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INSTRUCTIONS FOR AUTHORS

Editorial Policy

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Jones, M. A. 1986. Article title—lowercase after colon or dash unless it is a proper noun. Abbr. J. 00: 00–00.

1988a. Title. Abbr. J. 00: 00–00.

1988b. Title. Abbr. J. 00: 00–00.

Jones, M. A. & R. Burns. 1975. Title. Abbr. J. 00: 00–00.

Jones, M. A. & R. Burns. In press. Title. Abbr. J. 00: 00–00.

Jones, M. A. & A. B. Skyler. 1973. Title. Abbr. J. 00: 00–00.

Jones, M. A., A. B. Skyler & H. H. Monroe. 1973. Title. Abbr. J. 00: 00–00.

Jones, M. A., R. Burns & L. O. Curtin. 1979. Title. Abbr. J. 00: 00–00.

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Books

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

Borror, D. J., D. M. DeLong & C. A. Triplehorn. 1981. An introduction to the study of insects, 5th ed. Saunders, Philadelphia, Pennsylvania, 827 pp.

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 acaracide 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.

Rossignol, P. A. 1988. Parasite modification of mosquito probing behavior, pp. 25–28. *In* T. W. Scott and J. Grumstrup-Scott [Eds.], Proceedings of a symposium: The role of vector-host interactions in disease transmission. Miscellaneous Publication 68, Entomological Society of America, College Park, Maryland, 50 pp.

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Baker, W. H. 1972. Eastern forest insects. United States Department of Agriculture Forest Service Miscellaneous Publication 1175, Washington, D.C., 672 pp.

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.

International Rice Research Institute (IRRI). 1977. Title. International Rice Research Institute, Manila. Philippines, 336 pp.

Theses and Dissertations

Anway, C. L. 1982. Male-produced aggregation pheromone of the maize weevil and effect of diet on production and response. MS thesis, Univ. of Wisconsin, Madison, 66 pp.

Hogsette, J. A., Jr. 1979. The evaluation of poultry pest management techniques in Florida poultry houses. PhD dissertation, Univ. of Florida, Gainesville, 307 pp.

Abstracts and Translations

Barker, S. 1989. Toxicity of XXX. Chem. Abstr. 18: 193a.

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Shenderovskaya, L. P. 1979. Introduced insect enemies and microorganisms. Zash. Rast. (Kiev) 3: 52–56 (in Russian).

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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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Scientific Notes. The *Journal of Agricultural and Urban Entomology* will consider publication of research reports which are considered to be of a preliminary nature in the form of a scientific note. The format for a scientific note is as follows:

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3. There should be no abstract nor section divisions (e.g., Introduction, Materials and Methods, etc.)
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In light of this, the Committee requests that authors return revised manuscripts to their subject editor within three months of receipt. Manuscripts held for revisions for six months or more will, at the discretion of the editor, be subject to a 10% surcharge over the normal cost of publication.

The Committee recognizes that extenuating circumstances arise, and will consider special arrangements for authors in such situations.

Effect of Nonviruliferous Wheat Curl Mites on Yield of Winter Wheat[1]

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J. Agric. Urban Entomol. 17(1): 9–13 (January 2000)

ABSTRACT The effect of nonviruliferous wheat curl mites (*Aceria tosichella* Keifer) on yield of the hard red winter wheat cultivar 'Ike' was evaluated in field plots. Plots artificially infested with wheat curl mites reared in the greenhouse averaged 8,821 ± 3,814 mites per spike compared with l,166 ± 644 per spike in the naturally infested controls. The yield of the infested plots (3,707 ± 401 kg/ha) was significantly less (17%) than the naturally infested control plots (4,481 ± 349 kg/ha). Assuming a linear effect, which may or may not be valid, it is estimated that a 1% loss in grain yield may result from 450 wheat curl mites per spike. Test weights and 1,000 kernel weights were also significantly lower for the infested plots than for the naturally infested controls. There were no significant differences among treatments for numbers of kernels per spike or for any other variables associated with the May 1, 8, and 15 dates of infestation. These results indicate that wheat curl mites cause yield losses to wheat aside from that caused by the viruses they vector. The limited information available on natural infestations of wheat spikes indicates that yield losses due to wheat curl mites may range from less than 1% to 15%.

KEY WORDS wheat yield, wheat curl mite, Acari, Eriophyidae, *Aceria tosichella* Keifer, vector, nonviruliferous

The wheat curl mite, *Aceria tosichella* Keifer is the only known vector of wheat streak mosaic virus (Slykhuis 1955) and the high plains virus (Seifers et al. 1997, 1998), which are important diseases of wheat (*Triticum aestivum* L.). Direct damage to wheat by wheat curl mites in the greenhouse is characterized by leaf rolling, stunting, necrosis and even plant death, but signs of damage in the field are largely limited to minor rolling of leaf edges and occasional trapped leaves. Because of its microscopic size (250 × 75 μm) and the difficulty in evaluating its effect in the absence of associated viruses, it generally has been assumed that the wheat curl mite is of no economic importance on wheat other than in its capacity as a vector. However, when wheat reaches the heading stage the wheat curl mites move from the leaves into the spikes and feed on the glumes and kernels (Kantack & Knutson 1954). During a wheat streak mosaic epiphytotic in 1988 the wheat

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cultivar 'Arkan' averaged more than 18,000 wheat curl mites per spike (Harvey et al. 1990). Thus, the possibility exists that such large populations feeding on the developing wheat kernels may have an effect on grain production, especially since wheat curl mites cause a condition in corn known as kernel red streak which may be due to a salivary phytotoxin injected during feeding (Nault et al. 1967). No information is available on the effects of nonviruliferous wheat curl mites on wheat production, so our objective was to evaluate their effect on grain yield of the hard red winter wheat cultivar 'Ike'.

Materials and Methods

Wheat curl mites were from a collection of several hundred obtained from volunteer wheat in Ellis County, Kansas in September, 1996, and identified as *Aceria tosichella* Keifer by J. W. Amrine at West Virginia University. A nonviruliferous colony was established from this collection by egg transfer to wheat seedlings (Slykhuis 1955). The colony was maintained on 'Tomahawk' wheat grown in poly-cast tubes, 2.5-cm diameter and 14-cm long (Conetainer Company, Canby, Oregon), and covered with mite-proof cylinder cages (Seifers et al. 1997). Wheat leaves were tested in enzyme-linked immunosorbent assay against wheat streak mosaic virus and high plains virus antisera to verify the absence of these viruses (Seifers et al. 1996). Wheat curl mites were increased for transfer to the field by placing heavily infested leaves (excised from plants in the poly-cast tubes) on 'Ike' wheat seedlings grown in soil in rows in metal flats (51 × 35 × 9 cm). The wheat seeds were treated with Imidacloprid (1.2 g AI/kg) to prevent contamination by aphids and other insects (Harvey et al. 1998). There were about 25 two-leaf stage seedlings per row and 11 rows per flat at the time of infestation. The wheat curl mites were allowed to increase in a greenhouse (temperature 18° to 30°C) for three to four wks before being used to infest the field plots. 'Ike' wheat was seeded at 50 kg/ha on October 6, 1997. Each experimental unit consisted of a single one m row and rows were 30 cm apart. Treatments consisted of three infestation dates and one naturally infested control which was exposed to wheat curl mite migration from adjacent infested plots. The four treatments (infested on May 1, 8, and 15 and naturally infested control) were arranged in randomized blocks with eight replications. Wheat growth stages when infested on May 1, 8, and 15 were Feekes scale 8, 9, and 10.5 (Zadoks 37, 39, and 58), respectively. On each date, wheat curl mite infested leaves (excised from flats maintained in the greenhouse free of insects and diseases) were placed in contact with the plants within the rows. Seedlings, having several hundred mites per plant, from one flat were used to infest two plots. The wheat curl mites were allowed two days to move from the dying leaves to the field plants, then the dead plant material was removed. Plants in the control plots were exposed to natural infestations, including mites that may have moved from adjacent infested plots, but were not deliberately infested.

Wheat curl mite numbers were estimated from spikes collected from the plots on June 13 when they were still green and in the late milk stage (Feekes 11, Zadoks 75). One spike was randomly selected from each plot for a total of eight spikes per treatment. Populations were determined by the sticky-tape method that involved placing individual spikes on transparent tape trapping mites as they crawled from the drying spikes (Harvey & Martin 1988). Wheat curl mite numbers were estimated by measuring clusters of mites on the tapes under a

dissecting microscope at 45X one week after the spikes were placed on the tapes (Harvey & Martin 1988). Mature spikes were hand harvested on July 1 and passed through a Vogel thresher. Yield per plot (converted to kg/ha.), g/1000 kernels, and test weights (kg/hl) were determined. In addition, one spike was randomly selected from each plot (eight spikes/treatment) and numbers of kernels per spike were recorded. Values presented are means ± SE. Data were subjected to analysis of variance with treatment means separated by the Student-Newman-Keuls (Steel & Torrie 1960) multiple range test at $P<0.05$ with df = 3, 21 for all tests.

