infected individuals can limit their distribution within the colony. Moreover, there is as yet no published evidence of field efficacy with microbial agents except in mounds, and as temporary repellents. So far as bait development is concerned, either repellence or too-rapid termite mortality will lead to avoidance of the inoculation source and greatly limit any colony-wide effects from the pathogen. Other obvious technical issues that are more difficult to address with a biological agent than with an insecticide are those of quality control, shelf life, and field longevity of the product.

On the other hand, a number of ecological, social, political, and economic factors provide strong motivation for developing microbial control methods for termites. A major factor is the low toxicity of insect pathogens to nontarget organisms and people, which translates into reduced hazard to the applicator, the client, and the environment. From the developer and manufacturer's standpoint, biological agents will likely be candidates for streamlined EPA registration procedures. To the applicator, reduced hazard may mean fewer requirements for public notification prior to application and possibly lower insurance and legal costs.

The technical difficulties are daunting, and research in this area must be considered to be highly speculative. Thus, we should be cautious in our enthusiasm and in commitment of resources to such research. However, the potential payoff from development of a successful system of microbial termite control is so substantial, and the laboratory results to date sufficiently promising, that we must continue to explore its feasibility, albeit with realistic expectations.

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Alternative Control Strategies for Termites¹

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ABSTRACT The relative efficacy of five detection and 12 control methods for termites is reviewed. Chemical and nonchemical control methods are included. Discussions of the latest advances in detection and control focus on seven species: *Cryptotermes brevis* (Walker), *Incisitermes minor* (Hagen), *I. snyderi* (Light), *Coptotermes formosanus* Shiraki, *Heterotermes aureus* (Snyder), *Reticulitermes flavipes* (Kollar), and *R. hesperus* Banks. These species were chosen because they have great economic importance as pests and because studies have been published on alternative control strategies. Tables and discussions recount the historical development as well as strengths and limitations for most termite detection and control methods. Differences between whole-structure and localized treatments also are discussed. Chemical methods are the most predominant termite treatment application. However, uses of alternative methods that emphasize least-toxic and nonchemical applications are increasing. Technological advances in detection are needed to enhance all termite control methods, especially those directed at localized applications. Prospects for the development and public acceptance of alternative termite controls appear good, although population reduction of termites from structures may be a more attainable and realistic goal than elimination as new technologies are developed. The greatest challenges ahead in improving and developing existing and new termite detection and control strategies will be to secure funds for research and to identify mechanisms for rapid dissemination of evolving information to pest control operators and consumers.

KEY WORDS alternative termite control, chemical termite control, nonchemical termite control, Isoptera, Rhinotermitidae, subterranean termite, Kalotermitidae, drywood termite

Of the approximately 2,200 recognized species of termites worldwide, 45 occur in North America (Weesner 1965, Su & Scheffrahn 1990a). However, recent investigations involving cuticular hydrocarbons suggest that there may be considerably more species diversity, at least in *Reticulitermes* (Haverty et al.

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1996, Haverty & Nelson 1997). The economically important species in North America can be classified into three ecological groups: **dampwood termites**—*Zootermopsis angusticollis* (Hagen), *Z. nevadensis* (Hagen); **drywood termites**—*Cryptotermes brevis* (Walker), *C. cavifrons* Banks, *Incisitermes minor* (Hagen), *I. snyderi* (Light), *I. schwarzi* (Banks), *Kaloterme approximat* Snyder, *Marginitermes hubbardi* (Banks), *Neotermes castaneus* (Burmeister), *N. jouteli* (Banks), *Paraneotermes simplicicornis* (Banks), *P. occidentis* (Walker); and **subterranean termites**—*Coptotermes formosanus* Shiraki, *Heterotermes aureus* (Snyder), *Prorehinotermes simplex* (Hagen), *Reticulitermes arenicola* Goellner, *R. flavipes* (Kollar), *R. hageni* Banks, *R. hesperus* Banks, *R. tibialis* Banks, *R. virginicus* Banks, *Amitermes coachellae* Light, *A. emersoni* Light, *A. minimus* Light, *A. snyderi* Light, *A. wheeleri* (Desneux), *Gnathamitermes perplexus* (Banks), *G. tubiformans* (Buckley), and *Tenuirostritermes cinereus* (Buckley) (Su & Scheffrahn 1990a). The major difference among dampwood, drywood, and subterranean groups is the requirement of contact of either soil or moisture by subterranean species. Dampwood termites are primarily restricted to fallen logs rotting on the forest floor whereas drywood termites do not require contact with soil or moisture for survival. Only those species listed as having great economic importance and published studies on alternative control strategies (*C. brevis*, *I. minor*, *I. snyderi*, *C. formosanus*, *H. aureus*, *R. flavipes*, and *R. hesperus*) (Su & Scheffrahn 1990a) are included in this review.

The damage to wooden structures attributed to termites is significant and can exceed \$3 billion annually, although estimates vary considerably by region (Su & Scheffrahn 1990a). Subterranean termites account for at least 80% of the losses; drywood termites account for <20% (Su & Scheffrahn 1990a).

The intent of this paper is to review the important advances in termite detection and control and provide general information on their relative efficacy. For the purposes of this paper, only general references to efficacy – effective, ineffective, or mixed results – and original author citation will be made. Because the mode of action, application, and treatment protection time vary considerably among treatment methods, specific references to numeric efficacy levels can not be made at this time. For example, fumigation is tremendously effective but the structure is vulnerable as soon as the fumigant dissipates from the building, whereas some localized treatment strategies require continual vigilance, such as baits, for remedial and preventive control. From the pest control provider's perspective, the goal of a treatment is to protect the structure. This goal can be accomplished by elimination or simply by trimming back the termite population.

In the discussion on alternative control strategies, chemical and nonchemical methods are included. For the most part, selected academically oriented papers and review articles are cited. Some reports and trade magazine features also are mentioned, but testimonial and marketing documents were avoided. Efficacy of pressure-treated wood for termite prevention (Rust & Scheffrahn 1982, Tamashiro et al. 1988, Grace et al. 1993a, Grace 1997), effectiveness of termite-resistant woods (Grace et al. 1989a, Scheffrahn 1991, Grace & Yamamoto 1994, Delate & Grace 1995), and use of biological control agents (Mauldin &

Beal 1989, Su & Scheffrahn 1990a, Delate et al. 1995a) are not reviewed in this paper and the reader may consult the aforementioned citations on these topics.

Termite Detection

Before a termite-infested structure can be treated, some assessment of the extent of the infestation must be made. Visual searching and probing of wood is the dominant means of inspecting for termites (Scheffrahn et al. 1993). Within California alone, more than 1.5 million inspections are conducted yearly (Lewis & Lemaster 1991). However, the efficiency of visual searches is currently not known. The use of borescopes, a fiber optically based visual search aid, also is marketed. The efficiency of this visual search method for termite detection has yet to be scientifically tested.

Several nonvisual detection methods are being marketed. They include electronic stethoscopes, dogs, and methane gas detectors. All detection technologies have limitations, and care must be shown in their selection (Potter 1997a). Acoustic emission detection has shown promise in laboratory and field investigations (Fujii et al. 1990, Scheffrahn et al. 1993, Hyvernaud et al. 1996, Lemaster et al. 1997, Scheffrahn et al. 1997). Acoustic emission devices are currently not commercially available. Future detection devices that may allow for the nondestructive searching of entire walls could include the use of microwave, infrared, and laser technologies (Lewis et al. 1997).

For subterranean termites, ground-based monitoring devices have been developed and used experimentally to identify and delimit the extent of colonies (Esenther & Gray 1968; La Fage et al. 1973; Tamashiro et al. 1973; Esenther & Beal 1974, 1978; Beard 1974; Esenther 1980; Su & Scheffrahn 1986a; Grace 1989; French 1991a, b; Ewart et al. 1992; Lenz & Creffield 1993; Silvestri 1996; Potter 1997b). One of these ground-based subterranean termite monitoring devices is commercially available (Su 1994a).

Subterranean Termite Control

A list of alternative strategies for subterranean termite control and selected references are provided in Table 1.

Soil termiticides. Applications of liquid termiticides to the soil, forming a chemical barrier between the structure being protected and the termites below, have been the dominant means of subterranean termite control since the late 1940s. The standard measure of acceptable performance is that the chemical barrier must keep termites from penetrating 90% of the barriers for at least 5 yr (Kard 1996a). Laboratory efficacy studies have shown all currently available organophosphate and pyrethroid soil termiticides to be effective (Su & Scheffrahn 1990b, Kard 1996a). The newer soil termiticide imidacloprid suggests a different mode of action toxic to termites attributed to interactions of the termiticide and a mycopathogen (Boucias et al. 1996). Although all currently registered termiticides have undergone rigorous simulated field testing, efficacy results have been mixed (Tamashiro et al. 1989, Tamashiro et al. 1990, Grace et al. 1993b, Kard 1996a). A likely cause for ineffective

Table 1. Summary of subterranean termite control options.

Treatment	Selected references
REMEDIAL	
Soil termiticides	Tamashiro et al. 1989, 1990; Su & Scheffrahn 1990b; Smith & Rust 1990; Grace et al. 1993b; Forschler 1994; McDaniel & Kard 1994; Boucias et al. 1996; Gold et al. 1996; Forschler & Townsend 1996a; Kard 1996a; Forschler & Lewis 1997
Baits	Esenther & Gray 1968; Esenther & Beal 1974, 1978; Beard 1974; Esenther 1980; Su 1994b; Su et al. 1995; Forschler & Ryder 1996; Grace et al. 1996a; Pawson & Gold 1996; Potter 1997b
Topical liquids, dusts, and foams	Randall et al. 1934a; Grace & Abdallay 1990; Su & Scheffrahn 1991a, b; Grace & Yamamoto 1992; French 1991a, b; Myles 1996; Potter et al. 1991
Least Toxic & Nonchemical	
Asphyxiant gases	Delate et al. 1995b, Rust et al. 1996
Extreme temperatures	Woodrow & Grace 1997, Rust & Reiersen 1997, Rust et al. 1997
PREVENTIVE	
Nonchemical	
Particle barriers	Ebeling & Pence 1957, Smith & Rust 1990, Tamashiro et al. 1991, Su & Scheffrahn 1992, French & Ahmed 1993, Lewis et al. 1996
Metal barriers	Lenz & Runko 1993, Grace et al. 1996b, Kard 1996b
Shields	Su & Scheffrahn 1990a

chemical barrier performance, termiticide persistence (active ingredient in ppm), has been investigated; however, field results also have been mixed (Tamashiro et al. 1990, McDaniel & Kard 1994, Gold et al. 1996). Interactions between soils, termiticides, and termites can be complex and achieving a continuous and uniformly treated soil barrier can be difficult (Smith & Rust 1990, Smith & Rust 1991, Forschler 1994, Forschler & Townsend 1996a, Forschler & Lewis 1997).

Baiting. Simply put, successful baiting results after the introduction of a small amount of toxicant contained in a palatable matrix that subterranean termites pass among nest mates via foraging and trophallaxis feeding, leading to the death of the colony. Preliminary knowledge needed to implement any successful baiting program directed against subterranean termites is understanding their foraging behavior. The earliest studies on subterranean termite foraging behavior were for *Reticulitermes* spp. (Esenther & Gray 1968; Esenther & Beal 1974, 1978; Beard 1974; Esenther 1980; Howard et al. 1982) and for *H. aureus* from the desert southwest (Haverty et al. 1975). For these earlier works only indirect measures of colony size were possible. The development of an internal dye made colony census and levels of confidence possible (Lai 1977, Su et al. 1983). The result of this innovation saw the proliferation of mark-recapture-release studies estimating the abundance and foraging range of subterranean termites, both *Reticulitermes* and *Heterotermes* species, in wildland and urban locations (Su & Scheffrahn 1988, Grace et al. 1989b, Jones 1990, Haagsma & Rust 1995, Forschler & Townsend 1996b). Acceptance of mark-recapture-release methods for estimating subterranean termite population size and foraging range are mixed and vary from favorable (Grace 1992) to critical (Thorne et al. 1996).

The last decade has seen the rapid development of baiting technology. Laboratory screening trials have identified several toxicants as candidates to be incorporated into subterranean termite baits (Jones 1984, Haverty et al. 1989, Grace 1990, Su & Scheffrahn 1993, Su et al. 1994). The search for additional active ingredients to be incorporated into baits for subterranean termite control continues today.

The earliest field studies reporting successful control of subterranean termites by using a bait were by Esenther & Gray (1968), Beard (1974), and Esenther & Beal (1974, 1978). Wooden blocks treated with mirex suppressed *Reticulitermes* activity in field plots. Field successes with materials containing other active ingredients have been reported (Su 1994b, Forschler & Ryder 1996c, Grace et al. 1996a). Successful control of subterranean termites by using baits has been mixed, and it is not clear if lack of success has been due to inadequacies of the delivery system or active ingredient (Su et al. 1995, Pawson & Gold 1996). There is also considerable disagreement among researchers and companies that develop baits on whether elimination or suppression are realistic goals for subterranean termite control (Potter 1997b) and on what a termite colony is and measures of population reduction or colony elimination (Kistner 1996). For some products there is little or no published data indicating that the bait will perform as advertised and the disparity in performance among bait products may prove greater than with other product categories, such as conventional liquid termiticides (Potter 1997b). Areas of improved bait

performance include development of additional active ingredients (Potter 1997b), modifications of bait stations (Grace et al. 1995) and the incorporation of materials to increase their appeal and retention of foragers (French 1991a, b).

Topically applied termiticides. Historically, topically applied liquids and dusts have been reported to be effective (Randall et al. 1934a). The active ingredients reported in these earlier works were predominantly chlorinated hydrocarbons or arsenic materials formulated into dry dusts. More recently, active ingredients containing borates have dominated efficacy reporting (Grace & Abdallay 1990; Su & Scheffrahn 1991a, b; Grace & Yamamoto 1992). French (1991a, b) and Myles (1996) reported field success for subterranean termite control by topically applying insecticides onto foragers and releasing them back into the colony. Using topically coated foragers to introduce a toxicant into a colony is similar to baiting and assumes trophallactic passing of food throughout. A slight modification of liquid usage of termiticides, foam, also has been investigated (Potter et al. 1991). For remedial use, the effectiveness of topically applied liquids, foams, and dusts appears to be mixed for controlling subterranean termites (Grace & Abdallay 1990, Grace & Yamamoto 1992, Myles 1996, Potter et al. 1991).

Least toxic and nonchemical methods. Laboratory trials have been conducted on the use of asphyxiant gases (i.e., CO₂ and N₂) and extreme temperature to control subterranean termites (Table 1). These studies have focused on controlling aerial subterranean termite nests. Aerial nest formation does occur among species of several genera of subterranean termites (Rhinotermitidae). However, *C. formosanus* is frequently reported as forming aerial nests (Su & Scheffrahn 1990a). For asphyxiant gases and extreme temperatures, the reported efficacy results are very good; however, they represent laboratory studies for *C. formosanus* and *I. minor* only (Delate et al. 1995b, Rust et al. 1996, Woodrow & Grace 1997). Extreme temperatures derive from either the use of liquid nitrogen or convection heat. For liquid nitrogen, the core temperature of the individual termite must reach at least -19.5°C to cause 100% mortality for *C. formosanus* (Rust & Reiersen 1997, Rust et al. 1997). For heat, the minimum lethal threshold temperature for *C. formosanus* has been reported to be 44°C for 20 min (Woodrow & Grace 1997). Field verification tests for both asphyxiant and extreme temperatures are needed, especially for other species and genera of subterranean termites.

Physical barriers. Ebeling & Pence (1957) first reported the use of sand as a physical barrier for excluding subterranean termites from structures. Their findings were later reaffirmed by other laboratory (Smith & Rust 1990, Smith & Rust 1991, Tamashiro et al. 1991) and field studies (Tamashiro et al. 1991, Su & Scheffrahn 1992, Kard 1996b, Lewis et al. 1996). Field efficacy studies using sand barriers to exclude subterranean termites from structures have been mixed (Tamashiro et al. 1991, Su & Scheffrahn 1992, Kard 1996b, Lewis et al. 1996). Penetration of sand barriers by subterranean termites was attributed to use of incorrect particle size and presence of structural irregularities (Su & Scheffrahn 1992, Lewis et al. 1996). Frequent monitoring of sites also may be needed to ensure that termites do not construct foraging tubes over barriers (Lewis et al. 1996). Other barrier materials tried to exclude subterranean termites from structures include crushed granite (French &

Ahmed 1993), glass splinters (Pallaske & Igarashi 1991), and stainless steel mesh (Lenz & Runko 1993, Grace et al. 1996b, Kard 1996b). Metal shields have had mixed effectiveness and diatomaceous earth is not an effective barrier against subterranean termites (Su & Scheffrahn 1990a, Grace & Yamamoto 1993).

Drywood Termite Control

Drywood termite control can be classified as whole-structure or localized treatments (Table 2). Whole-structure treatment is defined as the simultaneous treatment of all wooden members, whereas localized treatment is restricted to a group of boards or locations within boards (Scheffrahn & Su 1994). For localized treatments, accuracy in detection of and determining the extent of drywood termite infestation is critical to optimizing pest control service and providing effective treatment (for discussion on detection methods see the Termite Detection section). The discussions presented in the following sections pertain to *C. brevis*, *I. minor*, *I. Snyderi*, and *M. hubbardi*, the most commonly encountered drywood termite pests in structures throughout the continental United States and Hawaii.

Whole-structure treatments. Two fumigants are currently registered for drywood termite control: methyl bromide and sulfuryl fluoride (Scheffrahn & Su 1994). However, many more were previously used (Randall et al. 1934b). Fumigants are very hazardous materials and require highly specialized training in their safe use and structural preparations to prevent or minimize any disruption or damage. Fumigation is considered effective and is supported by many studies (Su & Scheffrahn 1986b, Osbrink et al. 1987, Scheffrahn & Su 1992, Lewis & Haverty 1996a). The use of synergists, approximately 10% CO₂, also can enhance fumigant performance (Scheffrahn et al. 1995). Desorption and residual studies for at least one of the fumigants, sulfuryl fluoride, report its safety for many household commodities if properly used and the structure is adequately aerated after treatment (Kenaga 1957; Osbrink et al. 1988; Scheffrahn et al. 1987; 1989a, b). Asphyxiant gases (CO₂ and N₂) show promise as whole-structure treatments for some drywood termite species but only laboratory studies have been conducted thus far (Delate et al. 1995b, Rust et al. 1996).

Whole-structure heating for controlling drywood termites was first reported several decades ago (Ebeling 1975). However, laboratory and large-scale field validations have only recently been reported (Ebeling 1994, Lewis & Haverty 1996a, Woodrow & Grace 1997, Rust & Reiersen 1997). The heat tolerance for drywood termites appears higher than that of subterranean termites, 49°C versus 44°C, respectively (Rust & Reiersen 1997). Whole-structure treatments with heat appear to be effective. However, unsuccessful control using heat can be due to the occurrence of heat sinks. Heat sinks are areas within a structure that are more difficult to heat, for example, wood on concrete (Lewis & Haverty 1996b). The use of heat is unique in being both a whole-structure and localized treatment control method (Table 2). Pretreatment preparations to prevent and minimize structural and household item damage from heat treatment have been reported (Forbes & Ebeling 1986, Ebeling 1994).

Table 2. Summary of drywood termite control options.

Treatment	Selected references
WHOLE STRUCTURE	
Fumigation	Su & Scheffrahn 1986b, Osbrink et al. 1987, Scheffrahn & Su 1992, Scheffrahn et al. 1995, Lewis & Haverty 1996a
Asphyxiant gases	Delate et al. 1995b, Rust et al. 1996
Heat	Ebeling 1994, Lewis & Haverty 1996a, Woodrow & Grace 1997, Rust & Reiersen 1997
LOCALIZED TREATMENTS	
Chemical	
Topical liquids and dusts	Randall et al. 1934a, Ebeling 1975; Scheffrahn et al. 1979, Scheffrahn & Su 1994, Scheffrahn et al. 1997
Foams	?
Liquid nitrogen	Forbes & Ebeling 1986, Lewis & Haverty 1996a, Rust & Reiersen 1997, Rust et al. 1997
Nonchemical	
Microwaves	Lewis & Haverty 1996a
Electrocution	Ebeling 1983, Lewis & Haverty 1996a
Screens, caulk, and paint	?

? Indicates little or no published information.

Localized treatments. The earliest localized treatments directed against drywood termites relied on arsenic-containing dry dusts applied directly into drywood termite galleries (Randall et al. 1934a, Ebeling 1975). Most of these earlier materials are not registered. The effectiveness of newer chemicals appears to be mixed (Scheffrahn et al. 1979, Scheffrahn & Su 1994, Scheffrahn et al. 1997). Localized treatments with these chemicals can be enhanced by drilling into infested locations; however, this post-construction method of application is destructive to wall coverings and wood. For drywood termites, because of cheaper costs and possible avoidance of the disruption and expense of fumigation, localized treatments are increasingly being used (Lewis & Haverty 1996a).

A least-toxic chemical alternative to treating wood includes the use of liquid nitrogen (Table 2). Extreme cold $< -20^{\circ}\text{C}$ is used to kill drywood termites (Rust & Reiersen 1997, Rust et al. 1997). After drilling a hole near the top plate of wall voids, liquid nitrogen under pressure in large dewars is gravity fed directly into wall voids. Relative efficacy of localized treatments with liquid nitrogen is highly dosage dependent and varies from ineffective to effective (Lewis & Haverty 1996a). The limitations of this localized treatment include: many locations within structures are not treatable with this method, drilling holes damages wall coverings and wood, large amounts of liquid nitrogen may be needed, and accurately monitoring temperature changes is critical to success (Lewis & Haverty 1996a, Rust & Reiersen 1997, Rust et al. 1997).

Several nonchemical methods for locally treating drywood termites are commercially available (Table 2). Two are based on elevating levels of heat: convection heat and microwaves. The effectiveness of localized convection heating in controlling drywood termites for field conditions has yet to be published in refereed journals. Not unlike whole-structural heating, heat sinks may reduce the effectiveness of localized applications and care must be taken not to damage household items. The use of microwaves (700 watts), internally generated heat, to control drywood termites also has been reported (Lewis & Haverty 1996a). Effectiveness of using microwaves in controlling drywood termites was reported as mixed (Lewis & Haverty 1996a). However, more powerful devices ($>10,000$ watts) have been developed but their effectiveness is currently not known. Scorching can occur if the temperature of wood is not monitored during treatment (Lewis & Haverty 1996a).

High voltage electricity, or electrocution, is another nonchemical option for controlling drywood termites. The device currently marketed uses high voltage (90,000 volts) but low current (<0.5 amps). Reported efficacy has been mixed and highly dependent on applicator technique (Ebeling 1983, Lewis & Haverty 1996a). The administration of high levels of heat is the probable cause of mortality, although the exact mode of action is not known. For maximum effects, high-voltage bursts of electricity are directed at galleries containing termites. Effects can be enhanced by drilling holes and inserting metal pins into wood (Lewis & Haverty 1996a). Success also can vary depending on proximity to certain building materials (Lewis & Haverty 1996a). The drilling of wall coverings and wood for insertion of metal pins is a destructive treatment technique.

Other nonchemical methods experimentally tried against drywood termites are biological control and physical barriers, e.g., screens, caulk, and paint.

There is little published information of the relative efficacy of biological control, screens, caulks, or paints directed against drywood termites (Scheffrahn & Su 1994).

Conclusions

In the past, we have used pest control strategies that provided excellent, albeit excessive, termite control. We have come to expect that any new treatment will provide total elimination of termites. Clearly this is the goal of structural treatments when properties are sold (real estate transactions). However, some existing and past standards of termite control, i.e., chlordane, aldrin, dieldrin, heptachlor, and methyl bromide are or will soon be no longer available. Do we need to prepare the general public and pest control industry for the possibility that total elimination is not really feasible? Will the "cost" of total elimination be too great for some consumers, and are they willing to accept some degradation of their structures when using localized treatments and least toxic approaches to termite control? Will we eventually adopt an IPM approach to control of termites that includes monitoring and population reduction, not elimination? These questions and more will need to be addressed before innovations in termite detection and control can effectively proceed.

Optimistically, prospects for the development of new and improvements of existing technologies as well as public acceptance of alternative termite controls appear good. Least-toxic and nonchemical methods have and will continue to be developed. For subterranean termites, baits will play a major role in their control. However, materials that increase bait appeal and retention at stations are needed. Additional information needed includes a more detailed understanding of subterranean foraging behavior. Advances in detection, especially those viewing entire walls nondestructively, will improve all termite control methods; whole-structure and localized treatments. The greatest challenges ahead will be securing funds for research, and identifying mechanisms for disseminating rapidly evolving information to pest control operators and consumers.

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Biorational Approaches to Flea (Siphonaptera: Pulicidae) Suppression: Present and Future¹

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ABSTRACT Cat fleas (*Ctenocephalides felis felis* [Bouché]) are common pests of the urban environment, both in homes and around the premises. Successful management involves suppression of fleas indoors, outdoors, and on the pet. Alternative control methods for development of integrated management systems may include chemical, biological control, sanitation, mechanical, environmental modification, host animal resistance, semiochemical, and genetic techniques. Because of the close association of pets and flea habitat to humans, both on-animal and environmental chemical use may maximize opportunities for human exposure to pesticides. For adoption, alternative strategies must be easy to use, unobstrusive, and effective.

KEY WORDS cat flea, Siphonaptera, Pulicidae, *Ctenocephalides felis felis*, IPM, biorational, IGR, biological control, larvicide

Cat fleas (*Ctenocephalides felis felis* [Bouché]) are common pests of the urban environment, both in homes and around the premises. Fleas affect the home's occupants by causing itching and irritation. In addition, they produce flea allergy dermatitis in hyperallergic animals and serve as the obligate intermediate host of the dog tapeworm, *Dipylidium caninum* (L.). Their shared intimacy with pet owners makes ectoparasites of companion animals a public health menace (Koehler et al. 1994). Cat fleas can serve as bridges for zoonoses such as murine typhus and plague to move from wildlife into human habitations (Williams et al. 1992). Public health and personal comfort make integrated pest management (IPM) of pests of humans and animals problematic. Successful management involves suppression of fleas indoors, outdoors, and on the pet.

Children and adults hug and pet the companion animal, thereby exposing themselves to substances on their pet. Modes of insecticide application such as dusts, sprays, dips, and shampoos increase applicator exposure (Davis et al. 1992). Environmental treatment necessitates application of insecticides to virtually the entire carpeted area of the home, as carpet is the habitat of the developing flea

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(Byron 1987). The volume and rate of insecticide applied in flea treatments is typically greater than that for any other indoor pest (Dryden & Rust 1994). Consequently, the concern for both human and pet safety is the impetus behind the development of alternative methods of flea suppression.

Development of biorational controls for arthropod pests of companion animals has been limited by three main features. First, this is a niche market, with little economic incentive to develop specific products for pests of companion animals. Second, development of insecticide resistance in fleas has made many traditional insecticides ineffective (WHO 1992). Because the action threshold is so low, treatment for fleas is essentially continuous, with virtually 100% of the population under selection pressure. As only a few products are registered for fleas, and compounds from the same chemical class are often used against several life stages, selection pressure is intense. And third, clientele are demanding, simultaneously, a virtually flea-free environment and the avoidance of "hard" traditional pesticides.

Chemicals

Historically, flea control has been based on the use of traditional insecticides, including chlorinated hydrocarbons, cyclodienes, organophosphates, carbamates, and pyrethroids for both premise and on-animal treatment. Recent introductions include imidacloprid (Advantage[®]), a nitroguanidine that works by binding to nicotinic receptor sites on the postsynaptic neuron and thus disrupting the insect nervous system (Moffat 1993). Another new product is fipronil (Frontline[®]), a phenylpyrazole that acts on invertebrate gamma-aminobutyric acid receptors (Postal et al. 1995). Both Advantage[®] and Frontline[®] are formulated as on-animal adulticides.

In the last 2 decades, a major shift has occurred with the introduction of insect growth regulators for flea suppression (Brakke 1988). Products are formulated for animal treatment and environmental application. Juvenile hormone mimics such as methoprene (Moser et al. 1992), fenoxycarb (Marchiondo et al. 1990), and pyriproxyfen (Meola et al. 1996) have been used for suppression of immature flea stages in homes. Those exhibiting photostability, fenoxycarb and pyriproxyfen, also have been registered for outdoor use (Palma & Meola 1990).

Adulticides. Adulticides act directly on adult fleas and typically are formulated for rapid knockdown and kill. Their mode of action may target the nervous system or other vital body processes. Adulticides are applied to environmental surfaces where adult fleas will contact them (Koehler et al. 1986).

Traditional insecticides. Outdoor flea suppression remains one of the most challenging components of flea control programs, with many registered compounds having low efficacy, and even the most effective ones having limited residual activity (Metzger et al. 1996). Potential developmental sites must be identified and treated with an appropriate rate of insecticide, but then environmental conditions rapidly degrade most compounds.

On-animal products must have several features to be successful. These include ease of use (to ensure owner/user compliance), low mammalian toxicity,

and rapid efficacy against fleas. Treatment of the pet exploits the adult cat flea's dependency on its host, in effect making the host analogous to a "trap crop" (Kissileff 1962).

Botanical insecticides. The botanical compounds pyrethrum, sabadilla, and rotenone have been recommended for flea suppression (Bishopp 1921). Limonene (Hink & Fee 1986) and linalool (Hink et al. 1988) have been labelled for flea control. The potential exists for the development of other herbal extracts as pulicides, particularly those with folk-history such as the aptly named fleabane and pulegone from certain plants (Chatterjee et al. 1968, Sudekum et al. 1992, Nicholson 1995). Neem also has been found to be toxic to fleas (Kilonzo 1991).

Interestingly, egg, larval, and adult cat fleas may be killed with dusts containing small amounts of naturally occurring hexa-hydroxyl fragrance oils such as alpha-terpineol, benzyl acetate, benzyl alcohol, eugenol, phenylethyl alcohol, and others (D. A. R., unpublished data). Some of the fragrance substances most active against fleas have high insect specificity, and initial studies have shown that they apparently interfere with the insect octopamine system, resulting in paralysis that mimics the paralysis produced from exposure to pyrethroids. Closely related compounds such as benzyl benzoate, diethyl phthalate, and dipropylene glycol are not active. Some of the active substances are safe for humans, and are presently being used as Food and Drug Administration-approved flavor and odor additives in cosmetics, foods, and air fresheners. Dust diluents and aerosol propellants increase cuticular penetration, greatly enhancing the activity of the fragrance oils against fleas. At 98% RH, as little as 9.7 cc/m² of a proprietary calcium carbonate-based dust containing <1% alpha-terpineol (EcoSafe, Inc., Roswell, Georgia) provided 100% kill of adult fleas in deep shag carpeting within 24 h. At 60% RH, only 2.2 cc/m² was needed to provide complete kill (D. A. R., unpublished data). Mixtures and analogous fragrance substances probably will be proven to be even more efficacious.

The assumption is that hematophagous arthropods such as fleas, having had less evolutionary pressure to develop strategies for dealing with plant allelochemicals, are more likely to be susceptible to botanical insecticides than are phytophagous pests. However, botanical compounds are not free of toxicity to mammals and nontarget species and may offer no inherent advantage over synthetic pesticides (Hinkle 1995).

Larvicides. In general, larvicides act on the principle of suppressing immature fleas before they reach the adult biting-pest stage. We discuss three types of larvicides against cat fleas: insect growth regulators, insect development inhibitors, and borates and other stomach toxicants.

Insect growth regulators. Larvicides that mimic the effect of juvenile hormone have included methoprene, fenoxycarb, and pyriproxyfen. Pyriproxyfen, in addition to having activity against immature flea stages, has been demonstrated to produce mortality in fleas exposed as adults, due to histopathological damage to internal tissues (Meola et al. 1996).

The juvenile hormone analogs are active against larval cat fleas and effective in reducing development of larvae to adults (Palma & Meola 1990, Palma et al. 1993, Moser et al. 1992). Presumably this inhibition of adult emergence should

be reflected in fewer fleas infesting the hosts, with on-host numbers decreasing over time due to host grooming and flea population senescence (Silverman et al. 1981).

Insect developmental inhibitors. The two insect developmental inhibitors cyromazine and lufenuron have been registered for larval flea suppression. Both are given orally to the host animal, absorbed into the bloodstream, and ingested by feeding fleas. The specific mode of action of cyromazine is not clearly understood, but it appears to disrupt or inhibit ecdysis (Friedel 1986, Shipstone & Mason 1995).

Lufenuron is incorporated into the egg prior to oviposition, so that the affected embryo is unable to break through the chorion and emerge (Hink et al. 1991, Zakson et al. 1992). Larvae feeding on adult flea feces containing lufenuron also are affected, being unable to successfully molt due to the inability to form a new exoskeleton (Zakson et al. 1992). Lufenuron-contaminated flea feces thus serve as a bait for larval fleas. Other chitin synthesis inhibitors such as alsystin and diflubenzuron (el-Gazzar et al. 1988) have shown promise for indoor and outdoor uses because of their environmental stability and activity against larval fleas (Henderson & Foil 1993).

Development of insect growth regulators and other physiological mimics is a promising direction for biorational strategies against ectoparasites (el-Gazzar et al. 1986, Spindler-Barth 1992). Because they do not affect adult flea populations but function by reducing environmental infestation and subsequent host reinfestation, prophylactic insect growth regulator applications must be made prior to buildup in flea populations. Because of their photostability and prolonged residual efficacy, some of the insect growth regulators and insect development inhibitors such as pyriproxyfen and diflubenzuron may prove useful for treating outdoor infestations (Palma & Meola 1990, Henderson & Foil 1993). Insect growth regulators are active at low rates and have prolonged residual lives (Hinkle et al. 1995a, Kawada & Hirano 1996), so their use actually reduces the environmental insecticide load (Wright 1976).

Borates and stomach toxicants. Borate products also have been demonstrated to be effective larvicides at low rates (Hinkle et al. 1995b). They have additional benefits of long residual efficacy and low mammalian toxicity. Applications of 5% and 10% disodium octaborate solutions with a standard rental carpet cleaning machine to carpets provided >90% kill of larvae for at least 56 d (Rust & Reiersen, unpublished data). Applied in such a way as to contaminate the flea larval food, borates serve as a type of poison bait; formulated in a food matrix, the rate of toxicant could probably be decreased (Klotz et al. 1994).

Other compounds may be similarly used as larval toxicants. Although larval rearing media treated with 2%-5% sodium bicarbonate was not toxic to flea larvae, media containing 2%-5% calcium carbonate killed 85%-88% of larvae (Rust & Reiersen, unpublished data).

Biological Control

Biological control components are limited for fleas. Biological control is the antithesis of "eradication" because it assumes that some portion of the pest

population will be left to maintain the beneficial population. Most people are willing to accept this concept and practice in agricultural situations, but not in their own homes. In addition, the development of application technology may be even more challenging than finding possible biological control agents.

Biological control of fleas is problematical in that most of the effective pathogens of fleas also are pathogens of humans. For instance, the plague bacillus, *Yersinia pestis* (Yersin), is eventually fatal to virtually all infected fleas. However, the feeding activity of the infected flea prior to its death typically results in further propagation of the infection in mammalian hosts. Development of dog tapeworm cysticercoids can produce >90% mortality in infected fleas (Chen 1934, Marshall 1967). However, in these cases, the attempted solution with biological control is actually worse than the problem.

Fleas do have the added advantage of virtually assured indirect vertical transmission, in that a major portion of the larval diet is the excrement of the adult flea. Thus, infectious agents acquired by the adult will likely be passed along to the developing larvae. However, as the adult flea feeds only on vertebrate blood, there is little chance for infection other than via the host—either pets or humans.

Beard (1988) surveyed potential biological control agents for fleas and summarized the disappointing results by saying, "Biological control is in its infancy with respect to pest management of fleas." Of the possible infectious agents he identified, all appeared to have limited potential as pathogens. Parasitized hosts survived heavy infections and the most frequently observed microorganisms produced heavy infections with no apparent pathogenic effects (Beaucornu & Deunff 1976). Most investigators looking for potential parasites and pathogens, including protozoa and bacteria (Beard et al. 1990), have found only marginally harmful symbionts. No insect-pathogenic viruses have been reported from fleas (Beard 1988).

Other than *Bacillus thuringiensis* Berliner (*Bt*), few of the biological control agents observed have produced high rates of mortality in fleas (Castillo 1980). Although *Bt* parasporal crystals were not found to be toxic, the beta exotoxin produced both developmental abnormalities and mortality (Maciejewska et al. 1988). Some *Bt* products have been patented for other ectoparasites (Payne & Hinkle 1993), but development of effective delivery systems remains challenging.

Host grooming is the most significant mortality factor for adult fleas on the host, with most being removed by the animal within a week (Wade & Georgi 1988). Cats were found to vary in their grooming efficiencies, with poor groomers removing only 4.1% of their flea load per day whereas better groomers removed 17.6% (Hinkle 1992).

Generalized predators such as ants (Fox & Garcia-Moll 1961) and beetles (Fox & Bayona 1968), can have a significant impact on larval flea numbers. As with any on-host pest, there is little opportunity for establishing populations of beneficial arthropods to serve as either parasites, predators, or competitors of the ectoparasites. In general, any arthropod is objectionable on companion animals.

The only known parasitoid of any flea was found parasitizing squirrel fleas in the family Ceratophyllidae (Waterston 1929). The cryptic habits of

immature fleas, particularly pupae in the silken cocoon, reduces their susceptibility to arthropod invasion (Silverman & Appel 1984).

The singular biocontrol option currently marketed for flea control is the nematode *Steinernema carpocapsae* Weiser (Manweiler 1994). Although usage requirements (substrate, temperature, humidity) are restrictive, these parasitic nematodes are effective against the larval and pupal stages in specific outdoor settings (Silverman 1981, Henderson et al. 1995).

Sanitation

Sanitation is helpful in removing flea eggs before they hatch and in reducing the food available to developing larvae (Robinson 1995). The cleaning action of a standard rental carpet cleaning machine was shown to be inadequate to remove all larval rearing media from carpets and to prevent larval development (Rust & Reiersen, unpublished data). Although vacuuming may remove >90% of flea eggs in carpet, only 15%-27% of larvae are extracted via this method (Byron 1987). Additionally, cleaning pet bedding helps prevent and eliminate infestations because off-host stages are concentrated around areas where pets spend large portions of time (Kern 1993). Ewing (1929) recognized the significance of flea development in pet bedding, saying "if dogs or cats are allowed to sleep in the house, they should be given a mat or rug to lie upon. This mat or rug should be regularly taken out and shaken and left for a few hours in the sun."