Results and Discussion

Wheat curl mite populations and their effects on the yield of 'Ike' wheat in the field are presented in Table 1. Estimates of wheat curl mite numbers per spike for infestations initiated on May 1, 8, and 15 were not significantly different, but the controls had significantly lower mite populations than any of the three treatment dates ($F = 14.6$, $P = 0.00008$). Kernel weight in g/1000, test weight in kg/hl, and yield of grain in kg/ha did not differ significantly among the three treatments. This was expected since date of infestation did not significantly affect wheat curl mite numbers per spike.

However, all three parameters were significantly lower for infested treatments compared with the control plots (kernel weight $F = 18.5$, $P = 0.00003$; test weight $F = 14.7$, $P = 0.00007$; yield $F = 10.1$, $P = 0.0004$). The observed reduction in kernel weight explains most of the yield reduction since numbers of kernels per spike were not affected ($F = 2.3$, $P = 0.10$).

The average yield for the three treatment dates (3,707 ± 401kg/ha.) was 17% less than the yield of the naturally infested control (4,481 ± 349 kg./ha). Because the artificially infested plots averaged 7,655 ± 3,170 more mites per spike than the control this may indicate that 450 mites per spike cause a yield loss of 1% (7,655/17 = 450). If a linear effect of mites on yield is assumed (which may or may not be valid) then the naturally infested control may have had a loss of 2.6% and the average loss for the three infestation dates would approach 20%.

The three dates of infestation were designed to increase the probability of avoiding environmental conditions unfavorable for mite transfer. It was also expected that different population levels would occur since the early infestation would have a longer period for mite increase. In this study varying the time of infestation did not provide the expected differences, since all three dates of infestation provided uniformly high mite populations in the spikes. Since the natural infestation was 1,166 ± 644 mites/spike, it would have been desirable to have had control plots with plants free of wheat curl mites or at least with lower populations. This possibly could have been achieved by use of buffer rows and/or application of an insecticide such as carbofuran (Harvey et al. 1979, Hammon et al. 1993), but the use of insecticides entails the risk of killing mites in adjacent plots where they are needed. Other problems with chemical treatment are the possible effect of controlling other pests and the unknown direct effect of the insecticide on yield (Thompson & Harvey 1980).

Little information is available on the extent of natural infestations of wheat curl mites in wheat spikes. It was difficult or impractical to estimate wheat curl mite populations prior to the use of the sticky-tape method in 1986, and mite

Table 1. Effect of non-viruliferous wheat curl mites (WCM) on Ike wheat at Hays, Kansas, 1998.

	Mean ± SE[b]				
Treatments[a]	No. WCM spike^{-1}	No. kernels spike^{-1}	g/1,000 kernels^{-1}	Test wt. kg/hl^{-1}	Yield kg/ha^{-1}
May 1	10,263 ± 4,809a	27.1 ± 4a	31.2 ± 1b	72.2 ± 2.3b	3,561 ± 282b
May 7	7,888 ± 2,948a	28.3 ± 4a	31.3 ± 1b	73.2 ± 2.0b	3,716 ± 484b
May 15	8,313 ± 3,685a	30.8 ± 5a	31.6 ± 2b	73.9 ± 2.0b	3,843 ± 437b
Control	1,166 ± 644b	29.8 ± 2a	35.0 ± 1a	76.7 ± 0.3a	4,481 ± 349a

[a]Date WCM released in plots. Control was infested naturally.
[b]Means within a column followed by the same letter are not significantly different ($P > 0.05$) according to Student-Newman-Keuls multiple range test.

numbers were not considered important because only one or two mites are required to vector wheat streak mosaic virus (Slykhuis 1955, Orlob 1966). Numbers of wheat curl mites per spike collected from six cultivars in 1986, 1987 and 1988 were 113 ± 99, 1,056 ± 876, and 7,610 ± 4,676 respectively (Harvey & Martin 1988, Harvey et al. 1990). The wheat cultivar 'Karl 92' sampled at Hays and Garden City, Kansas in 1997 averaged 307 ± 171 and 442 ± 279 wheat curl mites per spike, respectively (Harvey et al. 1998). During 1995 and 1996 in Nebraska numbers of wheat curl mites per spike ranged from 3 to 2,958 (Mahmood et al. 1998). From this limited data (assuming that 450 wheat curl mites per spike cause a 1% reduction in yield) it appears that losses due to natural infestations of wheat curl mites may range from less than 1% to more than 15%.

Results suggest that wheat curl mites cause yield losses in wheat in addition to that caused by the viruses they vector. Additional tests are needed to further define the damage and the threshold of mite numbers needed to cause economic damage. Monitoring of mite populations in production fields also would help determine the magnitude of losses due to wheat curl mites, thereby indicating if improved control measures are needed. More research is also needed to improve methods used to determine the frequency and magnitude of wheat curl mite infestations in the field and to develop mite resistant cultivars and other methods of control.

Acknowledgment

Partial funding was provided by the Kansas Wheat Commission. Voucher specimen No 092 of wheat curl mites are deposit in the Research Collection of Insects, Department of Entomology, Kansas State University, Manhattan, Kansas. Contribution No. 99-301-J from the Kansas Agricultural Experiment Station. Mention of a proprietary product does not constitute a recommendation or endorsement by Kansas State University.

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Greenbug (Homoptera: Aphididae) Resistance in Sorghum: Characterization of KS 97[1]

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ABSTRACT The greenbug, *Schizaphis graminum* (Rondani), has been a major insect pest of sorghum, *Sorghum bicolor* (L.) Moench since 1968. Although sources of resistance have been identified to combat this pest, new and virulent biotypes have evolved to overcome most sources of genetic resistance in sorghum. KS 97 was developed and released in 1998 by the Kansas State University Agricultural Experiment Station as a new sorghum germplasm source of resistance to biotype I greenbug. The objectives of this study were to evaluate the seedling responses of KS 97 to greenbug biotype I infestation and to characterize the mechanisms of resistance that may be involved. KS 97 seedlings were evaluated for seedling responses to biotype I greenbug in experiments comparing all known resistant sorghum genotypes. The results of these studies indicated that KS 97 was as resistant as PI 550610 and PI 550607 to biotype I greenbug and was significantly more resistant than Cargill 607E. KS 97 was compared with a greenbug- susceptible sorghum hybrid to determine mechanisms of resistance that might be involved in expression of this trait. The results of these experiments indicated that KS 97 expressed significantly higher levels of antibiosis and tolerance than did the susceptible hybrid 550E. KS 97 should provide useful and potentially unique biotype I greenbug resistance genes in sorghum.

KEY WORDS *Schizaphis graminum,* greenbug, sorghum, host-plant resistance, antibiosis, antixenosis, tolerance

Sorghum, *Sorghum bicolor* (L.) Moench, is host to several important insect pests, including the greenbug, *Schizaphis graminum* (Rondani). Greenbugs are phloem feeders and frequently cause severe crop damage and economic loss. Although different sources of greenbug resistance have been identified (Harvey & Hackerott 1969, Harvey et al. 1991, Andrews et al. 1993), new and virulent greenbug biotypes have developed to overcome most sources of genetic resistance. Biotype C greenbug emerged in sorghum in 1968 (Hackerott et al. 1969). Resistant sorghum hybrids were developed to reduce damage by this pest; however, biotype E greenbug overcame resistance to biotype C and replaced biotype C in

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the field in the 1980s (Porter et al. 1982). Biotype I greenbug, virulent to biotype E-resistant sorghum hybrids, was identified in the early 1990s and is currently the most prevalent greenbug biotype in the central Great Plains (Harvey et al. 1991, Bowling et al. 1994). Biotype K, a clone capable of damaging PI 550610, which is resistant to biotype I (Andrews et al. 1993), was recently described (Harvey et al. 1997). The incidence of biotype K in the field (G.W., unpublished data) is very low.

The usefulness of resistance as a management tool can be extended by the presence of multiple genes for resistance. The objectives of our work were to characterize KS 97, a new sorghum germplasm source of resistance to biotype I in sorghum, and to determine the mechanisms of greenbug resistance (antibiosis, antixenosis, and tolerance) expressed in KS 97.

Materials and Methods

Plant material. KS 97 was developed from a heterogenous seed source of IS 2388, a South African accession resistant to greenbug biotype E (Wilde & Tuinstra, unpublished data). In 1995, seedlings of IS 2388 were evaluated for resistance to greenbug biotype I. A few highly resistant plants survived the initial evaluation. The seed produced from resistant plants was evaluated for greenbug resistance in five subsequent generations (1996-1998) until a uniform, greenbug resistant line was obtained.

Analysis of resistance to greenbug. All greenbug screening assays were conducted using biotype I greenbugs in a growth chamber at 25°C with a photoperiod of 12:12 (L:D)h. KS 97 was first contrasted for resistance with PI 550607, PI 550610, and Cargill 607 E (resistant) sorghum and with Wheatland (susceptible) sorghum. Seed of each entry was planted (two seeds per cone) in plastic Conetainers (4 by 20.5 cm) (Stewe, Corvallis, Oregon) containing Sunshine Mix blend No. 1 (Sweaker Knipp, Topeka, Kansas). Individual cones were placed in plastic holding racks. After emergence, plants were uniformly thinned to one plant per cone. Each plant at the 2- to 3-leaf stage was infested with 50 greenbugs per plant with the technique described by Harvey et al. (1985). Differences in resistance to greenbug were quantified by a resistance rating that ranged from 1 (no damage) to 10 (plant death). The experiment was conducted with a randomized complete block design with five replications.