Mechanical

One type of mechanical control includes keeping the animal isolated from a chance of infestation. This method may work with cats that can be confined indoors all the time, but it does not work well with pets that occasionally are allowed outdoors, even for brief intervals. The catholic taste in hosts exhibited by *C. felis* means that virtually any mammal venturing through the property may provide flea contamination to be acquired by a passing pet (Rust & Dryden 1997). Domestic pets often pick up ectoparasites from wild or feral animals, as well, amplifying the opportunity to pass along infections such as bubonic plague, murine typhus, and cat scratch fever. Animals that roam have opportunities to acquire and introduce into the home environment a variety of disease organisms. Thus, exclusion of wild and feral animals from property occupied by pets reduces the opportunity for transmission of flea infestations and their associated diseases.

Traps are physical or mechanical means of eliminating fleas. The concept of a lighted candle in a bowl of soapy water being used to lure in fleas and drown them is ancient (Gaaboub & Abu-Hashish 1974) but has been updated and made more sophisticated by blinking a light and optimizing the wavelength of light used (Dryden & Broce 1993).

Silica gel and diatomaceous earth are reportedly effective larvicides, resulting in lethal desiccation by adsorbing or abrading the protective epicuticular layer (Ebeling 1961). Insects exposed to these dusts lose body water faster than it can be replaced. This water loss is particularly critical for

flea larvae that require high humidities for development (Silverman et al. 1981). However, effectiveness diminishes at high humidity because of the low drying property (i.e., low saturation deficit) of air at high humidities.

Flea combs can be used to mechanically remove adult fleas from the animal's coat; however, their use requires time and effort and is limited by the animal's temperament and haircoat (Olkowski et al. 1991, Kuepper 1995). It is widely recognized that combing removes only a small percentage of the fleas on an animal. Infestation of hosts with known numbers of fleas and combing within 1 h of placement on the animal results in removal of <25%-30% of the original population (Kwochka 1987). Even with intensive combing under circumstances where the number of fleas was known, only 94% of the fleas were recovered from the animal (Dryden 1989).

Environmental Modification

Alteration of the habitat can make the environment inhospitable such that it cannot support larval survival. High temperature and low humidity are significant mortality factors, especially for immature flea stages (Silverman et al. 1981, Silverman & Rust 1983), so landscape modifications that expose potential developmental sites to drying will reduce survival.

Salt has historically been used as a "siccative" or desiccant in areas where immature fleas develop, by scattering salt on the area dry (Metcalf & Flint 1939) or then wetting it down thoroughly (Bishopp 1921, Furman 1971). Near the coast, soil around runways and kennels was treated with seawater (Ewing 1929).

Flooding can be used not only to drown larvae but also to dissolve the adult fecal material that serves as larval food (Kern 1993). Soil moisture >30% increased cat flea larval mortality to 90% (Silverman & Rust 1983).

Host Resistance

Host resistance may be either physiological or behavioral (Chandy & Prasad 1987). Physiological resistance may be either inherent or induced. Some hosts have been found to be less supportive of flea development than others. However, it is not known if such differences are due to physiological factors, genetic predisposition, or behavioral factors. Some hosts are better groomers than others (Silverman et al. 1981). Most people do not select their pets based on whether or not the animal can support fleas. In fact, flea-allergic animals exhibit intensified grooming efforts making it more difficult to find fleas on symptomatic animals (Kissileff 1962), and the intense grooming exhibited by some animals is a quality that makes them hyperactive and unaffectionate (Hart 1992, Dryden & Rust 1994). While devoting attention to scratching fleas, a dog or cat is less involved in interactions with the owner.

Physiological host resistance may be due to such attributes as skin pH and blood constituents, or to morphological features such as skin thickness, sebum production, and density of hair coat. Genetic engineering may be used to produce breeds that do not support fleas, as by preventing feeding, reducing viability, or lowering fecundity (Muller & Brem 1994).

Immunologic concepts are being explored for fleas (Opdebeeck & Slacek 1993, Heath et al. 1994). Although a flea would still have to feed on an immunized host, theoretically the subsequent reaction would either result in flea mortality or reduce its fitness, perhaps by lowering its reproductive capacity (Heath et al. 1994). The immunology of reactions to flea antigen is poorly understood. Flea bites typically produce severe urticaria, often leading to frenzied scratching and self-induced alopecia and excoriation. Attempts at inducing host immunity would necessitate isolation of the allergenic fraction to prevent such severe side effects.

Semiochemicals

Pheromones and other natural products might be used to alter flea behavior to make them less objectionable, as feeding deterrents, mating confusants, or by blocking host location by interfering with chemoreceptors (Slifer 1980). Repellents might be developed to prevent adults from either finding a host or entering a treated area. A better understanding of flea visual and chemical perception is needed before this can be optimally exploited (Benton & Yee 1965).

Genetic Control

As with any blood-sucking arthropod, the presence of the pest is sufficient to be objectionable. So it is difficult to introduce genetic characters because the modified individuals are as undesirable as the natural ones.

Summary

Dryden & Rust (1994) and Rust & Dryden (1997) have provided reviews of current control strategies for fleas, both on the host and in the environment. Overwhelmingly, these tactics are based on insecticides. Development of biorational strategies for suppression of fleas is in its infancy and additional research is needed to better understand the pest and to identify potential points of attack. The following are some obvious gaps in current knowledge:

- Understanding of host-ectoparasite interactions (including host-seeking behaviors);
- Vulnerabilities of the pre-emerged adult within the cocoon;
- Triggers and methods of adult flea emergence;
- Role of semiochemicals, pheromones, and other behavioral chemicals affecting host location, mating, and larval food location;
- Determination of susceptible stages in the life cycle;
- Investigation of flea ecology in outdoor settings, including microhabitats and use of alternative hosts;
- Method of flea overwintering;
- Delivery systems for insecticides; and
- Techniques for population monitoring.

Action thresholds for fleas extend beyond economic injury or even aesthetic injury to include "comfort level." Pet owners have their own internal (and variable) tolerance for what they think they and their pet should be exposed to. An animal that is being tormented by fleas does not perform well and may exhibit behavioral problems. In the show ring, an insect-induced welt or coat discoloration caused by insect feeding can be a major detraction. Arthropod-induced appearance defects such as depilation, hyperkeratosis, and depigmentation are significant detriments for show animals and family pets. Flea allergy dermatitis in cats and dogs is sufficiently distressing to some owners that they have the animal euthanized to alleviate the suffering (Fenster 1985).

Monitoring is one of the challenges of developing an effective flea suppression program. Typically, evaluation of the program is based on the homeowner's perceptions, but more objective assessment methods are needed. The "white sock" technique involves the investigator walking through an area wearing knee-high white socks and counting adult fleas clinging to them; this monitoring technique has been used to assess the numbers of adult fleas in homes (Osbrink et al. 1986). Light traps and glue boards may provide the pest control operator with a means of determining extent of control obtained (Dryden & Broce 1993). Hand-held vacuum cleaners have been used for sampling and modifications of this technique could be used for household monitoring (Osbrink et al. 1986).

The factors that make development of alternative pest management strategies for pests of companion animals most desirable are the same factors that make it most challenging. The intimacy of the environment, with pet-owners spending time in close proximity with their animals, exposes humans to both the pest and the chemicals used to treat for it. Clientele are demanding nonchemical alternatives and insecticide resistance is rendering current pesticides ineffective.

Currently, IPM programs for control of arthropod pests of companion animals are restricted by the limited availability of usable components. There is a tremendous need for research into basic biology and behavior of the pest, coupled with discovery and development of alternative strategies, including novel insecticides and innovative delivery systems, that might be employed in an integrated system.

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Alternative Methods for Suppression of Pantry Pests¹

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ABSTRACT There are many biorationals available for the control of pantry pests, including traditional biological control agents such as parasitoids, predators, and pathogens, as well as alternative biorationals such as temperature, pheromones, and protective packaging. Although most traditional biorationals are not acceptable due to the problems associated with food contamination, some of the alternative biorationals offer acceptable ways to prevent an infestation, rather than eliminate it after the fact. Temperature modification and protective packaging offer the consumer the best combination of food safety and reliability. Both the benefits and problems associated with traditional biocontrol agents and alternative biorational strategies are discussed as they pertain to use within the home pantry.

KEY WORDS stored-product insects, biorationals, temperature, pheromones, biological control, pantry pests

Biorationals used to suppress pantry pests include the traditional biocontrol agents such as predators, parasitoids, and pathogens, as well as the alternative biorationals such as temperature, pheromones, and packaging. The diversity of the pantry pest species complex must be considered when examining these control agents because, unlike other urban pest systems where only a few species of termites, fleas, or ants must be dealt with, a single item of food in a pantry can be confronted not only with multiple species within the same genera but also multiple families, and in many cases, multiple orders. For example, confused flour beetle (*Tribolium confusum* Jacquelin du Val), Indianmeal moth (*Plodia interpunctella* [Hübner]), sawtoothed grain beetle (*Oryzaephilus surinamensis* [L.]), and warehouse beetle (*Trogoderma variable* Ballion) may coexist within the same pantry or container of food. In a spice jar, cigarette beetles (*Lasioderma serricornis* [F.]) may be the primary infestor, but other pests then use their exit holes to reinfest the contaminated food. This complexity often results in the need for several control strategies.

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A final consideration in choosing a control strategy is how to deal with pests of directly consumable goods. Consumers have different restrictions on what they are willing to accept with a directly consumable good compared with the environment. Contamination of a food product, whether it is a beneficial or pest, is not acceptable.

Many predators are available to prey on stored-product insects (Arbogast 1984). There are representatives in the beetle families Carabidae, Staphylinidae, and Histeridae that attack and consume stored-product insects (Rees 1987, 1991; Brower et al. 1996). Unfortunately, most of these insects do not have a major impact on regulating population growth. One insect that has been extensively studied, the warehouse pirate bug, *Xylocoris flavipes* (Rueter), does appear to have some regulating ability when released into warehouse or bulk grain facilities (Press et al. 1975, Arbogast 1976, LeCato et al. 1977, Brower & Mullen 1990). This insect will attack most immature stages of moths and beetles (Jay et al. 1968). Thus, it could potentially control a multispecies complex. However, releasing a warehouse pirate bug into a homeowner's kitchen, pantry, or food storage container to eliminate a pantry pest infestation is not going to be well received, mainly because it involves a food item that could be consumed in the immediate future. If the consumer were willing to clean the food of a predator, he or she would have been willing to remove the pest. The most probable use of predators is farther back in the food production chain, such as raw ingredients that undergo additional cleaning prior to consumption. This application is certainly reasonable and has been used.

Options for the use of parasitoids for pantry pests also exist. Numerous parasitic Hymenoptera feed or oviposit on stored-product insects, including beetles, weevils, and moths (Press et al. 1982, Wen et al. 1994, van Huis et al. 1991, Brower et al. 1996). Most of the research on the efficacy of parasitoids has been targeted at moths (Pyrilidae) because they are the most destructive pests of stored food. *Trichogramma pretiosum* Riley, an effective egg parasite, can reduce Indianmeal moth populations by 57.4% (Brower 1988). The braconid wasp *Bracon hebetor* Say parasitizes late instars of all pantry pest moths and can reduce their populations by 97% (Press et al. 1982, Keever et al. 1986). However, as with predators, the most probable use of these biological control agents is with raw ingredients that will undergo additional cleaning.

Application of a pathogen in pantry pest control is similar to the use of a grain protectant. Pathogens can be applied as a dust or wettable powder to bulk stored food; however, application to processed food, except possibly popcorn, is undesirable. Many pathogens, including viruses, bacteria, protozoa, and fungi, infect stored-product insects (Arbogast 1984). Unfortunately, most pathogens of stored product insects do not cause high mortality (McGaughey 1975, Kinsinger & McGaughey 1976, McGaughey & Beeman 1988). They tend to cause a debilitating disease, and some individuals may not be affected. Whether an insect is sick, dead, or alive, most consumers do not want it in their food or pantry.

Consumers might philosophically support a biological control program because they do not want traditional residual chemicals applied directly to their food ingredients. They especially do not want chemicals directly mixed in with their food products at home. Thus, although consumers desire alternatives,

they do not want them applied in the pantry or directly on a food item. Parasitoids, predators, and pathogens, need to be applied directly to a food item to be effective. Beneficial biological control agents are regulated by the Federal Insecticide, Fungicide, and Rodenticide Act, and although exempt from tolerances in food products, the Food and Drug Administration still would uphold the standards for insect fragments in food. Thus, the biological control agent must be removed prior to packaging and sale. Another problem is that biological control agents are very slow to control insect pests. Most consumers want immediate resolution to their pest infestation so that other food products in the pantry are not affected.

In addition to the traditional biological control strategies of predators, pathogens, and parasitoids, other biorational approaches have promise in the home and urban environment, many of which are already used by consumers and pest control operators. They include pheromone trapping and monitoring, temperature modification, and improved sealing and packaging technology.

Several pheromones (both sex and aggregation) have been identified to monitor or control pantry pests (Table 1). Some pheromone traps also incorporate a food attractant in combination with, or apart from, a pheromone lure (Loschiavo 1965, Tamaki et al. 1971, Nara et al. 1981, Pierce et al. 1983). Traps are relatively simple in design, readily available, and small enough so that they can fit within even the most crowded pantry. One advantage of using pheromone traps is that they monitor the population size of the pest. Typically, the homeowner or pest control operator inspects the premises and attempts to remove all sources of infestation. To confirm the elimination of the infestation, a pheromone trap may be placed near the determined source and monitored for additional pest outbreaks.

One situation where pheromone traps are particularly effective is with infestations of moths. All the stored-product moths leave their food source to pupate. If only the infested product is removed, and not the larvae that have left the food to pupate, another pest infestation may ultimately develop. Pheromone traps can continue to remove adults from the population and hopefully prevent reinfestation. However, sex pheromones generally attract only males whereas aggregation pheromones can attract both sexes (Burkholder 1992). Thus, when using sex pheromones in population control, several traps should be employed (Sower et al. 1975). All males must be trapped, so that females are not successful in attracting a mate. If only one male is left in the population, it could (and probably will) mate with the remaining females and reinitiate the infestation.

The second biorational alternative acceptable for pantry pest control is temperature modification. It is an effective way to stop an existing infestation from growing or to prevent a new infestation from becoming established (Fields 1992; Maier 1992, 1994; Maier et al. 1996, 1997; Mason et al. 1997). The temperature can be either raised or lowered, but it must be applied quickly (Fields 1992, Sheppard 1992). The lower temperature that must be reached within the product is -17.8°C for 4 d. Such treatment does not imply that the kitchen needs to be chilled to this temperature; rather, it is the temperature of the food product that should be lowered. If a pantry pest problem has existed in the past in any spices, bulk food items, or items of food that are susceptible

Table 1. Stored-product insects for which pheromones have been identified.

Species	Male produced, long-lived adults	
	Common name	Reference
Coleoptera		
<u>Bostricidae</u>		
<i>Rhyzopertha dominica</i>	lesser grain borer	Williams et al. 1981
<i>Prostephanus truncatus</i>	larger grain borer	Dendy et al. 1989
<u>Tenebrionidae</u>		
<i>Tribolium castaneum</i>	red flour beetle	Suzuki & Sugawara 1979
<i>Tribolium confusum</i>	confused flour beetle	Suzuki & Sugawara 1979
<u>Curculionidae</u>		
<i>Sitophilus oryzae</i>	rice weevil	Schmuff et al. 1984
<i>Sitophilus granarius</i>	granary weevil	Faustini et al. 1982
<i>Sitophilus zeamais</i>	maize weevil	Schmuff et al. 1984
<u>Cucujidae</u>		
<i>Cryptolestes ferrugineus</i>	rusty grain beetle	Borden et al. 1979
<i>Cryptolestes pusillus</i>	flat grain beetle	Millar et al. 1983
<i>Oryzaephilus surinamensis</i>	sawtoothed grain beetle	Pierce et al. 1984
<i>Oryzaephilus mercator</i>	merchant grain beetle	Pierce et al. 1984
Female produced, short-lived adults		
<u>Dermestidae</u>		
<i>Trogoderma inclusum</i>	larger cabinet beetle	Cross et al. 1976
<i>Trogoderma variabile</i>	warehouse beetle	Cross et al. 1976
<i>Anthrenus verbasci</i>	varied carpet beetle	Kuwahara & Nakamura 1985
<i>Anthrenus flavipes</i>	furniture carpet beetle	Fukui et al. 1974
<i>Trogoderma glabrum</i>	glabrous cabinet beetle	Cross et al. 1976
<i>Trogoderma granarium</i>	Khapra beetle	Cross et al. 1976
<i>Attagenus unicolor</i>	black carpet beetle	Silverstein et al. 1967
<i>Attagenus brunneus</i>		Fukui et al. 1977
<u>Anobiidae</u>		
<i>Lasioderma serricorne</i>	cigarette beetle	Chuman et al. 1979
<i>Stegobium paniceum</i>	drugstore beetle	Kuwahara et al. 1978
Lepidoptera		
<u>Pyalidae</u>		
<i>Plodia interpunctella</i>	Indianmeal moth	Kuwahara et al. 1971b
<i>Cadra cautella</i>	almond moth	Kuwahara et al. 1971b
<i>Ephestia elutella</i>	tobacco moth	Brady & Nordlund 1971
<i>Anagasta kuehniella</i>	Mediterranean flour moth	Kuwahara et al. 1971a
<u>Gelechiidae</u>		
<i>Sitotroga cerealella</i>	Angoumois grain moth	Vick et al. 1974

to infestation, they can be put into the freezer before they are put into the pantry. The same process applies with heat; an infested product may be put into a shallow pan and heated at 54.4°C for 30 min to kill all insect life stages within it. At the end of the heat treatment the insects are still in the food, but they are now dead. Most consumers believe that the value of the product is low enough that they discard the entire package, insects and all. One disadvantage of heating a packaged food product to the recommended temperature is that some ingredients will be destroyed if heated prior to use (Hamid & Boulanger 1969, Watters 1991). Chilling a product rarely has this disadvantage.

Another factor that must be considered is freezer space. Many homes do not have the required freezer space to chill all products that will ultimately be placed in the pantry. Along with the concern of freezer size is freezer chilling capacity. It can take considerable time to get the internal temperature of the product to -17.8°C. To use cold temperatures, one needs to get the internal temperature of the product down to the temperature to be used to control insect pests (-17.8°C) and then hold it there for the recommended time, which does not include the time to get the center of the food to that temperature. The time that it takes for the center temperature to reach -17.8°C depends on the type of product and the amount of product (Table 2). The larger the bulk product, the longer it takes for the temperature threshold to be recorded at the center of the product (Mullen & Arbogast 1979).

The final alternative biorational available is protective packaging. It has not been fully explored, although there are several companies and entomologists developing and testing new packaging materials (Highland 1991, Watters 1991). One way to protect packaged food is to build a repellent into the packaging so that even if an insect is present and attempts to eat its way into the package, it is either repelled or killed (Highland & Merritt 1973, Loschiavo 1970, Watters 1966). Another way to deter pantry pests is packaging that a insect cannot eat through. There are several ways to build stronger packaging that prevent an insect from entering a package: better sealing of the outer packaging material, improved glue so that the flaps that seal the package are sufficiently sealed, and a stronger inner package lining (Highland & Jay 1965, Laudani et al. 1966, Highland 1977, Yerington 1979). These alternatives would provide protection from insects contamination throughout the food distribution channel, including retail outlets, until the package is opened by the consumer. Eventually packages will be opened and then all the protective packaging, resistant linings, and glued boxes cannot protect the processed food. Any existing insect-resistant barrier is destroyed and the insect is able to get in and infest the food. To protect the food after the manufacturers' seal is broken, consumers must be persuaded to put open packages into alternative storage containers with insect-resistant seals.

There are numerous clues that a pest control operator can use to determine if a pantry pest problem exists. The most obvious clue is the presence of a pet in the home. Pet food is notorious for becoming infested with pantry pests, especially if bought in bulk and not consumed rapidly. The majority of stored-product insects will infest dog food. If the house has a rodent infestation, the rodents may steal the pet food and stash it in wall voids. An insect pest infestation may develop within the walls, only to make itself evident in the

Table 2. Chilling times for selective commodities in a freezer (0.756 m³) filled to capacity (Mullen & Arbogast 1979).

Commodity	Freezer setting (0°C)	Time to 0°C	Time to equilibrium (h)
Cornflakes	-10	7	30
28, 1.45-kg cases	-20	5	35
Flour	-10	55	160
7, 45.45-kg bags	-20	25	145
Elbow macaroni	-10	29	130
15, 19.91-kg cases	-20	19	100
and blackeyed peas			
15, 10.91-kg cases			

vicinity of the pantry. Occasionally, no infested product is found within the pantry, and the homeowner and pest control operator are misled. Vertebrate control and effective structure sealing are generally recommended in this situation. The presence of bird seed on the premises is another major source that can lead to a pantry pest infestation. The major problem with bird seed is that there are very few protectants that are labeled for all the types of seeds within a packaged seed mix. One biorational alternative that can be applied to bird seed is diatomaceous earth (Protect-It, HTI Agritech [USA], Inc., Blaine, Washington). However, this control method is rarely practiced by homeowners. Another nonfood inspection clue is the presence of dried flower arrangements. These arrangements are popular, along with laminated or coated rolls or bread. These decorations can be infested for a long time without the homeowners' knowledge.

In summary, temperature modification of the product and changes in product packaging show the greatest promise for successful incorporation of a biorational approach to pantry pest control. Traditional biological control agents may reduce pest populations under the best circumstances; however, problems associated with removing the biological control agent prior to consumption are not likely to be easily resolved. If consumers would accept a level of infestation above a zero tolerance level, traditional biological control options might be feasible.

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Biorational Suppression of Pests in Landscapes¹

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ABSTRACT A biorational approach to pest suppression in urban landscapes includes alternative plantings, naturally occurring parasites and predators, and other alternatives to chemical pesticides. Managing pests in landscapes is part of a dynamic management system. Intelligent plant management (IPM), as defined for landscapes, recognizes that site and plant selection, construction of landscapes, and routine management practices such as proper planting, fertilization, and irrigation influence pest complexes. Prioritizing areas within a landscape, mapping pest populations, alternative plantings (including pest-resistant cultivars), and naturally occurring parasites and predators are some factors that make biorational pest suppression possible. Hindrances include the high visibility of landscapes, the fact that they may be heavily trafficked areas, that landscapes are labor intensive, and often profit oriented. Commercially, efficacy is measured in dollar savings; for homeowners, efficacy often is even more closely tied to landscape appearance. In addition to public perception, development of public acceptance of intelligent plant management strategies is essential.

KEY WORDS biorational, intelligent plant management, mapping, *Prosapia bicincta*, *Scapteriscus vicinus*, Scarabaeidae, *Solenopsis invicta*

There is a great diversity among and within urban landscapes. Landscapes in urban areas include home grounds, golf courses, parks, athletic fields, commercial property landscapes, and other settings in which turf, ornamentals, and trees are managed. Biorational suppression of pests in urban landscapes includes the use of alternative plantings; maximizing the effects of naturally occurring biocontrol agents; "chemical" alternatives (not alternative chemicals, but alternatives to chemical pesticides); and minimizing the use of chemical pesticides. Other practices that reduce plant stress, such as appropriate fertilization and irrigation practices, proper drainage patterns, and correct planting and plant placement for shade/sun requirements also are important.

Alternative plantings are beginning to be used in urban landscapes. Particularly, ornamental grasses are being used as nonhost plants as well as a labor-saving tactic. For example, taller ornamental or native grass grown on a bankside where tawny

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mole crickets, *Scapteriscus vicinus* Scudder, previously infested turfgrass reduces or eliminates chemical use because these grasses are nonhost plants. The use of these grasses in this manner also reduces labor costs once the grasses become established because they do not have to be mowed or mowing frequency is reduced.

There are situations in which alternative plantings are desired to reduce pest populations. Two-lined spittlebugs, *Prosapia bicincta* Say, are potential pests of home lawns throughout most of the eastern United States (Braman 1995). In the southeastern and the mid-Atlantic states, improved management and irrigation practices probably have encouraged these insects to reach pest levels. Reports from homeowners and lawn care professionals indicate that the "deadly combination" for stimulating two-lined spittlebug problems is a centipede grass turf, *Eremochloa ophiuroides*, and a small-leaved holly, such as Japanese holly, *Ilex crenata*. In parts of the southern United States, two-lined spittle bugs will likely become a pest where these plants are grown together and maintained properly. Thus, alternative plantings in urban landscapes are, in this case, a viable option that can currently be used.

Homeowners and landscape managers are encouraged to use cultural practices as control options. For example, pruning is done routinely to shape plants into a desired form. Existing pest populations, such as those of scale insects and whiteflies, can often be reduced significantly by pruning before other control options are exercised. If a landscape is scouted routinely, or an existing problem is otherwise identified, pruning can be used as a control option or as a suppression technique.

Naturally occurring biological control agents—parasites and predators—occur in urban landscapes. Use of alternatives to chemical pesticides can maximize the beneficial effects of these organisms. At present, the use of biological control products in urban landscapes usually is limited to environmentally sensitive areas, often because of regulations that prohibit or limit use of pesticides in these areas. Sometimes use of suppression tactics involves an educational process, particularly with homeowners and turf managers, to gain an understanding that suppression may be all that is needed, not complete pest elimination. For example, if soil insects such as tawny mole crickets that could be controlled with entomopathogenic nematodes are infesting turf at the edge of the water, a reduction of that population coupled with an increase in mowing height could mean that the damage really is not visible beyond acceptance levels. Use of biological control in landscapes currently is limited mainly to the use of *Bacillus thuringiensis* Berliner products on ornamentals and turf and insect-parasitic nematodes for certain soil insects. Other products, such as entomopathogenic fungi, are being developed. In addition to the use of biological agents, some horticultural oils that are highly purified can be used throughout the growing season, as can the "soaps" (potassium salts of fatty acids). These materials can be used early in the growing season to control such pests as spider mites, scale insects, and whiteflies. Where there is an awareness of the importance of monitoring (scouting), the efficacy and economic feasibility of using these alternatives is beginning to be appreciated by commercial managers, perhaps more than by homeowners. Realistic expectations of biological agents and alternatives to chemical pesticides by both these clientele groups are important.

Identification of pest life stage, in addition to pest identification, is extremely important in minimizing the use of insecticides. Knowledge of vulnerable pest stages and environmental conditions that minimize the use of insecticides is needed.

The use of water is critical to the efficacy of a biorational approach to suppression of pests in landscapes. Plants require water to grow. In some areas of the United States, water availability and water quality determine the type of landscape managed. Soil insect pests move closer to the surface in response to available moisture near the soil surface (Villani & Wright 1990); so manipulation of water can determine the success or failure of biocontrol agents and insecticides. The use of water—quality, quantity, and the timing of irrigation—is extremely important in a biorational approach to pest suppression.

New technology is being developed for the application of control products such as biocontrol agents and insecticides. Until recently, subsurface application has been limited to golf courses. Now, prototype equipment is being developed for use by commercial landscape managers, people who maintain home landscapes, and possibly even for homeowners. This technology can minimize the use of insecticides by placing chemicals in closer proximity to pests. In some cases, the rate of chemical used can be reduced (Vittum 1994) because putting materials below the soil surface means that they are subject to less breakdown by light and heat. Some of these systems work well for application of entomopathogenic nematodes because by placing these agents into the moist soil they are less likely to be fatally desiccated (Crowe & Madin 1975) and can move or become active almost immediately.

A holistic approach to biorational suppression of pests in landscapes is needed. By looking at IPM as "intelligent plant management" with pest management being a part of a dynamic system for managing a landscape, one can get a more realistic view of what suppression means and what this common-sense approach involves. Intelligent plant management actually begins when a person decides to put a landscape at a certain site. Selection of the site, establishment of drainage systems, and choices of plants, including turfgrass cultivars, all influence pest populations. Alternative plantings, as mentioned earlier, are options possible in new or existing landscapes in many parts of the United States.

The possibility as well as practicality of biorational suppression of pests in urban landscapes includes the fact that areas within landscapes can be prioritized; that pest populations often can be mapped; alternative plantings are possible; and biocontrol agents in nature can be maximized. Areas within a landscape can be prioritized according to visibility, use, and clientele expectations to develop realistic budget considerations for pest reduction strategies. The following ideas and examples illustrate this point.

Mapping involves recording the location of pest populations in a landscape, and usually is done for spot treatment of heavily infested areas and for recordkeeping of pest reservoirs in a landscape. Mapping systems have been developed for white grubs (Villani 1990), tawny mole crickets (Cobb & Lewis 1990), and red imported fire ants (Cobb & Cobb 1995). Often, mapping is tied directly to threshold-setting. For example, a map from a golf course was

developed for mapping tawny mole crickets. Adult mole cricket activity was mapped to facilitate the return to and treatment of the next generation at a younger, more vulnerable stage at the mapped locations. The golf course superintendent decided that fairways could sustain higher damage ratings (Cobb & Mack 1989) than greens; thus, damage acceptance levels (thresholds) on fairways differed from those on greens. Mapping for tawny mole crickets is now recommended on golf courses in the southeastern United States.

One of several pilot projects for red imported fire ant control involved setting some priority areas in a commercial landscape. Included were those areas that were most visible, most trafficked, and from which most sting reports and other complaints were reported. The landscape was mapped to record where the most fire ants in those priority areas were located, and in surrounding areas where fire ants were moving into the landscape. The first-year perimeter spot treatments with baits were done in late spring and in late summer. The ants were allowed to gather the bait particles. A few days later, in the heavily trafficked areas, a contact mound treatment was done to eliminate the stinging workers that would otherwise take several weeks to die as a result of the bait's disruption of the colony's reproductive potential. The site was remapped in the second and third years. Perimeter spot treatment was done only in the late spring. Mounds were treated with contact insecticides where needed. At the end of the third year, the expenditures (primarily labor costs) for fire ant control in priority areas were reduced by 90% of the cost before the project began. All the fire ants were not eliminated. A few ant colonies were found beside buildings and shrubs out of the heavily trafficked areas (Cobb & Cobb 1995). Perhaps the lack of resurgence of imported fire ants in this and similar landscapes that adopted this program can be explained by the fact that those fire ant workers that remained in less visible and/or accessible areas served as predators on new fire ant queens entering the landscape.

Factors that work against biorational suppression of pests in landscapes include high visibility and extensive site usage. Both of these factors are linked to clientele (and management) expectations. For example, competition for clientele accounts among commercial landscape maintenance companies is usually based upon the quality of their currently managed properties as well as bids for new accounts. Landscapes that include entry areas to office buildings, shopping complexes, clinics, and similar highly visible areas also are often highly trafficked. Scheduling landscape maintenance, including pest control options, may be more difficult because of high usage. Both factors, high visibility and usage, usually contribute to intensive management that is already a characteristic of urban landscapes. In addition, biorational suppression of pests in landscapes may be more difficult because management is often profit oriented. Landscape management companies must make a profit to stay in business. Efficacy is measured by the landscape manager in dollar savings. For homeowners, appearance of the landscape is linked closely with efficacy. Control of pests is site specific, as are thresholds.

In conclusion, management efforts, including biorational pest control, are often linked directly not just to public perception but also to public acceptance. Research and program development and implementation efforts must be tied to

the "people factor," which, in landscape management (including biorational pest control) can have positive as well as negative influences on results.

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Efficacy of Conventional Insecticide and Juvenoid Mixtures on an Insecticide-Resistant Field Population of German Cockroach (Dictyoptera: Blattellidae)¹

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ABSTRACT Insecticide and juvenoid mixtures were evaluated for 12 mo in the field against an insecticide-resistant field population of the German cockroach, *Blattella germanica* (L.), in infested apartments. Treatments consisted of three mixtures: (1) Empire 20[®] and Gentrol[®] (i.e., chlorpyrifos and hydroprene); (2) Commodore[®], NyLar[®], and PBO-8[®] (i.e., lambda-cyhalothrin, pyriproxyfen, and piperonyl butoxide); and (3) Diacap 300CS[®] and Torus[®] (i.e., diazinon and fenoxycarb). Sticky trap catch was used to monitor population numbers, and there were no significant differences between treatment regimes at any pre- or posttreatment evaluation periods. Trap catch reductions increased throughout the study to 95.3%, 90.7%, and 85.3% at the end of the test for Empire, Commodore, and Diacap treatments, respectively. Percentage of wing twist (an indicator of juvenoid-induced sterility) increased over time and reached over 80% at 6 mo for all treatments. In addition, the ratios of nymphs to adults (an indicator of population productivity) and gravid to nongravid females (a measure of population fertility levels) were reduced by all mixtures. Results of this study indicate the potential usefulness of mixtures of conventional insecticides and juvenoids in integrated pest management and resistance management programs for the German cockroach.

KEY WORDS Dictyoptera, Blattellidae, *Blattella germanica*, insect growth regulator, fenoxycarb, hydroprene, pyriproxyfen, juvenoids, PBO

Juvenile hormone analogs (juvenoids) are chemicals that control cockroach populations by producing morphogenic deformities in affected individuals (Bennett & Reid 1995). Specifically, these morphogenic deformities lead to a loss of reproductive ability through malformation of reproductive structures and/or supernumerary nymphal instars (King & Bennett 1989, Kramer et al. 1989, Reid et al. 1994). Until recently, there have been two juvenoids available for cockroach control that have proven effectiveness in controlling populations: fenoxycarb (Brenner et al. 1988, Ogg & Gold 1988, Reid et al. 1990) and hydroprene (Bennett et al. 1986, Reid & Bennett 1994).

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Pyriproxyfen is the most recent juvenoid to be introduced for use by the structural pest control industry. In the field, pyriproxyfen has been proven to be effective as a juvenoid control agent (Koehler & Patterson 1991).

Insecticide resistance can be a significant obstacle to German cockroach management. The use of juvenoids in conjunction with conventional neurotoxic spray insecticides is a management approach with great potential for controlling resistant populations of the German cockroach (Hemingway & Small 1993). This strategy is based on the concept that the conventional insecticide removes susceptible individuals from a population, and the juvenoid hinders development and reproduction in the more resistant survivors.

Recently, we discovered a field population of the German cockroach that was resistant to organophosphate and pyrethroid insecticides (Scharf et al. 1997). By topical application bioassays, this population was found to have 80-fold resistance to the pyrethroid insecticide cypermethrin and 5-fold resistance to the organophosphate insecticide chlorpyrifos. Additionally, cypermethrin resistance was reduced by 90% by the cytochrome P450 inhibitor piperonyl butoxide, suggesting an involvement of cytochrome P450 oxidation in pyrethroid resistance. Subsequent biochemical studies involving this population have identified elevated expression of esterase and cytochrome P450 detoxication enzymes (M. E. S., unpublished data) that are likely responsible for organophosphate and pyrethroid-resistance, respectively. In the present study, we monitored the effects of three conventional insecticide and juvenoid mixtures on this resistant field population. Our objective was to describe the efficacy of these mixtures as resistant management strategies.

Materials and Methods

Field trials were conducted from May 1994 through May 1995 in a German-cockroach-infested apartment complex (Munस्याna; Muncie Housing Authority, Indiana). Building construction practices, poor sanitary conditions, and lack of an effective pest management program at this site favored the development of large cockroach populations. Before this study, Demon WP insecticide (AI = cypermethrin; Zeneca, Inc., Wilmington, Delaware) had been used for 8 yr by the housing authority on a quarterly basis. Resistance was initially suspected as large cockroach numbers were easily observable in many regularly treated apartments. Insecticide bioassays on field-collected individuals later confirmed this suspicion (Scharf et al. 1997).

Within apartments, cockroach numbers were sampled in the kitchen and bathroom by using Lo-Line cockroach sticky traps (Agrisense, Inc., Fresno, California). Six trap placement sites were monitored: (1) under and (2) above the kitchen sink, (3) behind the refrigerator, (4) behind the stove, (5) in the utility/pantry area, and (6) on the floor beneath the toilet's water tank. Populations were sampled before the initial insecticide applications (0 wk) and at intervals thereafter (1 wk, and 1, 2, 3, 4, 5, 6, 9, and 12 mo).

At each sample interval, traps were left in place overnight, collected the next morning, and counted that day. For each trap, the number of small nymphs (nymphs did not emerge in the trap; 1st-3rd instar), large nymphs (4th-6th instar), adult males and females, gravid (egg-case bearing) females, and

morphogenically (juvenoid) affected adult males and females was recorded. Trap catch data were used to determine: (1) the efficacy of the treatment on reducing the population; (2) the percentage of adults with juvenoid-induced wing twist (an indicator of genital malformation that adversely affects population reproduction); (3) the ratio of nymphs to adults (an indicator of population productivity); and (4) the ratio of gravid to nongravid females (a measure of population fertility levels).

Treatments were randomly assigned to several buildings in the apartment complex before initial sampling. Each apartment in a building served as a replicate. After collecting the pretreatment traps, every apartment in a given building was treated whether or not it was determined to be a test site. This will assure that the percentage of population reductions was caused by one treatment only. A minimum catch of 12 cockroaches per apartment before treatment ($n = 6$ traps) was required for inclusion of an apartment in the test.

Treatments were made using 3.79-liter (1 gal) B&G compressed air sprayers (Plumbsteadville, Pennsylvania) equipped with multee-jet spray nozzles (Spraying Systems, Inc., Wheaton, Illinois). Sprays were applied using a general spray procedure (Bennett et al. 1988) throughout entire apartments with a coarse-fan nozzle (20 psi), except where nontarget contamination was a concern. Additionally, insecticide was applied to all accessible cracks and crevices by using a crack and crevice tip, and household items (kitchen utensils, garbage containers, toothbrushes, clothes, etc.) were moved to allow for a more thorough application. Applications required from 0.95–1.25 liters (0.25 to 0.33 gal) of finished spray per apartment. The amount of insecticide put into each apartment varied depending on apartment size, the amount of cockroach harborage, and whether the apartment was a test site; however, areas in the vicinity of the kitchen sink, utility areas and bathrooms were always treated. Details of initial and follow-up treatments for all insecticide and juvenoid mixtures are summarized in Table 1.

A range of 14–21 test apartments was established for the three mixtures at pretreatment counts. Ethical constraints did not permit us to conduct untreated controls during this study because the study was conducted in human dwellings and the cockroach populations were very large.

The decision rule for terminating follow-up treatments at 6 mo was based on the mean frequency of sampled, twisted-wing adults for all apartments. When the frequency of twisted-wing adults reached $\geq 80\%$ (i.e., at 6 mo), follow-up treatments were terminated. This decision was based on previous data presented by Koehler & Patterson (1984) and Reid et al. (1990) who showed that the juvenoids hydroprene and fenoxycarb, respectively, inhibited population reproduction significantly at a level of 80% wing twist.

The dependent variable in the analysis was the mean number of cockroaches per trap. Two approaches were taken to determine efficacy. One approach involved analysis within time to determine the comparative efficacy among the number of treatments independently at each sample interval. Percentage data were transformed by arcsine \sqrt{p} to normalize variance. Analysis was based on a completely randomized 1-way ANOVA and followed by multiple comparison tests among treatments by the least significant difference (LSD; $P < 0.05$) (SAS Institute 1990). The second approach involved analysis among time periods to

Table 1. Insecticide and juvenoid mixtures evaluated against the German cockroach in the field.