Mechanisms of resistance. All screening assays to evaluate mechanisms of resistance to greenbug were conducted on sorghum plants grown as described above. Mechanisms of greenbug resistance in KS 97 were compared with those of 550E, a commercial sorghum hybrid susceptible to biotype I greenbug. All experiments were conducted with sorghum seedlings at the 2- to 3-leaf stage. A randomized complete block with subsampling in each block was used.

Experiments to quantify differences in antibiosis were conducted by infesting individual plants of KS 97 and 550E with a 1-d-old biotype I greenbug nymph. Individual plants were isolated in a ventilated polyethylene cage (3.7 by 20.5 cm) with a foam top stopper. After 3 wk, the cage was removed from each plant, and the greenbugs were counted. This experiment was designed as a randomized complete block with three replications and seven subsamples per genotype in each block.

Differences in antixenosis were determined by growing one plant of each genotype (KS 97 and 550 E) in a single Conetainer. Each cone was infested with 20 greenbugs and the paired plants were isolated in a ventilated cage. The number of live greenbugs on each sorghum genotype was counted 24 and 48 h after infestation. This experiment was designed as a randomized complete block with four replications and seven subsamples per genotype in each block.

Differences in host-plant tolerance to greenbug feeding were determined with a cage to confine eight adult greenbugs to a small spot (4 by 4 mm) on the lowest leaf of each plant. After feeding for 2 d, aphids and cages were removed from the plants and a chlorophyll measuring SPAD-meter (Minolta Camera Co. Ltd, Japan) was used as described by (Deol et al. 1997) to assess injury to the spot where the aphids had been confined. This experiment was designed as a randomized complete block with three replications and four subsamples per genotype in each block.

Statistical analysis. Data for all experiments were analyzed by PROC GLM (SAS Institute 1989) and means were separated with the least-significant difference (LSD) test.

Results and Discussion

Analysis of greenbug resistance. Significant differences in resistance to biotype I greenbug were identified when damage to KS 97 was compared with that to standard greenbug-resistant and susceptible checks. Seedlings of KS 97, PI 550607, PI 550610, and Cargill 607E were significantly less damaged than Wheatland (Table 1). Among greenbug-resistant genotypes, KS 97 expressed significantly higher levels of resistance than did Cargill 607E, but was not rated significantly more resistant than PI 550607 and PI 550610.

Mechanisms of resistance. In antibiosis experiments, significantly fewer greenbugs were produced on KS 97 (97) than on the susceptible sorghum hybrid 550 E (147). These results indicated that antibiosis is an important component of resistance in this line. Bowling & Wilde (1996) also found a significant level of antibiosis in PI 550607, PI 550610, and Cargill 607E. Antibiosis is an important component of resistance because reduced greenbug fecundity and viability provides predators with a greater opportunity to suppress greenbug abundance before it reaches an economic threshold.

Analysis of greenbug feeding preference revealed no significant differences in preference between KS 97 and 550E at 24 and 48 h although differences approached significance at 48 h ($P = 0.07$). Antixenosis does not seem to be a significant component contributing to resistance in KS 97; however, antixenosis was previously reported to be an important component of resistance in PI 550607, PI 550610, and Cargill 607E (Bowling & Wilde, 1996).

Significant differences were noted in the SPAD reading (amount of chlorophyll loss) or plant tolerance to insect feeding where greenbugs had been confined on 550 E (.404) and KS 97 (.219) plants. These differences suggest that tolerance is also an important component of resistance in KS 97 to biotype I greenbug, at least in the seedling stage. Bowling & Wilde (1996) did not detect higher levels of tolerance in the seedling stage of the 3 biotype I resistant genotypes in their

Table 1. Seedling response of sorghum genotypes to greenbug biotype I.

Entry	Greenbug damage[a] rating
KS 97	2.4a
PI 550607	3.4ab
PI 550610	3.2ab
Cargill 607E	5.0b
Wheatland	8.2c

[a]Damage rating 1 to 10: 1, no damage; 10, plant death. Means followed by the same letter are not significantly different ($P < 0.05$, LSD).

study. However, Girma et al. (1999) found a high level of tolerance to greenbug biotype I in PI 550610 in a later growth stage.

In summary, KS 97 expressed high levels of resistance to greenbug biotype I and should provide a useful source of resistant germplasm. The analysis of the mechanisms of resistance indicated that antibiosis and tolerance are the most important components of resistance in KS 97. KS 97 may provide novel genes for resistance to greenbug in sorghum.

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Antifeedent Effect of Aqueous Extract of Neem (*Azadirachta indica* A. Juss) Leaves on *Oxya velox* F. (Orthoptera: Acrididae)[1]

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ABSTRACT The antifeedent effect of aqueous extracts of neem (*Azadirachta indica* A. Juss) leaves was assayed using an abundant and potentially pestiferous grasshopper (Orthoptera: Acrididae) of Indian grasslands, *Oxya velox* F. A neem extract of 0.00004% (40 mg powdered leaves in 1-l water) applied to the food plants of 4th instar nymphs decreased daily consumption of the grasshoppers by 22% (a significant reduction compared to untreated controls) and caused a significant (6 d) reduction in longevity, relative to individuals fed on untreated foliage. An extract of 0.00006% (60 mg in 1-l water) decreased daily consumption of the grasshoppers by 50% (a significant reduction compared to untreated controls and 0.00004% extract) and significantly reduced (12 d) their longevity, relative to individuals fed on untreated foliage. At the highest neem concentration of 0.0001% (100 mg in 1-l water), daily consumption was reduced by 77% (a significant reduction compared to untreated controls and lower concentrations of extract) and significantly reduced (20 d) their longevity, relative to individuals fed on untreated foliage. Thus, it appears that simple, water-based extractions of neem may provide substantial protection of treated foliage from damage by grasshoppers.

KEY WORDS grasshopper, phytophagy, consumption, pest management

Various extracts of plant materials have been used for insect control since ancient times, and nearly 2,000 species of plants are known to have allelochemicals that are biologically active against insects (Crosby 1966, Jacobson 1975). Ancient pest management practices on the Indian subcontinent include mixing dried leaves of the neem tree (*Azadirachta indica* A. Juss), now known to contain azadirachtin and other allelochemicals, with stored grains and placing the leaves in the folds of clothing and woolen blankets to repel insects (Chopra et al. 1956, Dymock et al. 1980). Neem trees are common throughout India, so their potential role in contemporary pest management is considerable. Although they are not native to the Northwest provinces, these trees are extensively planted throughout

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this region, as well in other areas ranging up to 1,500 m elevation in the Kumaon hills. The neem tree flowers in March to May, and the fruit ripens in July and August. The tree is never leafless, with old foliage persisting until the new leaves have grown to 23 to 38 cm in length.

In recent times, several laboratory and field studies have been conducted to explore the potential of neem extracts as safe substitutes for synthetic, chemical pesticides against a variety of agricultural, medical and veterinary pests. Within various contexts, neem has been reported to function as an antifeedent, repellent, toxicant, insect growth inhibitor and oviposition deterrent, and *A. indica* is possibly the world's most effective, natural insect antifeedent (Jhansi 1984, Singh 1987, Gujar 1972). Warthen (1979) listed 71 species of insects belonging to Coleoptera, Diptera, Hemiptera, Isoptera, Lepidoptera and Orthoptera which responded to neem extracts and pure compounds. In particular, azadirachtin has been isolated, identified, and found to be an effective feeding deterrent for the desert locust, *Schistocerca gregaria* (Forskal) (Butterworth & Morgan 1968, 1971; Butterworth et al. 1972).

The present study was carried out to investigate the effects of neem treated plants on the consumption rate and longevity of the grasshopper, *Oxya velox* F., which is a particularly wide ranging and abundant pest species of various cropping systems in India. In particular, we were interested in using a laboratory assay as a preliminary screening method to assess the potential of simple, aqueous extractions of neem for pest management. If laboratory trials using a highly palatable food plant suggest significant biological activity with this extraction method, then it may be possible to extend these results in developing a technique that could be conducted with minimal technology by rural agriculturalists to reduce the extent of grasshopper damage to forages and crops.

Materials and Methods

To prepare the neem extract, fresh new leaves were collected October 1998 from trees growing on the Gurukul Kangri University campus (Hardwar, India). The leaves were oven-dried at 60° C until they attained a constant weight. The dried leaves were ground into a powder using a mortar and pestle. Extracts of three different concentrations were prepared by adapting the method of Singh (1987). Initially 100 mg of powder was added to 1-l distilled water at 22 ± 2°C for 24 h and strained through a piece of cotton cloth. This preparation was termed a 0.0001% extract (0.1 g of powder added to 1,000 g of water). To prepare 0.00006 and 0.00004% extracts respectively, 60 and 40 mg of powder were dissolved in 1-l distilled water.