Treatment	Trade name	Manufacturer (address)	AI ^a	% (AI)	Initial treatment		Follow-up treatment (1, 2, 3, 4, 5, and 6 mo)	
					Desired dilution (%)	Amount / 3.79 L of water	Desired dilution (%)	Amount / 3.79 L of water
1	Empire 20 +	DowElanco (Indianapolis, IN)	chorpyrifos	20.0	0.5	95 ml	0.25	48 ml
	Gentrol	Zeocon Industries (Dallas, TX)	hydroprene	9.0	0.07	30 ml	0.07	30 ml
2	Commodore WP+	Zeneca Inc. (Wilmington, DE)	lambda-cyhalothrin	10.0	0.03	11 g	0.03	11 g
	Nylar +	MGK Inc. (Minneapolis, MN)	pyriproxyfen	10.0	0.187	71 ml	0.015	7 ml
	PBO-8	Prentiss Inc. (Sandersville, GA)	piperonyl butoxide	91.3	0.1	4 ml	-	-
3	Diacap 300CS +	Ciba-Geigy (Basle, Switzerland)	diazinon	30.0	1.0	126 ml	0.5	63 ml
	Torus	Ciba-Geigy (Greensboro, NC)	fenoxy carb	24.4	0.187	30 ml	0.125	20 ml

^a AI, active ingredient.

determine whether or not the average trap catch (sample density) was reduced by the treatment. The mean number of cockroaches trapped before treatment was compared with the mean numbers at each interval after treatment by using the UNIVARIATE Procedure of SAS (SAS Institute 1990).

Results and Discussion

There were no significant differences between the three treatment regimes at the pre- and all posttreatment evaluation periods (Table 2). The mean \pm SE number of cockroaches captured per apartment before treatments was 55.9 ± 7.9 , 27.9 ± 5.0 , and 18.0 ± 4.3 for the Empire + Gentrol, Commodore + Nylar, and Diacap + Torus treatments, respectively. Percentage reduction in trap catch increased for all treatments in a fairly continuous manner over the course of the study (UNIVARIATE Procedure of SAS). The mean \pm SE number of cockroaches captured per trap at the end of the study (i.e., at 12 mo following initiation of the treatments) was 2.6 ± 0.4 , 2.6 ± 2.6 , and 2.6 ± 0.5 for the Empire + Gentrol, Commodore + Nylar, and Diacap + Torus treatments, respectively. Population samples at each posttreatment were significantly ($P < 0.05$) different from those obtained in pretreatment samples (i.e., the reduction at posttreatment interval was significant). It is very unlikely that seasonal population fluctuations were responsible for the population decline as cockroach numbers were significantly less in May 1995 than in May 1994.

The critical threshold of 80% wing twist, in which the reproductive potential of a German cockroach population is significantly affected by a juvenoid treatment (Koehler & Patterson 1984, Reid et al. 1990), was first observed at 6 mo with a percentage of twisted-wing adults of 80.2, 86.5, and 83.5 for the Empire + Gentrol, Commodore + Nylar, and Diacap + Torus treatments, respectively (Table 3). However, percentage of wing twist decreased slightly (but not significantly) for Commodore + Nylar and Diacap + Torus treatments at 12 mo (70.6% and 71.2%, respectively), indicating that the population was somehow escaping juvenoid effects. The slight reduction in the effectiveness of the juvenoid is possibly due to natural environmental degradation of the residue following the final treatment at month 6 (although the effects of insect migration cannot be refuted) or that the residual of two mixtures became repellents after they aged in the field.

Changes in the age structure of treated populations were determined by mean nymph:adult ratios and gravid:nongravid female ratios. Nymph:adult ratios decreased over time, ranging from 3.34 to 0.28 (Fig. 1a) and indicated a substantial decrease in the population's productivity by 6 mo following the initial treatment. However, in conjunction with the slight population recovery from juvenoid effects at 12 mo, nymph:adult ratios increased to a range of 0.72 to 1.3. Gravid:nongravid female ratios fluctuated during the experimental period (Fig. 1b). Nevertheless, this ratio also dropped sharply at 6 mo following treatments of Empire + Gentrol and Commodore + Nylar, which coincided with the decreases in nymph:adult ratios (Fig. 1a) and the increase in population reduction at this time (Table 1). The 9–12-mo increase in nymph:adult and gravid:nongravid ratios was associated with the slight population rebound from juvenoid effects at the end of the test.

Table 2. Mean trap catch and percentage of cockroach reductions for three insecticide + juvenoid mixtures against an insecticide-resistant field strain of the German cockroach.

		Mean \pm SE no. of cockroaches per trap (% reduction in parenthesis ^a at each date)									
Treatment	N ^b	Pre-treatment	1 wk	1 mo	2 mo	3 mo	4 mo	5 mo	6 mo	9 mo	12 mo
Empire + Gentrol	21	55.9 \pm 7.9a	23.3 \pm 3.1a (58.3)	23.8 \pm 3.3a (57.4)	16.1 \pm 1.9a (71.2)	15.1 \pm 1.8a (73.0)	12.6 \pm 1.3a (77.5)	7.8 \pm 1.2a (86.0)	4.9 \pm 0.4a (91.2)	4.1 \pm 0.5a (92.7)	2.6 \pm 0.4a (95.3)
Commodore + Nylar	17	27.9 \pm 5.0ab	16.3 \pm 3.3a (41.2)	19.0 \pm 3.2a (31.9)	12.1 \pm 2.3a (58.5)	11.6 \pm 1.6a (60.0)	12.8 \pm 1.5a (54.2)	9.5 \pm 1.1a (64.5)	5.6 \pm 0.7a (80.0)	4.3 \pm 0.7a (84.6)	2.6 \pm 0.5a (90.7)
Diacap + Torus	14	18.0 \pm 4.3b	11.9 \pm 2.1a (33.9)	14.6 \pm 2.2a (18.9)	8.0 \pm 0.9a (55.6)	7.8 \pm 0.8a (56.7)	7.3 \pm 0.7a (59.4)	4.8 \pm 0.3a (73.3)	3.7 \pm 0.3a (79.4)	2.9 \pm 0.3a (83.9)	2.6 \pm 0.4a (85.3)
<i>F</i>		2.8	1.2	0.5	1.2	1.3	1.2	1.6	0.6	0.4	0.001
<i>P</i>		0.07	0.3	0.6	0.3	0.3	0.3	0.2	0.5	0.7	0.99

Means in the same column followed by the same letter are not significantly different (LSD, $P \geq 0.05$).

^a Numbers in parentheses represent the overall percentage reduction in trap catch compared with time zero.

^b Number of test apartments at pre- and posttreatment (i.e., no missing apartments during the entire 12-mo study).

Table 3. Mean and percentage of twisted-wing adults for three insecticide + juvenoid mixtures against an insecticide-resistant field strain of the German cockroach.

Treatment	N ^b	Mean ± SE no. of twisted-wing adults per trap (% twisted-wing adults in parenthesis ^a at each date)									
		Pre-treatment	1 wk	1 mo	2 mo	3 mo	4 mo	5 mo	6 mo	9 mo	12 mo
Empire + Gentrol	21	0.02 ± 0.01a (0.16)	0.7 ± 0.2a (9.8)	1.5 ± 0.3a (15.1)	3.8 ± 0.5a (40.3)	4.5 ± 0.6a (69.5)	3.9 ± 0.4a (70.5)	2.2 ± 0.3a (78.5)	2.4 ± 0.2a (80.2)	2.0 ± 0.3a (92.5)	1.0 ± 0.2a (85.0)
Commodore + Nylar	17	0.01 ± 0.01a (0.16)	0.3 ± 0.01a (5.9)	1.1 ± 0.2a (17.6)	2.6 ± 0.6a (46.4)	4.5 ± 0.9a (66.7)	4.1 ± 0.6a (65.6)	3.3 ± 0.5a (70.5)	2.6 ± 0.4a (86.5)	2.0 ± 0.4a (90.5)	0.9 ± 0.2a (70.6)
Diacap + Torus	14	0.01 ± 0.01a (0.24)	0.08 ± 0.03a (2.1)	1.7 ± 0.5a (13.0)	2.3 ± 0.4a (38.3)	3.4 ± 0.4a (60.2)	2.6 ± 0.3a (62.3)	2.0 ± 0.2a (76.0)	1.8 ± 0.2a (83.5)	1.4 ± 0.2a (81.2)	0.9 ± 0.2a (71.2)
<i>F</i>		0.8	0.9	0.2	0.7	0.3	0.7	1.0	0.4	0.3	0.1
<i>P</i>		0.5	0.4	0.8	0.5	0.7	0.5	0.4	0.6	0.7	0.9

Means in the same column followed by the same letter are not significantly different (LSD, $P \geq 0.05$).

^a Numbers in parentheses represent the overall percentage twisted-wing adults as calculated by the formula: (no. of twisted-wing adults/number of adults) × 100.

^b Number of test apartments at pre- and posttreatment (i.e., no missing apartments during the entire 12-mo study).

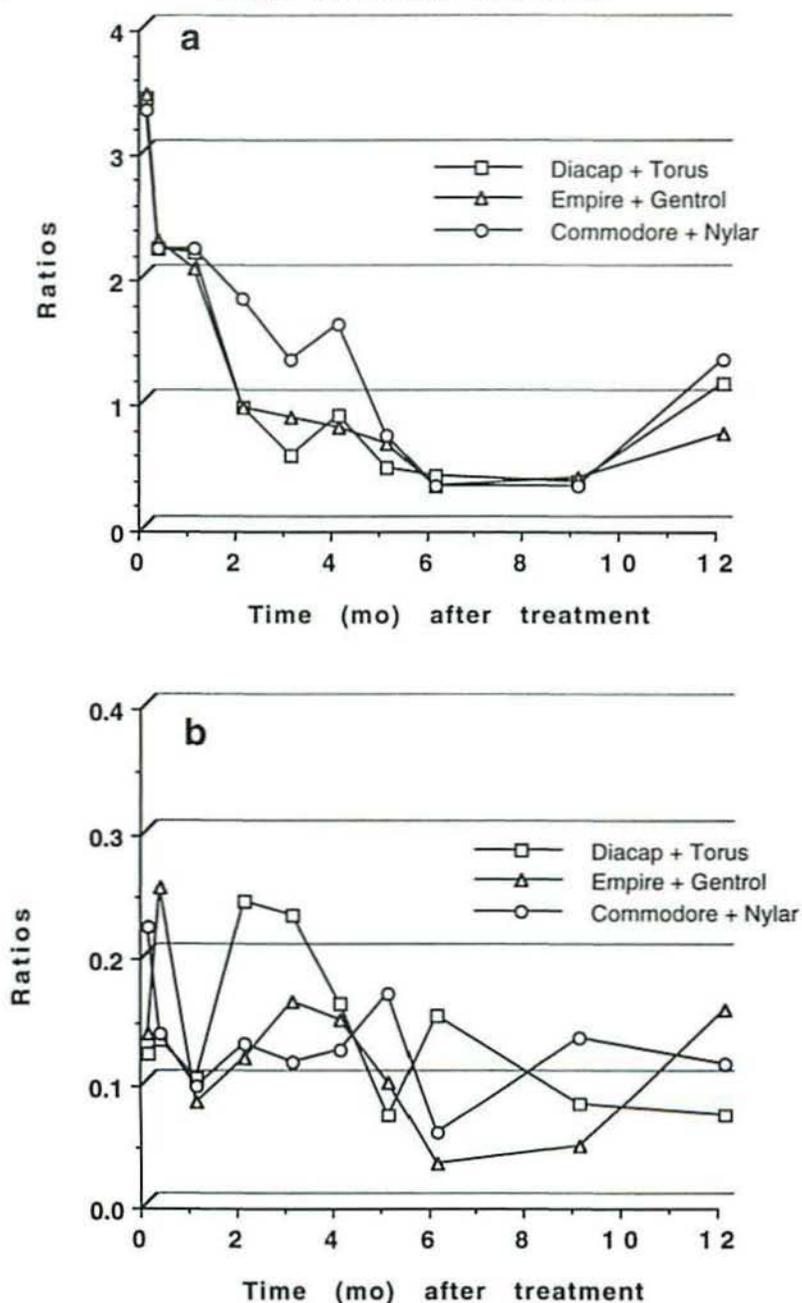


Fig. 1. Changes in (a) total nymph:adult and (b) gravid:nongravid female ratios over time for a resistant field population of German cockroach observed following exposure to Empire + Gentrol, Commodore + Nylar, and Diacap + Torus treatments (see text for details).

Because juvenoids act slowly on populations (Bennett & Reid 1995), the use of the "companion" insecticides may have been crucial in achieving initial population reductions. Koehler & Patterson (1991) observed similar patterns of population reduction by using a mixture of the organophosphate acephate + pyriproxyfen, but not for acephate alone, reinforcing the effectiveness associated with using companion insecticides in conjunction with juvenoids. In the laboratory, Kramer et al. (1990) demonstrated that the juvenoid hydroprene synergized the effectiveness of both propoxur and chlorpyrifos for a multiresistant cockroach strain. In this regard, one explanation for the apparent effectiveness of this treatment on reducing the population could be that some type of previously unidentified synergistic relationship exists between these mixtures.

Reduced juvenoid effects were observed through this study. The percentage of wing twist in surviving adult cockroaches decreased from a range of 81.2%-92.5% at month 9 to a range of 70.6%-85.0% at month 12. This decrease in juvenoid effectiveness in each mixture may be attributed to increased metabolic activities but is most likely the result of decreased residual activity of juvenoids caused by natural environmental degradation. Koehler & Patterson (1991) reported a similar pattern of decreasing juvenoid effects, but this was at 12 mo following five consecutive monthly treatments of exclusively pyriproxyfen.

In this study we have reported the impact of three insecticide plus juvenoid mixtures in controlling a resistant German cockroach population. Each of the three treatments examined significantly reduced trap catch (and presumably population numbers) indicating that these mixtures have applications as IPM and resistance management tools. However, having a diverse array of mixture-based management tools to use in alternation or rotation would seem necessary to prevent the further selection for resistance. In light of this observation, additional research examining the efficacy of other insecticide and juvenoid combinations on resistant populations would provide longer-term benefits to the structural pest control industry.

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NOTE

Evaluation of Commercial Sticky Traps Used for German Cockroach (*Dictyoptera: Blattellidae*)¹

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Sticky traps are primarily used as sampling, detection, and monitoring tools for the German cockroach, *Blattella germanica* (L.). However, there has been an interest in the use of sticky traps as control tools (Ballard & Gold 1983, 1984; Moore & Granovsky 1983). Traps have been advocated as an alternative to chemical methodologies (Kaakeh & Bennett 1996, 1997), and their use has increased with the implementation of IPM programs (Gold 1995). This preliminary study was initiated to obtain quantitative data on the effectiveness of seven types of commercially available sticky traps in reducing German cockroach populations.

The study was conducted in a 11 by 14.5-m controlled environment room (27°C; 50% RH; 12:12 [L:D] h photoperiod). Test arenas were constructed of 2-cm-thick particle board floor (1 m² surface area) with a white painted epoxy polymer floor. To this floor, Plexiglas™ sheets (38 cm high by 6.35 mm thick) were fastened to form the walls. All wall and floor junctures were sealed with latex caulk to form a tight, escape-proof enclosure. Arenas were made escape-proof by an impassable barrier of petrolatum and mineral oil (1:2) applied to the arena walls. This barrier was applied to within 4-5 cm of the chamber floor to prevent test cockroaches from climbing the arena walls, thereby forcing all cockroach exploratory behaviors to the arena floor and the arena's provisions. Refugia were restricted to a single harborage unit, positioned in the center of the arenas, and consisted of five masonite panels (separated by 5-mm spacers) that provided 250 cm² of the horizontal surface space. A water vial was placed at one corner of the arena and a laboratory diet of Wayne rodent blox (Continental Grain, Chicago, Illinois) was placed in the opposite side. Each trap type was evaluated separately. Trap types (number of openings, sticky surface area, and manufacturer) were: Lo-Line (4, 185 cm², AgriSense), Victor Roach Pheromone

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(4 openings for 2 attached square-shaped traps, 29 cm², Woodstream), Victor Roach Pheromone 2 (3, 77 cm², new version with food attractant from Woodstream), Mr. Sticky (6, 104 cm², LTP Int.), Trapper (8 openings for 3 attached traps, 120 cm², Bell Laboratories), Catchmaster (8 openings for 3 attached rectangular-shaped traps, 130 cm², Atlantic Paste & Glue), and Trap Stik (2, 165 cm², Lipha Tech). Two traps of each type were placed in the remaining opposite corners of the box and kept there for 14 d. German cockroach populations were established in boxes by releasing mixed-age groups of 160 cockroaches (30 2nd- and 3rd instars, 30 4th- and 5th instars, 20 1-wk-old gravid females, 40 newly eclosed females, and 40 newly eclosed males). Cockroaches were allowed 2 d to acclimate and establish within the harborage before introduction of traps. Each treatment was replicated three times in a completely randomized design. The trap catch was recorded at 1, 3, 7, 10, and 14 d. After recording of trapping data, traps were placed back in the arena. Cockroaches within traps were counted, classified by age and sex, and kept in the traps after counting. The number, age, and sex of trapped cockroaches in the two traps per arena were analyzed as a cumulative trend at each sampling interval. Data from sampling at day 1 and 14 were subjected to analysis of variance (ANOVA), and Duncan's multiple range test ($P < 0.05$) was used to separate means.

The percentages of cumulative trap catch in the seven sticky traps ranged from 5% to 46.7% during the first 24 h of exposure ($F = 15.5$, $P < 0.01$), with the greatest percentage of trapped cockroaches in Victor 2, followed by Lo-Line traps (Fig. 1A). Trap catches increased gradually over time: by the end of the 14-d test period, the percentage of trapped cockroaches ranged from 18.1% to 87.3% ($F = 18.1$, $P < 0.01$) with greatest percentages recorded from Victor 2 and Lo-Line traps. The high trap catch in Victor 1 and Victor 2 during the test period is likely due to the presence of German cockroach aggregation pheromones on the ceiling of both traps and the presence of a food attractant in the center of Victor 2 traps. The lower number of cockroaches caught on larger traps was probably due to suboptimal trap design and quality of sticky materials.

When efficacy of traps was further compared as the number of cockroaches trapped per square centimeter, the smallest traps in their sticky surface area such as Victor 1 and Victor 2 caught significantly higher numbers of cockroaches than other larger traps after 14 d ($F = 49.6$, $P < 0.05$, Fig. 1B). Although Trap Stik had sticky material on all interior sides, few cockroaches were found on the bottom surface and on the lower portion of the sides.

The proportions of population reduction caused by the seven sticky traps varied by sex and developmental stage (Table 1). Males, gravid females, and nymphs were caught in larger numbers in Victor 2 than in Lo-Line traps at day 1, but there were no significant differences between the numbers of all developmental stages and sexes caught in both traps at the end of the test period.

The ability of a trap to catch large numbers of cockroaches is important if control is desired. Our results showed that some traps caught significantly greater numbers of German cockroaches than others. Trap efficacy in reducing cockroach populations is likely dependent on a number of factors such as trap

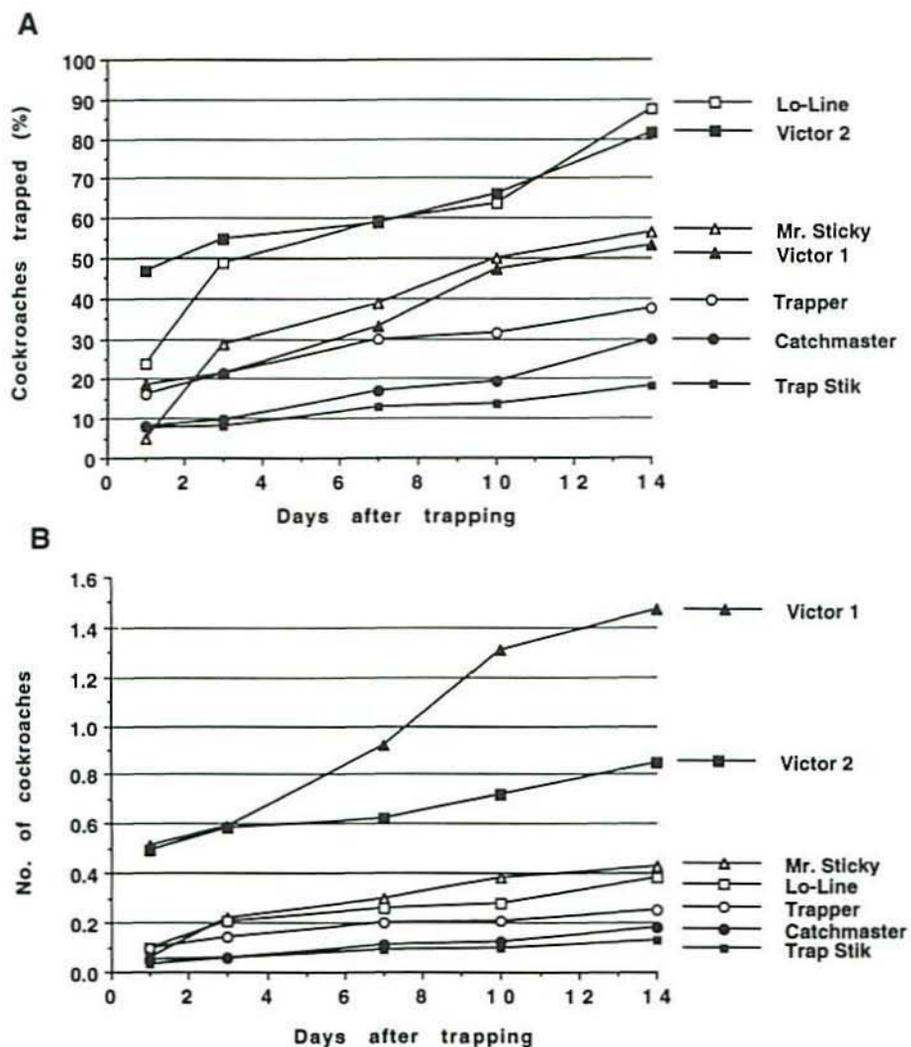


Fig. 1. (A) Percentage of cumulative sticky trap catches on cockroaches in arenas, and (B) number of trapped cockroaches per square centimeter of sticky surface.

Table 1. Sex and developmental stage comparisons of German cockroaches trapped after 1- and 14-d exposure to commercial sticky traps.

Trap brand	Mean \pm SE of cockroaches at day									
	1					14				
	Males	females	Gravid females	Nymphs	Total	Males	females	Gravid females	Nymphs	Total
Lo-Line	6.0 \pm 1.5bc	16.7 \pm 3.2a	3.3 \pm 1.6b	12.0 \pm 2.3b	38.0 \pm 4.2b	46.3 \pm 3.1a	27.3 \pm 1.6a	33.3 \pm 5.3a	32.7 \pm 3.2a	139.7 \pm 7.7a
Victor 2	18.3 \pm 4.0a	22.7 \pm 3.3a	8.7 \pm 2.7a	25.0 \pm 5.8a	74.7 \pm 13.2a	37.3 \pm 5.2ab	32.7 \pm 3.3a	25.3 \pm 3.0ab	34.7 \pm 3.3a	130.0 \pm 12.0a
Victor 1	9.7 \pm 1.9b	3.3 \pm 1.3cd	8.0 \pm 1.0a	8.7 \pm 1.2bc	29.7 \pm 4.5bc	30.7 \pm 2.7b	10.7 \pm 3.6bc	17.7 \pm 2.2bc	26.0 \pm 4.5a	85.0 \pm 9.8bc
Mr. Sticky	2.0 \pm 1.0c	7.3 \pm 1.7b	0.3 \pm 0.3b	2.7 \pm 1.5d	12.3 \pm 3.4d	25.3 \pm 2.0bc	23.3 \pm 1.8a	20.0 \pm 2.5bc	21.7 \pm 5.0ab	90.3 \pm 4.7b
Trapper	9.0 \pm 2.7b	6.7 \pm 1.0bc	3.0 \pm 1.1b	7.3 \pm 3.6bcd	26.0 \pm 5.1bc	20.0 \pm 3.7cd	14.3 \pm 2.8a	11.0 \pm 2.8cd	14.7 \pm 6.3bc	60.0 \pm 11.7cd
Catchmaster	6.3 \pm 2.2bc	3.0 \pm 0.7bcd	2.3 \pm 0.8b	1.3 \pm 0.9d	13.0 \pm 2.4d	17.0 \pm 2.8cd	6.0 \pm 1.9c	18.0 \pm 5.2bc	7.0 \pm 3.1cd	48.0 \pm 11.5de
Trap Stik	2.0 \pm 1.5c	1.7 \pm 0.6d	1.0 \pm 0.4b	3.3 \pm 1.4cd	8.0 \pm 1.7d	12.7 \pm 2.5d	4.7 \pm 1.2c	7.7 \pm 2.9d	4.0 \pm 1.5d	29.0 \pm 6.1e
<i>F</i>	6.7	17.6	6.3	8.2	15.5	11.8	14.5	6.6	11.0	18.1
<i>P</i>	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Means in the same column followed by the same letters are not significantly different (Duncan's multiple range test; $P > 0.05$; SAS Institute 1990).

design (number, location, and size of openings on the sides and top of the traps), trap placement near infested sites and food sources, the ability of a trap to hold and attract cockroaches (i.e., relative quality of sticky materials and the presence of attractants such as food or pheromones), cockroach exploratory behavior during foraging, and cockroach learning through contact with sticky materials.

The research discussed here was conducted under a manageable and observable laboratory environment in a small testing arena. Thus, field trials to support this laboratory research are needed and can be accomplished using these traps, alone or in conjunction with other nonchemical methods (e.g., vacuuming and sanitation) or with low-impact pesticide techniques (e.g., baiting) to determine the effectiveness of the traps in controlling or reducing German cockroach populations. Placing Victor 1 traps in infested apartments reduced cockroach populations in homes to levels comparable to those obtained through the use of insecticides alone (Kaakeh & Bennett 1997). Development of strategies for using trapping technology in an IPM program is needed to enable the implementation of environmentally sensitive control methods and for achieving acceptable, cost-effective cockroach control in infested residences.

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Toxicity of azinphosmethyl, methyl parathion, and oxamyl against the boll weevil (Coleoptera: Curculionidae) in Texas, Mexico, and Guatemala¹

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Rio Bravo, Tamaulipas, Mexico

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ABSTRACT The toxicity of azinphosmethyl, methyl parathion, and oxamyl was tested against boll weevil populations from Texas, Mexico, and Guatemala. The LD₅₀ values of topically applied azinphosmethyl against field-collected boll weevil, *Anthonomus grandis* Boheman, from the Lower Rio Grande Valley of Texas were 0.16, 0.42-0.74, 0.43, 0.23-0.25, 0.043, 0.059-0.13, and 0.0011-0.14 µg per weevil in 1983, 1985, 1986, 1988, 1989, 1990, and 1991, respectively. Even though LD₅₀ values showed 127-fold difference in 1991, only variation in susceptibility was indicated during the 8 yr of testing. The LD₅₀ value of topically applied oxamyl was 0.31 µg per weevil against field-collected weevils in 1990 from the Lower Rio Grande Valley. The LD₅₀ values of topically applied methyl parathion were 0.081-0.0091 µg per weevil in Lower Rio Grande Valley and in Mexico (Rio Bravo, Tamaulipas) in 1990 and 1991, respectively. These LD₅₀ values indicate variation in susceptibility. A strain from Tiquisate, Guatemala, had a LD₅₀ of 0.44 µg of methyl parathion per weevil in 1991. This LD₅₀ is the first shown from that country, and it was greater than LD₅₀ values for weevils collected from the Lower Rio Grande Valley and Mexico in 1991.

KEY WORDS Methyl parathion, azinphosmethyl, oxamyl, *Anthonomus grandis*, Coleoptera, toxicity, insecticide resistance

Azinphosmethyl and methyl parathion are recommended (Norman & Sparks 1992) and widely used insecticides for control of the boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae), in the Lower Rio Grande Valley of south Texas. Producers of cotton in the Lower Rio Grande Valley have indicated that azinphosmethyl, at its maximum recommended use rate of 0.28 kg/ha (Norman & Sparks 1992), is not as effective today in the field as it was in the early 1960s when it was first introduced.

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Methyl parathion was shown to be effective against the boll weevil in the Lower Rio Grande Valley (McGarr & Wolfenbarger 1970). In the coastal bend area of south Texas, Benedict et al. (1983) reported that oxamyl and azinphosmethyl were equally effective against the boll weevil. Hines (1989) showed that from 0.14 to 0.28 kg/ha (0.125 to 0.25 lb/acre) of oxamyl and azinphosmethyl were equally effective against the boll weevil in Alabama and Texas in 1983 and in Mississippi and Georgia in 1987. Maximum LD₅₀ values of 0.13 and 0.1 g per weevil of azinphosmethyl applied topically to weevils collected in 1972 to 1983 from the Lower Rio Grande Valley were determined by Teague et al. (1983) and by Wolfenbarger et al. (1986), respectively.

Methyl parathion is the insecticide of choice by producers for boll weevil control in Mexico and Guatemala. Methyl parathion and oxamyl are used in the United States for control of this insect. No information in the literature was found on long-term toxicity of azinphosmethyl to boll weevils from Texas since 1983. This study was conducted to determine if resistance to azinphosmethyl had indeed developed as suggested above. For these reasons, toxicity of azinphosmethyl, methyl parathion, and oxamyl was tested against field-collected strains of boll weevil from the Lower Rio Grande Valley and Corpus Christi in south Texas; Uvalde and Brazos Valley in south central Texas; Mexico; and Guatemala. The "ebony" insecticide-susceptible strain also was tested each year for comparison.

Materials and Methods

Methyl parathion (Monsanto Co., St. Louis, Missouri) and oxamyl (Dupont, Inc., Wilmington, Delaware) were obtained as 97% pure technical grades for all laboratory tests. Azinphosmethyl (Bayer, Inc., Kansas City, Kansas) was obtained as 94% technical grade. Laboratory tests were conducted in 1983 and from 1985 to 1991.

Boll weevils were reared from squares of cotton, *Gossypium hirsutum* L., collected in the Lower Rio Grande Valley and in sites in Texas and Guatemala. Weevils also were reared from bolls of cotton collected in Rio Bravo, Tamaulipas, Mexico. Squares or bolls were collected once during the cotton midseason in one or more fields (50 km apart) at each site in Texas, Mexico, and Guatemala. Weevils from each field were tested separately. "Ebony" weevils were obtained from the Gast Rearing Facility, United States Department of Agriculture, Agricultural Research Service, Boll Weevil Research Laboratory, Mississippi State, Mississippi. This strain has been reared in the laboratory without selection by any insecticide for the past 10 yr.

Azinphosmethyl was topically applied to the dorsum of the thorax of weevils in the laboratories of Miles, Inc., located in Kansas City, Missouri; Vero Beach, Florida; and Weslaco, Texas. In 1990 and 1991, methyl parathion was topically applied as described for azinphosmethyl in Weslaco, Texas, and Rio Bravo, Tamaulipas. Weevils from Guatemala were tested in Weslaco, Texas. In 1990, oxamyl was applied as described for azinphosmethyl in Weslaco, Texas. All insecticide concentrations were prepared in acetone, and 1 µl of solution was applied. Weevils were given water and larval diet as described by Anonymous (1968) for 3–7 d prior to treatment. Anonymous (1968) recommended that

weevils be tested at 2 d of age; however, no significant differences in LD_{50} values for azinphosmethyl (Wolfenbarger et al. 1986) and fenvalerate (Bariola & Bergman 1982) have been found on weevils 3–7 d old. Eleven dosages (1, 0.6, 0.4, 0.2, 0.1, 0.08, 0.06, 0.04, 0.02, 0.01, and 0.005 g per weevil) of azinphosmethyl and methyl parathion were applied. Seven doses (10, 5, 2.5, 1.25, 0.625, 0.31, and 0.1 g per weevil) of oxamyl were applied. Weevils from all locations were held individually in capped 28-ml plastic cups following treatment.

Mortalities were determined after 48 h and subjected to Probit (SAS 1985) analysis. Food was not offered to weevils during the holding period. To correct for mortalities of treated insects for all insecticides, mortalities of untreated controls were analyzed with $C = \text{rate}$ option by SAS (1985), where rate specifies a constant threshold rate of control mortality between 0 and 1. Differences between LD_{50} values were indicated when 95% confidence intervals (CI) did not overlap.

Results and Discussion

The LD_{50} values for azinphosmethyl against field-collected boll weevils from infested squares collected in the Lower Rio Grande Valley were 0.16, 0.42–0.74, 0.43, 0.23–0.25, 0.043, 0.059–0.13, and 0.0011–0.14 g per weevil in 1983, 1985, 1986, 1988, 1989, 1990, and 1991, respectively (Table 1). In 1991, there was a 127-fold difference between the highest and lowest LD_{50} values. This variation is the greatest observed in LD_{50} values shown for this insect with azinphosmethyl at one location in a year examined. The highest and lowest LD_{50} values were observed in May 1985 and 1991, respectively. In addition, the LD_{50} value for azinphosmethyl of 0.16 g per weevil collected in 1983 from the Lower Rio Grande Valley near Weslaco was similar (95% CI overlapped) to that of another field strain collected near Brownsville, Texas, in 1983 (Wolfenbarger et al. 1986). In 1990, LD_{50} values for azinphosmethyl were equal (95% CI overlapped), but an extremely low and significantly different LD_{50} of 0.0011 g per weevil was observed for weevils collected from one Lower Rio Grande Valley field in 1991. One explanation for this low LD_{50} value is that weevils can readily disperse during a given season from any of the up to 10,000 fields of cotton planted each year in the Lower Rio Grande Valley and Mexico. Any LD_{50} is possible because adjacent fields are within 60 km north and south of the Rio Grande River.

The LD_{50} values for azinphosmethyl were determined for weevils from Corpus Christi in 1985, 1986, 1987, 1989, and 1991 as 0.42, 0.19, 0.11, 0.038, and 0.048, respectively (Table 1). At this location, LD_{50} values generally decreased each year from 1985 to 1991. The LD_{50} values determined for weevils from Uvalde in 1985, 1986, 1987, 1989, 1990, and 1991 were 0.67, 0.28, 0.15, 0.11–0.27, 0.17, and 0.078, respectively (Table 1). A similar trend to that observed for Corpus Christi was shown for Uvalde over the same time period. Although the LD_{50} values for Uvalde in 1985, 1986, 1987, 1989, and 1991 were greater than those for Corpus Christi, they were not always statistically different than those for the Lower Rio Grande Valley in corresponding years.

The LD_{50} values were determined from the Brazos Valley in 1985, 1986, 1988, 1989, and 1991 as 0.29, 0.37, 0.17–0.19, 0.081, and 0.17 g per weevil,

Table 1. Toxicity of azinphosmethyl against field-collected and ebony laboratory-reared boll weevils in Texas (1983 and 1985-1991).

Year	Location in Texas	Number tested	Control mortality (%)	Micrograms per adult after 48 h		
				Slope \pm SE	LD ₅₀	(95% Confidence interval)
1983	LRGV ^a	600	0	2.25 \pm 0.49	0.16	0.058-0.25
	Ebony strain	400	9.6	7.68 \pm 2.06	0.12	0.084-0.17
1985	LRGV	120	0	0.95 \pm 0.23	0.74	0.39-2.97
	LRGV	124	0	3.29 \pm 1.37	0.42	- b
	Corpus Christi	124	0	2.58 \pm 0.41	0.42	0.32-0.55
	Uvalde	120	0	2.92 \pm 0.93	0.67	0.32-5.89
	Brazos Valley	124	0	2.89 \pm 0.66	0.29	0.13-0.54
	Ebony strain	700	5.0	3.07 \pm 0.74	0.37	0.17-0.64
1986	LRGV	600	0	2.96 \pm 0.61	0.43	0.24-0.74
	Corpus Christi	400	0	2.68 \pm 0.44	0.19	0.12-0.70
	Uvalde	600	0	1.46 \pm 0.23	0.28	0.15-0.47
	Brazos Valley	800	0	3.23 \pm 0.27	0.37	0.27-0.5
	Ebony strain	380	0	3.34 \pm 0.27	0.23	0.20-0.27
1987	Corpus Christi	60	0	2.02 \pm 0.71	0.11	0.06-0.17
	Uvalde	140	0	2.66 \pm 0.62	0.15	0.06-0.26
	Ebony strain	140	0	6.47 \pm 3.34	0.066	- b
1988	LRGV	140	0	5.13 \pm 1.19	0.23	0.15-0.29
	LRGV	140	0	3.40 \pm 0.85	0.25	0.089-0.43
	Brazos Valley	120	0	2.54 \pm 0.75	0.19	0.017-0.47
	Brazos Valley	140	0	2.68 \pm 0.63	0.17	0.081-0.34
	Ebony strain	320	7.2	3.25 \pm 0.95	0.09	0.027-0.17
1989	LRGV	700	0	1.86 \pm 0.28	0.043	0.024-0.065
	Corpus Christi	600	0	2.51 \pm 0.60	0.038	0.01-0.062
	Uvalde	700	0	1.37 \pm 0.37	0.11	0.032-0.35
	Uvalde	200	0	1.33 \pm 0.64	0.27	0.15-10.2 \times 10 ⁶
	Brazos Valley	500	0	3.99 \pm 1.20	0.081	0.00034-0.15
1990	Ebony strain	700	0	2.20 \pm 0.50	0.043	0.020-0.079
	LRGV	105	0	1.61 \pm 0.55	0.06	0.038-0.092
	LRGV	105	0	1.39 \pm 0.51	0.059	0.034-0.094
	LRGV	290	0	0.78 \pm 0.053	0.13	0.068-0.26
	Uvalde	180	0	2.85 \pm 1.31	0.17	0.094-0.27
	Ebony strain	479	3.6	1.09 \pm 0.014	0.024	0.016-0.033
1991	LRGV	105	0	1.90 \pm 0.61	0.14	0.10-0.21
	LRGV	70	0	2.10 \pm 0.80	0.088	0.056-0.14
	LRGV	180	3.2	0.34 \pm 0.13	0.0011	9 \times 10 ⁻⁸ -0.0065
	Corpus Christi	105	0	2.62 \pm 0.88	0.048	0.035-0.066
	Uvalde	105	0	2.07 \pm 0.79	0.078	0.054-0.11
	Brazos Valley	70	0	1.65 \pm 0.68	0.17	0.10-0.30
	Ebony strain	140	4.8	1.95 \pm 0.58	0.044	0.031-0.060

^aLRGV, Lower Rio Grande Valley, Texas.^bProbit calculation shows infinity for both high and low confidence intervals.

respectively (Table 1). These values were generally similar to those from the Lower Rio Grande Valley in corresponding years. Although the trend for decreasing LD_{50} values over time was evident, it was not as clear as for field-collected strains from Corpus Christi and Uvalde.