Fourth instar nymphs of the test grasshopper, *O. velox*, were collected from the Rajaji National Park (29°15′ to 30°15′ N and 77°55′ to 78°30′ E) in October, 1998. Their principal host in this area, the grass *Cynodon dactylon* L., was collected from the same area and used throughout the experiments as the sole food plant. Four replicates of each extract and an untreated control were used to assess the antifeedent effects in the laboratory at 29 ± 2°C, 77% RH, and 9:15 (L:D). Each replicate consisted of four grasshoppers (three males and one female) placed in a 500-ml glass container with a cotton cloth top, to which we added 50 mg of fresh *C. dactylon* of fresh food. Before the food was introduced, it was sprayed with 8 ± 1 ml of water or neem extract, and the grass was then placed in a small plastic

tube filled with water for the purpose of hydration. Every 24 h, the remaining food was removed and the treatment process was repeated. The relatively intense (daily) treatments of the foliage were chosen to ascertain whether aqueous neem extracts had detectable biological activity. This strategy represents the first phase of a screening process designed to subsequently narrow the conditions of efficacy and apply these results to the field, if there is a basis for concluding that this botanical extract has sufficient biological activity to suggest potential as a pest management tool.

The amount of food consumed each day was calculated by subtracting the amount of food unconsumed from the food provided. A wet:dry mass ratio was determined for the food provided and consumed. The experiment was continued until the grasshoppers reached the adult stage or died.

The effects of treatment on daily food consumption and time of survival were assessed using analysis of variance with Fisher's protected least significant difference test (LSD) for mean separations (Lund 1986). Differences were considered significant at $P \leq 0.05$, with an appropriate adjustment of P using Bonferoni's inequalities (Snedecor & Cochran 1980).

Results

The average amount of food consumed each day and the total number of days that grasshoppers survived differed significantly as a function of the treatments (Table 1; Fig. 1). Grasshoppers supplied with untreated food material survived for the greatest number of days, feeding continuously for 30.0 ± 1.1 d (range 27 to 32 d). The mean survival of these grasshoppers was significantly greater than that of any of the treated groups, and the shortest survival (28 d) of any individual in that treatment was greater than the maximum survival of any treated individual (26 d). The amount of food consumed each day increased with time, as would be expected with increasing body size in this species (Chapman 1998). During the first week, consumption increased by 48% and continued to increase, albeit at a slower rate thereafter. The amount of food consumed ranged from 8.2 to 27.5 mg $insect^{-1}$ day^{-1}, and the daily mean consumption was significantly greater than the values for all of the treatments, with the exception of the 0.00004% treatment on day 26.

The survival and development of grasshoppers exposed to the 0.00004% neem extract were consistently reduced, relative to the untreated controls. All of the subjects survived to the adult stage, and average longevity was 23.8 ± 0.9 d (range 21 to 25 d). The mean longevity was significantly greater than that of the two treatments at higher concentrations of neem, and the shortest lived individual at 0.00004% neem (21 d) exceeded the maximum longevity of grasshoppers in the higher neem concentrations (19 d). The amount of food consumed by grasshoppers exposed to 0.00004% neem ranged from 7.9 to 16.3 mg $insect^{-1}$ day^{-1}. The rate of consumption in the first week of treatment increased by 22% and continued to increase thereafter at a rate similar to the control. Daily mean consumption was significantly greater at all times in this treatment than in the treatments with higher rates of neem.

Treatment with 0.00006% neem extract generated a noticeable effect on all feeding parameters. Half of the subjects survived to the adult stage, and average longevity was 18.0 ± 0.7 d (range 16 to 19 d). The mean survival was significantly

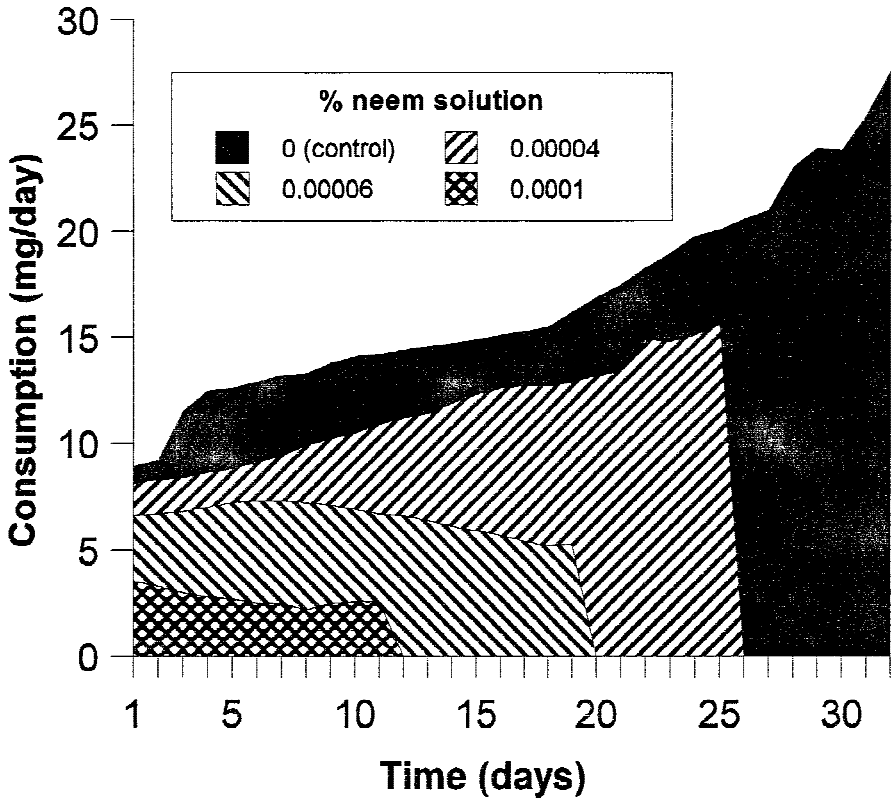

Fig. 1. Daily consumption by *O. velox* of grass treated with a range of concentrations of an aqueous extract of neem. Mean consumption rates for treatments within each day differ significantly ($P < 0.01$) according to Fisher's protected least significant difference test, except for the control and 0.00004% neem at 26 d.

greater than that of the treatment at a higher concentration of neem, and the shortest longevity at 0.00006% neem (16 d) was greater than the longest life-span at the highest neem concentration (11 d). The amount of food consumed by grasshoppers ranged from 4.8 to 7.5 mg insect^{-1} day^{-1}. In the first week of treatment, consumption increased by 11%, and there was a decreasing rate of consumption afterwards, in marked contrast to the trend seen in the untreated controls and the lower rate of neem. Daily mean consumption was significantly greater at all times in this treatment than in the treatment with highest rate of neem.

Treatment with 0.0001% neem extract severely reduced all feeding parameters. None of the subjects survived to the fifth instar, and average longevity was 9.8 ± 0.8 d (range 9 to 11 d). The mean survival was significantly less than that of all lower rates of treatment. The amount of food consumed by grasshoppers ranged from 1.1 to 4.1 mg insect^{-1} day^{-1}. In the first week of treatment, consumption decreased by 28% and continued to decrease thereafter. Daily mean

consumption was significantly less at all times in this treatment than in the all lower rates of neem.

Discussion

Jhansi (1984) compared the antifeedent property of ethanolic and aqueous extracts of de-oiled neem seed kernel against the desert locust. A 0.003% ethanolic extract completely inhibited feeding of locusts on cabbage leaves, and the aqueous extract gave the same degree of inhibition at 0.006%. In experiments on *S. gregaria,* Singh (1987) extracted neem seed kernel, seed coat and fallen leaves with water and ethanol. Ethanolic extract was re-extracted successively with hexane, chloroform, and methanol. Neem seed kernel was found to have the greatest antifeedent activity followed by seed coat and leaves, and water was determined to be nearly as effective as any organic solvent in the bioactivity of extracts. In Singh's (1987) study, the aqueous extract of neem leaves gradually reduced the rate of food consumption with increasing concentrations. Absolute deterrency was found with a 1.6% leaf extract, which is a markedly greater concentration than was reported by Jhansi (1984) for the same effect. Although none of the concentrations that we investigated completely suppressed feeding, the 0.0001% extract reduced consumption by 77%. A higher concentration may well have further reduced feeding, but greater toxic effects would probably obscure such a trend. Indeed, contrary to the work with *S. gregaria*, it appears that the toxic effects of neem on *O. velox* may be more relevant than the antifeedant properties, and these former effects warrant more intensive investigation.

In tests of North American grasshopper species, Mulkern & Mangolkiti (1977) reported that the neem extracts had no effect on feeding. In fact, there was a slight enhancement of feeding at certain concentrations. This apparent feeding stimulation was similar to the initial results that we obtained with 0.0006% neem, suggesting that short-term results may not be a reliable indicator of chronic responses to neem by grasshoppers. That is, after a 1-wk period of slightly enhanced (i.e., a daily increase) feeding, we found a dramatic reduction in feeding for the next 10 d with 0.00006% neem. Thus the concentration of neem extract and the duration of the bioassay may both be important elements in assessing the viability of this material as an antifeedent for grasshoppers.