The "ebony" laboratory strain showed LD_{50} values of 0.06–0.13, 0.12, 0.37, 0.23, 0.066, 0.09, 0.043, 0.024, and 0.044 g of azinphosmethyl per weevil from samples of this population in 1978, 1983, 1985, 1986, 1987, 1988, 1989, 1990, and 1991, respectively (Table 1). The LD_{50} values for this insecticide against the ebony strain differed 15-fold between the highest and the lowest value during the 8 yr of testing and were 0.09 from 1987 to 1991. However, from 1983 to 1986, LD_{50} values ranged from 0.12–0.37. Such high values have never been reported for this strain (D.A.W., unpublished data). These results show about the same variability for this strain as is shown for the field strains collected each year from the Lower Rio Grande Valley.

Slope values ranged from 7.68–0.34 for probit regressions of azinphosmethyl topically applied to field-collected and laboratory-reared weevil strains in 1983 and from 1985 to 1991. However, slope values for azinphosmethyl against field-collected weevils were the flattest from 1989 to 1991 and the steepest from 1983 to 1988. This coincides with the approximate time periods for higher LD_{50} values in 1983 to 1988 and lower LD_{50} values in 1989 to 1991. A frequency distribution of the percentage of slopes that ranged 0.1–0.9, 1–2, 2.1–3, 3.1–4, and 4.1 were 10.3%, 25.6%, 38.5%, 15.4%, and 7.6%, respectively. Standard errors of slopes were variable.

Data reported by this investigation show great variation in the response of boll weevils from field-collected cotton squares to azinphosmethyl in the Lower Rio Grande Valley in 1983 and from 1985 to 1991. Similar variation was reported by Wolfenbarger et al. (1986) in 1972 to 1982. How are resistance and susceptibility defined for this insecticide when LD_{50} values differ over 100-fold and cycle from high to low during this time period? Resistance should be consistent with field control failure occurring in all or most all fields of cotton in a defined area each year. This concept is different from the idea that resistance to azinphosmethyl can be random and of local adaptation. This concept also suggests that the first resistant populations, when detected, will be rare, patchy, and not homogeneous, thus causing randomness of response as observed in this study. In addition, weevil dispersal flights between fields in an area or between areas and mating among resistant and susceptible forms make detection of adaptation difficult. Resistance cannot be a field failure with a high LD_{50} one year and not the next in the same or adjacent fields.

In 1991, weevils collected from Rio Bravo, Tamaulipas, Mexico, were susceptible to methyl parathion with a LD_{50} of 0.0091 g per weevil (Table 2). In 1990 and 1991, we obtained LD_{50} values of 0.081 and 0.01 (Table 2) g per weevil, respectively, in the Lower Rio Grande Valley. All three LD_{50} values for the strains from the Lower Rio Grande Valley of Texas and Rio Bravo, Tamaulipas, Mexico, were significantly less than those collected from Tiquisate, Guatemala. The LD_{50} values of methyl parathion to boll weevils from Tiquisate, Guatemala, were intermediate to those from Nicaragua in 1983–1985 (Laboucheix & Gonzalez 1987, Swezey & Salamanca 1987). The LD_{50} values reported by these authors showed a normal distribution from the

Table 2. Toxicity of methyl parathion and oxamyl against field-collected and ebony strain boll weevils in Texas, Mexico and Guatemala (1990-1991).

Insecticide	Strain and year	Number Tested	Control mortality (%)	Micrograms per adult after 48 h		
				Slope \pm SE	LD ₅₀	(95% Confidence Interval)
Methyl parathion	LRGV, 1990 ^a	410	0	3.89 \pm 0.51	0.081	0.073-0.093
	Ebony 1990	540	7.4	1.74 \pm 0.061	0.12	0.1-0.14
Oxamyl	LRGV, 1990	390	1.6	1.07 \pm 0.021	0.31	0.22-0.46
	Ebony 1990	330	3.1	1.45 \pm 0.039	0.16	0.11-0.21
Methyl parathion	LRGV, 1991	163	0	1.16 \pm 0.22	0.01	0.0051-0.16
Methyl parathion	Mexico, 1991 ^b	145	9.8	2.22 \pm 0.3	0.0091	0.0066-0.013
Methyl parathion	Guatemala, 1991 ^c	611	0	1.31 \pm 0.11	0.44	0.36-0.54

^aLRGV, Lower Rio Grande Valley, Texas.

^bRio Bravo, Tamaulipas, Mexico.

^cTiquisate, Guatemala.

high value of 1.73 g per weevil to a low of 0.09 g per weevil. Thus, LD₅₀ values for methyl parathion, the standard insecticide used against the boll weevil in Central America, were generally higher than those observed in strains from Texas and Mexico.

Topical applications of both azinphosmethyl and methyl parathion to field-collected weevils from the Lower Rio Grande Valley gave LD₅₀ values in this investigation that were similar (95% CI values overlapped) in 1990 (Tables 1, 2) to those reported in 1981 by Teague et al. (1983). In 1990, slope values for methyl parathion were variable. In 1981, Teague et al. (1983) showed a LD₅₀ for azinphosmethyl for the Brazos Valley of 0.12 g per weevil, which was similar (95% CI values overlapped) to the LD₅₀ value determined in the present study in 1985 (Table 1).

In 1990, the LD₅₀ value for oxamyl was 0.31 g per weevil (Table 2) and greater than any shown for both azinphosmethyl and methyl parathion against the same population of boll weevils. The LD₅₀ values for oxamyl and methyl parathion against the ebony strain were similar but greater than those observed for azinphosmethyl.

No comparison of field control versus any LD₅₀ shown here was made with any insecticide. It would be most difficult because boll weevil populations can be susceptible and yet have LD₅₀ values greater than the controls. If insecticide treatments are applied after the economic threshold for the Lower Rio Grande Valley of Texas (Norman & Sparks 1992) of 15% damaged squares is determined, weevils can be almost impossible to control if the field is large or many fields in an area have high damage levels.

In summary, LD₅₀ values from 1983 and from 1985 to 1991 show variation in the susceptibility but not resistance of field-collected boll weevils to azinphosmethyl and methyl parathion. This variation in toxicity and the failure to initiate spray applications on time accounts for the verbal reports by producers of suspected resistance of boll weevils to azinphosmethyl.

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Feeding Preferences of Larval and Adult *Microthecha ochroloma* (Coleoptera: Chrysomelidae) for Crucifer Foliage¹

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ABSTRACT Feeding preferences of first and third instars, and the adult yellowmargined leaf beetle, *Microthecha ochroloma* Stål, for the foliage of cabbage, *Brassica oleracea* var *capitata* L.; collard, *B. oleracea* var *acephala* L.; mustard, *B. juncea* Cosson; turnip, *B. rapa* L.; and radish, *Raphanus sativus* L., were evaluated with leaf disk choice tests. Each of the three developmental stages showed statistically significant ($P < 0.05$) feeding preference for some crucifer hosts over others. In general, turnip and mustard were the more preferred plants, and cabbage, the least preferred. Although first instars strongly preferred turnip to all other plants, third instars showed equal preference for turnip and mustard, and adult beetles consumed about the same amount of turnip, mustard, and radish. Beetles' choice of leaf disk was not significantly influenced ($P > 0.05$) by the host plant on which they were conditioned prior to feeding preference evaluation.

KEY WORDS *Microthecha ochroloma*, Coleoptera, Chrysomelidae, Cruciferae, feeding preference, leaf disk, consumption

The yellowmargined leaf beetle, *Microthecha ochroloma* Stål, is a serious foliar pest of cruciferous crops in Louisiana (Oliver & Chapin 1983). This beetle, indigenous to South America, was accidentally introduced into the United States around 1945 (Chamberlin & Tippins 1948). The beetle is presently restricted in distribution to the Gulf Coast states and has been collected on cabbage, *Brassica oleracea* var *capitata* L.; collard, *B. oleracea* var *acephala* L.; mustard, *B. juncea* Cosson; turnip, *B. rapa* L.; radish, *Raphanus sativus* L.; and watercress, *Nasturtium officinale* R. Brown (Chamberlin & Tippins 1948, Haeussler 1951, Woodruff 1974, Balsbaugh 1978, Oliver & Chapin 1983). These plants belong to the family Cruciferae, whose principal allelochemical is mustard oil glucosides (Hicks 1974, Feeny 1977). Both adult and larval *M. ochroloma* damage hosts by feeding on the foliage. The adults chew holes in

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the leaves and sometimes feed upon leaf margins, whereas the larvae feed frequently in groups and when abundant, consume large portions of foliage of infested plants (Chamberlin & Tippins 1948). Because the foliage of these plants is harvested for sale in local fresh markets as "greens," their marketability is subject to an aesthetic damage threshold in which there is a near zero tolerance for insect feeding holes on the leaves.

The biology of this beetle has not been well studied, despite its wide distribution in the southeastern United States and its damaging impact to crucifers. Laboratory experiments were conducted to evaluate the preferences of *M. ochroloma* for the foliage of cabbage, collard, mustard, radish, and turnip, partly to aid our understanding of its biology and also to enable an assessment of its potential for field crop damage.

Materials and Methods

Host plants and insects. The cultivars 'Early Round Dutch' cabbage, 'Georgia' collard, 'Florida Broadleaf' mustard, 'Scarlet Globe' radish, and 'Purple Top White Globe' turnip were used. These plants were raised in the greenhouse to ensure that a source of insecticide and disease-free foliage was available to feed the beetles. Plants were raised on a Jiffy-mix[®] medium (Jiffy Products of America, Inc., Batavia, Illinois), and fertilized on alternate days by using Miracle-Gro[®] (Stern's Miracle-Gro Products, Inc., Port Washington, New York), a 20:20:20 water soluble NPK fertilizer mixture. New plantings were made every 10 d to ensure that young foliage (3 to 5 wk) was continuously available.

A laboratory colony of the yellowmargined leaf beetle was started in the fall of 1992 from beetles collected on mustard (cv 'Florida Broadleaf') at the St. Gabriel Research Station, Louisiana State University Agricultural Experiment Station, Iberville Parish, Louisiana. A total of 291 specimens was collected at five weekly intervals between 23 September and 30 October 1992. In the spring of 1993, a total of 87 beetles was collected from a second mustard plot at the same location on 13 November 1992, and 18 January and 25 January 1993. These beetles were maintained continuously for >11 generations, initially on mustard foliage obtained from the field until plants raised in the greenhouse became available. Beetles were held in filter-paper-lined Petri dishes (100 mm × 15 mm) in growth chambers maintained at 20°C and a photoperiod of 14:10 (L:D) h with 50% RH.

Feeding preference studies. To begin the study, about 500 eggs were collected from the beetle colony (described above), when it contained about 400 beetles (with approximately 60% females). These eggs were randomly divided into five groups and each group was randomly assigned to each of the five host plants. Feeding preference evaluation was carried out after beetles had been conditioned on the assigned host plant for two generations. The experiment was set up as a randomized complete block design and replicated at five weekly intervals. For each developmental stage, twenty 1-d-old test insects were exposed to five randomly generated arrangements of host plant leaf disks. Leaf disks (15 cm²) were excised from young foliage of host plant (2 to 3 wk old) obtained from the greenhouse and were circularly arranged in filter-paper-lined

Petri dishes (140 mm × 50 mm). The dishes were placed on trays and the trays were arranged on benches in a growth room maintained at $20 \pm 1^\circ\text{C}$ with a photoperiod of 14:10 (L:D) h with 70% RH. Because leaf consumption by first instars was minuscule, preference was measured as the number of larvae found feeding on each leaf disk after 24 h. Feeding preferences of third instars and adult beetles were quantified separately as the amount of foliage consumed after 24 h, and for the third instars also as the number of larvae associated with the leaf disks. Foliage consumption was quantified as the difference between the initial and final weight of the leaf disks (to the nearest milligram) after test insects were removed. To account for the possibility of water loss in the leaf disks in the experimental arena, the initial and final weight of five leaf disks of each host plant to which beetles were not exposed, were recorded at each replication.

Statistical analyses. Data were analyzed as a randomized block design (PROC GLM, SAS Institute 1990) for the number of test insects associated with a leaf disk of a host plant (first and third instars) and leaf consumption (third instars and adults). The number of third instars associated with the leaf disk of each plant also was correlated with leaf consumption (PROC CANCORR, SAS Institute 1990). A multivariate analysis (PROC GLM with MANOVA statement, SAS Institute 1990) was carried out to test whether beetles' choice of leaf disk was significantly influenced ($P = 0.05$) by the host plant on which they were conditioned prior to feeding preference evaluations. To determine whether there was water loss in the leaf disks to which beetles were not exposed, a paired comparison of initial and final weight of these leaf disks was carried out (PROC MEANS with *t*-test option, SAS Institute 1990).

Results and Discussion

Each of the three developmental stages of *M. ochroloma* showed strong feeding preference for some crucifer plants over others as revealed by the number of larvae associated with the leaf disks of the plants (first and third instars) and the amount of foliage consumed (third instars and adults). Significant differences were found in the number of first ($F = 44.22$, $df = 4$, $P = 0.0001$) and third instars ($F = 9.76$, $df = 4$, $P = 0.0003$) associated with the leaf disks of host plants. The amount of foliage of each plant consumed also was significantly different for the third instars ($F = 15.23$, $df = 4$, $P = 0.0001$) and adult beetles ($F = 4.90$, $df = 4$, $P = 0.0090$).

First-instar yellowmargined leaf beetles showed strong preference for turnip and least preference for cabbage and collard (Table 1). The preferences of third instars and adult beetles were similar because both consumed a significantly higher amount of the foliage of turnip or mustard over those of collard or cabbage (Table 2). Consumption of turnip by the third instars was three times higher than consumption of collard or cabbage. A positive and significant correlation ($r = 0.98$, $P = 0.0035$) was found between the number of third instars associated with the foliage and the amount of foliage of each plant consumed. Unlike the larval instars, the feeding preference of adult beetles was not as clear cut. Although cabbage was the least preferred, consumption of turnip, mustard, or radish foliage was not significantly different (Table 2). Beetles choice of leaf disks was not significantly ($P > 0.05$) influenced by the host plant

Table 1. Feeding preferences of first- and third-instar *M. ochroloma* as determined by the number of larvae associated with leaf disk of cabbage, collard, mustard, radish, and turnip after 24 h.

Mean ^a (\pm SE) number of insects associated with leaf disk of host plant		
Host plant	First instar	Third instar
Cabbage	1.12 \pm 0.26c	1.88 \pm 0.31c
Collard	1.52 \pm 0.34c	2.32 \pm 0.44c
Mustard	5.96 \pm 1.07b	4.80 \pm 0.49b
Radish	2.32 \pm 0.42c	3.36 \pm 0.58bc
Turnip	9.16 \pm 1.05a	7.04 \pm 0.85a

Means within columns followed by same letter(s) are not significantly different ($P > 0.05$, Tukey test [SAS Institute 1990]). For each column, $df = 4$.

^aBased on 5 replications, $n = 20$ insects per replicate.

on which they were conditioned prior to feeding preference evaluations. The Wilk's Lambda test statistics and the respective p -values were 0.4048 and 0.3520 for first instars, 0.4394 and 0.7463 for third instars, and 0.4696 and 0.8170 for adult beetles, respectively.

We did not detect any significant differences ($P > 0.05$) in the initial and final weight of leaf disks to which beetles were not exposed. The associated p -values were 0.1876, 0.1029, 0.6700, 0.2715, and 0.7589 for cabbage, collard, mustard, radish, and turnip, respectively. These results suggest that there was minimal water loss in the leaf disks in the experimental arena. Perhaps because the leaf disks were placed on water-saturated filter papers and the duration of the experiment was short.

In general, larval and adult yellowmargined leaf beetles showed strong preference for turnip and mustard foliage, followed by radish, collard, and cabbage, respectively. These results suggest field plantings of turnip and mustard will be more attractive to beetle infestations than field-planted cabbage and collard. At present, there are no quantitative data on the relative population densities of the beetle on field plantings of any of these crops. Nevertheless, many authors have observed and reported that this beetle was more commonly found in the field associated with turnip and mustard rather than on the other crucifers (Chamberlin & Tippins 1948, Haeussler 1951, Rohwer et al. 1953, Oliver 1956, Spink 1959, Anonymous 1976). Chamberlin & Tippins (1948) suggested that the beetle was found on the less preferred plants in the field only when the preferred plants were not available. When insects are forced to feed on less preferred plants, egg output by the female may decline leading to a decrease in population density (Dethier 1954).

Table 2. Feeding preferences of third instars and adult *M. ochroloma* for cabbage, collard, mustard, radish, and turnip determined by leaf consumption (in milligrams) after 24 h.

Host plant	Mean ^a (\pm SE) leaf consumption	
	Third instar	Adult
Cabbage	16.16 \pm 2.21c	24.15 \pm 3.52c
Collard	19.84 \pm 2.65c	36.19 \pm 5.36bc
Mustard	45.55 \pm 2.95a	50.04 \pm 2.63a
Radish	32.61 \pm 2.77b	43.89 \pm 4.05ab
Turnip	55.51 \pm 3.61a	49.68 \pm 3.21a

Means within columns followed by same letter(s) are not significantly different ($P > 0.05$, Tukey test [SAS Institute 1990]). For each column, $df = 4$.

^aBased on 5 replications, $n = 20$ insects per replicate.

The preference shown by the yellowmargined leaf beetle for these crucifer plants has some relationship to the fecundity of the females. Ameen (1996) reported that beetles fed cabbage and collard, on average, laid fewer eggs (271 and 198, respectively) than beetles fed mustard (424) and turnip (490). In general, females of phytophagous insects have been shown to be able to select and feed on host plants based on characteristics associated with reproductive performance, hence host preference and fecundity are expected to be correlated (Ferguson et al. 1991, Ramnath et al. 1992). In addition, the physical characteristics of the foliage of the crucifer plants also seem to have some relationship to the feeding preference of the beetle. The preferred plants have relatively soft foliage whereas the foliage of the less preferred plants are relatively tough and waxy. On the basis of this observation, we speculate that leaf texture might have played a role in the beetles' feeding preference. The physical toughness of plant tissues has been suggested to influence the degree to which they can be exploited by phytophagous insects (Tanton 1962, Theagwam 1981).

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Larval Rearing Density Effects on Lipid Reserves and Wing-loading in Fall Armyworm Adults (Lepidoptera: Noctuidae)¹

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ABSTRACT A laboratory study was conducted to determine if a high larval rearing density produces premigrant traits in the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), and if these traits can be used as criteria for separating non-premigrant from premigrant groups. The putative premigrant traits of high lipid reserves and low wing-loading were measured in newly emerged adult fall armyworm. An increase in rearing density significantly increased adult whole-body lipid content, but the increase may be due, in part, to enhanced nutrition through cannibalism. Although larval rearing density did not affect wing-loading, the fall armyworm as a migrant species showed lower than theoretically expected wing-loading values. The lack of density-dependent increases in lipid reserves and the lack of density effects on adult activity, developmental time, and size (documented herein and in previous studies) may indicate that a premigrant phase induced by high larval density does not exist in the fall armyworm.

KEY WORDS Lepidoptera, Noctuidae, *Spodoptera frugiperda*, migration, lipid reserves, larval density, wing-loading

A laboratory study was conducted to determine if a high larval rearing density produces premigrant traits in the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), and if these traits can be used as criteria for separating non-premigrant from premigrant groups. Premigrant moths, which are morphologically and physiologically capable of migratory flight and have a high propensity for migratory flight but have not yet flown, may possess certain traits such as small size, high flight potential, low wing-loading (ratio of body weight to wing area), and high lipid reserves (Angelo & Slansky 1984). Previous research has shown density to have an inverse effect on pupal weight (an indicator of adult size), but density did not affect developmental time or adult flight activity (Ferguson et al. 1994, H. J. F., unpublished data).

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Lipids are a more economical energy source than carbohydrates for long-range flight because lipids yield more energy calories per gram than carbohydrates (Weis-Fogh 1968), release more metabolic water per mole oxidized than carbohydrates, and take up less storage with water than carbohydrates (Beenackers et al. 1985). In addition, lipids are a vital resource for egg production, which becomes important for a colonizing migrant moth when it reaches its destination.

Wing-loading is the ratio of whole body weight to total wing area (derived from the aeronautical term W/A , where W is the gross weight of an airplane or glider and A is the gross wing area). A moth with a lower wing-loading is one that would carry less weight per unit of wing area over long distance flights and burn less fuel than one with a relatively higher wing-loading.

The objective of this study was to determine the effects of larval rearing density on lipid reserves and wing-loading. By increasing the larval rearing density of the fall armyworm in the laboratory, premigrants may be produced that have higher lipid reserves and lower wing-loadings.

Materials and Methods

Insect rearing. Fall armyworm eggs were obtained from the Insect Biology and Population Management Research Laboratory, USDA, ARS, Tifton, Georgia. This colony was originally collected from volunteer corn and sorghum in Georgia, and it is assumed to have genetic similarity to Pashley's (1988) corn strain. Voucher specimens have been placed in the Museum of Biological Diversity, The Ohio State University, Columbus, Ohio.

For the laboratory studies, first instars were placed in 30-ml plastic cups with approximately 15 ml of diet (an excess) at an initial density of one, two, or three larvae per cup. One hundred to 150 cups were used for each density, and each one was replicated three times. Larvae were reared on a modified artificial diet (Guy et al. 1985, brewer's yeast was used instead of torula yeast). Cups were kept in an environmental growth chamber with a photoperiod of 14:10 (L:D) h (summer conditions) at 26°C. Newly formed pupae were placed individually in clean 30-ml cups. Adults were allowed to emerge in the cups.

High population density probably acts in conjunction with a decrease in food quality and quantity (Johnson 1969). Although both food quality and quantity decline over time in the field and in the 30-ml cups, the quality and quantity of the insect diet used in this study were sufficient to support numerous larvae at density levels of two or three larvae to a cup.

The fall armyworm is a naturally cannibalistic noctuid in both the laboratory and the field (Luginbill 1928). Therefore, cannibalism is considered part of the density effect. Fall armyworm larvae prefer to feed inside closed leaves of a corn plant such as the whorl or the furl (Morrill & Greene 1973a) because of an apparent protective advantage possibly influenced by thigmotaxis (Morrill & Greene 1973b). Only one additional larva apparently results in a crowded condition, and cannibalism often occurs. Cannibalism may not be specifically linked to a decline in food quality or quantity for it may occur anytime during the feeding stage of the fall armyworm larva (H. J. F., personal observation).

However, it is assumed that it is more prevalent as the larvae increase in size and become more crowded in the whorl or the small cup.

Lipid studies. Moths were frozen on the day of emergence for lipid analyses. Whole-body lipid content was determined by using the following protocol adapted from Judge (1988). Individual moths were freeze-dried in 5-ml glass vials for at least 3 d. Vials were weighed with moths; the moths were removed and placed individually in preweighed 1.5-ml microcentrifuge tubes. Vials were weighed without moths for calculation of their dry body weight. One steel ball (3 mm in diameter) was placed into each microcentrifuge tube. Two tubes were fastened to a Wig-L-Bug[®] amalgamator (Model # 3110-3A, Crescent Dental Manufacturing Co., Lyons, Illinois). Moths were ground for at least 1 min or until the moth was crushed to a fine powder. The steel balls were removed from the tubes containing moths and 400 μ l of a 2:1 (vol:vol) chloroform:methanol solution was added to each tube. Tubes were vortexed and then centrifuged for 3 min. Supernatants containing the lipids were pulled off and placed in a second set of corresponding preweighed 1.5-ml microcentrifuge tubes. The chloroform/methanol extraction of the pellet was repeated. Uncapped tubes containing the combined extracts were placed in a sand bath at 40°C, and the solvent was allowed to evaporate overnight under the hood, leaving the lipid. Tubes containing dried pellets and supernatant (lipid extract) were weighed. The proportion of whole-body lipid content was determined by dividing the weight of the lipid extract by the initial dry body weight. Verification analyses using spikeovers with Mazola[®] corn oil revealed a mean recovery of 98.8% by using this method.

Analyses to determine the kinds of neutral lipids (those without a reactive sugar or phosphate group attached) found in the fall armyworm were accomplished by using the high-performance thin-layer chromatography (HPTLC) methods developed by Judge (1988) with one modification. The second developing solution was 70:30 vol:vol hexane:ether to enhance the separation of cholesterol ester and triacylglycerol (D. N. Judge, personal communication).

Wing-loading studies. The right forewing and hind wing of each moth were taped to white paper. The length and width of these wings were measured to the nearest 0.01 mm with a hand-held micrometer. The length was measured at its maximum from the tegula to the end of the fringe. The width was measured at the widest point of the wing perpendicular to its long axis (the length).

Several methods for estimating wing area were evaluated, including passing photographic negatives of enlarged wings through a leaf area meter, tracing outlines of wings on graph paper, and using the triangle area formula, but the following method proved to be the most reliable and time efficient. Outlines of wings were traced on weighing paper, cut out, and weighed on an enclosed microbalance. These weights were converted to area using a known area's weight. Forewing area was regressed on forewing width, and hind wing area was regressed on hind wing width to evaluate the wing area estimates. The width was chosen over length because part of the length of the wing was often torn during removal from the body. Total wing area was obtained by adding the forewing area to the hind wing area and multiplying by two.

Many different approaches have been used to evaluate wing-loading (Danthanarayana 1976, Miller 1977, Angelo & Slansky 1984, Parker & Gatehouse 1985). We followed the methods of Angelo & Slansky (1984) and regressed total wing area on adult dry body weight.

Statistical methods. To test for a rearing density effect, analysis of variance ($\alpha = 0.05$) was performed on adult dry body weight and percent whole-body lipid data (transformed to arcsine of the square root of the proportion) with the GLM procedure of SAS (SAS Institute 1985). Regressions to evaluate the wing area estimates and to determine wing-loading were accomplished with the REG procedure of SAS ($\alpha = 0.05$). Total wing area and dry body weight data were log-transformed (base 10) before wing-loading analysis. Regression lines among different density treatments or between sexes were compared by using tests for heterogeneity of slopes and analysis of covariance (PROC GLM, $\alpha = 0.05$).

Results and Discussion

Lipid studies. Percent lipid in fall armyworm adults ranged from 20.1 to 51.2% in females and 19.1 to 56.55% in males (Table 1). Although the dry body weight of females did not differ among density treatments ($F = 1.74$; $df = 2, 182$; $P = 0.1784$), mean percent whole-body lipid in females was significantly greater in the higher density treatments of two and three larvae per cup ($F = 9.92$; $df = 2, 182$; $P < 0.001$) (Table 1). In males, both mean dry body weight ($F = 7.48$; $df = 2, 161$; $P < 0.001$) and percent whole-body lipid ($F = 13.37$; $df = 2, 161$; $P < 0.001$) were significantly greater in the two higher density treatments.

There was a tendency for body weight to increase as rearing density increased in both males and females. In contrast, previous studies showed an increase in pupal weight with decreasing larval rearing density (Ferguson et al. 1994). Cannibalism, considered part of the density effect, may have contributed to the increase in body weight. Cannibalism, as one of several causes of mortality, cannot be isolated and analyzed as a single effect. However, the effect of mortality of accompanying larvae in crowded cups may be examined. To determine if mortality of larvae in a crowded cup affected dry body weight and whole-body lipid of survivors, the data from individuals in the density treatment of two larvae per cup were split into two groups according to the number of larvae per cup at the prepupal stage (in the case of two larvae remaining, when both reached the prepupal stage). Thus, one group consisted of individuals that were crowded with two larvae per cup during the entire larval feeding period (two larvae per cup, Table 2), and the other group consisted of larvae from cups in which mortality, including cannibalism, occurred (one larva per cup, Table 2). Similarly, data from individuals in the density treatment of three larvae per cup were split into three groups according to the number of larvae per cup at the prepupal stage (Table 3). Data from the density treatment of two larvae per cup and of three larvae per cup were analyzed separately.

Within the density treatment of two larvae per cup, dry body weight and whole-body lipid of females and males did not differ when mortality of accompanying larvae was taken into consideration (females—dry body weight

Table 1. Adult dry body weight and percent whole-body lipid in the fall armyworm reared at different densities.

Sex	No. larvae per cup	Dry body wt. (mg)		% Whole-body lipid	
		Mean \pm S.E. (<i>n</i>)	Range	Mean \pm S.E. (<i>n</i>)	Range
Female	1	54.29 \pm 1.79a (61)	23.70–79.70	36.06 \pm 0.82b (61)	20.06–44.84
	2	58.71 \pm 1.55a (46)	37.90–89.70	38.76 \pm 0.65a (46)	25.86–51.08
	3	57.54 \pm 1.55a (78)	22.00–82.50	40.07 \pm 0.56a (78)	27.33–51.24
Male	1	47.14 \pm 1.31b (62)	18.90–71.40	38.96 \pm 0.98b (62)	19.13–51.66
	2	53.51 \pm 1.16a (35)	39.90–64.90	44.91 \pm 0.71a (35)	35.03–56.55
	3	52.61 \pm 1.16a (67)	31.10–80.50	43.51 \pm 0.66a (67)	27.56–54.12

Means within the same column and sex followed by the same letter are not significantly different, Tukey's Studentized Range Test, $\alpha = 0.05$.

Table 2. Effect of early larval mortality on adult dry body weight and percent whole-body lipid in the fall armyworm reared at an initial density of two larvae per cup.

Sex	No. larvae per cup at prepupal stage	Dry body wt. (mg)		% Whole-body lipid	
		Mean \pm S.E. (<i>n</i>)	Range	Mean \pm S.E. (<i>n</i>)	Range
Female	1	61.13 \pm 2.30a (26)	38.20–89.70	39.47 \pm 0.71a (26)	33.53–48.88
	2	55.57 \pm 1.75a (20)	37.90–70.90	37.86 \pm 1.18a (20)	25.86–51.08
Male	1	55.04 \pm 1.27a (23)	43.10–64.90	44.82 \pm 0.92a (23)	35.03–56.55
	2	50.58 \pm 2.19a (12)	39.90–64.20	45.09 \pm 1.15a (12)	39.02–51.69

Means within the same column and sex followed by the same letter are not significantly different, Tukey's Studentized Range Test, $\alpha=0.05$.

Table 3. Effect of early larval mortality on adult dry body weight and percent whole-body lipid in the fall armyworm reared at an initial density of three larvae per cup.

Sex	No. larvae per cup at prepupal stage	Dry body wt. (mg)		% Whole-body lipid	
		Mean \pm S.E. (<i>n</i>)	Range	Mean \pm S.E. (<i>n</i>)	Range
Female	1	65.68 \pm 1.78a (30)	40.00–82.10	41.32 \pm 0.81a (30)	32.39–51.24
	2	52.98 \pm 2.09b (42)	22.00–82.50	39.53 \pm 0.76a (42)	27.33–50.49
	3	48.70 \pm 5.28b (6)	32.30–62.40	37.68 \pm 2.57a (6)	29.19–47.76
Male	1	56.74 \pm 2.31a (19)	34.10–70.90	44.76 \pm 1.21a (19)	33.27–52.97
	2	51.71 \pm 1.51a (37)	35.20–80.50	43.43 \pm 0.96a (37)	27.56–54.12
	3	48.46 \pm 2.21b (11)	36.20–58.20	41.57 \pm 1.12a (11)	36.70–46.64

Means within the same column and sex followed by the same letter are not significantly different, Tukey's Studentized Range Test, $\alpha=0.05$.

[$F = 3.33$, $df = 1, 44$; $P = 0.0750$] and lipid [$F = 1.57$, $df = 1, 44$; $P = 0.2164$]; males—dry body weight [$F = 3.56$, $df = 1, 33$; $P = 0.0680$] and lipid [$F = 0.03$, $df = 1, 33$, $P = 0.8579$] (Table 2). However, within the density treatment of three larvae per cup, females from cups in which two larvae died weighed significantly more than females from cups in which one larva or none died ($F = 11.23$, $df = 2, 75$; $P = 0.0001$) (Table 3). Males from cups in which one or two larvae died weighed significantly more than males from cups in which no mortality occurred ($F = 3.23$, $df = 2, 64$; $P = 0.0463$) (Table 3). Larval mortality did not affect whole-body lipid in either females or males from the density treatment of three larvae per cup (females: $F = 2.01$, $df = 2, 75$; $P = 0.1414$; males: $F = 1.19$, $df = 2, 64$; $P = 0.3112$). Females that came from cups in which two larvae died (one larva per cup at prepupal stage, 65.68, Table 3) weighed 21% more than females that were reared by themselves during the entire immature period (one larva per cup, 54.29, Table 1). Also, females that came from cups in which two larvae died (one larva per cup at prepupal stage, 41.32, Table 3) contained 14.6% more whole-body lipid than females reared by themselves (one larva per cup, 36.06, Table 1), a difference greater than the increase in lipid from females reared at one larva per cup (36.06) to females initially reared at three larvae per cup (40.07, 11.1% increase, Table 1). Although other similar comparisons may be made, these examples are sufficient to indicate that cannibalism may have enhanced the nutrition of the cannibals, resulting in increased body weight and lipid reserves in some individuals in the higher-density groups.

When males are compared to females within the density treatment of one larva per cup, the males weighed significantly less and had a significantly greater percent lipid content than females in all three density treatments (dry body weight [$F = 10.43$, $df = 1, 121$; $P = 0.0016$] and lipid [$F = 4.77$, $P = 0.0309$]). Male fall armyworm adults contained approximately 8% more whole-body lipid than female fall armyworm adults. This difference may not be biologically significant between the sexes because it is relatively small compared with those detected in *S. exempta* (Gunn & Gatehouse 1986). Laboratory- and field-reared female *S. exempta* contained 55.9% and 25.6% more abdominal lipids, respectively, than their male counterparts. However, later studies with *S. exempta* showed no appreciable differences between the sexes within a density and phase (Gunn & Gatehouse 1987, 1993).

Analysis of the lipid extracts with HPTLC showed the presence of fatty acid, cholesterol ester, cholesterol, triacylglycerols, diacylglycerols, and traces of monoacylglycerols. The predominant lipid class was triacylglycerol, averaging 33.2% of whole body lipid ($n = 14$). Diacylglycerols made up less than 10% of the total lipid. An abundance of triacylglycerols was expected in these moths because these lipids are the primary fat source in the whole insect and are stored in the fat body (Beenackers et al. 1985). Triacylglycerols are converted to diacylglycerols in the fat body and transported through the hemolymph to the flight muscles and the developing ovaries.

The amount and proportion of lipid observed in the fall armyworm adults of this study are comparable to those reported for the migratory noctuid *Anticarsia gemmatilis* Hübner (Teo et al. 1987, Fescemyer & Hammond 1988, Fescemyer 1993), and by van Handel (1974) for the fall armyworm. In the

present study, total lipid content in newly emerged moths ranged from 18.4 to 24.0 mg, amounting to 36.1 to 44.9% of total dry body weight. For example, Teo et al. (1987) reported a range of 12.2 to 13.6 mg of lipid in 8-d-old fed moths, which amounted to 29.6 to 37% of the dry body weight of *A. gemmatalis*. van Handel (1974) reported total lipid content ranging from 12.5 to 13.5 mg per 100 mg of wet weight. These values are slightly lower than those found in the present study because the moths analyzed by van Handel (1974) were unfed and 3 to 7 d old at the time of lipid analysis.

The differences in the whole-body lipid content of fall armyworm adults from the different larval densities were not as great as those found for *S. exempta* (Gunn & Gatehouse 1987). With an average of 33.2% triacylglycerol in the whole body lipid in fall armyworm, female adults from the one larva per cup density treatment contained approximately 6.5 mg of triacylglycerol whereas female adults from the three larvae per cup density treatment contained approximately 7.7 mg of triacylglycerol, an increase of only 18.5%. In contrast and assuming that *S. frugiperda* and *S. exempta* are comparable in weight, *S. exempta* female adults of the high-density migrant phase contained 10.9 mg/100 mg wet adult weight or 500% more abdominal glyceride than female adults of the low-density nonmigrant phase (1.8 mg/100 mg wet adult weight [Gunn & Gatehouse 1987]). Gunn & Gatehouse (1987) argued that the increase in lipid reserves in the moths reared under crowded conditions indicated their dispersal potential, as larvae reared at unfavorable density levels stockpile lipid reserves in the larval stage for use in prolonged flight as adults. Their hypothesis was substantiated by data that demonstrated that larvae reared at a higher density are more likely to produce adults that are "long fliers" (Woodrow et al. 1987). Although data in the present study showed increased lipid reserves as a result of a higher larval rearing density, possibly indicating a premigrant condition, the increases in lipid reserves were not large and may have been due, in part, to cannibalism. Furthermore, previous studies lent little evidence that crowded fall armyworm larvae emerge as adults with higher flight activity potentials (Ferguson et al. 1994, H. J. F., unpublished data).

Wing-loading studies. Rearing density did not influence the regressions of wing width on estimated wing area in females nor did it affect the regression of forewing width on estimated forewing area in males (Figs. 1-3; female forewing width vs. forewing area, $P = 0.3752$, $y = -46.24 + 19.10x$, $r^2 = 0.72$, $n = 73$; female hind wing width vs. hind wing area, $P = 0.5476$, $y = -57.99 + 16.05x$, $r^2 = 0.77$, $n = 69$; male forewing width vs. forewing area, $P = 0.0768$, $y = -25.03 + 15.71x$, $r^2 = 0.74$, $n = 99$). However, rearing density affected the relationship of male hind wing area on hind wing width ($P = 0.0303$) (Fig. 4) (one larva per cup: $y = -41.36 + 13.95x$, $r^2 = 0.81$, $n = 23$; two larvae per cup: $y = -37.95 + 13.65x$, $r^2 = 0.69$, $n = 34$; three larvae per cup: $y = -60.06 + 16.34x$, $r^2 = 0.77$, $n = 37$). This statistically significant difference may not be biologically significant, considering the narrow range of hind wing area and hind wing width values (Fig. 4). These significant regressions ($P < 0.001$; Figs. 1-4) indicate that the wing area estimates obtained gravimetrically were valid.

Estimates of forewing and hind wing area were used to obtain total wing areas for the calculation of wing-loading. There were no significant differences in wing-loading among density treatments for either sex (female fall

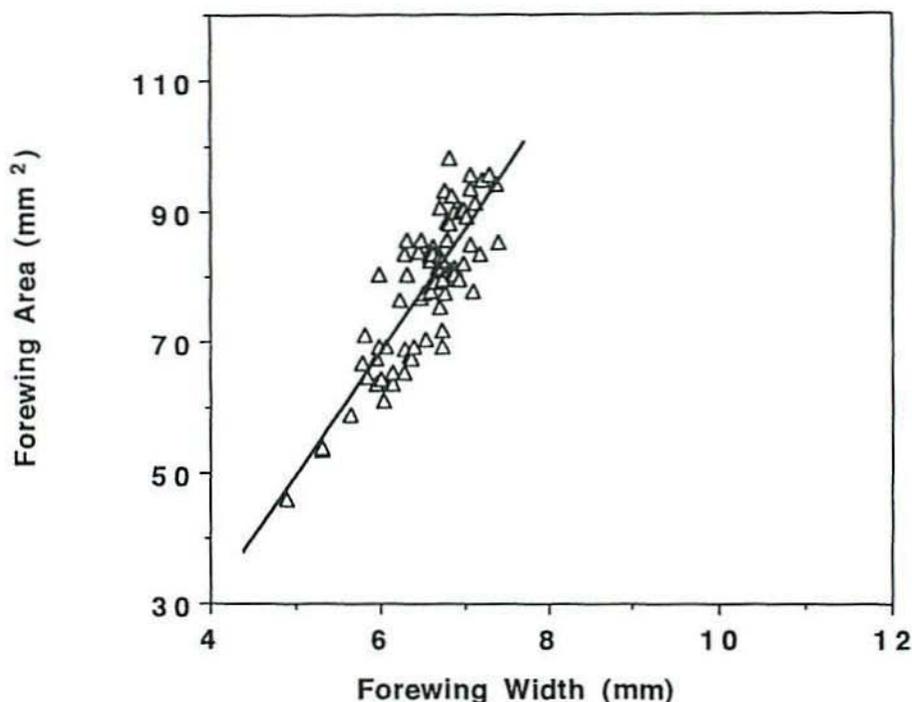


Fig. 1. The relationship between estimated forewing area and measured forewing width of female fall armyworm moths; $y = -46.24 + 19.10x$, $r^2 = 0.71$, $n = 73$.

armyworm: $F = 2.02$, $df = 2, 66$; $P = 0.1406$; male fall armyworm: $F = 2.45$, $df = 2, 92$; $P = 0.0922$). It can be concluded from this study that rearing density did not affect wing-loading in fall armyworm adults.

Angelo & Slansky (1984) determined that *S. frugiperda* subjected to varying degrees of starvation during the last instar have lower than theoretically predicted wing-loading ratios based on the following logic. If wing area is the square and body weight is the cube of linear dimensions (i.e., the wing length), then linearizing wing area against body weight through a logarithmic (base 10) transformation would yield a straight line with a slope of 0.67. Indeed, Miller (1977) determined empirically that biomass is related to forewing length cubed in a pooled group of olethreutid moth species. The wing area to body weight log-log relationship of the fall armyworm in Angelo & Slansky's (1984) study yielded a slope of 0.22, which is considerably lower than the theoretical slope of 0.67. This led Angelo & Slansky (1984) to conclude that as body weight is reduced in the fall armyworm, a relatively larger wing area than theoretically

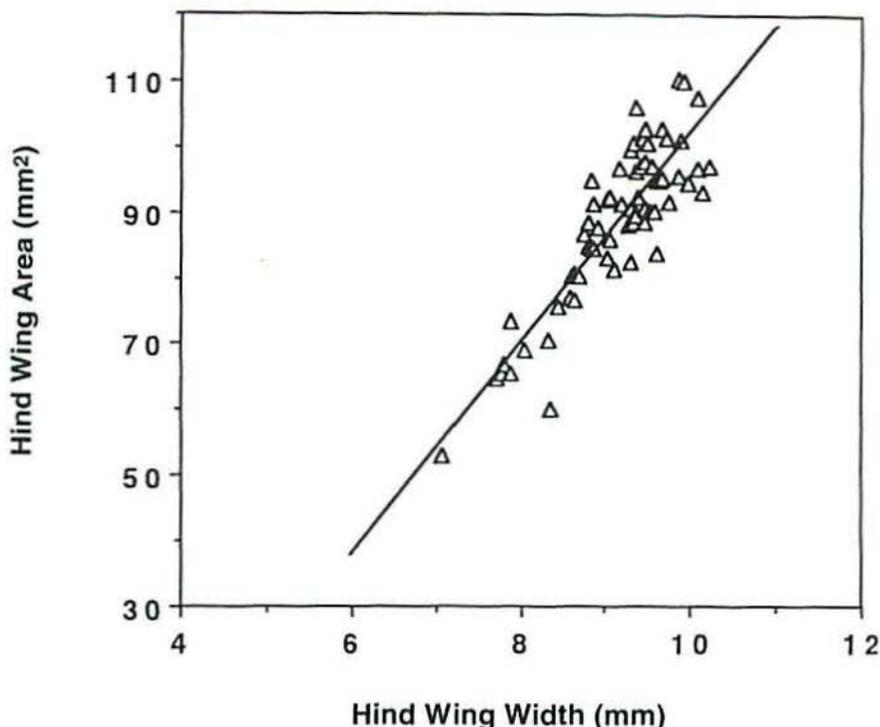


Fig. 2. The relationship between estimated hind wing area and measured hind-wing width of female fall armyworm moths; $y = -57.99 + 16.05x$, $r^2 = 0.77$, $n = 69$.

expected occurs. In the present study, slopes of both female and male wing area to body weight regressions were significantly less than the theoretical slope of 0.67 (Fig. 5, $P < 0.001$, using the \log_{10} theoretical line from Angelo & Slansky 1984: $y = 1.59 + 0.67x$; females: $y = 1.83 + 0.41x$, $r^2 = 0.62$, $n = 70$; males: $y = 1.75 + 0.46x$, $r^2 = 0.56$, $n = 96$). However, all the data lie below the theoretical line, indicating that the fall armyworm moths in our study had a higher than expected wing-loading. If another arbitrarily defined theoretical line in Fig. 5 is drawn with a slope of 0.67 but with a lower intercept, then it could be concluded that the fall armyworm moths had a lower than expected wing-loading, supporting the hypothesis that migrant species have a low wing-loading for more efficient flight. Thus, in determining the relative wing-loading of an insect by comparison with a theoretical relationship, only the slopes may be compared and not the intercepts, which appear to be dependent on the sampling population in question. Lower wing-loading has been recorded for the presumed migrants of other migratory species, such as *Plusia gamma* L. (Long

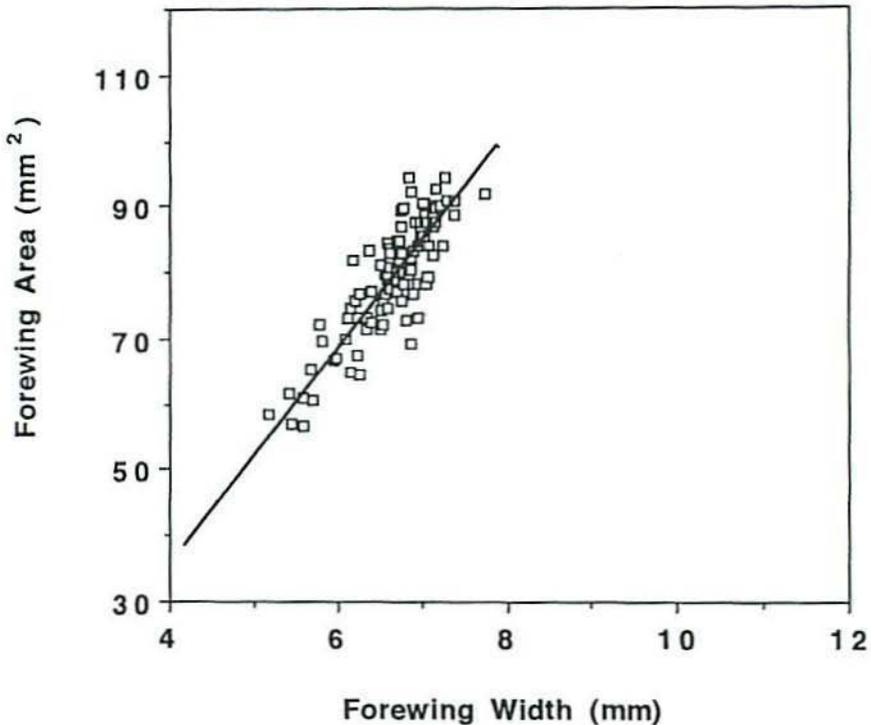


Fig. 3. The relationship between estimated forewing area and measured forewing width of male fall armyworm moths; $y = -25.03 + 15.71x$, $r^2 = 0.74$, $n = 99$.

1959), *Epiphyas postvittana* (Walker) (Danthanarayana 1976), and *A. gemmatalis* (Fescemyer 1993).

In summary, the apparent increase in lipid reserves in fall armyworm as a result of an increase in larval rearing density may have been due, in part, to enhanced nutrition through cannibalism in the higher density treatments and not because of a premigrant condition. Triacylglycerols, the primary storage lipids used for flight energy, were the dominant neutral lipids detected in whole-body lipid samples. Larval rearing density did not affect wing-loading in the fall armyworm. Yet, as a migratory species, it showed a lower than theoretically expected wing-loading, based on the fact that wing area to body weight relationships had significantly smaller slopes than a theoretical slope of 0.67.

The "identification of physiological and behavioral mechanisms conducive to initiation . . . of flight" (Stinner et al. 1983) is of particular importance in the study of fall armyworm dispersal. Crowding, or high population density, produces physiological and behavioral changes in the crowded individuals and

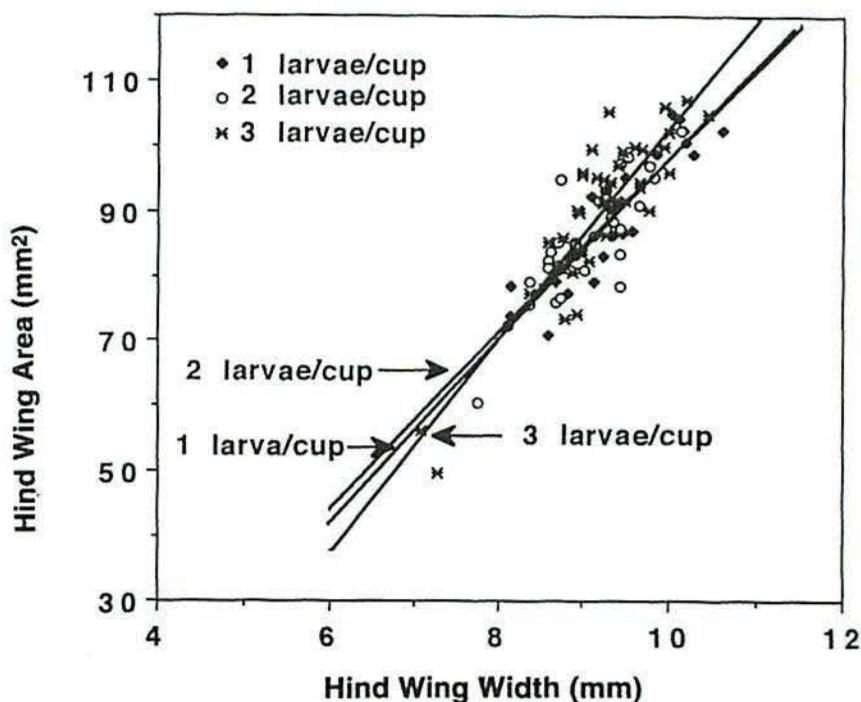


Fig. 4. The relationship between estimated hind wing area and measured hind-wing width of male fall armyworm moths: One larva per cup, $y = -41.36 + 13.95x$, $r^2 = 0.81$, $n = 23$; two larvae per cup, $y = -37.95 + 13.65x$, $r^2 = 0.69$, $n = 34$; and three larvae per cup, $y = -60.06 + 16.34x$, $r^2 = 0.77$, $n = 37$.

may lead to the premigrant condition. Thus far, we have measured the effect of crowding on several premigrant traits—pupal weight, developmental time, adult activity (Ferguson et al. 1994, H. J. F., unpublished data), adult dry body weight, and whole-body lipid (present study) and have found little effect of density on these factors and hence, little evidence that density is involved in producing a premigrant condition in the fall armyworm as seen in other migratory noctuids. There are other biochemical and hormonal effects of high population density associated with migration that need to be studied in the fall armyworm. However, from field studies, the effects of weather (wind, temperature, photoperiod, etc.), host plant, or both may be more important than larval density in initiating fall armyworm migratory behavior (Pair et al. 1986, Westbrook & Sparks 1986, Johnson 1987), and controlled research examining these factors should be undertaken.

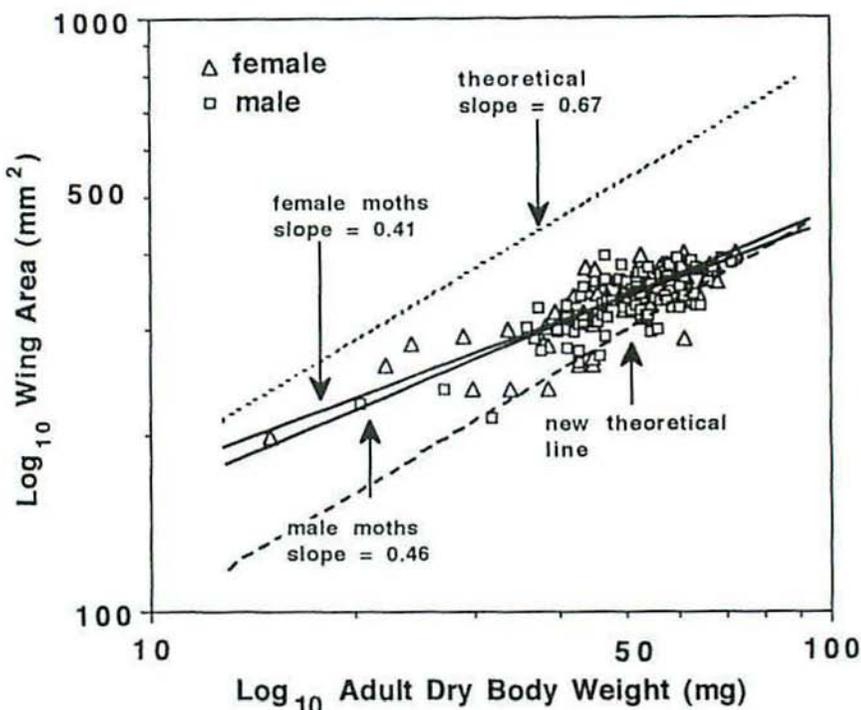


Fig. 5. Comparison of the relationship between wing area and dry body weight of fall armyworm moths to a theoretical relationship ($y = 1.59 + 0.67x$ [Angelo & Slansky 1984]). Males: $y = 1.75 + 0.46x$, $r^2 = 0.56$, $n = 96$. Females: $y = 1.83 + 0.41x$, $r^2 = 0.62$, $n = 70$. New theoretical line: $y = 1.33 + 0.67x$.

Acknowledgment

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Effect of Imidicloprid Seed Treatment and Planting Time Applications of Insecticides on Chinch Bug (Hemiptera: Lygaeidae) and Resulting Yields of Sorghum¹

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ABSTRACT Imidicloprid seed treatment was compared with standard soil-applied insecticides for chinch bug (*Blissus leucopterus leucopterus* [Say]) control on seedling sorghum (*Sorghum bicolor* [L.]) to determine the usefulness of this new seed treatment in Kansas where the chinch bug is a primary pest of sorghum. Seeds of treated and untreated sorghum were planted over a 6-yr period in plots at various locations in eastern Kansas where chinch bugs move from maturing wheat or barley into seedling sorghum. During all years and tests, the imidicloprid treatment (2.5g AI/kg of seed) compared favorably with carbofuran applied as a granule or liquid or with aldicarb applied as a granule in-furrow in controlling chinch bugs and protecting sorghum yields. Thus, the imidicloprid seed treatment gives growers another option to reduce losses from chinch bugs.

KEY WORDS Sorghum, *Blissus leucopterus leucopterus*, imidicloprid, seed treatment

The chinch bug, *Blissus leucopterus leucopterus* (Say), has been a major pest of grain sorghum, *Sorghum bicolor* (L.) Moench, in Texas, eastern Kansas, and southeastern Nebraska for many years. Annual losses caused by chinch bugs in outbreak years have been estimated at \$11.3 and \$10 million in 1990 and 1991, respectively, in Nebraska (Spike et al. 1991). Chinch bugs have been managed by a combination of cultural practices (planting sorghum away from wheat) and chemical control by using planting time treatments of granular insecticides, primarily carbofuran (Wilde et al. 1984). The recent cancellation of registration for granular carbofuran by the Environmental Protection Agency has necessitated the evaluation of alternative insecticides. The new insecticide imidicloprid is in the chloronicotinyl chemical group, the compounds of which act on the nicotinic acetylcholine receptor (Pike et al. 1993). Imidicloprid has been shown to control the Russian wheat aphid (*Diuraphis noxia* [Mordvilko]) on wheat and barley (Pike et al. 1993, van der Westhuizen et al. 1994) when applied as a seed treatment and the green peach aphid

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(*Myzus persicae* [Sulzer]) on lettuce (Palumbo & Kerns 1994) when applied as a soil treatment. The purpose of this study was to test and compare the effectiveness of an imidicloprid seed treatment (registered as Gaucho™ by Gustafson, Inc., Plano, Texas) with that of soil-applied insecticides for controlling chinch bugs on seedling sorghum and for maintaining the grain-yielding potential of sorghum hybrids.

Materials and Methods

Field-plot treatments were established over a 6-yr period (1990–1995) at several locations in eastern Kansas where chinch bugs cause a perennial problem. In the 10 experiments established in 1990–1994, the sorghum hybrid NC+271 was used. In 1995, three sorghum hybrids (Taylor Evans Hardy, Taylor Evans Elite, and DP 1506) were used. Field plots were established next to wheat or barley fields infested with chinch bugs. Sorghum was planted in early June so that seedlings would be emerging when chinch bug nymphs moved away from maturing small grains. Field plots were two rows wide, 9.1 m long, and planted perpendicular to the small grain in a randomized complete block design with four replications. Sorghum was planted at a depth of 0.5 cm with a John Deere 7100 Max Emerge planter. In-furrow, granular insecticide treatments were applied with a v-belt seeder mounted on the tractor. Liquid insecticide was applied in 27.4 liters of water per hectare through a microtube system. Detailed information for each location is presented in Table 1.

Treatments included imidicloprid (Gaucho™) applied to the seeds, carbofuran (Furadan™) applied as a 15G granular or 4F liquid form, aldicarb (Temik™) applied in a 15G granular form, and an untreated control. Treatments were evaluated by assessing a damage rating based on stand loss and seedling vigor to the individual plots after maximum damage occurred (usually about 3 wk after planting) on a scale of 1–9, where 1 represents no damage and 9 represents all plants dead. Yield data were taken by hand or machine harvesting the plots at maturity and are expressed as kilograms per hectare at 12.5% moisture. All data were analyzed based on the main effects. Mean separation was based on an LSD test at a probability level of 0.05 (SAS Institute 1988).

Results

1990–1994 Tests. Data from the 10 locations using one sorghum hybrid in 1990–1994 were categorized into three groups based upon the reduction in yield caused by chinch bugs in untreated plots. Group A consisted of moderate damage (about 50% yield reduction in the untreated control) and occurred in three of the tests. Group B consisted of heavy damage (100% loss in untreated plots) and occurred in five of the tests. A severe infestation in which all plots eventually were destroyed by chinch bugs (Group C) occurred in two tests. In the tests showing moderate and heavy infestations (Groups A and B), all treatments differed significantly from the untreated control in damage ratings and yield but did not differ from each other except for the Gaucho seed treatment and Temik 15G damage rating in Group B (Table 2). In the two tests

Table 1. Relevant planting and agronomic practices for evaluation of insecticides to control chinch bugs in grain sorghum in eastern Kansas (1990-1995).

Year	Location ^a	Planting date	Damage evaluation date
1990	Manhattan 1	5 June	26 June
1990	Manhattan 2	5 June	17 June, 24 June
1992	Manhattan 1-1	9 June	21 June, 24 June
1992	Manhattan 1-2	16 June	2 July
1992	Manhattan 2-1	9 June	2 July
1992	Manhattan 2-2	16 June	2 July
1992	Chapman	21 June	15 July
1993	Manhattan 1	11 June	4 August
1994	Manhattan 1	11 June	4 August
1994	Chapman	14 June	13 July
1995	Manhattan 1	13 June	13 July

^aThe number following the site location refers to specific fields at the Manhattan location.

showing severe infestations (Group C where all plots were destroyed), significant differences in damage ratings occurred between all treatments and the untreated control and between the Gaucho seed treatment and Furadan 15G treatment at the first evaluation (Table 2). However, there were no significant differences at the second evaluation. These results indicate that the insecticides were effective in controlling chinch bugs initially, but the infestation was so severe or persisted so long that none of the insecticides provided continuous plant protection.

1995 Test. Data from the 1995 tests with three hybrids indicated that significant differences in chinch bug damage and sorghum yield occurred between all treatments and the untreated control. Again, no significant differences occurred among the various insecticides and formulations tested. (Table 3) except for the Furadan 15G and Furadan 4F damage ratings. There was a significant difference between hybrids in yield. Hybrid DP 1506 yielded significantly more ($2,171 \pm 545$ kg/ha) than hybrids T-E-Elite ($1,073 \pm 270$ kg/ha) or T-E-Hardy (957 ± 224 kg/ha), suggesting it is less susceptible to chinch bugs than the other two hybrids tested. These results, if confirmed, suggest that growers could reduce yield losses caused by chinch bugs by growing the appropriate hybrid.

Table 2. Summary of comparisons of insecticides to control chinch bugs on grain sorghum in eastern Kansas (1990-1994).

A. Moderate Infestations - 3 tests - ca. 50% loss in yield											
Treatment	Form	Rate ^a AI	Chap-1994		Man-1994		Man-1993		Avg ^b ± SE		Avg ^b ± SE
			kg/ha	DR ^c	kg/ha	DR ^c	kg/ha	DR ^c	kg/ha	DR ^c	DR ^c
Gaucho	ST	2.5	4,135	1.0	4,617	1.6	5,491	1.0	4,747 ± 396a		1.2 ± 0.2a
Furadan	4F	1.12	4,141	1.0	4,885	1.0	4,998	2.6	4,674 ± 268a		1.5 ± 0.5a
Furadan	15G	1.12	3,959	1.0	2,898	3.3	6,133	1.0	4,330 ± 952a		1.8 ± 0.8a
Temik	15G	1.12	3,937	2.0	3,808	2.0	6,010	1.0	4,585 ± 713a		1.6 ± 0.3a
Untreated	—	—	2,057	5.3	3,428	4.3	2,078	5.6	2,521 ± 453b		5.1 ± 0.4b

B. Heavy Infestation - 5 tests - ca. 100% loss in yield															
Treatment	Form	Rate ^a AI	Chap 1992		Man 2-2 1992		Man 2-1 1992		Man 1-2 1992		Man 1 1990		Avg ^b ± SE		Avg ^b ± SE
			kg/ha	DR ^c	kg/ha	DR	kg/ha	DR ^c	kg/ha	DR ^c	kg/ha	DR	kg/ha ^c	DR ^c	DR ^c
Gaucho	ST	2.5	2,266	7.2	2,833	5.0	1,650	6.8	5,341	3.0	369	8.7	2,491.8 ± 821a		6.1 ± 1.0b
Furadan	4F	1.12	—	—	4,398	3.0	2,432	5.3	4,708	3.0	0	9.0	2,884.5 ± 1,085a		5.1 ± 1.4ab
Furadan	15G	1.12	4,317	5.2	4,585	3.0	2,726	4.9	4,323	3.5	0	9.0	3,190.2 ± 862a		5.1 ± 1.1ab
Temik	15G	0.56	4,575	2.2	4,312	4.0	2,587	5.0	5,705	2.0	6,267	7.2	3,561.0 ± 887a		4.1 ± 1.0a
Untreated	—	—	0	9.0	0	9.0	0	9.0	59	9.0	0	9.0	12.0 ± 12b		9.0 ± 0.0c

Table 2 continued.

C. Severe Infestation - 2 tests - all treatments - no yield

Treatment	Form	Rate ^a AI	Damage Rating ^c				DR Avg ^b ± SE	
			Man 2		Man 1-1		1st	2nd
			1990		1992			
			1st	2nd	1st	2nd		
Gaucho	ST	2.5	1.2	9.0	2.3	8.5	1.8 ± 0.5a	8.8 ± 0.3a
Furadan	4F	1.12	2.5	9.0	2.5	9.0	2.5 ± 0.0ab	9.0 ± 0.0a
Furadan	15G	1.12	2.5	8.7	4.3	9.0	3.4 ± 0.9b	8.9 ± 0.2a
Temik	15G	0.56	2.2	8.5	4.0	9.0	3.1 ± 0.9ab	8.8 ± 0.3a
Untreated	—	—	6.0	9.0	7.3	9.0	6.7 ± 0.7c	9.0 ± 0.0a

^aGranular or flowable formulations expressed as kilograms per hectare; seed treatment expressed as grams per kilogram of seed.

^bMeans followed by same letter in a column are not significantly different at 0.05 level (LSD).

^cDamage rating scale of 1-9: 1 represents no damage; 9 represents severe damage.

Table 3. Summary of damage and yield data from evaluation of insecticides to control chinch bug on sorghum hybrids (Manhattan, Kansas, 1995).

Treatment	Form	Rate ^a AI	Yield of Hybrids						Avg ^b ± SE	
			T-E-Elite		T-E-Hardy		DP 1506			
			kg/ha	DR ^c	kg/ha	DR ^c	kg/ha	DR ^c	kg/ha	DR ^c
Gaucho	ST	2.5	1,510.7	4.2	1,141.1	3.8	3,005.4	2.7	1,885 ± 569a	3.6 ± 0.4ab
Furadan	15G	1.12	1,253.5	3.8	1,253.5	2.5	3,696.4	1.0	2,066 ± 814a	2.4 ± 0.8a
Temik	15G	0.56	1,462.5	3.3	1,301.8	4.3	1,919.6	3.2	1,561 ± 185a	3.6 ± 0.4ab
Furadan	4F	1.12	1,108.9	4.3	1,012.5	4.8	1,687.9	4.5	1,269 ± 210a	4.5 ± 0.1b
Untreated	—	—	32.1	8.8	80.4	8.8	546.4	8.8	219 ± 163b	8.8 ± 0.0c

^aGranular or flowable formulation expressed as kilograms per hectare; seed treatment expressed as grams per kilogram of seed.

^bMeans followed by the same letter in a column are not significantly different at 0.05 (LSD).

^cDamage rating scale of 1-9: 1 represents no damage, 9 represents severe damage.

Discussion

The results of these tests over a 6-yr period indicate that seed treatment with imidicloprid compares favorably with in-furrow applications of granular or liquid insecticides in controlling chinch bugs and maintaining grain sorghum yield potential. Also, two tests indicated that chinch bug infestations on seedling sorghum can be so severe and/or persistent that planting time or seed treatments would not protect against stand loss. Additional foliar applications of insecticides would be needed to protect against stand loss in these situations (Wilde & Morgan 1978). In most cases, the data suggest that imidicloprid seed treatment as well as other planting-time treatments can reduce yield losses caused by chinch bug infestations on seedling sorghum.

The cost of using Gaucho is about two-thirds that of the commercially available FuradanTM 4F or TemikTM15G. Recent price quotations from dealers indicate the cost of using Gaucho equates to about \$3.20 per hectare whereas the granular or liquid formulations are priced at \$4.80 per hectare. Gaucho is available only through commercially treated seed and is not available for grower application or planter box treatment.

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NOTE

Susceptibility of Adult Alfalfa Leafcutting Bees (Hymenoptera: Megachilidae) to *Beauveria bassiana* Infection¹

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KEY WORDS *Beauveria bassiana*, *Megachile rotundata*, Hymenoptera, Megachilidae, conidia

Beauveria bassiana (Balsamo) Vuillemin is a deuteromycete fungus that is being developed as a bioinsecticide of grasshoppers and other insects. Asexual spores or conidia of *B. bassiana* are the infective form of the fungus, which can be mass produced and aerially applied over large areas by using conventional spray equipment. Soil is the natural reservoir for conidia, and provides protection from photodegradation and dessication. Marcandier & Khachatourians (1987) were able to infect grasshoppers with *B. bassiana* under low-humidity conditions in the laboratory. Results from the study by Marcandier & Khachatourians (1987) suggest that *B. bassiana* infection in grasshoppers may be possible during dry periods on semiarid rangeland.

Strain-specific differences may exist in fungal virulence toward a single species of insect (Khachatourians 1992, Kosir et al. 1991). Brinkman et al. (1997) used a grasshopper-derived strain of *B. bassiana* in spraytower bioassays and observed high mortality (72.5%) of grasshoppers, yet yellow mealworms, *Tenebrio molitor* L., exhibited much lower levels of mortality in similar studies. Grasshoppers and yellow mealworms were sprayed with *B. bassiana* treatments equivalent to a field application rate of 1×10^{13} conidia/3.785 liter/0.405 ha (Brinkman et al. 1997). Vandenberg (1990) found that honey bees, *Apis mellifera* L., were susceptible to *B. bassiana* infection in spray tests. Vandenberg (1990) tested three concentrations (10^6 - 10^8 spores per bee) of *B. bassiana* on honey bees and observed reduced longevity and mycosis in treated bees.

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The alfalfa leafcutting bee, *Megachile rotundata* (F.), is the most important pollinator of seed alfalfa, *Medicago sativa* L., in western North America (Goettel et al. 1993). As a beneficial insect, the alfalfa leafcutting bee must be considered in evaluations of *B. bassiana* for use against grasshoppers on western rangeland (Goerzen et al. 1990). The purpose of our research was to determine the effects of a grasshopper-derived strain of *B. bassiana* on adult alfalfa leafcutting bees in a spraytower bioassay. Studies on the effects of aerially applied *B. bassiana* on adult alfalfa leafcutting bees are needed because adults may be more likely to come in contact with conidia than immature bees within pupal cells.

Pupal alfalfa leafcutting bees (in "plugs" or "cells") were obtained from laboratory colonies at Oregon State University. For each test, about 100 plugs containing pupae were placed in a 302.4 cm² covered container with shredded paper towels. Pupae were incubated at room temperature (29°C) for 18–21 d. Not all bees emerged from plugs on the same day. On each day following the first day of emergence, available adults were divided into three groups and randomly assigned to treatments. A total of 360 adult alfalfa leafcutting bees (120 per treatment) was used in the laboratory experiments.

A spraytower was used to simulate the aerial application of *B. bassiana* according to procedures described by Brinkman et al. (1997). Fungal conidia (batch # 921114GHA) and an oil carrier solution were supplied by Mycotech (Butte, Montana). The oil carrier was an inert paraffin that is registered with the Environmental Protection Agency as an insecticide carrier (Johnson et al. 1991).

Spray equipment was calibrated to deliver a spray pattern equivalent to a field application rate of 1×10^{13} conidia/3.785 liter/0.405 ha in practice tests by using the oil carrier and oil sensitive paper (TeeJet® Spraying Systems Co., Wheaton, Illinois). Prior to each spray test, clean newsprint was placed on the floor below the spraytower in the spray room. Test insects were immobilized by cooling to 1.7°C and then placed on the newsprint. Bees in groups of 10 or less were either sprayed with air (control), oil carrier, or oil containing *B. bassiana* conidia. Treatment of bees with air was conducted first by turning on the air pump for 15 s. Bees were then removed and placed in containers. For oil treatments, the air pump was turned on and 0.09 ml of oil carrier was injected into the airstream by using a 1-ml syringe. Oil (0.09 ml) containing 2.64×10^9 conidia per milliliter of oil was injected into the air nozzle in a manner similar to oil-only treatments. One oil-sensitive card was placed adjacent to the group of insects to confirm reception of spray treatment. After *B. bassiana* treatments, the spray room and spray equipment were cleaned and disinfected with bleach. Treatments were replicated four times, and the experimental design was a randomized complete block with repeated measures. Results were analyzed using the PROC MIXED procedure (Littell et al. 1996).

Following treatment, alfalfa leafcutting bees were placed in 0.25-liter styrofoam cups (five individuals per cup) with clear plastic lids. Temperature and humidity of the holding room were maintained at 27°–29°C and 40%–50%, respectively. A small rubber serum stopper containing undiluted honey was placed in each cup along with three 4-cm² pieces of paper towel. The paper towel provided a substrate and also absorbed excess honey on bees and in

containers. A 2-mm-diameter hole was burned into the plastic lid to allow for air exchange. Honey was replenished with a syringe through the opening. Each day, dead bees were removed from containers, and the sex of dead bees was determined. Dead bees were individually placed in 20-ml scintillation vials with a moist cotton ball and observed for external development of *B. bassiana* hyphae for 14 d following death.

Mortality of alfalfa leafcutting bees sprayed with *B. bassiana* was significantly higher ($F = 10.92$, $P = 0.0001$) than mortality in oil or control treatments. Mean mortality of bees treated with *B. bassiana* dramatically increased 5 d after treatment. After 10 d, 84.8% of alfalfa leafcutting bees in *B. bassiana* treatments had died (Fig. 1). Sporulation of *B. bassiana* was observed in more than 99% of the dead bees that were treated with this bioinsecticide. The male to female ratio was approximately 3:1, and no differences in susceptibility due to sex were observed.

Brinkman et al. (1997) used the same rate (1×10^{13} conidia/3.785 liter/0.405 ha) and strain in tests of *B. bassiana* virulence towards yellow mealworms and found low mortality of treated beetles after 10 d. In a study conducted by Goerzen et al. (1990), immature alfalfa leafcutting bee mortality was 56% at day 7 and 96% at day 10, and *B. bassiana* was pathogenic to prepupae at all doses tested. Results from our study and those of Goerzen et al. (1990) indicate that this species is extremely susceptible to *B. bassiana* infection.

Because of the importance of alfalfa leafcutting bees on rangeland, precautions should be taken to minimize damage that may result from field applications of *B. bassiana*. Alfalfa leafcutting bees are easily managed and will readily accept artificial nesting structures. Potential injury can be reduced by correct timing of applications or movement of artificial nests. Nesting structures could be replaced within days of spray treatments because conidia do not persist on exposed surfaces (Steinkraus et al. 1991).

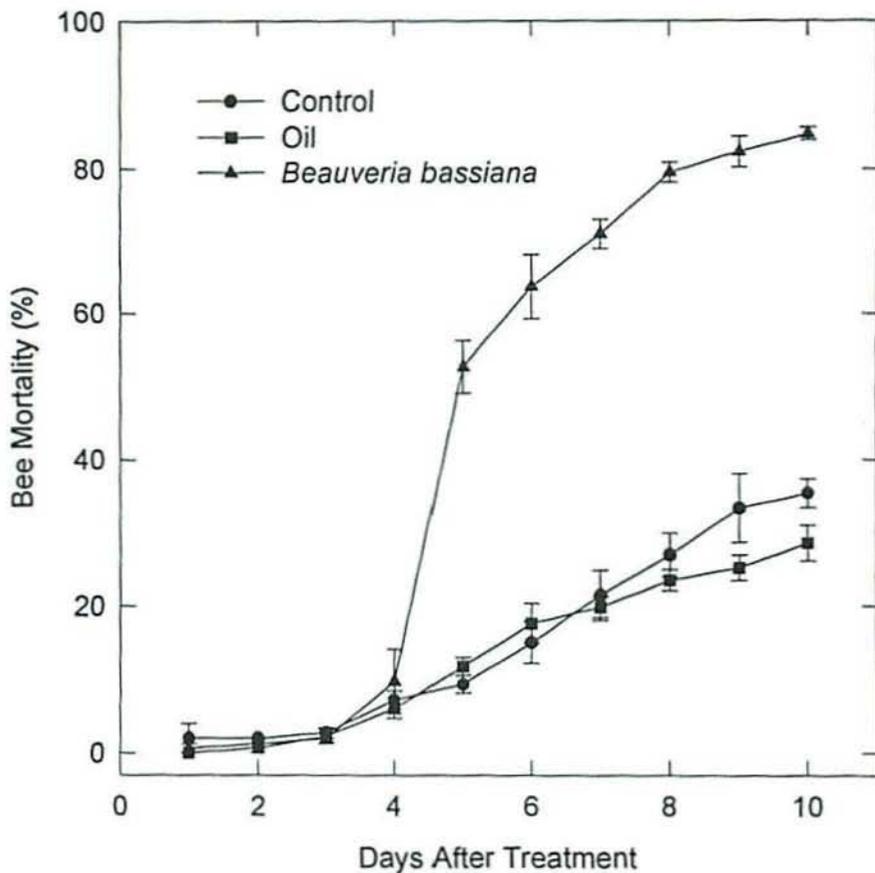


Fig. 1. Percent cumulative mortality (\pm SE) of alfalfa leafcutting bees sprayed with air, oil carrier, and oil containing *Beauveria bassiana* conidia. The spray equipment in the laboratory was calibrated to deliver a spray pattern equivalent to a field application rate of 1×10^{13} conidia/3.785 liter/0.405 ha.

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Soil-Surface Collection of Volatilized Insecticides in Relation to Corn Rootworm Management¹

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ABSTRACT Soil-applied granular insecticides vary in their efficacy for controlling corn rootworms. In a laboratory study, volatility-related losses of these insecticides away from the area of application were shown to occur. These findings assist in explaining the reduced insecticide efficacy that occasionally occurs when applications have failed to control corn rootworms. Our research investigated the volatility of the two insecticides terbufos and chlorethoxyfos and its potential implications on corn rootworm management. Soil-surface collection of volatilized insecticides was carried out by using a technique designed to offer portability and to serve as a low-cost alternative to the aerodynamic method. Our technique uses portable pumps and glass bottles that easily can be installed or retrieved, and the battery-powered vacuum pumps are lightweight, thus adding to their transportability. Additionally, an onboard computer can be programmed to operate at various predetermined sampling intervals. Aerodynamic or momentum balance methods require complex equipment to obtain supporting data as compared with our technique. Pre-study evaluations indicated >94% of the terbufos or chlorethoxyfos was captured in commercially made (Supelco, Inc.) sorbent tubes. Volatilization amounts captured for chlorethoxyfos and terbufos were monitored over a 22-d period. Recovery of terbufos was 0.56% and 15% from dry and water-saturated soil, respectively, representing an approximately 27-fold greater volatility for water saturated soils. Similarly, we observed a 16-fold (from 1.83% to 29%) increase in chlorethoxyfos volatility between dry and moistened soil, respectively. The greatest amounts of chemical captured occurred immediately after initial application (day 0) in all treatments. Captured terbufos was 43% and 21% of the total recovery from dry and saturated soil treatments, respectively, on day 0. In addition, 49% and 55% of recovered chlorethoxyfos was captured on day 0 from dry and saturated soil

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treatments, respectively, and >75% of the total volatilized material of both insecticides had been captured by day 4. Our results demonstrate that volatilized terbufos and chlorethoxyfos can be trapped and recovered from dry and saturated soil by using these methods. The portability of this system can be useful in assessing volatilization losses in either controlled chambers or from the field.