Based on our findings, it appears that a simple, aqueous extract of minimally processed neem leaves (dried and ground) can significantly reduce grasshopper feeding and longevity under laboratory conditions. Whether less frequent foliar treatments could deter feeding or induce mortality under field conditions remains to be seen. However, extending this line of research into the next phase (small, replicated field plots with foliar treatments) is clearly justified in context of our preliminary results.

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Insecticidal Components in the Meal of *Crambe abyssinica*[1]

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ABSTRACT The defatted seed meal of crambe, *Crambe abyssinica* Hochst ex. R. E. Fries, was systematically analyzed for insecticidal activity against the house fly, *Musca domestica* L., and the active components were isolated and characterized. 2-(S)-1-Cyano-2-hydroxy-3-butene (SCHB) and phenylethyl cyanide (PEC) were identified as active components, whereas diacetone alcohol (DAA), which was identified in the extracts and tested, was not toxic to house flies. The presence of DAA and PEC in the extracts was confirmed by using gas chromatographic and mass-spectral (GC-MS) comparison with purchased reference compounds. 1,3-Benzodioxole-5-carboxaldehyde (piperonal, PIP) was also identified as a possible minor component, but was not tested. GC-MS analysis determined that the dichloromethane extract of defatted crambe seed meal contained SCHB and crambe oil at a ratio of 5:2, while DAA and PEC were present in trace amounts. Topical LD_{50} values for *M. domestica* were calculated for SCHB, PEC, DAA, crude crambe extract, crambe oil and an "artificial crambe extract" composed of SCHB and crambe oil in the proportions found in the crude crambe extract. SCHB was found to be the most toxic major component of the crambe extract. Although SCHB concentration accounted for the toxicity of the artificial extract, the natural crambe extract was significantly less toxic than would be expected based on SCHB concentration alone. 2-(R)-1-Cyano-2-hydroxy-3-butene (RCHB), an enantiomer of SCHB which does not occur in crambe, was extracted from canola, *Brassica napus* L., seed meal and tested as well. RCHB was found to be significantly less toxic to house flies than SCHB.

KEYWORDS *Crambe abyssinica, epi*-progoitrin, glucosinolate, insecticidal activity, SCHB

Many projects have examined the insecticidal activities of glucosinolates and their aglucones isolated from the Cruciferae, and these have been reviewed in the literature (Duncan 1991, Chew 1988, Fenwick et al. 1983). Seed meal and extracts of crambe, *Crambe abyssinica* Hochst ex. R. E. Fries (Cruciferae), have been shown to display insecticidal (Tsao et al. 1996), nematicidal (Donkin et al. 1995) and phytotoxic (Vaughan & Berhow 1998) properties. Tsao et al. (1996) found that crambe seed meal was toxic to house fly, *Musca domestica* L., larvae

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when the meal was incorporated into the larval media, as well as to the stored grain pests the sawtoothed grain beetle, *Oryzeaphilus surinamensis* (L.) and the red flour beetle, *Tribolium castaneum* (Herbst), when the meal was incorporated into the adult media. Organic and aqueous solvent extracts of the crambe seed meal were toxic to yellow fever mosquito, *Aedes aegypti* L., larvae and western corn rootworm *Diabrotica virgifera virgifera* (LeConte), larvae (Tsao et al. 1996). The enzymatic hydrolysis products of crambe's major glucosinolate, 2-(S)-hydroxyl-3-butenyl glucosinolate (*epi*-progoitrin), 2-(S)-1-cyano-2-hydroxy-3-butene (SCHB) and 5-vinyl oxazolidine-2-thione (goitrin), displayed modest toxicity to house flies, *M. domestica* (Peterson et al. 1998).

In this study, we aimed to isolate and identify active components in the meal of crambe. Bioassay-directed fractionation was used to isolate components from crambe seed meal extracts, and topical bioassays were used to determine fraction toxicity. The active extracts were purified to single chemical components. Our objective was to determine if SCHB adequately accounted for the toxicity displayed by the crambe meal or if other factors significantly contributed to toxicity.

We also examined 2-(R)-1-cyano-2-hydroxy-3-butene (RCHB) from canola, *Brassica napus* L., seed meal for comparison to SCHB in order to determine if configuration about the chiral center was related to toxicity. Progoitrin and *epi*-progoitrin, the parent glycosides of RCHB and SCHB, respectively, differed in their cytotoxic activity to human erythroleukemic K562 cells upon *in vitro* hydrolysis by myrosinase, the difference in activity being ascribed to the R or S configuration about the chiral center (Nastruzzi et al. 1996).

Materials and Methods

Plant material. Defatted seed meal of crambe (*C. abyssinica*) was obtained through the Center for Crops Utilization Research at Iowa State University, Ames, Iowa from National Sun, Inc., Enderlin, North Dakota. Seed meal of canola (*B. napus*) was obtained from Agro Oils, Carrington, North Dakota.

General GC Procedures. GC-MS analyses were performed by using a Hewlett-Packard 5890 gas chromatograph interfaced to a Hewlett-Packard 5972 mass selective detector (electron impact 70 eV) (Wilmington, Deleware). Aliquots of the plant extract (1μl) were injected in splitless mode onto a 30-m × 0.32-mm ID DB-1 fused silica capillary gas chromatographic column (J & W Scientific, Folsom, California). Column conditions were as follows: helium carrier gas flow of 1.5 ml/min, injector temperature 250°C, and a column temperature program of 4 min at 35°C, ramped at 15°C/min to 320°C, and 15 min at the final temperature setting. Identifications were based on a Wiley 138K mass spectrum library (Wiley 1990) as well as comparisons of mass spectra and retention times of the isolated materials with those of the authentic standards. Open-column chromatography was conducted on 2.5 × 40-cm glass columns wet-packed with J. T. Baker silica gel, 40 – 140 mesh (Phillipsburg, New Jersey). All solvents used were ACS certified or HPLC grade, purchased from Fisher Scientific (Pittsburg, Pennsylvania). A Fisher Versa-Bath S, Model 236, was used to heat the soxhlet apparatus. Samples were removed of solvent by using a Buchi R-114 rotary evaporator.

Extraction procedure. Six 100-g portions of crambe meal were extracted independently with 500 ml of dichloromethane for 24 hr in a soxhlet continuous-

extraction apparatus at 40°C. The extract was vacuum-filtered by using a glass fiber filter (Whatman G6, Hillsboro, Oregon). The six portions were combined, and concentrated under vacuum to 12 ml at 25°C. A portion (1 ml) of concentrated extract was mixed with water (1 ml). The mixture separated into three distinct layers: organic (top, designated T), aqueous (middle, designated M) and what appeared to be starchy particulates (bottom, designated B). The three layers and the crude extract were analyzed by thin-layer chromatography (TLC) and visualized with 0.1M $KMnO_4$ spray. Two solvent systems were used; hexane: ethyl acetate (1: 1) and hexane: ethyl acetate (4: 1). The Rf values of observed spots were compared to qualitatively compare extractions.

Insect bioassays. All bioassays were conducted on adult house flies (*M. domestica* L.) (Muscidae) of the Orlando regular strain, reared in our laboratory for fifteen years. The flies were initially anesthetized with carbon dioxide and kept inactive by placing them in an aluminum pan which was on an ice-water bath. One μl of test compound (pure or in a minimum amount of acetone or hexane), was applied to the thoracic venters of the house flies with a topical applicator (Model PB-600, Hamilton Co., Inc., Whittier, California). The flies were then placed in a paper cup supplied with a 2-cm section of cotton dental roll soaked with water and about 0.5 g of 1: 1 dry sucrose: powdered milk mixture. The top of the cup was covered with nylon mesh secured in place with a paper ring to allow direct observation of the flies.

Systematic isolation of active components from crambe. A schematic representation of the extraction process is given in Figure 1. Bioassay-directed fractionation of the extracts was conducted in order to determine which components displayed insecticidal activity. Two μl from each T, M and B (undiluted) layer was applied to the house flies and mortalities were recorded at 0.5 and 18 hr. Open-column silica gel chromatography was conducted on T. Fractions were collected in 25-ml portions, with a solvent system of 4: 1 hexane: ethyl ether for fractions 1 to 15, then the system was changed to 7: 3 hexane: ethyl ether (fractions 15 to 37), and then 1: 1 hexane: ethyl ether (37 to 56, at which compounds were no longer observed). The column was washed with 500 ml 100% acetone to remove any compounds remaining on the column. Five portions were collected from this column: portion TA contained fractions 1 to 11; TB, 12 to 21; TC, 22 to 40; TD, 41-55; and TE, the final acetone wash. TLC was conducted on each portion to compare contents. Each portion was tested on house flies as described above, and mortalities were recorded at 0.5 and 22 hr. Portion TA was subjected to column chromatography, with 9: 1 hexane: ethyl ether as the solvent to fraction 30, at which time the solvent was changed to 1: 1 hexane: ethyl ether. Three portions were collected, portion TAa consisted of fractions 3-7, TAb consisted of 8-13, and TAc of 14-38. Each portion was tested on house flies as described above, and mortalities were recorded at 0.5 and 20 hr. Portion B from the original solvent extraction, found by using TLC to contain TAc (data not shown), was purified with open-column chromatography to obtain a larger amount of TAc. Seven portions were collected from B: BA consisted of fractions 5-20, BB of 21-50, BC 51-65, BD 66-73, BE 74- 88, BF 89-97, and BG 98-115. Each portion, as well as TAc, was tested on house flies as described above. Hexane was added to portion BC to dissolve crystals, and to BD and BE to obtain a testable volume. The appropriate hexane control was conducted, as well as an acetone control, to compare solvent effects (no mortality was observed for the solvent controls, data not shown). Mor-

talities were recorded at 0.5 and 23 hr. Testing to the house flies was repeated on TAc, BA, BB, BE, and BG. One μl was used to determine the most active portion(s). Acetone was added to BB to dissolve crystals, and to BE to bring it to a testable volume. Mortalities were recorded at 0.5 and 24 hr. Preparatory thin-layer chromatography (prep-TLC) was run to separate the contents of BB. Hexane to ethyl ether (7: 3 by volume) was used as the solvent. Individual components (seven in all) were scraped off the plate, and washed from the silica with dichloromethane. The components were designated as BB(1), BB(2), . . . BB(7) until proper characterization could take place. The solvent was removed and 1 μl was tested on the house flies as described above. Mortalities were recorded at 0.5 and 24 hr. The three most active components, BB(5), BB(6), and BB(7), were subjected to GC-MS to establish the identity of the chemical components.

Chemicals and extracts. Authentic samples of diacetone alcohol (DAA) and phenylethyl cyanide (PEC) were purchased from Aldrich Chemical Co., Milwaukee, Wisconsin. SCHB was isolated in a previous study, reported elsewhere (Peterson et al. 1998), and RCHB was isolated from canola seed meal via the same method. Portion TAa, a non-SCHB-containing fraction obtained from the preliminary bioassay-directed fractionation, was given the convenient name of "crambe oil" due to its similarity to other plant-derived oils. Crude crambe extract, DAA, PEC, extracted crambe oil and SCHB were subjected to GC-MS at the conditions mentioned above in order to determine the ratio of SCHB: crambe oil: PEC: DAA present in the crambe extract. "Artificial crambe extract" was prepared by mixing SCHB and crambe oil in a 2.5: 1 ratio.

Determination of LD_{50}. DAA, PEC, crambe oil, SCHB, natural crambe extract, artificial crambe extract and RCHB were tested topically on house flies and LD_{50} values, expressed in μg/fly, were calculated by using probit analysis (proc probit) on SAS (SAS Institute, 1991). Determination of LD_{50} values was made based on three replications, with ten flies per replication, and six concentrations.

Results and Discussion

Identities of components in active portions. Extensive extraction and purification of extracts of crambe meal yielded 2-(S)-1-cyano-2-hydroxy-3-butene (SCHB) in some active fractions. Fractions that did not contain SCHB were active also. These contained (determined by using GC-MS) the following compounds: diacetone alcohol (DAA); phenylethyl cyanide (PEC); piperonal (PIP); hexadecanoic acid; (Z,Z)-9-12-octadecadienoic acid; (Z)-9-octadecanoic acid and 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester. Toxicities of products obtained from preparative TLC are displayed in Figure 1, and a summary of the probable components as identified by GC-MS analysis is provided in Table 1.

Diacetone alcohol (DAA; 4-hydroxy-4-methyl-2-pentanone) has been isolated from sleepy grass, *Stipa vaseyi* Scribn. (Epstein et al. 1964), licorice, *Glycyrrhiza glabra* L. (Frattini et al. 1977) and watercress, *Rorippa nasturtium-aquaticum* L. Hayek, headspace volatiles (Spence & Tucknott 1983a) and epicuticular waxes (Spence & Tucknott 1983b), as well as from cotton, *Gossypium* spp., where it exists as an intermediate in mesityl oxide production causing "catty" odors (Hron & Kuk 1989). Although it is sometimes found as a contaminant in commercial acetone (Hefetz & Lloyd 1983), no acetone was used in the current extraction

Table 1. Probable identity of components in prep-TLC bands, determined by GC-MS.

Band[a]	Retention time (min.)	Probable identity	Probability of match (%)
BB(5)	5.6	4-Hydroxy-4-methyl-2-pentanone [diacetone alcohol]	78
	11.21	Benzenepropanenitrile [phenylethyl cyanide]	81
	12.3	1,3-Benzodioxole-5-carboxaldehyde [piperonal]	96
	17.76	Hexadecanoic acid [palmitic acid]	93
	18.87	(Z,Z)-9,12-octadecadienoic acid [linoleic acid]	90
	18.97	(Z)-9-octadecanoic acid [oleic acid]	86
	21.27	1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester	80
BB(6)	5.4	4-Hydroxy-4-methyl-2-pentanone [diacetone alcohol]	56
	11.19	Benzenepropanenitrile [phenylethyl cyanide]	87
	18.96	(Z,Z)-9,12-octadecadienoic acid [linoleic acid]	95
BB(7)	5.58	4-Hydroxy-4-methyl-2-pentanone [diacetone alcohol]	74

[a]See Materials and Methods, Systematic isolation of active components from crambe, and Fig. 1 for extraction process.

procedure. In arthropods, it is present in Douglas-fir beetles, *Dendroctonus pseudotsugae* Hopkins (Madden et al. 1988), and in the alarm pheromone of *Tapinoma simrothi* phoenicea Emery, a species of ant common to Israel (Hefetz & Lloyd 1983). It mildly improves attraction of *Glossina* spp. to carbon dioxide-baited electrocution traps (Vale 1980).

Phenylethyl cyanide (PEC; hydrocinnamonitrile; benzenepropanenitrile) is the nitrile aglucone of gluconasturtiin, a glucosinolate found in garden cress, *Nasturtium officinalis* R. Br. (Fenwick et al. 1983). Tsao et al. (1996) did not report this glucosinolate as being present in crambe.

Piperonal (PIP; 1,3-benzodioxole-5-carboxaldehyde) is a methylenedioxyphenyl found in black pepper, *Piper nigrum* L. (Debrauwere et al. 1975), and as a minor component of dill, *Anethum graveolens* L. (Schreier et al. 1981), and rabbiteye blueberry, *Vaccinium ashei* Reade (Horvat et al. 1983). It has been found to inhibit larval development of *Drosophila* spp. (Ohigashi et al. 1983), reduce food consumption of, but not repel, honey bees, *Apis mellifera* L. (Atkins et al. 1975) while being moderately attractive to the males of certain euglossine bee species (Williams & Whitten 1983). Methylenedioxyphenyl compounds (piperonyl butoxide being the most commercially important) are highly biologically active, and serve as synergists for pyrethroid and carbamate insecticides (Matsumura 1985). They have been shown to inhibit rat nasal cytochrome P-450-dependent monooxygenases (Dahl 1982) and house fly tyrosinase (Metcalf et al. 1966).

Hexadecanoic acid, 9,12-octadecadienoic acid and 9-octadecanoic acid are palmitic acid, linoleic acid, and oleic acid, respectively, commonly found as components of plant lipids (Voet & Voet 1995). The compounds of concern here are lipophilic; therefore it is not surprising to isolate fatty acids as co-extractives with these active components.

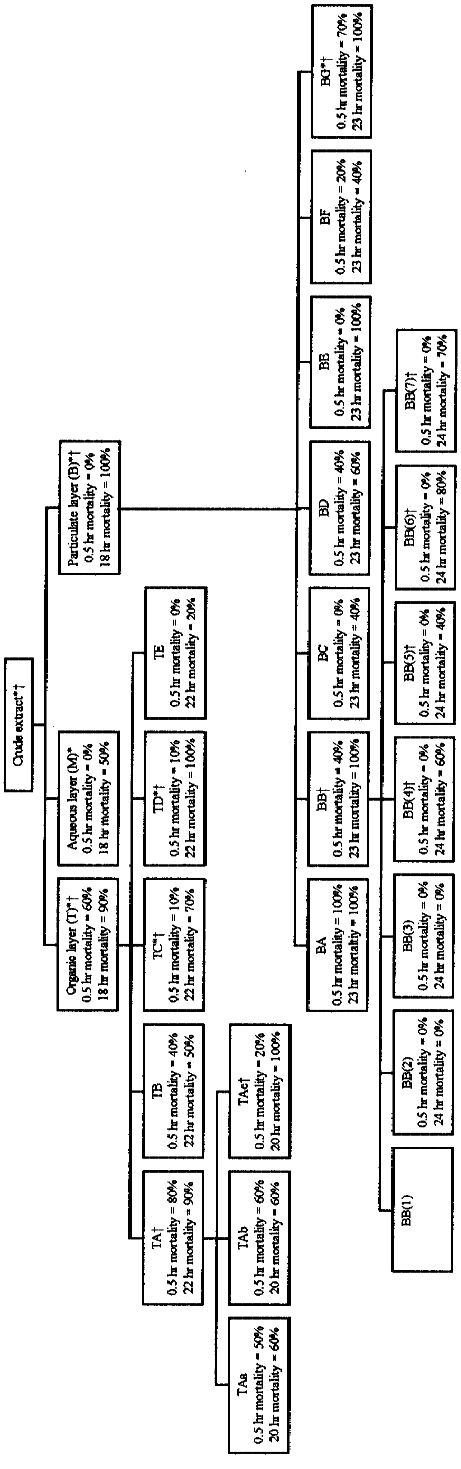

Fig. 1. Extraction scheme for isolation of components from crambe meal. *Portions containing SCHB. †Portions displaying toxicity.