KEY WORDS Volatility, insecticide, terbufos, chlorethoxyfos, corn rootworm

Corn rootworms are regarded as the most serious insect pest of the Midwestern corn belt. Annual losses in the United States from rootworm damage and associated management costs have been estimated to be in the range of \$1 billion (Metcalf 1986). There are many soil-applied granular insecticides used to manage these pests; however, even with insecticide treatments, farmers occasionally have unexplained damage in excess of the economic injury level. Root pruning, lodging, and subsequent yield reductions are often observed too long after the at planting-time insecticide applications; therefore, efforts to explain these rare failures are not possible. One plausible explanation could involve the premature or excessive loss of the insecticide from the crop protection target zone (T-band or in-furrow), and volatilization is one of the means of pesticide movement. For example, volatility losses of the herbicide trifluralin have been studied extensively in laboratory and greenhouse experiments (Glotfelty et al. 1984, Taylor 1978, White et al. 1977, Spencer & Cliath 1974). Also, several volatility investigations of the insecticide dieldrin have been conducted under field conditions (Taylor et al. 1971, 1976, 1977; Farmer et al. 1972; Igue et al. 1972; Spencer et al. 1969). Other insecticide volatility studies of heptachlor, lindane, chlordane, and dacthal were conducted by Glotfelty et al. (1984). However, Caro et al. (1971) and Parmele et al. (1972) are credited with the pioneering work that described various micrometeorological techniques for making field-scale measurements of pesticide volatilization rates.

The most widely used collection technique is the aerodynamic, or momentum balance method, that employs an air sampling mast with exponentially spaced ports to collect pesticide vapor. Pesticide diffusivity coefficients and transfer coefficients are used to predict volatilization losses. This method measures the pesticide atmospheric concentration gradient at different heights above the soil surface. Using this method, Willis et al. (1972) observed dieldrin losses within 24 h from irrigated fallow soil that were 24% and 51% greater than from dryland fallow soil at 10 and 30 cm above the soil surface, respectively. Taylor et al. (1976) estimated seasonal losses of dieldrin and heptachlor incorporated into soil of a growing corn crop to be 2.8% and 3.9%, respectively, and Harper et al. (1976) observed a 90% loss of trifluralin in the first 35 d after application when studying soil and microclimate effects on volatilization.

Aerodynamic methods have been used to measure volatility of broadcast-applied liquid pesticides at various collection heights above the soil or plant surface. However, volatility studies for soil-surface-applied granular formulations that typically are used for managing corn rootworms (*Diabrotica*

spp.) have not been investigated. Thus, our primary objective was to develop a technique to quantify the volatilization losses for granular insecticides by using the two insecticides chlorethoxyfos and terbufos. Also, we sought to determine the impact of moisture on soil-surface volatilization for these insecticides under laboratory conditions. Improved volatility assessment would have implications for future field studies by providing farmers with information necessary for them to choose the most appropriate soil-applied application method. Thus, our findings would aid in reducing the likelihood of premature or excessive insecticide volatilization that might occur prior to rootworm damage.

Materials and Methods

Experiments were conducted in a SHERER model CEL 37-14 growth chamber maintained at $25 \pm 1^\circ\text{C}$. Contamination was prevented by using fresh-air intake hoses and an electric fan to evacuate exhaust air. Granular (G) formulations of chlorethoxyfos (Fortress 5G; E. I. Du Pont De Nemours & Co., Wilmington, Delaware) and terbufos (Counter 15G; American Cyanamid Co., Wayne, New Jersey) were applied at 1.1 kg (AI)/ha to saturated and dry soil. Both of these materials are currently labelled for placement in-furrow or T-band (ca. 17.8 cm to 22.9 cm) application over the row at planting time. In-furrow placement offers superior incorporation into the soil; however, banded applications cover a greater soil-surface treated area and only minimal (ca. 0 cm to 2.5 cm depth) incorporation is afforded by a planter's drag chains. Banded applications of terbufos can be made immediately before or after the press wheels. In contrast, chlorethoxyfos is placed in front of the press wheels for T-band or in-furrow applications. Each insecticide/soil-moisture (dry or saturated) condition was investigated by using four replications with four subsamples.

Lismore silty clay loam soil (fine loamy mixed pachic udic haploboroll) was obtained from the East Agronomy Farm, South Dakota State University, at Brookings, South Dakota. Soil was sieved through a screen (1 cm by 1 cm) to remove debris and to achieve uniformity. Subsequently, it was placed in pans and stirred over a 4-d period until soil became air dried. Following drying, soil was placed into sealed plastic containers.

Sampling procedure. Round aluminum pans (314 cm², 20 cm diameter) were filled with 700 g of dry soil (21%, 44%, and 36% sand, silt, and clay, respectively, and 3% organic matter). In dry-soil treatments, granular insecticide was applied to the soil surface. However, for saturated soil treatments, 250 g of distilled water was applied evenly over the soil surface with a 500-ml atomizing bottle to obtain saturation following insecticide application. This water application would result in insecticide movement into the soil, thus simulating a newly insecticide-treated cornfield following irrigation or rainfall.

Formulated chlorethoxyfos (116 mg) and terbufos (78 mg) were placed on 180 cm² of soil surface area in the central portion of each pan. These insecticide placements provided 11.69 mg of the active ingredient per treatment. Immediately following treatment, each pan was placed inside the chamber and fitted with an air sampling apparatus (Fig. 1) that enclosed a simulated band (18 cm width) application.

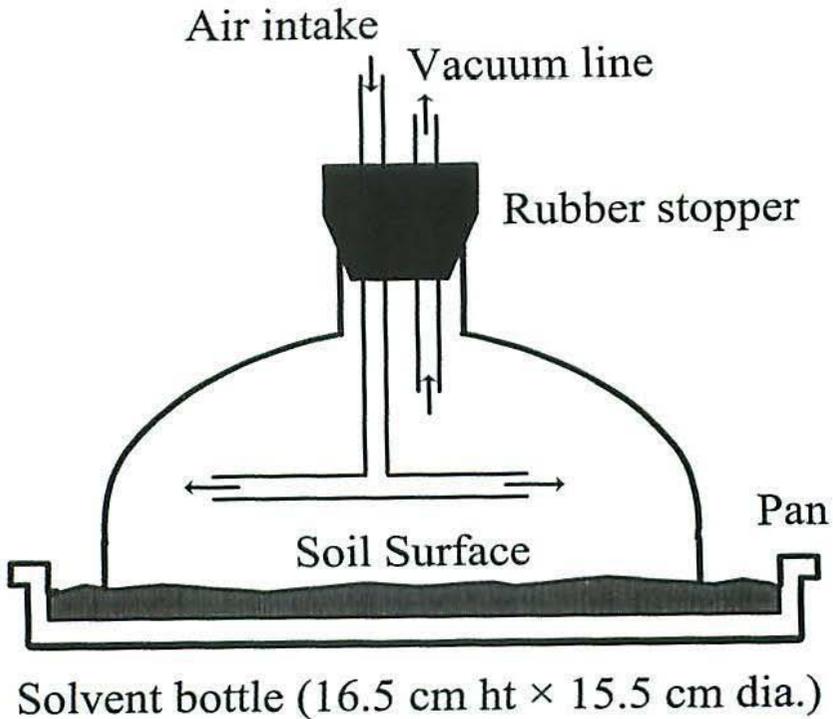


Fig. 1. Apparatus (Illustration not to scale) used to collect volatilization losses of chlorethoxyfos and terbufos applied at 1.1 kg (AI)/ha at 25°C in dry and water-saturated soil.

Air samples were taken for the 6-h periods beginning at 0900 h and ending at 1500 h when solar radiation causes elevated air and soil temperatures that are most likely to result in increased levels of insecticide volatilization. These collections were made after the initial treatment (day 0) and at 1, 2, 3, 4, 8, 12, 15, 18, and 22 d following application. After samples were collected, pans were uncovered and stored inside the chamber until the next sampling period. At the beginning of subsequent periods, chamber air was purged to prevent contamination. Soil within pans was moistened to simulate an irrigation or rainfall event sufficient to establish field capacity (saturation), but below a level that would result in runoff or standing water. All pans were subsequently fitted with air-sampling equipment.

Air sampling apparatus. Brown-glass solvent bottles were used to reduce the likelihood of increased greenhouse effects on insecticide volatility. Internal and external soil temperatures were assessed for bottles by using a temperature sensor diode. The upper portion of each bottle was cut to establish an internal volume of 1 liter. Four portable vacuum pumps (model 224-PCXR7;

SKC, Inc., Eight Four, Pennsylvania) were attached to four adjustable 4-way manifolds that were calibrated at 0.5 liter/min airflow for each port by using a rotometer (Cole-Parmer Instrument Co., Vernon Hills, Illinois). This volume, combined with a pump flow rate of 0.5 liter/min, allowed for 30 air exchanges per hour. Airflow was held constant so that accurate recoveries would be possible. Each port was connected to the top of the 1-liter glass bottle by equal lengths of 6-mm (i.d.) R-3603 Tygon tubing fitted to a No. 5 1/2 rubber stopper. Two 4-mm (i.d.) glass tubes were inserted through each stopper (one for a vacuum port and the other for a fresh-air intake). A commercially prepared (Supelco, Inc., Bellefonte, Pennsylvania) sorbent sample tube containing 150 mg of XAD-2 resin beads was mounted in-line in the vacuum tubing to trap volatilized terbufos and chlorethoxyfos. A stopper-held "t"-shaped glass tube was inverted within the bottle to provide fresh air. The upper fresh-air port was connected to an intake manifold constructed from equal lengths of 6-mm (i.d.) R-3603 Tygon tubing, and polypropylene T-connectors. This intake manifold was joined with a fresh-air hose and routed outside the chamber.

Extraction procedure. Sorbent tubes were collected after air sampling, and extraction of volatilized insecticides from resin beads was carried out as follows. Three 1-ml rinses of pesticide-grade toluene were followed by an additional three 1-ml rinses of pesticide-grade iso-octane (2,2,4-trimethyl pentane). Each rinse had a duration of about 2 min, and following the final rinse, any residual solvent was removed from the tube by air pressure. These combined rinses were collected in a test tube (16 mm by 125 mm) and brought to 10 ml with iso-octane. Test tubes were sealed with Teflon-lined caps and stored at -18°C pending chromatographic analysis.

Sorbent tube extraction efficiency. Extraction efficiency of the sorbent tubes was measured before all sampling procedures. Thus, 1 mg of liquid formulations of the technical material for each compound was applied directly to the open glass end of these tubes. Air was drawn through each tube for 6 h at a rate of 0.5 liter/min. Tubes were then rinsed as previously described.

Chromatographic analysis. Chemical analysis was performed via gas-liquid chromatography by using an electron capture (EC) detector. A 1.5-ml aliquot of extract was placed into each sample vial and then loaded into a Varian model 8000 autosampler that injected a 1.5- μ l aliquot into a Varian model 3700 gas chromatograph. Under these conditions, it is possible to detect amounts of these insecticides as low as 5 pg. This amount would correspond to a detection limit of about 0.05 μ g per tube analyzed. Oven, injector, and detector temperatures were 200°, 220°, and 350°C for terbufos and 180°, 220°, and 350°C for chlorethoxyfos, respectively. A 1.8-m by 4-mm (i.d.) column was packed with 1.5% OV-17 and 1.95% QF-1 with nitrogen as the carrier gas delivered at 82.8 kPa. Results were printed on a Spectra-Physics model SP4290 integrator that calculated peak areas and retention times for comparison with known standards. Retention times for terbufos and chlorethoxyfos were 4.05 ± 0.03 and 3.64 ± 0.03 min, respectively. All values were converted into micrograms per cubic meter, and data were analyzed with the SAS Means Procedure (SAS Institute 1985), which provided means and standard errors for each sampling day.

Results and Discussion

Pre-study evaluation of the sorbent tubes extraction efficiency exceeded 94% recovery of terbufos and chlorethoxyfos that was available for collection from the open glass end of these tubes. This recovery rate indicated that tubes could collect at least 1 mg of insecticide and retain it irrespective of continuous airflow. This efficiency trial showed that solvent desorption would be complete, and demonstrated a highly efficient means for measuring volatility from soil-surface applied insecticides that are used to control corn rootworms. Also, prior to the laboratory investigation, soil temperature assessments to ensure against potential greenhouse effects from the soil beneath the brown glass bottle were compared with the soil temperatures outside the apparatus under field conditions. The soil temperatures were found to be the same only when the pumping system was providing the constant air exchange that occurs while the mechanism is functioning; however, increased temperatures were noted when these air exchanges were halted. Therefore, any field implementation with this sampling procedure will require having the glass bottles added to the system only when actual volatility assessments are being taken to avoid any temperature changes to the soil surface.

The volatilization amounts captured for chlorethoxyfos and terbufos during a 22-d period are presented in Table 1. Measurable reductions in the amount of volatilized pesticides were apparent for all treatments as the experiment progressed. Recovery of terbufos was 0.56% and 15% from dry and water-saturated soil, respectively, representing an approximately 27-fold greater volatility rate from water-saturated soils. Similarly, a 16-fold increase in volatility was observed for moistened soil, in that total chlorethoxyfos recovery was from 1.83% to 29% from dry and saturated soil, respectively. The greatest amounts of captured chemical occurred within the first sampling interval after application (day 0) in all treatments. Captured terbufos was 21% and 43% of the total recovered for dry and saturated soil treatments, respectively, on day 0. Captured chlorethoxyfos was 49% and 55% of the total recovered for dry and saturated soil treatments, respectively, on day 0. Furthermore, by day 4 more than 75% of the total volatilized amounts of terbufos and chlorethoxyfos had been captured.

Daily measurements of terbufos indicated 14 to 310-fold greater volatility for saturated soil when compared with dry soil conditions, respectively. Chlorethoxyfos was from 9 to 33-fold more volatile in saturated soil than in dry soil. These substantial increases in volatility in relation to soil moisture are similar to those observed by Spencer & Cliath (1974) who showed that trifluralin was from 3,000 to 5,000-fold more volatile in moist soil than in dry soil in laboratory studies. Initially (day 0), we observed a 1.3-fold greater response with chlorethoxyfos from saturated soil compared with dry soil than that noted with terbufos. However, on subsequent days terbufos showed a 1.6 to 21-fold greater response from saturated soil than that observed with dry soil for chlorethoxyfos. These disparities in volatility may be due to chlorethoxyfos having a vapor pressure that is 15-fold greater than terbufos (1.7×10^{-3} compared with 1.15×10^{-4} , respectively). Therefore, more rapid levels of chlorethoxyfos volatilization from dry soil would be expected. This relationship

Table 1. Volatility of chlorethoxyfos and terbufos applied at 1.1 kg (AI)/ha at 25°C in dry and water-saturated soil.

Day post- application	Amount ($\mu\text{g}/\text{m}^3$) recovered (Mean \pm SEM)			
	Chlorethoxyfos		Terbufos	
	Dry soil	Saturated soil	Dry soil	Saturated soil
0	105 \pm 3.87	1,900 \pm 27.57	28 \pm 4.41	380 \pm 6.49
1	56 \pm 3.11	580 \pm 10.13	19 \pm 3.68	340 \pm 4.89
2	21 \pm 2.00	200 \pm 8.38	9 \pm 2.17	270 \pm 3.66
3	5.6 \pm 0.72	180 \pm 4.43	3 \pm 0.72	240 \pm 4.65
4	5.0 \pm 0.67	140 \pm 3.55	2 \pm 0.72	120 \pm 3.85
8	4.8 \pm 0.69	110 \pm 2.70	1 \pm 0.33	92 \pm 3.72
10	4.2 \pm 0.51	87 \pm 2.92	0.67 \pm 0.17	85 \pm 3.41
12	3.5 \pm 0.36	86 \pm 1.94	0.48 \pm 0.12	81 \pm 3.56
15	3.2 \pm 0.29	78 \pm 2.47	0.30 \pm 0.11	71 \pm 2.38
18	2.6 \pm 0.24	48 \pm 2.49	0.19 \pm 0.08	54 \pm 2.35
22	2.2 \pm 0.19	32 \pm 1.05	0.16 \pm 0.06	49 \pm 2.12

between insecticides and moisture is supported by a lower rate of change between dry and saturated soil volatility of chlorethoxyfos compared with terbufos. Decrease in terbufos volatility was 99% and 87% less on day 22 as compared with that found initially (day 0) for dry and saturated soil, respectively. Similar decreases in chlorethoxyfos volatility were observed as a 98% reduction occurred on day 22 as compared with day 0, for both dry and saturated soil.

Our findings suggest the potential for some of the insecticide failures to be associated with volatility losses away from the treatment zone. In addition, soil moisture and temperature (air and soil-surface) are likely to be important volatility-related factors. Premature losses on insecticide due to volatility-related environmental factors could be better assessed with our technique compared with an aerodynamic method. Also, our technique was developed to use portable pumps and glass bottles that can be transported to a field site and deployed easily. It should be noted that our laboratory study was under controlled conditions, and field studies will require multiple sampling events at random from within the same treatment area to avoid the sampling apparatus becoming a hindrance to solar radiation, wind, or rainfall effects. In instances where extended or continuous volatility assessments are desired, we would suggest that multiple samplings be attained by moving the glass bottle after

short durations (6 to 8 h) and then added together to provide long-term estimates. Portability of the lightweight and battery-powered vacuum pumps would facilitate these multiple deployments. The onboard computer can be readily programmed to operate at various short-term sampling intervals making our system superior for assessing insecticide volatility under a wide range of environmental conditions.

In summary, the aerodynamic or momentum balance method requires complex equipment to obtain supporting data as summarized by Taylor & Glotfelty (1988). Our study, however, uses portable pumps and low-cost collection equipment. The results of this study demonstrate that volatilized terbufos and chlorethoxyfos can be efficiently trapped and recovered from dry and saturated soils. This system can be used in controlled chamber environments and potentially for field studies. Therefore, our findings would allow for a more thorough understanding of the role volatility plays in insecticide persistence and its subsequent removal from the treatment zone. Furthermore, this technique would offer an effective means to assess volatility losses in relation to insecticide placement (in-furrow or banded applications) under field conditions. As previously mentioned, the amount of soil incorporation with an at planting time insecticide is greater with in-furrow treatments than that found with a standard band or "T" band application. Consequently, farmers (in some cases) also should choose an application method that is appropriate for a specific insecticide's volatility characteristics. This would help to ensure that excessive volatility would not occur prior to the desired corn rootworm mortality. Finally, this technique would offer an excellent tool for further insecticide volatility assessments that not only include the corn rootworm but also other insect pests where insecticide applications are placed on the soil surface.

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Toxicity of Pesticide Residues to Citrus Thrips (Thysanoptera: Thripidae)¹

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ABSTRACT Bioassays were conducted with second-instar citrus thrips, *Scirtothrips citri* (Moulton), to investigate the residual activity of several pesticides that are registered (abamectin and cyfluthrin) or nearing registration (chlorfenapyr and spinosad) for California citrus. Results were similar on fully expanded leaves and moderate-sized fruit of Valencia orange, but when treatments were applied to small fruit, toxicity against citrus thrips declined more rapidly. Cyfluthrin was the most persistently toxic material with nearly 100% mortality until day 19 (fruit) or day 35 (leaves), followed by chlorfenapyr and spinosad, respectively. Abamectin was the least persistent pesticide. Abamectin at one-half the field rate with sugar added was as effective as the field rate without sugar. Because residual efficacy of several of these materials (abamectin, chlorfenapyr, spinosad) is shorter than for most chemicals (cyfluthrin, dimethoate, formetanate) used in the recent past against citrus thrips in California, growers and pest control advisors will need to more carefully monitor citrus thrips and optimally time treatments to provide effective control.

KEY WORDS *Scirtothrips citri*, Thysanoptera, citrus pest management, bioassay, pesticide residues, pesticide toxicity

The citrus thrips, *Scirtothrips citri* (Moulton), has been an important pest of California citrus since the early 1900s (Horton 1918). Chemical control has been the primary method used to manage economic populations during the 4 to 6-wk critical period following petal-fall when fruit are susceptible to damage (Rhodes & Morse 1989, Morse & Klonsky 1994.) Despite considerable research to develop nonchemical means of suppressing citrus thrips populations, the only advice currently available to growers is to minimize the use of broad spectrum pesticides that reduce citrus thrips biological control agents such as the predaceous mite *Euseius tularensis* (Congdon & McMurtry) (Haney et al. 1992, Morse 1995).

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For the near future, it appears that chemical control of citrus thrips will remain the major option for management of economic populations. The two relatively persistent, broad spectrum chemicals dimethoate and formetanate have dominated citrus thrips control in California since the mid-1970s. Because foliar and fruit residues of these two materials are quite persistent, growers have not needed to monitor citrus thrips populations carefully or to time treatments to optimally control the most damaging life stage, the second instar (Wiesenborn & Morse 1986). However, due to the development of citrus thrips resistance to dimethoate and formetanate, a Section 18 Emergency Exemption was granted for use of cyfluthrin on navel oranges in the San Joaquin Valley for each of the past 7 yr (1991–1997). Cyfluthrin also is relatively persistent but resistance is expected to develop rapidly to this material (Immaraju & Morse 1990).

Abamectin, a short residual chemical with limited harmful effect on natural enemies (Morse et al. 1987, Bellows & Morse 1993), was first registered in 1994 for use on California citrus. Other chemicals such as chlorfenapyr and spinosad are likely to be registered in the near future. Several chemicals available for citrus thrips control (abamectin, sabadilla) or nearing registration (chlorfenapyr, spinosad) appear to be much less persistent than chemicals such as cyfluthrin, dimethoate, and formetanate based on limited field trials. With these less persistent chemicals, it is likely that growers will need to monitor citrus thrips populations more carefully and time treatments more precisely to achieve satisfactory levels of control.

In this study, we compared the residual activity of abamectin, chlorfenapyr, cyfluthrin, and spinosad applied for citrus thrips control. These data will be useful in determining the relative field persistence of each material and will aid in developing treatment recommendations for growers and pest control advisors.

Materials and Methods

The residual toxicity of four pesticides applied to Valencia orange (*Citrus sinensis* [L.] Osbeck) was evaluated against early second-instar citrus thrips in two trials, one each in 1995 and 1996 at the University of California Citrus Research Center in Riverside, California. In 1995, residues on both young fruit (3.5 cm initial average diameter) and young but fully expanded leaves from Valencia orange were studied. The 1996 trial was done only on fruit (1.5 cm initial average diameter).

1995 Trial. Six treatments were compared (rates are amount per 935.3 liters of water, equivalent to the amount commonly applied per hectare, exclusive of air treatments): (1) abamectin (Agri-Mek[®] 0.15 EC [emulsifiable concentrate], 0.15 lb [AI] [active ingredient]/ gal, Merck, Three Bridges, New Jersey) + oil (Narrow Range 415 Spray Oil) was applied at 13.13 g [AI] + 0.01%; (2) abamectin + oil + sugar at 13.13 g [AI] + 0.01% + 4.71 kg; (3) chlorfenapyr (Alert[®] 2 SC [soluble concentrate], 2 lb [AI]/ gal, American Cyanamid, Wayne, New Jersey) at 168.1 g [AI]; (4) cyfluthrin (Baythroid[®] 2 EC, 2 lb [AI]/ gal, Bayer, Kansas City, Missouri) at 112.1 g [AI]; (5) spinosad (Success[®] 80% [AI] WDG [water dispersible granules] [formulation NAF-127], DowElanco,

Indianapolis, Indiana) at 150 g [AI]; and (6) untreated control. Treatments were applied on 17 July 1995 by tagging and then dipping individual leaves and fruit on the periphery of a tree in the appropriate pesticide suspended in 1 liter of water.

Leaves and fruit chosen for treatment were selected on the basis of similar initial size and appearance. Six trees of similar appearance were chosen and the chemical to be applied to each was randomly assigned. Forty fully expanded but young leaves (age chosen based on leaf texture and color; younger leaves are lighter green) on the northeast quadrant of the tree were tagged, the pesticide solution was restirred, and each leaf was dipped in the solution for 15 s. Similarly, 40 northeast quadrant fruit of 3.5 ± 0.05 cm initial diameter were selected, tagged, and dipped. On various days post-treatment, up to 94 d, three leaves and fruit were harvested from each tree (treatment) and laboratory bioassays were conducted with young, second-instar citrus thrips (chosen based on the width of their abdomen, which is thinner than late second instars) collected from our greenhouse colony. Leaf selection was random within those tagged on each tree but fruit were selected based on size. The smallest, largest, and median fruit (within the tagged fruit remaining on the tree) on each tree were harvested for the bioassay and their diameter was measured.

A greenhouse colony of citrus thrips was established using adults collected in July 1987 from wild laurel sumac plants, *Rhus laurina* (Nuttal), in a semidesert region near Jesus Maria, Baja, Mexico (same as Baja87, Immaraju et al. 1989). Since that time, the thrips were reared on *R. laurina* plants by using methods as described by Tanigoshi & Nishio-Wong (1981).

The experimental arena for fruit bioassays consisted of two cups, one small (approximately 4 cm diameter) and one large (9 or 14 cm diameter). The small cup was glued to the bottom of the large cup in an inverted position. A small hole, approximately 4 mm in diameter, was made in the base of the small cup through which the stem of the fruit was immersed in water. Fruit were picked with 2.5 cm of stem remaining. The stem was wrapped either in parafilm or was inserted into a rubber tube. A tangle-foot barrier (Insect Trap Coating, The Tanglefoot Co., Grand Rapids, Michigan) was applied around the stem and below the calyx to prevent escape of thrips. Individual thrips were transferred directly from greenhouse colony plants to the fruit with a camel hair brush. Bioassays were conducted with 10 second-instar thrips on three fruit per treatment ($3 \times 10 =$ total of 30 thrips). The number of dead and surviving individuals was recorded after 72 h, mortality was corrected using the formula of Abbott (1925), and data were statistically analyzed using Procedure REGWQ within SAS Version 6.03 (SAS Institute 1988).

For the leaf bioassays, three leaves were collected from each tree (treatment) on various days post-treatment and were placed individually into Munger cells (Munger 1942, Morse et al. 1986), adaxial surface up, that exposed a 3.2-cm-diameter section of the leaf surface. Opposite edges of the cells contained screened holes (5 mm diameter) to provide ventilation; one side of the cell was attached to a plenum providing forced air movement of approximately 5 ml/s to each cell (Morse et al. 1986). Ten young, second-instar citrus thrips were aspirated from the laboratory colony into clear plastic straws and transferred to

the Munger cells after anesthetization with carbon dioxide ($3 \times 10 =$ total of 30 thrips). Because of limited numbers of the appropriate age citrus thrips from the laboratory colony, tests on fruit and leaves could not always be run on the same day.

1996 Trial. The same chemicals and rates were evaluated on fruit, for 56 d, in a similar manner in 1996 (treatment applied May 27) except for the following: chlorfenapyr was tested at double the 1995 rate (336.2 g [AI]) and abamectin was tested at half the field rate (6.57 g [AI]) with oil (+ 0.01%) and with and without sugar (4.71 kg) in addition to the full rate (13.13 g [AI]) with oil (0.01%) and without sugar.

Results

1995 Trial. Average fruit diameter was 3.5 cm at the start of the trial and grew to 5.8 cm by the end of the trial (Fig. 1). Weather during the trial was hot. Daily minimums and maximums over the 94 d of the test ranged from 11–23 and 22–43°C, respectively, with averages of 16.6 ± 2.7 and $34 \pm 4.5^\circ\text{C}$ (SD). The only precipitation during the trial occurred on day 46 (0.025 cm).

Bioassay data are shown in Fig. 2 A and B. Control mortality was low (<15%) except late in the trial on leaves after the leaves had started to harden and on the last date of the fruit test. With some exceptions, bioassays yielded similar results on leaves and fruit.

In both tests, cyfluthrin was the most persistently toxic material with nearly 100% mortality through day 19 (fruit) or day 35 (leaves). During the later part of the trial, the decline in cyfluthrin toxicity was gradual and by day 94, thrips mortality was still >50%. Spinosad caused 100% mortality through day 7. Decline in toxicity was sharp in the second and third week post-application. Mortality resulting from chlorfenapyr treatment was somewhat less than that observed with spinosad except late in the trial on leaves. The decline in chlorfenapyr toxicity on leaves on day 19 appeared spurious, based on the recovery of toxicity on days 27 and 35.

Abamectin toxicity was the least persistent of the treatments evaluated and reached approximately 30% mortality by day 7. The addition of sugar to the abamectin plus oil mixture resulted in equal or higher mortality on each date compared with abamectin plus oil, but this difference was statistically significant only on day 4 and on leaves.

1996 Trial. The 1996 trial was conducted on much smaller fruit compared with 1995 and the weather was cooler. Average fruit diameter was 1.5 cm at the start of the trial and grew to 4.2 cm by day 56 (Fig. 1). Daily minimums and maximums over the 56 d of the test ranged from 10–26 and 21–40°C, respectively, with averages of 15.5 ± 2.4 and $31.5 \pm 4.5^\circ\text{C}$ (SD). No precipitation occurred during the trial.

Results in 1996 were similar to those on fruit in 1995 (Fig. 2C) except for the following: (1) less persistent toxicity was observed with the cyfluthrin treatment in the third and fourth week post-application; (2) abamectin with sugar at half the field rate was more toxic than abamectin without sugar until day 19 (this difference was statistically significant only during the first week post-application); and (3) chlorfenapyr (applied at twice the rate used in 1995) was as toxic as spinosad.

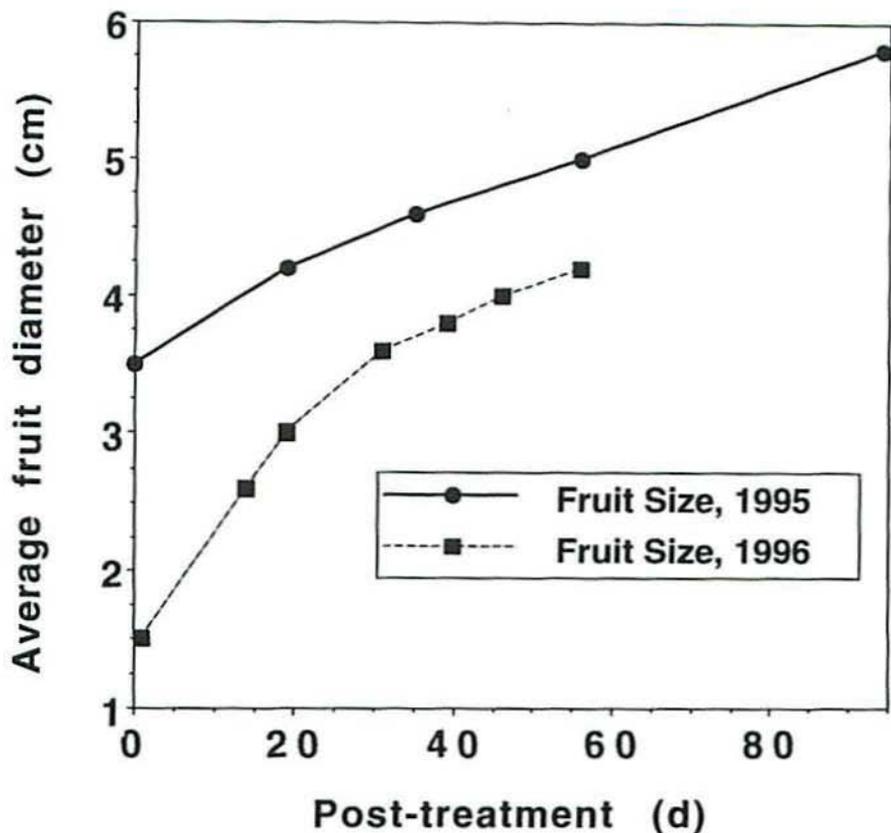


Fig. 1. Average fruit (Valencia orange) size versus days post-treatment for fruit used in pesticide bioassays in 1995 versus 1996.

Discussion

Concentration of oil with abamectin. The amount of oil that was added to abamectin in both years of the trial was lower than that usually applied with this chemical. We used 0.01% oil (10 μ l per liter of water), whereas the Agri-Mek label recommendation for use of abamectin against citrus thrips in California specifies a minimum of 0.2% oil and not less than 9.35 liters of oil per hectare (i.e., 1% oil if 953.3 liters of water were used per hectare as simulated in our trial).

Oil is added to abamectin not because of its activity against citrus thrips but because it extends the residual activity of abamectin, presumably because it increases the penetration of abamectin into leaves and fruit (McCoy et al. 1982, Dybas 1989). Leaf and fruit residues of oil alone have very low toxicity to citrus thrips (J. G. M., unpublished data). Although the contact toxicity of oil against

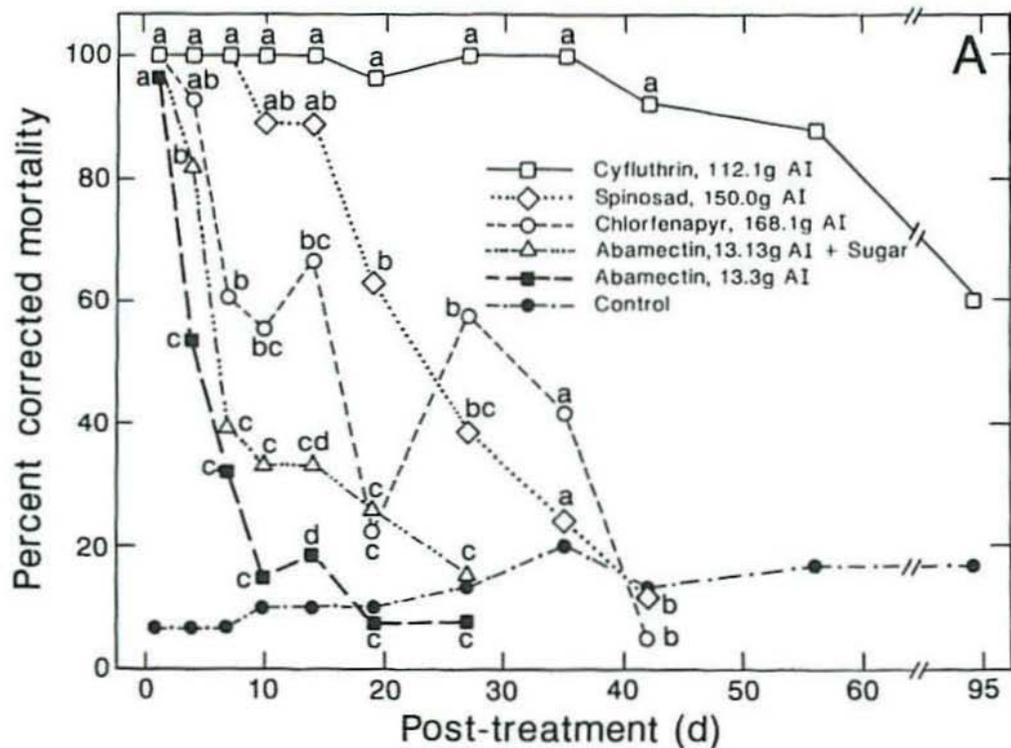
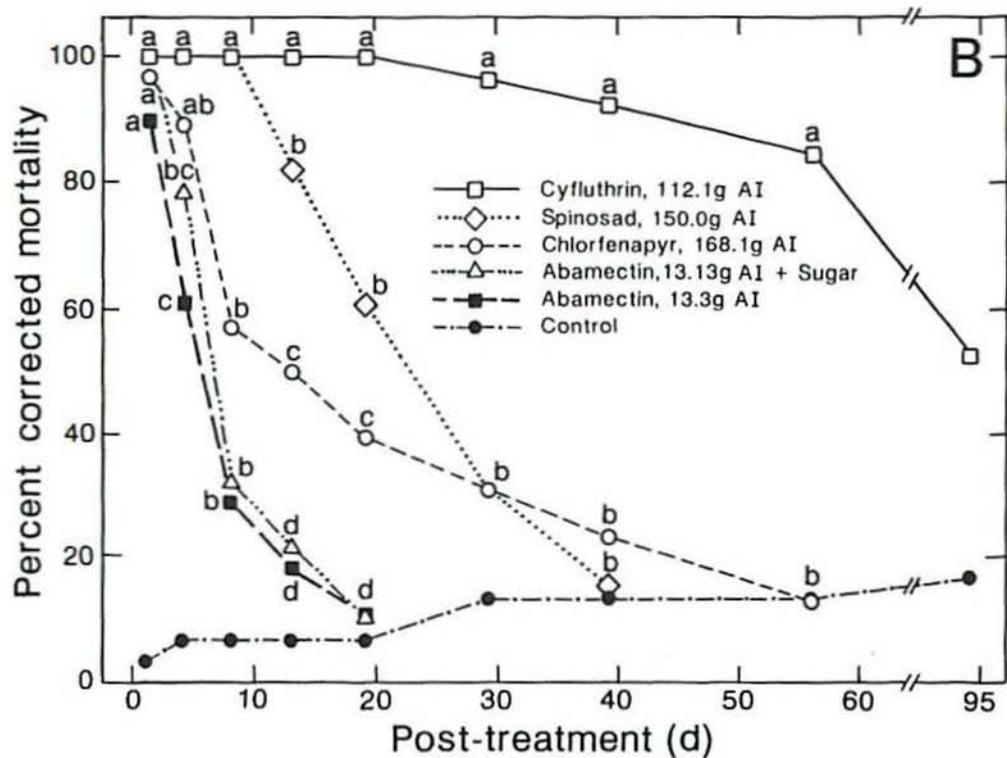
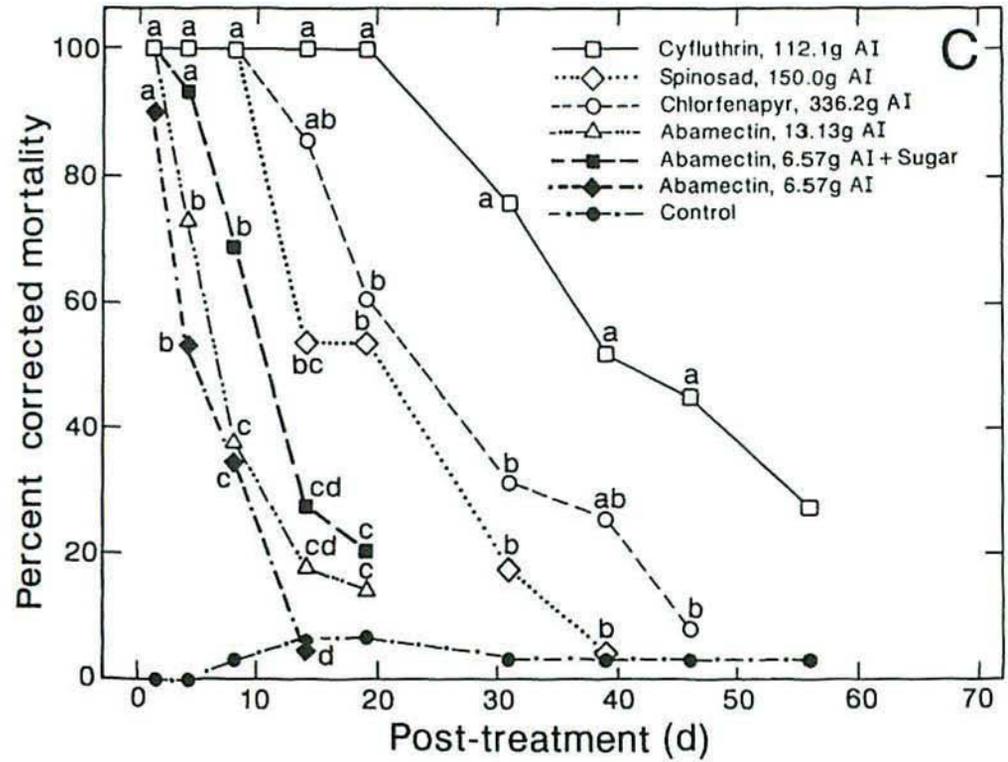


Fig. 2. Mortality of second-instar citrus thrips exposed to harvested orange fruit and leaves on various days post-treatment: (A) 1995 trial on leaves; (B) 1995 trial on fruit; and (C) 1996 trial on fruit. Means followed by the same letter on a particular date are not significantly different ($P = 0.05$, REGWQ multiple range test in SAS Version 6.03, SAS Institute 1988).





citrus thrips is quite high (Tanigoshi 1981; J. G. M., unpublished data), field applications of oil have limited impact on citrus thrips populations (Morse et al. 1992a).