1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl ester), also known as bis(2-ethylhexyl) phthalate, is often used as a plasticizer in the manufacture of certain plastics (Budavari et al. 1996). Our laboratory has detected this compound (via ^{1}H-NMR) in crambe meal in the past while isolating and confirming the identity of SCHB (unpublished). At the time, we assumed it to be a contaminant resulting from conducting dichloromethane extractions in high-density polyethylene (HDPE) bottles. We modified our extraction procedure and HDPE products were not used at any point in this study. This leaves a few possibilities: (1) this compound occurs naturally in crambe, (2) we have isolated a product which according to MS and NMR is very similar to this phthalate, (3) previous handling of the crambe meal (harvest, defatting or storage) has resulted in contamination, or (4) an unknown source of this contaminant exists in our method. The latter two possibilities are the most probable in the current situation.

Reference standards of DAA and PEC, along with isolated crambe oil and extracted SCHB, were subjected to GC-MS. DAA and PEC were confirmed as present in the crude crambe extract through comparison of GC retention times and mass spectra between the crude extract and reference samples. A ratio of 5: 2: trace: trace was determined by using GC-MS for SCHB: crambe oil: PEC: DAA in the crude extract. SCHB and crambe oil were mixed in this ratio to constitute an "artificial crambe extract" to be used in toxicity testing. PIP exists at too low of a concentration in the extract to be detected by GC-MS.

Mass spectral data. 2-(S)-1-Cyano-2-hydroxy-3-butene (SCHB): EIMS (70 eV) m/z 96 [$M^+ - 1$] (0.5), 69 (4), 57 (100), 55 (16), 51 (5), 41 (25), 40 (14), 39 (16). This is in agreement with published spectra (Vaughan & Berhow 1998). Diacetone alcohol (DAA): EIMS (70 eV) m/z 116 [M^+] (0.01), 101 (11), 59 (36), 43 (100). Piperonal (PIP): EIMS (70 eV) m/z 150 [M^+] (80), 149 [$M^+ - 1$] (100), 122 (4), 121 (38), 91 (19), 65 (54), 63 (85), 38 (23). Phenyl ethyl cyanide (PEC): EIMS (70 eV) m/z 131 [M^+] (14), 91 (100) 65 (21). 1,2-Benzenedicarboxylic acid, bis(2-hexylethyl) ester: EIMS (70 eV) m/z 279 (6), 167 (25), 148 (100), 103 (16), 57 (60), 41 (54).

Topical toxicity tests. Topical bioassays were conducted with PEC, DAA, SCHB, crambe oil, natural crambe extract and artificial crambe extract against *M. domestica* and the following LD_{50} (95% fiducial limit) values were calculated (μg/fly): 268 (215, 325) for PEC, 17.4 (11.7, 23.0) for SCHB, 304 (247, 362) for natural crambe extract, and 25.8 (20.8, 31.0) for the artificial extract. Determination of LD_{50} values for DAA and crambe oil was not possible since the highest dose tested (1000 μg/fly) resulted in 20% mortality for DAA and no mortaility was observed for crambe oil at this dose.

PEC, PIP and DAA occur in small amounts in the crambe extract and we do not believe they significantly contribute to the toxicity of the natural crambe extract. It was only through repeated concentration of large amounts of extract that we were able to isolate these components.

Testing of the artificial crambe extract resulted in the LD_{50} values we expected based on the toxicities of the components, SCHB and crambe oil. The artificial extract contained about 72% SCHB, and had an LD_{50} value of 25.8 μg/fly. This corresponded to 18.6 μg/fly pure SCHB, which was within the 95% fiducial limit of pure SCHB's LD_{50} value of 17.4 μg/fly. SCHB alone explained the toxicity of the artificial extract. The dichloromethane crambe extract contained between 40 and 50% SCHB, and its LD_{50} value of 304 μg/fly corresponded to an SCHB dose of 122

μg/fly, much higher than the LD_{50} value observed for pure SCHB. Even if we assumed only 40% SCHB in the natural extract, and use the lower 95% FL, that would give an LD_{50} of 99 μg/fly, which is much less toxic than SCHB in pure form.

SCHB and RCHB were tested in a head-to-head comparison of toxicity. An LD_{50} value of 27.7 μg/fly (24.8, 30.8) was found for SCHB and 143.2 μg/fly (120.9, 164.1) for RCHB. These two compounds are structurally identical except for configuration about the chiral center, but have significantly different toxicities. RCHB does not occur in crambe, and was tested here due to the extensive literature related to its presence in canola (Brown & Morra 1995, and references therein).

SCHB was the most toxic compound found in large proportion in natural crambe extract. When mixed with the non-toxic crambe oil, the toxicity to flies decreased as expected based on dilution effects. The natural crambe extract, however, was much less toxic than was predicted based on its SCHB content. It is possible that other compounds in the extracts had the effect of lowering the toxicity of SCHB to house flies, perhaps by interfering with SCHB penetration or by inducing detoxifying enzymes within the insect's body. It seems that the insecticidal efficacy of crambe extracts may be improved by purification to maximize SCHB content. For reasons as yet unclear, SCHB is significantly more toxic to house flies than its enantiomeric counterpart RCHB.

The use of glucosinolate aglucones from crucifer seed meal against insects and other pests may be economically feasible for at least two reasons. First, removal of the glucosinolates and their aglucones from the seed meal may enhance the value of the meal as a potential livestock feed, and secondly, any bioactive (e.g. insecticidal) extract or compound obtained may be of value itself.

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NOTE

Helicoverpa armigera Larval Growth Inhibition in Artificial Diet Containing Freeze-dried Pigeonpea Pod Powder[1]

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Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) is a key insect pest of pigeonpea, *Cajanus cajan* (L.) Millspaugh, in the semi-arid tropics and one of the primary production constraints (Lateef & Pimbert 1991; Shanower et al. 1999). *Cajanus scarabaeoides* (L.) Thouars, a wild relative of pigeonpea, is reported to be resistant to *H. armigera* (Lateef et al. 1981; Saxena et al. 1990; Shanower et al. 1997). Larvae feeding on flowers and green pods of *C. scarabaeoides* grow slower, take longer to pupate, and form smaller pupae than those that feed on *C. cajan* (Lateef et al. 1981; Shanower et al. 1997). A high density of pod surface trichomes, relatively tough pod wall, and differences in the structure of pod tissue may contribute to the poorer growth of *H. armigera* and the lower level of pod damage in *C. scarabaeoides* compared with *C. cajan* (Lateef et al. 1981; Romeis et al. 1999). In addition to these physical factors, chemicals in or on the pods may also contribute to *C. scarabaeoides* resistance to *H. armigera*. Compounds that elicit feeding responses from *H. armigera* larvae have been extracted from *Cajanus* spp. pod surfaces. An acetone-soluble feeding stimulant was found on *C. cajan* pods and a water-soluble feeding deterrent was observed on *C. scarabaeoides* pods (Shanower et al. 1997). These feeding responses were detected using a 24 h filter paper feeding test (Blaney et al., 1987). The purpose of this study was to evaluate the impact of these compounds, in the absence of physical features, using longer-duration feeding tests with semi-artificial diets containing freeze-dried powder of *Cajanus* spp. pods or their extracts.

Tender, green pods with small developing seeds of *C. cajan* and *C. scarabaeoides* were harvested from pesticide-free field plots on the research farm of International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), located near Hyderabad, India. The pods were frozen at -20ºC and lyophilized. The dried pods were milled in a blender. *Helicoverpa armigera* larvae used in this study were obtained from a laboratory culture established from and regularly supplemented with field-collected eggs. Larvae were reared on a chickpea flour-based diet (Armes et al. 1992). Seven-day-old larvae were transferred to artificial

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diets consisting of 53 g milled pod powder, 20 g wheat germ, 18 g yeast, 1.8 g L-ascorbic acid, 1.1 g methyl-4-hydroxybenzoate, 0.57 g sorbic acid, 0.83 g Aureomycin (Cyanamid India, Valasad, Gujarat, India), 5.3 g agar, 1.1 ml 40% formaldehyde, and 380 ml water. Control diet was prepared with 53 g chickpea flour in place of the pod powder. Forty-eight 7-d-old larvae were used in each treatment (diet), and individual larvae were held separately and considered replicates. After feeding for 6 d at 27°C under a photoperiod of 12:12 [L:D] h, larval weights were recorded. Larvae reared on *C. cajan* and *C. scarabaeoides* freeze-dried pod powder diets from 7-d-old to 13-d-old were significantly smaller than those reared on the control diet (Table 1). Most of the larvae on the pod powder diets did not pupate.