On the other hand, abamectin with oil is more effective against citrus thrips than abamectin alone (McCoy et al. 1982, Morse et al. 1990b). The critical question is whether or to what degree, our lower than intended concentration of oil (0.01%) reduced the persistence and efficacy of abamectin. Based on chemical analysis of abamectin residues on citrus fruits (Maynard et al. 1989a, b; a surfactant was added at 0.5%) that showed somewhat slower patterns of decline compared with the decline in citrus thrips mortality seen in our study, we believe the reduced concentration of oil did have a slight impact, reducing the efficacy of abamectin by a week or less. Past field trials with abamectin plus oil (Morse & Brawner 1983; Morse et al. 1986, 1990a, 1992b; Immaraju et al. 1988) are consistent with this estimation.

Addition of sugar to abamectin. The Agri-Mek label recommendation for use of abamectin against citrus thrips in California specifies a rate of 13.1–26.3 g [AI]/ha, but growers are somewhat reluctant to use this material at these rates because of its relatively high cost in comparison with available alternatives such as cyfluthrin. In the context of an evolving IPM program on citrus, which emphasizes biological control (Haney et al. 1992, Morse 1995), we were interested in evaluating lower than label-recommended rates of abamectin because of its relatively low toxicity against important predators and parasitoids (Morse et al. 1987, Bellows & Morse 1993). Sugar is used as a bait against citrus thrips in combination with sabadilla (Hare & Morse 1997), and we hypothesized that it might add to the efficacy of low rates of abamectin. It is encouraging to see that 6.6 g [AI]/ha with sugar was just as effective as the 13.1 g [AI]/ha label rate without sugar. Further trials are warranted (e.g., with a higher concentration of oil) but until other selective materials (chlorfenapyr, spinosad) are registered for citrus thrips control, this half-label rate of abamectin plus sugar provides, in addition to sabadilla, an alternative for growers who wish to reduce the impact of broad spectrum pesticides on natural enemies.

Rates used for other chemicals. Neither chlorfenapyr nor spinosad are registered for use on citrus and the recommended rate or rate range has yet to be determined based on the results of additional field trials. With chlorfenapyr, we used 168.1 g [AI]/ ha in 1995 and doubled this rate in 1996. Our best estimate is that chlorfenapyr may be registered for citrus thrips control at the higher rate (336.2 g [AI]/ ha) or as two applications of 84.1–168.1 g [AI]/ ha. With spinosad, it is likely that the citrus thrips label will specify two applications and our best estimate is that the specified rate will be 70–100 g [AI]/ha, which is somewhat lower than the rate we evaluated (150 g [AI]/ha).

Extrapolation to field control. Although our experiments were designed to simulate field conditions as much as possible, there were several features of this study that resulted in citrus thrips exposure to the pesticides in a manner somewhat different from what they might receive in the context of commercial pesticide applications. The most obvious of these is that leaves and fruit were dipped in pesticide solutions rather than receiving treatment by using a speed-sprayer or aircraft.

A second factor affecting the efficacy of a particular treatment is growth of the treatment substrate leading to dilution of pesticides residues. Growers seldom apply citrus thrips treatments to mature leaves or fruit greater than 5 cm in diameter (Rhodes et al. 1986); thus, growth dilution of the target substrate may be a common problem. Leaves used in this study were fully expanded. As a result, thrips mortality observed in this study on leaves (Fig. 2A) might be somewhat higher and more persistent than that expected when similar treatments are applied to rapidly growing young foliage.

Average fruit diameter at the beginning of the 1995 and 1996 trials was 3.5 and 1.5 cm, respectively (Fig. 1). Assuming that fruit shape approximates a sphere (a reasonable approximation for young Valencia oranges), the surface area of the fruit can be determined ($4\pi r^2$) and percentage pesticide dilution calculated based solely on expansion of the fruit (ignoring other causes of pesticide residue dissipation such as volatilization, breakdown, etc.). Using this method, dilution factors of 49% and 12.8% result from day 0 to day 56 in 1995 and 1996, respectively (i.e., the surface concentration of each material on day 56 in 1995 would be 49% of that which was present on the fruit after treatment if factors other than fruit expansion are ignored). Because of smaller fruit size at the beginning of the trial in 1996 (small fruit grow more quickly), fruit grew at a much faster rate during the period of the trial compared with 1995 (see Fig. 1). With similar calculations, ignoring factors that reduce pesticide residues other than fruit expansion, and assuming the same treatments were applied both years, pesticide residues in 1995 would be 2.8- and 3.8-fold higher than those in 1996 on days 19 and 56, respectively.

Another factor that might differentially affect results in 1995 and 1996 was the weather during the period of the trials. We believe the small amount of rain (0.025 cm) on day 46 of the 1995 trial had little impact. The slightly higher temperatures during the 1995 trial might have led to slightly more rapid volatilization of the chemicals compared with 1996 (Bellows & Morse 1988) but because temperatures were similar, this difference was probably minor. Overall, we were surprised at how similar results were on fruit in 1995 versus 1996. The similar results with leaves versus fruit in 1995 also were surprising given that leaves were fully expanded at the beginning of the trial.

Implications to growers and pest control advisors. Prior to the appearance of pesticide resistance, the chemicals that have been popular for citrus thrips control since the mid-1970s (dimethoate, then formetanate, and more recently, cyfluthrin) provided residual control of citrus thrips populations for 3 wk or longer after treatment. As a result, treatments could be applied in advance of the appearance of economically damaging citrus thrips populations and intensive field monitoring was not needed to carefully time treatments.

Based on the data presented in this study, chlorfenapyr, spinosad, and abamectin treatments will have to be timed carefully, especially if low rates of each chemical are used. Optimal use of these materials will require that growers and pest control advisors monitor citrus thrips populations carefully and frequently after petal-fall to determine the optimal time for treatment and that aircraft or spray rigs are mobilized quickly after notification that it is time for treatment in a particular grove. In many cases, this may not be easy to accomplish. Two advantages of the short residual persistence of these chemicals, however, is that these materials will have less impact on beneficial

species and selection for citrus thrips resistance will occur over a relatively short span of time following treatment, hopefully leading to a slower evolution of resistance than has been the case with more persistent materials.

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Systemic Toxicity and Field Efficacy of Imidacloprid, Pymetrozine, and Triazamate Against *Myzus persicae* (Homoptera: Aphididae) on Spinach¹

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ABSTRACT Spinach, *Spinacia oleracea*, L., plants infested with green peach aphids, *Myzus persicae* (Sulzer), were used in laboratory and field studies to determine the systemic activity and field efficacy of three recently developed aphicides. Outer leaves of laboratory test plants were treated with various rates of the compounds. Aphid mortality was recorded for treated outer leaves and nontreated inner leaves to determine translaminar and systemic efficacies, respectively. Concentrations equivalent to suggested rates of triazamate, through systemic movement in the plant, were sufficient to control $\geq 90\%$ of aphids on nontreated leaves. Imidacloprid also produced a level of aphid control through systemic activity; however, the suggested rate was not as effective against aphids on nontreated leaves as that of triazamate. Mortality from pymetrozine was erratic on both treated and nontreated plant parts. In the field study, imidacloprid and a high rate of triazamate were more effective than other treatments. A low rate of triazamate typically was not different from pymetrozine. All aphicide treatments included in the field study resulted in fewer aphids than in nontreated control plots.

KEY WORDS spinach, systemic insecticide, aphicide, triazamate, imidacloprid, pymetrozine

The most common insect pest of spinach, *Spinacia oleracea* L., in the Arkansas River Valley is the green peach aphid, *Myzus persicae* (Sulzer) (Homoptera: Aphididae). Although this aphid rarely causes decreases in yields, it is a potential contaminant of the finished product. Isely & Miner (1946) mentioned how the tolerance for aphids in the canned product was so low that control must be nearly absolute. At present, acceptable levels of aphids on raw preprocessed spinach are not

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clearly defined (McLeod 1987a) but are extremely low. An aphid infestation, if uncontrolled, can make an entire field of spinach unsuitable for processing, thus resulting in a substantial loss to the grower. Adding to the problem, the spinach varieties preferred and grown by most producers in the area are savoy-leaved with highly convoluted and wrinkled leaf surfaces. The convoluted leaves, along with the prostrate growing habit of spinach, hamper aphid management by preventing contact of aphids with insecticides (Isely & Miner 1946, Walton & Howell 1954, McLeod 1991).

Some recently developed compounds possess aphid toxicity, systemic activity, and reduced adverse effects against beneficial insects compared with commonly used insecticides (Jacobson & Thriugnanam 1991, Moffat 1993, Mullins 1993, Minton et al. 1994). Such chemicals, with effective systemic activity against aphids, would be a boon to the development of a pest management program in spinach.

One objective of this study was to compare the systemic and translaminar efficacies of three recently developed aphicides against green peach aphids on a commercial variety of spinach. Another objective was to determine the field efficacy of the aphicides by monitoring aphid populations on marked leaves and plants through time.

Materials and Methods

Laboratory study. Spinach plants used in the laboratory portion of the study were 'Grandstand' variety and were grown in Redi-earth® (Scotts®-Sierra Horticultural Products Company, Marysville, Ohio). Plants were grown in a greenhouse under natural day length with the temperature maintained at $22 \pm 4^\circ\text{C}$. Seeds were planted in flats, and seedlings were transplanted to 10-cm-diameter plastic pots after development of one true leaf. Growth was maintained with regular applications of a solution of 15 ml of Peters® Professional 20:20:20 water soluble fertilizer (Grace-Sierra Horticultural Products Company, Milpitas, California) in 3.8 liters of water. Plants used in the study were produced from October through April.

Aphids used in the study were from a laboratory culture of *M. persicae* parthenogenetically maintained on 'Grandstand' spinach in cages at the Insect Toxicology Laboratory at the University of Arkansas. The original aphid source was from spinach produced in the Arkansas River Valley near Fort Smith, Arkansas. Cages were made from 0.5-liter plastic cups with cup bottoms replaced with a fine screen. Cups were inverted and pushed into the top soil surface of the potted plant. Cages with aphids were held in environmental chambers at $12 \pm 2^\circ\text{C}$ and a photoperiod of 12:12 (L:D) h. For testing, plants that had developed approximately eight leaves were infested with alate virginoparae of *M. persicae* from the laboratory culture. Initially, aphid infestation was accomplished by placing an infested spinach leaf from the aphid culture onto each test plant. Leaves were left for 24 h to allow aphids to move to the test plant. Subsequently, more uniform infestations were obtained by transferring aphids to several leaves on a test plant with a camel's hair brush. Following infestation, aphid numbers on the test plants were allowed to build to an appropriate level of approximately 50 per leaf. This generally required

about 1 wk. Two outer and two inner leaves of each test plant were selected and labeled with a marker (Sharpie[®], Sanford Corporation, Bellwood, Illinois) based on the level and uniformity of aphid infestation. Pretreatment aphid counts were made on the labeled outer and inner leaves of each test plant. Plants were then randomly assigned to treatments.

Aphicides tested were triazamate (Aphistar[™] 25 WP, Rohm and Haas Company, Philadelphia, Pennsylvania), imidacloprid (Provado[®] 1.6 F, Miles, Inc., Kansas City, Missouri), and pymetrozine (Fulfill[™] 50 W, Ciba Crop Protection, Greensboro, North Carolina). Initially, various concentrations from 0.002-2X (X referring to field rates suggested by the respective company representatives) were tested for each chemical. Thereafter, respective rates were chosen to bracket a range of aphid mortalities. Kinetic[™] surfactant (Setre Chemical Company, Memphis, Tennessee) was included in each test solution at 0.1%. Treatments were applied by swabbing the upper (adaxial) surface of the outer leaves with a saturated piece of cheesecloth. Inner leaves were not treated. In preliminary applications, measurement of volume applied per area of treated leaf surface indicated an equivalent application rate of 416 liters/ha. Approximations of field rates were, therefore, based on this figure. Nontreated controls received water plus 0.1% Kinetic[™].

The number of outer leaves treated was arbitrarily selected but was estimated to be greater than one-half and no more than two-thirds of the total leaf area of the plant. The actual number of treated leaves varied from 3 to 9, depending on plant and leaf size, with an average of 6.2 leaves. Care was taken to prevent contacting nontreated inner leaves with treatment mixtures. Following applications, all plants were held in a growth chamber (Percival Model E-30B, Boone, Iowa, 50036) at 10°C and a photoperiod of 12:12 (L:D) h. Lighting was from four GRO-LUX F20T12/GRO bulbs (Sylvania, Danvers, Maryland) and two GE 25-w incandescent bulbs (General Electric Co., Fairfield, Connecticut). Vapor toxicity within the cabinet had previously been shown to be insignificant with triazamate (McLeod 1987b). Preliminary tests also indicated low vapor toxicities for imidacloprid and pymetrozine. These test conditions were an attempt to approximate average spinach growing conditions in the Arkansas River Valley. Also, the 10°C temperature resulted in less aphid movement than at higher temperatures. Aphid counts were made periodically from 1-4 d after treatment from each of the two marked inner and outer leaves.

Data were pooled for the two treated outer leaves and for nontreated inner leaves. POLO-PC (LeOra Software 1994) was used for probit analysis of the data, including the relevant statistics, such as the indices of significance for potency estimations (*g*) (Finney 1971).

Field study. Spinach used in the field study was 'Fall Green' variety and was planted on 5 September 1995 at the University of Arkansas Agricultural Experiment Station, Fayetteville. Row spacing in the field was 0.97 m. Alate virginoparae of *M. persicae* from the laboratory culture maintained at the station were released in the spinach field on 5, 13, and 19 October 1995 by scattering aphid infested leaves across the study area.

On 15 and 16 November 1995, the field study area was divided into plots two rows wide by 4.88 m long. By this time, most plants in the field were greater than 20 cm in diameter and had greater than eight total leaves. Ten aphid-infested

plants were randomly selected in each plot to be used in the study. Selected plants were marked by inserting a 10-cm-long wooden stake into the soil beside the plant. On 16 November 1995, each of the selected plants was given a pretreatment rating for severity of aphid infestation. Ratings ranged from 0 for none to 3 for many green peach aphids. A rating of 1 indicated fewer than 100 aphids whereas a rating of 3 indicated greater than 300 aphids. Plots were ranked in order of average aphid infestation rating for the 10 selected plants. Based upon the rank of these aphid ratings, plots were grouped into five replications (each with five plots).

One leaf, on which to make a total aphid count, was selected from each plant in the three most heavily infested replications. These leaves were selected based upon the following criteria: the leaf was infested, but not so severely as to make counting difficult (i.e., typically less than 50 aphids per leaf), and the leaf petiole was long enough to allow the leaf to be examined for aphids without damaging the leaf or removing it from the plant. Each selected leaf was marked on the upper (adaxial) surface with a felt-tip marker. The number of aphids on each labeled leaf was recorded as the pretreatment count for that particular leaf.

The experiment was conducted as a randomized complete block with aphid infestation level as a blocking factor. Plots within each replication were randomly assigned to a treatment. Treatments included imidacloprid (Provado® 1.6 F) at 53 g AI/ha, pymetrozine (Fulfill™ 50 W) at 100 g AI/ha, triazamate (Aphistar™ 50 W) at 36 g AI/ha and 105 g AI/ha, and a nontreated control. Imidacloprid and pymetrozine treatments also included 0.1% Kinetic™ surfactant. Triazamate treatments also included 0.25% Crop Oil Concentrate® (Setre Chemical Company, Memphis, Tennessee). Aphicide applications were made on 16 November 1995 with a CO₂-powered backpack sprayer with a single 8003VS nozzle delivering 191 l/ha at 207 kPa.

Posttreatment aphid ratings and counts were taken at 1, 4, 6, 11, 14, and 21 d after treatment. These ratings and counts were always made on the selected plants and leaves that had been examined for pretreatment counts, such that changes on an individual leaf through time could be documented. Aphid movement from leaves was not a problem during the field examination due to low temperatures and gentle leaf handling. Plants also were rated at 28 d after treatment, but by that time falling temperatures on 9 and 10 December 1995 had damaged some of the labeled leaves, making accurate counts on those leaves unavailable. Percentage change in aphid numbers through time was determined by simply subtracting the respective pretreatment count from the aphid count on the sample date, dividing by the pretreatment count, and multiplying by 100%. Percentages and ratings were transformed with arcsine and data were analyzed with PROC GLM of SAS (SAS Institute 1989).

Results and Discussion

Laboratory study. Because almost all aphids were observed on the underside of leaves, mortality recorded for treated outer leaves was probably due to translaminar toxicity. Mortality on nontreated inner leaves was likely due to systemic activity in the plant.

The approximations of recommended field rates of triazamate (36–105 g AI/ha) were sufficient to include the LC_{90} values for aphids on both treated outer leaves and nontreated inner leaves. Interestingly, the LC_{90} for systemic activity against aphids on nontreated leaves (43.84 g AI/ha) was lower than the LC_{90} for treated leaves (63.80 g AI/ha) (Table 1). Both nontreated and treated leaf data resulted in adequate indices of significance for potency estimations ($g[0.95]$) (Finney 1971) of 0.120 and 0.136, respectively. The reason for the unexpected relationship between results for nontreated and treated leaves is probably due to the difference in slopes of the dose-response lines. Triazamate applied to outer leaves resulted in a LC_{50} of 9.84 g AI/ha on treated leaves and 10.26 g AI/ha by systemic action on nontreated inner leaves. The slope of the dose-response line, however, was 1.58 for treated leaves compared with 2.03 for nontreated leaves. The steeper predicted slope for nontreated leaves resulted in lower rate predictions for levels of greater mortality than on treated leaves. One possible explanation for such a result is that after some threshold amount of triazamate is applied to the plant, additional triazamate results in a considerable increase in mortality of aphids on nontreated plant parts through systemic action. Visual observations of whole plants, including leaves not counted, also indicated that triazamate had the greatest systemic activity of the three materials tested. A rate of triazamate equivalent to 56.1 g AI/ha, often included in the range of rates tested, was in several cases sufficient to provide 100% control of aphids on treated and nontreated leaves at 7 d after treatment.

Imidacloprid resulted in the lowest LC_{90} on treated leaves of any of the materials tested (5.47 g AI/ha) (Table 1). This LC_{90} was well below its recommended rate of approximately 53 g AI/ha. On nontreated leaves, the LC_{90} due to systemic activity was equivalent to 85.65 g AI/ha but was questionable based on variation in the data and an index of significance for potency estimation ($g[0.95]$) of 0.446. Application of the recommended rate of imidacloprid to outer leaves did not result in the level of control on nontreated inner leaves as did that of triazamate. Nevertheless, imidacloprid did offer a level of aphid control through systemic activity. Higher rates of the compound, within possible usage rates according to one source (Thomson 1994), may, if acceptable, offer more consistent systemic performance. In many cases, imidacloprid resulted in an alteration of aphid behavior at 1 to 4 d after treatment. Although green peach aphids on spinach typically remain on the underside of leaves, those on imidacloprid-treated plants were often observed roaming over the upper surface of leaves. The reported repellency and reduction or cessation of pest feeding by imidacloprid (Mullins 1993, Nauen & Elbert 1994) may explain this unusual aphid activity. Such aphid movement may have contributed to the variation of the data from nontreated inner leaves of imidacloprid treated plants.

Based upon estimates from concentration-response lines for data collected at 1, 2, 4, and 7 d after treatment, both triazamate and imidacloprid were fast acting against aphids on treated leaves (Fig. 1). A rate of triazamate, equivalent to its LC_{90} at 7 d after treatment, would be expected to produce approximately 42.1, 83.4, and 85.1% mortality at 1, 2, and 4 d, respectively. Rapidity of action for imidacloprid was similar to that of triazamate. The LC_{90} at 7 d after treatment for imidacloprid-treated leaves should result in

Table 1. Concentration-mortality responses of *Myzus persicae* on aphicide-treated outer leaves and nontreated inner leaves of spinach at 7 d after treatment.

Leaves ^a	Total		95% limits ^b			95% limits ^b		slope ± SEM	g(0.95) ^c	
	plants	aphids	LC ₅₀ ^b	lower	upper	LC ₉₀ ^b	lower			upper
Treated outer/										
Triazamate	31	1978	9.84	6.10	16.42	63.80	32.81	230.47	1.58 ± 0.06	0.136
Imidacloprid	26	2086	0.79	0.39	1.39	5.47	2.93	15.43	1.53 ± 0.05	0.128
Nontreated inner/										
Triazamate	29	1284	10.26	6.38	15.32	43.84	27.44	96.57	2.03 ± 0.10	0.120
Imidacloprid	29	1871	8.04	1.84	22.10	85.65	28.47	7273.71	1.25 ± 0.06	0.446

^aMethodology explained in the text.

^bEquivalent to g AI/ha.

^cg = index of significance for potency estimation (Finney 1971).

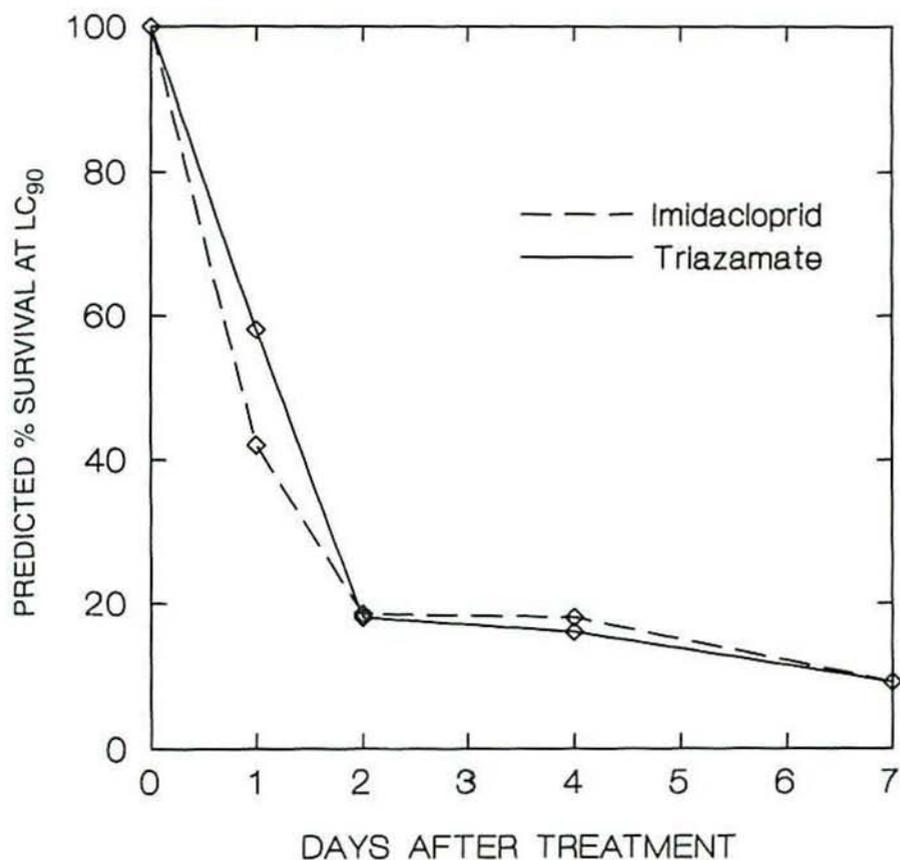


Fig. 1. Predicted survival of *Myzus persicae* on spinach following treatment with an amount of triazamate or imidacloprid equivalent to their respective LC_{90} values. Points were estimated from probit analyses of laboratory data taken 1, 2, 4, and 7 d after treatment.

approximately 56%, 82.4%, and 83.2% mortality by 1, 2, and 4 d, respectively. Whereas variation in the data for nontreated inner leaves at 1 and 2 d after treatment prevented reasonable estimates of concentration-response lines for those counts, data from 4 d after treatment did provide some indication of the speed of systemic action for these two materials. The LC_{90} at 7 d after treatment for triazamate against aphids on nontreated inner leaves was estimated to produce an average of 78.2% mortality at 4 d after treatment. Imidacloprid, at its 7 d after treatment LC_{90} for aphids on nontreated leaves,

was estimated to result in 71.4% mortality at 4 d after treatment. Visual observations of whole plants at 4 d after treatment suggest triazamate has a greater advantage in speed of systemic activity over imidacloprid than these dose-response estimates indicate.

Pymetrozine gave erratic results on both treated and nontreated plant parts. No LC values are reported due to the variation in outcomes for the various rates tested. Pymetrozine reportedly stops the feeding of sucking insects, resulting in their death after 2–4 d (Minton et al. 1994). When rates equivalent to 100–200 g AI/ha failed to provide acceptable aphid mortality on most plants at 7 d after treatment, some of these plants were held for additional observations and examined at various times up to 14 d after treatment. The suggested field rate for this material is approximately 100 g AI/ha. Additional time, exceeding 1 wk, did not result in increased aphid mortality. Pymetrozine offered little activity on nontreated inner leaves. This material is reportedly absorbed by plants and moved acropetally within the plant (Minton et al. 1994). The inner leaves of spinach are apical to the outer leaves and might, in theory, be capable of receiving substances moving by acropetal fashion from outer leaves. The rosette of spinach, with broad leaves situated low on the plant, however, may not be conducive for movement of systemic materials applied to outer leaves unless the substance is phloem mobile. In preliminary field studies, pymetrozine application resulted in fair control of aphids on spinach (P.J.M., unpublished data). Further studies are needed to determine if this chemical will be of value to spinach producers.

In the laboratory, triazamate and imidacloprid both offered aphid control on spinach through systemic activity. Under field conditions, such activity is necessary for a compound to be truly effective against aphids on the underside of inner leaves.

Field study. Based upon aphid infestation ratings, imidacloprid and the high rate of triazamate typically were more effective than the other treatments (Table 2). This effectiveness was true specifically at 4, 6, 11, 14, and 28 d after treatment when these two materials resulted in lower aphid ratings than all other treatments ($F = 20.75$ to 76.72 , $df = 4,241$, $P = 0.0001$ in all cases). Nontreated controls always had a greater average aphid infestation rating than any of the treatments ($F = 3.93$ to 76.72 , $df = 4,241$, $P = 0.0001$ to 0.0041) (Table 2). In most instances, aphid ratings were not different between the pymetrozine treatment and the low rate of triazamate.

Based upon the average percent change (Table 3) and average number of aphids per leaf (Table 4), triazamate and imidacloprid were the fastest acting of the tested materials. The higher rate of triazamate resulted in its greatest decrease in aphid numbers at 6 d after treatment (Table 3). The lowest average number of aphids per leaf for both triazamate treatments occurred at 6 d after treatment (Table 4). With the lower rate of triazamate, the least increase in aphid numbers was observed at 4 d after treatment (Table 3). The average percentage change in aphid numbers and the average number of aphids per labeled leaf were never different between the pymetrozine and the low rate of triazamate treatments (Tables 3, 4). Greatest aphid reductions (Table 3) and lowest aphid numbers (Table 4) for imidacloprid both occurred at 11 d after treatment.

Table 2. Average \pm standard deviation of aphid infestation ratings (0 = none, 1 = 100 or fewer, 3 = 300 or more *Myzus persicae*) of labeled spinach plants (the same plants were rated on each occasion) following aphicide treatments.

Aphicide	Rate (g AI/ha)	Days after treatment ^a						
		1	4	6	11	14	21	28
Imidacloprid	53	1.08 \pm 0.27b	0.62 \pm 0.49c	0.68 \pm 0.47d	0.52 \pm 0.50c	0.46 \pm 0.50c	0.58 \pm 0.54d	0.90 \pm 0.46d
Pymetrozine	100	1.38 \pm 0.64a	1.12 \pm 0.69b	1.32 \pm 0.74b	1.22 \pm 0.76b	1.24 \pm 0.72b	1.32 \pm 0.62bc	1.36 \pm 0.66c
Triazamate	36	1.24 \pm 0.43ab	0.94 \pm 0.42b	0.98 \pm 0.59c	1.28 \pm 0.76b	1.26 \pm 0.53b	1.56 \pm 0.70b	1.80 \pm 0.76b
Triazamate	105	1.20 \pm 0.40b	0.70 \pm 0.51c	0.54 \pm 0.54d	0.42 \pm 0.50c	0.50 \pm 0.58c	0.95 \pm 1.69cd	0.82 \pm 0.48d
Control	—	1.38 \pm 0.53a	1.46 \pm 0.68a	1.84 \pm 0.74a	2.12 \pm 0.77a	1.94 \pm 0.77a	2.34 \pm 0.66a	2.60 \pm 0.57a

^aMeans within a column not followed by the same letter are significantly different (LSD, $\alpha = 0.05$, $n = 50$ plants).

Table 3. Average percentage^a change in number of *Myzus persicae* ± standard deviation on labeled spinach leaves following aphicide treatments.

Aphicide	Rate (g AI/ha)	Days after treatment ^b					
		1	4	6	11	14	21
Imidacloprid	53	+3.9 ± 153.8a	-81.0 ± 23.2a	-85.9 ± 21.2a	-87.2 ± 21.8a	-78.1 ± 28.3a	-74.0 ± 49.2a
Pymetrozine	100	+56.7 ± 252.2a	-32.7 ± 59.6ab	-31.5 ± 80.7ab	-30.6 ± 91.9a	-38.7 ± 111.6a	-21.9 ± 174.4ab
Triazamate	36	+87.9 ± 161.1a	+3.8 ± 131.2b	+10.7 ± 178.6b	+23.8 ± 169.2a	+31.6 ± 214.4a	+135.5 ± 343.4b
Triazamate	105	+15.4 ± 51.1a	-66.5 ± 35.3a	-76.4 ± 43.5a	-70.8 ± 53.9a	-50.3 ± 113.9a	-47.6 ± 95.6ab
Control	—	+58.6 ± 66.3a	+92.5 ± 182.3c	+82.3 ± 190.5c	+263.6 ± 510.5b	+235.7 ± 481.4b	+597.1 ± 787.6c

^a $[(\text{Aphid count at X d after treatment} - \text{pretreatment count}) / \text{pretreatment count}] \times 100\%$.

^bMeans within a column not followed by the same letter are significantly different (LSD, $\alpha = 0.05$, $n = 30$ plants).

Table 4. Average number \pm standard deviation of *Myzus persicae* per labeled spinach leaf (the same leaves were examined on each occasion) following aphicide treatments.

Aphicide	Rate (g AI/ha)	Days after treatment ^a					
		1	4	6	11	14	21
Imidacloprid	53	15.1 \pm 25.2a	2.8 \pm 7.6c	1.6 \pm 2.5c	1.3 \pm 2.0b	1.7 \pm 1.7b	2.3 \pm 3.7b
Pymetrozine	100	30.2 \pm 37.6a	17.4 \pm 24.3b	12.5 \pm 14.8b	9.9 \pm 10.5b	7.0 \pm 9.1b	9.9 \pm 14.6b
Triazamate	36	21.4 \pm 17.1a	10.9 \pm 10.1bc	9.6 \pm 12.4bc	11.9 \pm 15.6b	11.9 \pm 15.6b	23.0 \pm 29.8b
Triazamate	105	36.1 \pm 34.3a	7.4 \pm 8.2c	4.1 \pm 6.1bc	4.7 \pm 7.2b	7.5 \pm 11.0b	9.1 \pm 16.1b
Control	—	30.1 \pm 33.0a	29.4 \pm 28.0a	28.2 \pm 31.1a	52.9 \pm 73.4a	48.7 \pm 74.2a	86.2 \pm 105.7a

^aMeans within a column not followed by the same letter are significantly different (LSD, $\alpha = 0.05$, $n = 30$ plants).

Oddly, the greatest decrease and lowest average number of aphids resulting from the pymetrozine treatment occurred at 14 d after treatment (Tables 3, 4). This time after treatment is interesting because pymetrozine reportedly causes aphids to stop feeding (Minton et al. 1994). Perhaps the cool temperatures conducive to spinach growth delay the death of aphids on pymetrozine-treated plants.

By 4 d after treatment and throughout the remainder of the study, the nontreated controls had more aphids per labeled leaf than any of the other treatments ($F = 9.29$ to 14.68 , $df = 4, 143$, $P = 0.0001$ in all cases). During the study, the number of aphids per labeled leaf on nontreated plants increased from an average of 18.7 ± 18.9 , before treatments were applied, to an average of 86.2 ± 105.7 , 3 wk after insecticide treatment. The average percentage increase for labeled, nontreated leaves at 21 d after treatment was 597.1 ± 787.6 (Table 3). The greater average percentage increase (Table 3), as compared with increases in number (Table 4), suggests that leaves with a lower aphid population experienced the greatest increases in aphids on a percentage basis.

In summary, of the three materials tested, imidacloprid and triazamate appear to offer the most promise for spinach growers. When both of these materials become available to growers, they could reduce much of the uncertainty currently associated with the production of spinach. Pymetrozine, although yielding erratic results, did provide a significant reduction of aphids in field plots. Therefore, pymetrozine also could benefit spinach production, albeit only under more specific circumstances than the two other aphicides. Research is forthcoming to determine how any of the three tested chemicals might fit into an integrated pest management program for spinach.

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Mortality of Diamondback Moth, *Plutella xylostella* (L.), Larvae Treated with Insecticides as a Function of Temperature¹

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ABSTRACT Leaf-dip bioassays were conducted at four constant temperatures by using diamondback moth (*Plutella xylostella* [L.] larvae and the two insecticides permethrin and methamidophos at various rates. Predicted LC₅₀ and LC₉₀ values were calculated based on results from the bioassays and used to determine the effect of temperature on the toxicity of the insecticides. Results indicated that permethrin toxicity to the larvae decreases with temperature whereas methamidophos toxicity increases with temperature. We discuss the implications of these results for a management strategy for diamondback moth larvae in the southern United States.

KEY WORDS Lepidoptera, Plutellidae, *Plutella xylostella*, brassica, insecticide toxicity, temperature response

The diamondback moth, *Plutella xylostella* (L.), is a key pest of brassica crops throughout the world and is of significance to producers of brassica crops in the southern United States (Cartwright et al. 1987). Consumers in the United States, the United States Food and Drug Administration, and the United States Department of Agriculture have established low tolerances for insects, contaminants, and damage on brassica produce bound for the fresh and processing markets. Pesticides have proven to be the most effective and reliable tools for minimizing the number of insect pests, including the diamondback moth, in brassica crops (Edelson et al. 1993). Reduction of insect pest populations to meet tolerances has been difficult and is dependent upon the efficient use of the proper insecticides.

Although numerous studies have proven the effectiveness of various insecticides in reducing diamondback moth populations (Chalfant 1997, Mau et al. 1997, Sorensen

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& Holloway 1997), results achieved by producers have often been variable and attributed to occurrence of insecticide resistance (Magaro & Edelson 1990). Alternative explanations for these failures include poor application and delivery of the insecticides, differences in activity of the insecticides due to host plant or climate or to the destruction of the natural enemy complex. The activity of insecticides may differ as a function of temperature (Grafius 1986, Chalfant 1973): some insecticides increase in activity as measured by increased mortality as temperatures increase, and others decrease in activity as measured by decreased mortality as temperatures increase.

We report the activity of two commonly used and generally effective insecticides—permethrin (pyrethroid) and methamidophos (organophosphate)—as measured by mortality of diamondback moth larvae exposed to the insecticides, over a range of temperatures. Based on our results we suggest management methods to increase the effectiveness of the pesticides for *P. xylostella* control under production conditions in the southern United States.

Materials and Methods

Insecticides. The formulated insecticides used in our experiments were permethrin (Pounce 3.2 EC, FMC Corporation, Philadelphia, Pennsylvania) and methamidophos (Monitor 4 EC, Mobay, Kansas City, Missouri). For all trials the formulated insecticides were diluted with distilled water. New dilutions were made immediately prior to each trial.

Insects. The diamondback moth larvae were taken from a culture maintained at the Texas Agricultural Experiment Station, Weslaco, from 1987 through 1992. This colony has been used as a reference "insecticide susceptible" population in previous research (Magaro & Edelson 1990) and was initiated from larvae collected from commercial cabbage production fields in south Texas that had not been treated with insecticides. The culture was maintained on cabbage, *Brassica oleracea* var. *capitata* L., seedlings in a laboratory under conditions similar to those described by Liu & Sun (1984). Individual insects used in all bioassays were of approximately the same size and instar having been raised in a similar manner and removed from the same rearing container. Voucher specimens associated with this research project are located in the Texas A&M University museum.

Bioassay. Bioassays were conducted by cutting 6.5-cm-diameter disks of tissue from the midsection of cabbage leaves that had not been infested with insects nor treated with pesticides. The disks were submerged in varying dilutions of insecticide solutions for 10 s, removed, and allowed to dry prior to placing them in glass petri dishes (8 cm diameter). Control disks were dipped in distilled water, removed and allowed to dry prior to inoculation with larvae. Ten large, approximately third instars were placed in each dish with a tissue disk and held at specific temperatures for 24 h. Mortality was determined after 24 h by examining larvae for movement. Larvae were noted as dead if they did not respond to a probe by moving at least one body length. Bioassays were conducted at four constant temperatures (10°, 20°, 25°, 35°C) as maintained in environmental chambers with a 12:12 (L:D) h photophase.

Initially bioassays were conducted using a log series of dilutions. The log series of dilutions prepared for both permethrin and methamidophos was 10, 100, 1,000, and 10,000 ppm formulated insecticide in distilled water. A geometric series of dilutions also was prepared such that dilutions ranged between the log dilutions that provided 10% to 90% mortality. The bioassays were conducted again using the geometric series of dilutions. The geometric series of dilutions of permethrin was 3, 9, 27, 81, and 243 ppm of formulated permethrin in distilled water. The geometric series of dilutions of methamidophos was 3, 9, 27, 81, 243, and 729 ppm formulated methamidophos in distilled water.

Analysis of data. Concentration-responses were estimated with the probit option of POLO (Russell et al. 1977). Responses for each insecticide were compared at LC_{50} and LC_{90} values. The criterion of failure of the 95% confidence limit to overlap was used to determine significant differences at each temperature for each insecticide. The \log_{10} values for the estimated LC_{50} and LC_{90} values for each insecticide were regressed against the test temperatures by using the PROC REG option of SAS (SAS Institute 1981) to develop a model for predicting toxicity at a given temperature. Values for slopes of the probit lines for each insecticide were regressed against test temperatures to determine the possibility of any physiological changes in the larval response to the insecticides at different temperatures.

Results and Discussion

The relationship between temperature and toxicity of formulated permethrin to diamondback moth larvae by using the leaf-dip bioassay is indicated in Table 1. Permethrin was the most toxic at 10°C and the least toxic at 35°C. Increasing temperatures from 10° to 35°C resulted in an 8.9-fold increase in dose required to achieve 50% mortality and a 1.4-fold increase in dose required to achieve 90% mortality. Our results indicate that permethrin has a negative temperature coefficient of 8.9 at the LC_{50} and a negative temperature coefficient of 1.4 at the LC_{90} . Slopes and intercepts were compared to determine significant differences for doses at all temperatures and the hypothesis that the slopes and intercepts were the same was rejected ($P = 0.05$).

The relationship between temperature and toxicity for formulated methamidophos was determined by using the leaf-dip bioassay (Table 2). In contrast to permethrin, methamidophos was the most toxic at 35°C and the least toxic at 10°C. Decreasing temperatures from 35° to 10°C resulted in a 5.5-fold increase in dose required to produce 50% mortality and a 12.4-fold increase in dose to achieve 90% mortality. Slopes and intercepts were compared and determined to be significantly different ($P \leq 0.05$).

Regression equations were determined for the temperature toxicity relationships (Table 3). Significant linear relations ($P \leq 0.05$) were indicated for the regressions of the mortality as predicted by temperature. Extrapolation or interpolation, or both, along the regression line can be used to calculate predicted doses necessary to achieve mortality at the LC_{50} or LC_{90} level for either insecticide at a given temperature.

These results provide clear evidence of the effects that temperature can have on the toxicity of two commonly used compounds in *P. xylostella* control in

Table 1. Temperature/toxicity relationships of formulated permethrin to third instars of diamondback moths by using a leaf-dip bioassay technique.

Temp. (°C)	Slope (+ SEM)	LC ₅₀ (ppm) (95% FL)	LC ₉₀ (ppm) (95% FL)	Temp. coefficients ^{a, b}	
				LC ₅₀	LC ₉₀
10	1.1 (± 0.27)	10.9 (4.9–17.5)	147.8 (68.7–1031.9)	-8.9	-1.4
20	1.6 (± 0.25)	24.8 (17.4–32.9)	158.4 (102.9–333.9)	-3.9	-1.3
25	1.7 (± 0.36)	33.7 (24.5–60.0)	183.1 (89.3–927.2)	-2.9	-1.1
35	3.8 (± 0.84)	97.1 (74.0–121.6)	210.7 (159.6–380.2)	1.0	1.0

^aTemperature coefficient = LC at 35°C / LC at comparison temperature.

^bPositive (+) or negative (-) relationship.

brassica crops. Grafius (1986) noted that the functional relationships between temperature and toxicity depend not only on the class of insecticide used but also on the temperature range and the specific insect species' physiological and biological responses to temperature. In studies conducted with the Colorado potato beetle (*Leptinotarsa decemlineata* [Say]), Grafius (1986) found a negative toxicity response to pyrethroid insecticides as temperatures increased. In contrast, the Colorado potato beetle (Grafius 1986) and cabbage looper (*Trichoplusia ni* [Hübner]) (Chalfant 1973) exhibited a negative toxicity response to organophosphate insecticides as temperatures decreased. We found these same type responses in this study over a similar range of temperatures by using the diamondback moth.

We suggest that insecticide recommendations for the most effective control of diamondback moth be based on knowledge of action thresholds, population abundance in production fields, comparative efficacy of insecticides available for controlling the larvae, and consideration of the temperature regime under which insecticide applications will be made.

Brassica crops, including cabbage, kale, collards, mustard, and turnips, are grown throughout the south-central region of the United States, which incorporates an area between Tulsa, Oklahoma (Arkansas River bottoms), to the lower Rio Grande Valley in Texas. The diamondback moth is a common and key pest of these crops. Within this region, these crops are typically planted from August through April and harvested from September through June. Because of the extended crop production time period (August to June),

Table 2. Temperature/toxicity relationships of formulated methamidophos to third instars of diamondback moths by using a leaf-dip bioassay technique.

Temp. (°C)	Slope (+ SEM)	LC ₅₀ (ppm) (95% FL)	LC ₉₀ (ppm) (95% FL)	Temp. coefficients ^{a, b}	
				LC ₅₀	LC ₉₀
10	1.8 (± 0.43)	136.7 (61.6–200.5)	715.4 (466.0–1912.7)	1.0	1.0
20	2.3 (± 0.36)	160.5 (127.5–200.2)	574.3 (407.8–1033.0)	+0.9	+1.2
25	3.4 (± 0.49)	91.9 (74.4–109.0)	220.5 (174.4–319.5)	+1.5	+3.2
35	3.5 (± 0.57)	24.8 (19.7–30.4)	57.5 (44.7–87.3)	+5.5	+12.4

^aTemperature coefficient = LC at 10°C / LC at comparison temperature.

^bSee Table 1.

temperature regimes to which the crop and pests are exposed vary tremendously. Temperatures are usually high (>30°C) during August, September, May, and June. Moderate temperatures (10°–25°C) prevail from October through April with very low temperatures (<0°C) in the northern areas during December, January, and February.

We conclude that insecticide application decisions should include knowledge of efficacy as affected by temperature, especially for those crop production systems where large variations in temperatures may be incurred when pesticides are necessary for pest control. For brassica crop production in the south-central United States, permethrin and other similar pyrethroid insecticides may provide optimal effectiveness for controlling diamondback moth larvae when applied under low temperature conditions ($\leq 10^\circ\text{C}$). In contrast, methamidophos and similar organophosphate insecticides may provide optimal effectiveness when applied under high temperature conditions ($\geq 35^\circ\text{C}$). Within intermediate temperature regimes (20°–30°C) the two materials may provide similar relative effectiveness.

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Table 3. Regression equations ($P = 0.05$) describing relationship between temperature and dose mortality for diamondback moth larvae and two insecticides as described using a leaf-dip bioassay.

Insecticide	LC value	Regression equation	r^2
permethrin	50	$y = -34.5 + 3.4x$	0.85
	90	$y = 116.4 + 2.6x$	0.93
methamidophos	50	$y = 1022 - 28.0x$	0.91
	90	$y = 212 - 4.8x$	0.71

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Susceptibility of Male Codling Moth (Lepidoptera: Tortricidae) to Azinphosmethyl in Missouri¹

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ABSTRACT Male codling moth, *Cydia pomonella* (L.), from four commercial apple orchards and one experimental research orchard located in central and west-central Missouri were examined for susceptibility to azinphosmethyl during the 1993 and 1994 field seasons. Moths were captured in sex pheromone traps and assayed by topical application of technical grade material. The LD₅₀ of the most susceptible population, located in the experimental research orchard, was 0.08 µg per moth, and the LD₅₀ of the most resistant population was 1.10 µg per moth. Three of the four commercial orchard sites had populations of codling moth with a resistance ratio significantly greater than that of the susceptible population.

KEY WORDS Lepidoptera, Tortricidae, *Cydia pomonella*, resistance, azinphosmethyl

The codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), is a key apple and pear pest in some of the major fruit-producing regions of North America. During the past 35 yr, the organophosphate insecticide azinphosmethyl has been the most commonly used product for codling moth control. Within the past several years, however, low-level cases of codling moth resistance to azinphosmethyl have been reported in California, Washington, Oregon, and Utah (Welter et al. 1991, Varela et al. 1993, Knight et al. 1994). Apple producers in the Pacific Northwest typically apply 3-4 applications of azinphosmethyl per year to control two generations of codling moth (1996 Crop Protection Guide for Tree Fruits in Washington, Washington State Univ. Coop. Ext. Guide EB0419). In Missouri, growers often apply 5-7 applications of an organophosphate insecticide, typically azinphosmethyl, per season to control three generations of codling moth (B.A.B., unpublished data).

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One aim of current apple integrated pest management programs is to reduce the frequency and extent of pest resistance (Croft & Bode 1983). The threat of widespread resistance development by codling moth to azinphosmethyl, and the subsequent change by growers to another class of insecticide, such as pyrethroids, would disrupt extensive biological control programs for secondary pests, such as mites and aphids, that are based on the conservation of organophosphate insecticide-resistant predators (Croft & Hoyt 1978, Croft 1982, Croft & Riedl 1991). The goal of this study was to assess the susceptibility of codling moth to azinphosmethyl in central and west-central Missouri apple orchards.

Materials and Methods

Orchard sites. Monitoring for azinphosmethyl resistance in codling moth was conducted at the following commercial orchards located in central and west-central Missouri: Huffstutter (Howard County), Peters (Saline County), and Rasa and Kerr (Lafayette County). Moths also were sampled from experimental orchard blocks located at the University of Missouri, Horticulture and Agroforestry Research Center (Howard County).

Sprays directed at codling moth in each of the commercial orchard sites during the 1993 and 1994 seasons consisted of organophosphate insecticides, primarily azinphosmethyl, with occasional applications of phosmet and methyl parathion. Insecticides used for codling moth control at the research center were azinphosmethyl and phosmet.

Applications of insecticides at the selected commercial sites are applied on a nearly weekly basis by using the alternate row spraying technique: the cover sprays were applied to every other row 1 wk, and the following week, sprays were applied to the previously skipped rows. Pesticide sprays at the experimental research center were applied approximately every 2 wk as complete sprays (all rows treated at the same time).

Trapping procedures. Adult male codling moths were captured in wing-style pheromone traps (Trécé, Salinas, California) that had a paper-thin layer of adhesive on the inside trap bottom. Approximately 100 traps, spaced 30–40 m apart, were placed throughout each site, 1–3 times each season. When moth populations were low, traps were placed in the orchards for 3–7 consecutive days during the peak emergence (B.A.B., unpublished data) of an adult flight period. Traps were positioned in the trees at a height of about 2 m in the late afternoon each day of the trapping period and then collected early the following morning.

Care was taken to place the traps at the orchard sites before or at least 2 d after the scheduled spray dates (Knight & Hull 1989). In cases where moths were inadvertently trapped right after an insecticide application (<48 h), the data were not incorporated in the final analysis and conclusions.

Bioassay procedures. Trap bottoms with captured moths were transported from the orchards to the laboratory in chilled, insulated containers. Equal numbers of live, vigorous moths were assigned to the treatment concentrations. Nine concentrations of technical grade azinphosmethyl (92% purity, Bayer, Kansas City, Missouri) were prepared from a stock solution by

using serial dilution procedures. The concentrations ranged from 12 to 3,200 ppm active ingredient in acetone. The control consisted only of acetone. The prepared dilutions were used no more than 7 d, after which time, new solutions were prepared. Prior to application to moths, the solution containers were removed from freezer storage and allowed to warm to room temperature.

A 1- μ l drop of solution was applied to the ventral side of each moth's abdomen by using a microsyringe mounted on a repeating dispenser (Hamilton, Reno, Nevada). Moths trapped dorsal side up were turned over to expose the ventral side of the abdomen and then treated. Treated moths were kept in a rearing chamber set at 15°C with a 16:8 (L:D) h photoperiod. After 48 h, mortality was assessed and a moth was considered dead if no consistent leg movement was discernible after tactile stimulation with a probe. Data were not included in the final analysis if the control mortality exceeded 20%.

If moth captures were light (<20 moths per night) at the beginning of a trapping period, the treatment doses were reduced from nine to five. This reduction was done because Robertson & Preisler (1992) indicated that as few as 60 to 120 total test subjects, divided among five application doses where no more than one of the doses would cause 100% mortality, can still provide reliable dose-response data. If at some of the sites where <60 moths were captured during a specific trapping period, the data were not incorporated in the final analysis and conclusions.

Data analysis. Mortality data were analyzed by logit regressions (SAS Institute 1985). Resistance ratios were calculated by dividing the lethal dose for 50% of the population (LD_{50}) by the lowest LD_{50} of all the populations assayed. The resistance ratios were tested for significance by calculating 95% confidence limits, and if the confidence limit included 1, then the LD_{50} was not significantly different from the susceptible population (Robertson & Preisler 1992).

Results and Discussion

The lowest LD_{50} of all populations sampled in both years, and the value used to calculate the resistance ratios for the other sites, was 0.08 μ g per moth from the third adult flight period (1994) at the research center site in Howard County (Table 1). This LD_{50} value is comparable to the LD_{50} values of susceptible codling moth populations found in California, Oregon, Utah, Washington, and New York (Howell & Maitlen 1983, Riedl et al. 1986, Varela et al. 1993).

The LD_{50} values for moths collected at the research center site during both years ranged from 0.08 to 0.23 μ g per moth, and were for the most part, the lowest values found in the study. Only one of the four sampling periods at the site had a significantly greater resistance ratio than the susceptible population (Table 1). The susceptibility of moths at the research center compared with those at the commercial orchards may be due, in part, to the center's relatively irregular and more infrequent spray program.

The highest LD_{50} values found in Howard County, both in 1993 and 1994, were at the Huffstutter orchard, 0.98 and 0.71 μ g per moth, respectively (Table 1). All resistance ratios from the Huffstutter site were significantly greater than the

Table 1. Logit regressions for azinphosmethyl to male codling moth adults collected with female sex pheromone baited traps at five central and west-central Missouri apple orchard locations, 1993-1994.

Site (County)	Year	Flight ^a	No. of moths tested	Slope (± SE)	LD ₅₀ ^{b,c} (95% CL)	LD ₉₀ ^b (95% CL)	RR ^d (95% CL)
Research Center (Howard)	1993	1	122	3.17 (± 0.60)	0.23* (0.15-0.38)	1.16 (0.64-3.47)	3.06 (1.33-7.04)
	1993	2	115	2.60 (± 0.51)	0.16 (0.09-0.26)	1.08 (0.55-4.10)	2.02 (0.85-4.81)
	1994	2	145	2.16 (± 0.38)	0.12 (0.07-0.21)	1.27 (0.61-4.77)	1.59 (0.66-3.84)
	1994 ^e	3	97	1.94 (± 0.44)	0.08 (0.03-0.16)	1.04 (0.41-8.20)	1.00 (0.37-2.70)
Huffstutter (Howard)	1993	1	198	4.63 (± 0.82)	0.98* (0.73-1.33)	2.92 (1.98-5.95)	12.75 (5.97-27.23)
	1994	1	193	3.32 (± 0.54)	0.71* (0.49-1.04)	3.24 (1.97-7.65)	9.21 (4.18-20.31)
	1994	2	115	1.88 (± 0.38)	0.30* (0.15-0.61)	4.35 (1.64-33.43)	3.86 (1.49-10.03)
	1994	3	115	1.27 (± 0.31)	0.37* (0.15-1.10)	19.91 (4.15-1270.0)	4.84 (1.57-14.87)

Table 1. Continued.

Site (County)	Year	Flight ^a	No. of moths tested	Slope (± SE)	LD ₅₀ ^{b,c} (95% CL)	LD ₉₀ ^b (95% CL)	RR ^d (95% CL)
Kerr (Lafayette)	1994	1	197	2.75 (± 0.41)	0.34* (0.24–0.51)	2.16 (1.24–5.46)	4.47 (2.01–9.95)
	1994	2	193	4.07 (± 0.76)	1.10* (0.81–1.47)	3.81 (2.55–8.20)	14.32 (6.70–30.57)
	1994	3	540	1.83 (± 0.19)	0.61* (0.45–0.86)	9.77 (5.32–23.62)	7.97 (3.69–17.24)
Rasa (Lafayette)	1994	1	81	2.33 (± 0.54)	0.23* (0.11–0.49)	2.06 (0.85–15.49)	3.06 (1.16–8.08)
Peters (Saline)	1994	1	328	2.13 (± 0.25)	0.15 (0.10–0.21)	1.58 (0.93–3.46)	1.91 (0.87–4.17)

^aMoths were trapped during the peak emergence of each flight period.

^bLD (lethal doses expressed as micrograms per moth).

^cThe asterisk symbol denotes that the 95% confidence limit of the resistance ratio (RR) did not include 1; hence, the LD₅₀ was significantly different from the susceptible population LD₅₀ (Robertson & Preisler 1992).

^dResistance ratio: LD₅₀/most susceptible site LD₅₀.

^eSite used as the susceptible population.

resistance ratio of the susceptible research center population. In fact, moths of the first adult flight (spring emergence) of both years showed levels of resistance that were 9.21 and 12.75 times greater than that of the susceptible population.

In Lafayette County, LD₅₀ values from moths assayed in 1994 at the Kerr site ranged from 0.34 to 1.1 µg per moth. All resistance ratio values were significantly greater than the susceptible population (Table 1). Moths assayed from the second adult flight at the Kerr orchard had the highest resistance ratio of the study. These moths were 14 times more resistant than those of the susceptible population. The LD₅₀ values found at the other Lafayette County site (Rasa) and the Saline County site (Peters) were 0.23 and 0.15 µg per moth, respectively. Only the Rasa site had a resistance ratio significantly greater than that of the susceptible population.

The assay data from all sites indicate that codling moth susceptibility to azinphosmethyl, i.e., lower resistance ratios, tended to be greater toward the latter part of the season (third adult flight period). This greater susceptibility by the moths later in the season is most likely due to age, as there is a more heterogeneous makeup of younger and older moths at that time (Riedl et al. 1985).

Considering the orchards assayed during the study, the two highest resistance ratio values, 12.75 and 14.32 (Table 1), were associated with the two orchard sites Huffstutter and Kerr, respectively, that historically have had codling moth problems during the past several years (B. A. B., unpublished data). It appears that the control problems were a result of azinphosmethyl resistant codling moth populations.

Since 1991, codling moth resistance to azinphosmethyl in orchard populations have been reported in California, Oregon, Washington, and Utah (Welter et al. 1991, Varela et al. 1993, Knight et al. 1994). This study revealed that some populations of codling moth in central and west-central Missouri apple orchards also have developed significant low to moderate levels of resistance to azinphosmethyl.

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Disruption of Sex Pheromone Communication in the Blackheaded Fireworm in Wisconsin Cranberry Marshes by Using MSTRS™ Devices¹

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ABSTRACT The results of experiments in Wisconsin cranberry marshes by using a novel, controlled release system called the Metered Semiochemical Timed Release System, or MSTRS™, for disrupting pheromone-source location by males of the blackheaded fireworm, *Rhopobota naevana* (Hübner), are described. During the first flight, disruption (trap catch reduction) of males' ability to locate synthetic sex pheromone lures containing 10 µg of the *R. naevana* pheromone blend averaged 95.7% in the first grower location and 99.6% in a second grower location, regardless of the MSTRS deployment pattern. However, disruption averaged only 81.7%, 80.7%, and 56.4% for a 12 MSTRS-per-ha cross pattern, a 5 MSTRS-per-ha perimeter pattern, and a 12 MSTRS-per-ha perimeter pattern, respectively, in the third grower site. During the second flight, in which the night-only emission of pheromone was tried, disruption of trap catch averaged 86.7% in the first location overall for all MSTRS configurations, 85.4% in the second location, and 53.8% in the third and poorest disruption location. Significant levels of disruption were achieved season-long regardless of the MSTRS array, but there was no significant difference in disruption efficacy among the three arrays. No significant effect on larval infestation following the first flight was observed in the MSTRS-treated plots, but there was high sampling variability and very low infestation in the check plots, making it difficult to discern effects of MSTRS on larval populations.

KEY WORDS Sex pheromone, *Rhopobota naevana*, blackheaded fireworm, Tortricidae, mating disruption, controlled release dispensers, *Vaccinium macrocarpon*

There has been much progress over the past 10 yr or so in improving the release-rate characteristics of some of the most commercially successful pheromone mating disruption formulations. However, none of the existing controlled-release technologies allow the user to actively alter the release rate. The existing systems are

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all passive systems that emit pheromone continuously according to ambient wind and temperature conditions.

We recently described a new system called Metered Semiochemical Timed Release System, or MSTRS™ (Mafrá-Neto & Baker 1996), in which an aerosol canister containing pheromone is placed in a machine and an aerosol spray-burst is emitted onto a large pad on a timed basis (e.g., every 15 min). Pheromone is then emitted from the pad at extremely high rates, ca. 20 times higher than most existing dispensers. Fewer dispensers are therefore needed for effective disruption, and pheromone is not wasted by being passively emitted from the reservoir during periods of the day when the insects are inactive. In addition, the pheromone is protected from oxidation and UV degradation because it is housed in pressurized canisters.

Significant work on mating disruption of the blackheaded fireworm, *Rhopobota naevana* (Hübner), a serious pest of cranberries (*Vaccinium macrocarpon* Aiton), has been undertaken by Fitzpatrick et al. (1995). Her work has shown much promise for using this technique for control of blackheaded fireworm by using either Shin-Etsu ropes (Pacific Biocontrol, Ltd.) or Ecogen Spirals (Scentry/Ecogen, Billings, Montana) with a total application rate of ca. 70 g of pheromone per acre. One problem with these dispensers, however, is that they must be retrieved at the end of the season due to the potential for the buildup of environmentally unacceptable levels of plastic in the cranberry marshes. The placement and retrieval of a high number of point sources on the cranberry beds also would result in unacceptably high foot traffic that would damage the delicate, slow-growing plants.

We hypothesized that a relatively few MSTRS stationed mostly around the perimeter of marshes, using the same total amount of pheromone per hectare as existing formulations tested by Fitzpatrick et al. (1995), might provide effective levels of disruption of pheromone source location that could reduce damage by the blackheaded fireworm and suppress populations of this species. We sought to begin our investigation in 1997 by first determining whether different arrays of MSTRS machines are effective in reducing captures of males in traps baited with the synthetic pheromone blend of this species to the same degree achieved by Fitzpatrick et al. (1995). We also sought to achieve levels of disruption comparable to those achieved by Fitzpatrick and colleagues during 1996 who were conducting concurrent experiments in neighboring cranberry marshes in Wisconsin (Fitzpatrick 1997).

Materials and Methods

We used MSTRS devices and affixed them to wooden stakes at a height of 20 cm above the cranberry plant canopy. The canisters contained *R. naevana* pheromone, a blend of (*Z*)-11-tetradecenyl acetate, (*Z*)-11-tetradecen-1-ol, and (*Z*)-9-dodecenyl acetate in a ratio of 9:3:1 (McDonough et al. 1987, Slessor et al. 1987). These components were purchased from Bedoukian Research, Inc., Connecticut, diluted in reagent ethanol to a weight of 40 g of solution, and formulated with propellant in the canisters for a total weight of 160 g inside each canister.

There were three MSTRS treatments (deployment patterns) plus a check in each of three grower locations within 50 km of each other near Babcock, Wisconsin. Two of the treatments used MSTRS containing 8 g of pheromone in the canisters (8-g canisters) and the third treatment used MSTRS outfitted with canisters containing 20 g of pheromone (20-g canisters). At each location, treatments were clustered such that the three plots containing MSTRS arrays occupied adjacent beds, whereas check plots that were not treated with pheromone disruptant were located at least 100 m from the MSTRS-treated beds. We hypothesized that any of the three MSTRS arrays would significantly reduce trap capture of males (pheromone source location) compared with the check plots. We also hypothesized that none of the arrays of MSTRS would be better than the others in disrupting pheromone source location.

At grower location 1, the check plot consisted of two beds having an area of 0.8 ha each for a total area of 1.6 ha. At locations 2 and 3 the check plots consisted of one bed of 1.4 ha and 1 bed of 1.7 ha, respectively. In the first MSTRS treatment, 8-g canisters were deployed at a density of 12 MSTRS per ha around the perimeter of two 0.6-ha beds at location 1, one 1.7-ha bed at location 2, and six 0.2-ha beds at location 3. The second MSTRS treatment again used 8-gm canisters and 12 MSTRS per ha, but with three of the devices transecting the center of the plot and the rest placed around the perimeter. For this treatment, at location 1 a single 0.8-ha bed was used, at location 2 a single 1.6-ha bed was used, and three 0.6-ha beds were used at location 3. The final MSTRS contained 20-g canisters and these were deployed around the perimeters of one bed of 0.8 ha at location 1, one bed of 1.5 ha at location 2, and six beds of 0.2 ha at location 3 at a density of 5 MSTRS per ha at each location.

Disruption was assessed by counting the number of males captured in wing traps (IPM Technologies, Inc., Portland, Oregon) baited with 10 μg of the pheromone blend on a rubber septum, a lure considered to be comparable in attractancy to females (Fitzpatrick 1997). The wing traps were placed, three per plot, in the interiors of the beds, and not closer than 30 m from the nearest machine. The number of males captured was assessed weekly, the males removed, and trap bottoms replaced as needed. At the end of the season, mean weekly male trap catch for each plot was calculated, and then these means were used to calculate a first-flight and second-flight mean trap catch per treatment. These means were subjected to a two-way ANOVA with three locations (complete blocks) as replicates. Means were compared using Tukey's HSD test. (Sokal & Rohlf 1981) Percent disruption in treatment plots also was calculated at each location by first dividing mean weekly male trap catch from treatment plots by mean weekly male trap catch from the check plot at the same location. This trap capture proportion relative to the check was then subtracted from 1 and multiplied by 100 to obtain the percentage reduction (disruption) of trap catch caused by the MSTRS.

Sweep samples were taken for several weeks after the first flight. Every week, a scout walked two randomly chosen straight-line transects within a given bed in each plot (one in the interior and the other near the edge) and made 100 sweeps per transect of the vegetation (one sweep per step) with a standard insect sweep net. The net was examined for blackheaded fireworm larvae that were then counted. Each week, mean larval counts per 100 sweeps

for each plot were analyzed using a two-way ANOVA with three locations (complete blocks) as replicates.

Both the check plots and the disruption plots were subject to standard grower practices of spray irrigation and applications of pesticides, including insecticides. When any of the three growers did apply pesticides, they treated both the check and the disruption plots with the same materials at the same time. The growers each made three applications of insecticide during the 1996 season. During the first flight of moths, the MSTRS were programmed to discharge every 15 min, 24 h per day. At the end of the first flight, the machines were switched off and the canisters were then all replaced because their contents were nearly depleted. In the week before the beginning of the second flight, the MSTRS were switched on again and programmed to discharge in the night-only mode, in which a light-sensor triggers them to begin discharging every 15 min only around sunset and to stop at sunrise. Discharging in this mode gave the MSTRS canisters a longevity of >75 d.

Results and Discussion

During both the first and second flights, all three MSTRS arrays caused significant disruption of pheromone source location (Table 1). However, none of the three MSTRS arrays were significantly better at disrupting pheromone source location season-long than the others (Table 1).

During the first flight, disruption averaged 95.7% in the first grower location, and 99.6% in the second grower location (Fig. 1) regardless of the MSTRS deployment pattern. However, disruption averaged only 81.7%, 80.7%, and 56.4% for the 12-dispenser-per-ha cross pattern (low cross), the 5 dispenser-per-ha perimeter pattern (high perimeter), and the 12 dispenser-per-ha perimeter pattern (low perimeter), respectively, in the third grower site (Fig. 1), which had a history of very high populations of fireworm and low yields compared with the industry average in the region.

Thus, the overall levels of disruption, averaging ca. 90% during the first flight across all three locations and all MSTRS treatments combined (Table 1), were affected by the poor disruption at the third grower location. At this third site, captures in the check plot averaged 98.9 males per trap per week over the 5-wk period from 27 June to 25 July, and 18.1 (± 11.2 SD; $n = 5$), 19.1 (± 12.8 SD; $n = 5$), and 43.1 (± 35.5 SD; $n = 5$) males per trap per week in the low-cross, high-perimeter, and low-perimeter patterns, respectively. The MSTRS at the other two grower locations during this 5-wk period resulted in high and similar levels of disruption compared with the third location. Captures in the check plots averaged 102.5 (± 68.4 SD; $n = 3$) and 52.1 (± 48.4 SD; $n = 3$) males per trap per week in location 1 and 2, respectively, whereas captures in the disruptant-treated plots (all MSTRS deployment patterns combined) in these locations averaged 4.4 (± 6.8 SD; $n = 15$) and 0.2 (± 0.3 SD; $n = 15$) males per trap per week, respectively.

For the first, second, and third grower locations the larval infestation rates were not significantly different in the MSTRS-treated plots than in the check plots following the first flight (Table 2). The check plot sweep samples were at

Table 1. Mean number (\pm SD) of blackheaded fireworm males captured during the first and second flights in the different MSTRS-treated and check plots from the same locations over the 1996 season in Wisconsin cranberry marshes. Data were square-root-transformed and a two-way ANOVA was conducted. Asterisks indicate significant *F* values. Means from the same flight having no letters in common are significantly different according to Tukey's HSD test ($P < 0.05$, $df = 64$; Sokal & Rohlf 1981).

Mean no. of males per trap (\pm SD)	
<u>1st flight (13 June - 1 Aug.)</u>	
Control	50.7 \pm 58.0a
MSTRS TM low, perimeter	9.7 \pm 23.1b
MSTRS TM low, cross	5.0 \pm 9.2b
MSTRS TM high, perimeter	5.1 \pm 10.1b
<i>F</i> statistics	15.3*
<u>2nd flight (8 Aug. - 25 Sept.)</u>	
Control	42.7 \pm 45.8a
MSTRS TM low, perimeter	13.3 \pm 20.6b
MSTRS TM low, cross	11.1 \pm 14.3b
MSTRS TM high, perimeter	12.4 \pm 17.4b
<i>F</i> statistics	7.9*

or near zero in most cases, and so it would be difficult to reveal any effect of the disruptant on larval density during this particular year in these beds. The apparent lack of reduction in larval populations may be due to the high variability inherent in this sampling technique due to the highly aggregated nature of the larval infestations (Fitzgerald 1997). Another factor may be the unknown level of migration of gravid females from one cranberry bed to another. An alternative way to get a more direct assessment of the efficacy of MSTRS-based disruption than using pheromone trap catch reduction would be to treat a wider area to reduce the effect of gravid female migration, and to examine freely flying females captured in disruption plots versus check plots for the presence of spermatophores. Collection of *R. naevana* females without undue foot-traffic on the beds is difficult, however.

During the second flight, in which the night-only emission of pheromone was tried, disruption was not as good as during the first flight in most plots but still

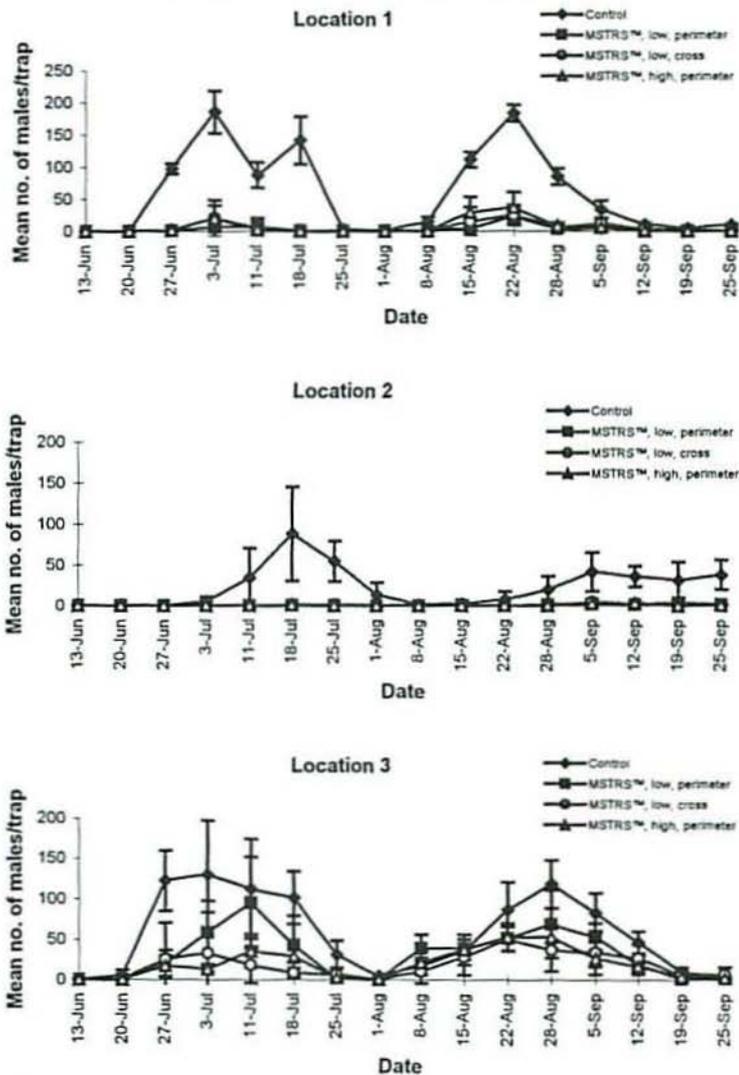


Fig. 1. Mean capture per trap ($n = 3$) of male blackheaded fireworm in wing traps containing 10 μg of synthetic pheromone at three locations at which either 5 (high perimeter) or 12 (low perimeter and low cross pattern) MSTRS™ devices per hectare were deployed in cranberry beds. The devices were activated before the first flight began and continued to release pheromone throughout the season (ending 25 September) from either 20-g canisters (high perimeter) or 8-g canisters (low perimeter and low cross). During the second flight, the MSTRS were programmed to release pheromone onto the pads only at night. Bars above and below the means ($n = 3$) indicate standard deviations.

Table 2. Mean number of blackheaded fireworm larvae (\pm S.D) sampled per 100 sweeps in cranberry beds following the first flight in three grower locations. These locations were the same grower locations that were used in assessing the disruption of trap catch (Fig. 1; Table 1). There were no differences among means (insignificant F value for treatment mean square) following a two-way ANOVA ($F = 0.45$, df 16, $P = 0.71$). No sample was available at grower location 2 on 5 Aug. ($n = 2$).

Treatment	Date			
	15–18 July	22–25 July	29–31 July	5 Aug.
Control	2.10 \pm 2.83	2.67 \pm 4.02	0.0 \pm 0.0	0.0 \pm 0.0
MSTRS TM Low Perimeter	2.50 \pm 2.78	3.10 \pm 5.11	0.67 \pm 1.16	0.85 \pm 1.20
MSTRS TM High Perimeter	2.27 \pm 2.37	3.83 \pm 3.75	0.00 \pm 0.00	0.0 \pm 0.0
MSTRS TM Low Cross	0.60 \pm 0.53	2.40 \pm 2.35	0.67 \pm 1.16	1.25 \pm 1.77

averaged 86.7% in the first location overall for all MSTRS configurations, 85.4% in the second location, and 53.8% in the third, poorest disruption location (Fig. 1). As during the first flight, capture levels in the MSTRS-treated disruption plots were significantly lower during the second flight than in the check plots (Table 1), but no MSTRS treatment produced significantly lower captures than another. The poor disruption at the third grower site (Fig. 1) was the main contributor to the relatively poor (ca. 75%) disruption averaged across all MSTRS arrays and all three sites during this flight (Table 1).

Our measurements of the emission rates from the pads during the daytime when they are not being recharged showed that after 14 d of night-only emission, the pads from the MSTRS containing canisters with 8 g of pheromone released Z11-14:Ac at 8 μ g/min during the first 3 h of daylight, and then by nightfall this rate diminished to 2.5 μ g/min. *Rhopobota naevana* appear to have a broad mating periodicity during daylight hours, commencing in late morning and extending to dusk (Sheila Fitzpatrick, Agriculture Canada, Agassiz, British Columbia, unpublished data). Thus, it is possible that the night-only discharge and slow diminution of emission rate from the pads during the day during the second flight may have caused the somewhat lower disruption efficacy compared with the 24-h discharge used during the first flight.

Nevertheless, our results are encouraging in this first attempt at using MSTRS on this species, in that they show that a relatively few MSTRS per hectare can, in some locations, effectively disrupt pheromone source location by *R. naevana* at levels of 95%–99% disruption for an entire flight period on ca. 1.2-ha plots consisting of several cranberry beds. The machines proved to be highly durable, and examinations of the batteries and the ability of the machines to produce sprays during the entire season showed that all but one of the machines and batteries were unimpaired and functioning perfectly all season long. This level of durability was encouraging because most of the beds were spray-irrigated and the irrigation regularly drenched the machines and pads. In addition, thunderstorms with high winds occurred in the area several times over the course of the summer and buffeted the MSTRS devices.

In all three locations, the MSTRS devices were deployed at the same time that a sprayable formulation of pheromone (microencapsulated, called MEC; Scentry/Ecogen) was applied directly to neighboring cranberry beds. Monitoring traps and lures used in the MEC plots were identical to those used in the MSTRS plots. Disruption levels achieved were 85%–93% during the first flight in MEC plots and 78%–91% during the second flight (Fitzpatrick 1997). Thus, the MSTRS gave levels of disruption of pheromone source location comparable to the sprayable formulation, and comparable to those achieved with other low-emission sources placed on the beds at ca. 1,000/ha (Fitzpatrick et al. 1995).

The geometry of deployment of such a low number of MSTRS devices per hectare is important, and it must be considered that the smaller the plot, the greater the amount of edge there is to protect relative to the interior area of crop. In principle, the MSTRS technology should work better over a very large, regularly shaped area where there will be fewer pheromone-plume-free holes along the edges. Experiments with high-emission-rate aerosol devices similar to MSTRS in California over very large areas of orchards or fields (16–256 ha) against various tortricid and noctuid species demonstrated that this type of device does indeed work very effectively over large areas (Shorey & Gerber 1996a, b; Shorey et al. 1996). A 256-ha block of tomatoes was effectively protected by a density of only one aerosol device per 13.5 ha (Shorey & Gerber 1996b). Thus, in the future, against the blackheaded fireworm on these relatively small blocks of cranberries comprising a much higher edge-to-area ratio, a slightly greater number of MSTRS should be used to fill holes that will occur along the edges of the beds, especially on the upwind side. Also, aerial transport of the pheromone plumes over multiple beds will probably be aided by deploying the devices higher up on the grassy banks of the dikes rather than lower, on the edges of the beds themselves, as was done in this study.

Finally, it must be considered that the efficacy of widely spaced dispensers such as described herein, whose plumes need to sweep for tens, and perhaps hundreds of meters horizontally over the crop canopy to both attract and habituate males sufficiently so that they are prevented from mating, will likely be more dependent upon ambient meteorological conditions than will be numerous lower-emission-rate point sources spaced only meters apart throughout the crop. This vulnerability may be accentuated for species that mate during the daytime, when adiabatic lapse rates are highest, and unstable,

rising air can potentially carry plumes from disruptant dispensers up and away from the crop canopy.

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