One hundred grams of freeze-dried pod powder was sequentially extracted with n-hexane, ethyl acetate, acetone, 70% aqueous methanol, and distilled water, according to increasing polarity of the solvent by soaking the powder in the solvent at room temperature for 2 h. Each extract, except water extract, was mixed with chickpea flour after evaporating the solvent under reduced pressure. They were then added to hot (50–60°C) agar aqueous solution with other ingredients to make the artificial diet. The water extract was made up to 380 ml with distilled water and substituted for the water in the artificial diet. Forty-eight neonates were fed on each treatment (diet) in separate containers, and individual larvae were used as replicates. After feeding for 8 d at 27°C under a photoperiod of 12:12 [L:D] h, larval weights were recorded. Strong growth inhibition was observed on the acetone and 70% methanol extracts of *C. scarabaeoides* pod powder and on all but n-hexane extract of *C. cajan* pod powder (Table 2). Larval survival was low on the ethyl acetate extract of *C. cajan* and acetone extract of the both species.

It was anticipated that *H. armigera* would grow more slowly on an artificial diet containing *C. scarabaeoides* pod powder than *C. cajan* pod powder due to antifeedant or growth inhibiting compounds, and/or poorer nutritional quality of the wild species. But if the lower growth rate observed on whole pods of this wild species (Shanower et al. 1997) was due primarily to physical barriers such as hard pod walls and dense trichomes, then it was expected that larvae on both pod diets would grow similarly well since trichomes and tough pod walls would be destroyed by milling. Contrary to expectations, larval growth was much lower on both diets as compared to control diets which did not contain freeze-dried pod powder. These results indicate the presence of growth inhibitors in the pod powder diets of both *C. scarabaeoides* and *C. cajan*.

These results do not necessarily conflict with earlier findings (Shanower et al. 1997). The acetone-soluble feeding stimulant and water-soluble feeding deterrent described in the earlier study were extracted from the pod surface by soaking intact pods in the solvents. In the present study, whole pods were freeze-dried, milled, and then extracted. Many more compounds were extracted by the latter method. Different bioassay methods were also used in the two studies. The earlier study compared behavioral feeding preferences in a "choice" situation using filter paper. Square pieces of filter paper were made palatable with a sucrose solution and treated with concentrated extract solutions from the pod surfaces. The amount of feeding that occurred over a 24 h period on treated paper was compared to untreated control paper containing sucrose solution only. In contrast, the present study compared growth rates using artificial diets prepared with freeze-dried pod powder, first from whole pods and then with various solvent extracts of

Table 1. Weight (mean ± SE) of *Helicoverpa armigera* larvae after feeding six days on artificial diets prepared from freeze-dried pods of two *Cajanus* spp.

Diet	Larval wt (mg)	n[a]
C. cajan	53.4 ± 4.0 b[b]	45
C. scarabaeoides	58.5 ± 4.0 b	48
Control	168.3 ± 8.8 a	47

[a]n = number of larvae surviving at weighing.
[b]Means within a column followed by the same letter are not significantly different at $p < 0.01$ (Student's multiple comparison test).

the powder. Pod powder was extracted sequentially with solvents of increasing polarity from n-hexane (most non-polar) to water (most polar). Growth inhibition was observed in ethyl acetate, acetone, 70% methanol and water extracts of *C. cajan* pod powder. For *C. scarabaeoides*, growth inhibition was observed in acetone, 70% methanol and water extracts. Compounds in ethyl acetate and acetone extracts are less polar than those in water extracts, indicating that growth inhibitors of different polarity are present in the pod powder diets of both *C. scarabaeoides* and pigeonpea.

These data suggest that both *C. cajan* and *C. scarabaeoides* pod powder diets contained larval growth inhibitors. Considering that *H. armigera* larvae grow well on fresh, intact *C. cajan* pods (e.g., Bilapate, 1987; Shanower et al., 1997), the growth inhibitors may have been synthesized by reactions of enzymes that meet the substrate only after the destruction of the tissue by freezing, drying and milling. Another possibility is that the growth inhibitors were synthesized by reaction of pod compounds with components of the artificial diet such as yeast extract or vitamins.

It is difficult to compare the results of the two experiments. Control larvae were smaller, though older, in the first experiment (Table 1) than control larvae in the second experiment (Table 2). The reason for this is not known. The eggs used in each experiment came from different cohorts approximately six months apart and may have differed genetically. Another possibility was infection by microsporidian. Sub-lethal infection by *Nosema* spp. can produce extended development times and reduced larval weights in *Helicoverpa* spp. without obvious external symptoms (Armes et al., 1992).

Additional studies are needed to elucidate the compounds inhibiting larval growth and the reason(s) why these compounds are apparently not present or available in fresh pigeonpea pods. The identification of effective larval antifeedants in pigeonpea or its wild relatives would be an important advancement in the effort to develop pigeonpea cultivars less susceptible to *H. armigera*. Given the worldwide importance of this pest and the rising use/misuse of pesticides to control it, especially in the less developed countries where pigeonpea is most widely grown (Shanower et al. 1999), a collaborative project between ICRISAT, the Natural Resources Institute (Greenwich University), and the Royal Botanic Garden (Kew, UK) was recently initiated to isolate and identify larval feeding stimulants and inhibitors in *Cajanus* spp.

Table 2. Mean (± SE) weight (mg) of *Helicoverpa armigera* larvae after feeding 8 days on artificial diets prepared with different extracts of freeze-dried pod powder.

	Extracting solvent				
Diet	n-Hexane	Ethyl Acetate	Acetone	70% Methanol	Water
Control	—	262.1 ± 14.2 a (46)[b]	277.1 ± 14.8 a (47)	292.8 ± 14.3 a (48)	277.1 ± 14.8 a (47)
Solvent alone	378.2 ± 12.4 a[a] (46)	255.9 ± 14.9 a (48)	318.1 ± 16.1 a (48)	216.3 ± 8.8 b (48)	—
C. cajan	380.9 ± 10.8 a (48)	2.0 ± 0.6 b (11)	3.5 ± 0.4 b (20)	43.0 ± 3.4 c (46)	176.0 ± 10.8 b (47)
C. scarabaeoides	364.1 ± 11.8 a (46)	215.8 ± 11.9 a (48)	4.5 ± 1.7 b (11)	82.0 ± 5.7 d (48)	224.7 ± 14.9 b (48)

[a]Means within a column followed by the same letter are not significantly different at $p < 0.01$ (Student's multiple comparison test).
[b]Number in parentheses equals number of larvae surviving to Day 8 from initial n = 48 larvae.

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Impact of Red Imported Fire Ant Infestation on Northern Bobwhite Quail Abundance Trends in Southeastern United States[1]

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ABSTRACT Northern bobwhite quail (*Colinus virginianus* L.) populations are declining throughout their range. One factor contributing to the decline in the southeastern United States may be the red imported fire ant (*Solenopsis invicta* Buren). Recent research in Texas has documented that red imported fire ants can have a significant impact on northern bobwhite quail. That research was conducted in areas where fire ants are predominately polygynous (multiple queen). Polygynous infestations have much higher mound densities than the monogynous (single queen) form. In most of the southeastern United States, fire ants are predominately monogynous. We determined if there was a relationship between the invasion of monogynous red imported fire ants and abundance trends in northern bobwhite quail in the southeastern United States. For Florida, Georgia, and South Carolina we compared average northern bobwhite quail abundance based on Christmas Bird Count data for each county before and after fire ant invasion, and conducted regression analyses on bobwhite quail abundance and year preinvasion, and abundance and year postinvasion. Regionally, northern bobwhite quail were more abundant before (0.067 ± 0.018 bobwhite quail per observer hour) than after fire ants invaded (0.019 ± 0.006; $Z = -3.746$, df = 18, $P < 0.001$). There was no significant regional population trend for northern bobwhite quail ($r^2 = 0.24$; df = 9, $P = 0.13$) before fire ant invasion. Post-invasion, northern bobwhite quail populations significantly declined regionally ($r^2 = 0.76$, df = 15, $P < 0.001$), and in Florida ($r^2 = 0.71$, df = 15, $P < 0.01$) and South Carolina ($r^2 = 0.50$, df = 9, $P = 0.01$). The number of years that a county had been infested by fire ants explained 75% of the yearly variation in northern bobwhite quail abundance after invasion, despite >30-yr variation in invasion dates.

KEY WORDS *Colinus virginianus,* Formicidae, Galliformes, Hymenoptera, northern bobwhite, Phasianidae, red imported fire ant, *Solenopsis invicta*, wildlife

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The northern bobwhite quail (*Colinus virginianus* L.) is declining throughout its range (Brennan 1991). The reasons for the decline remain unresolved and controversial. Numerous hypotheses, some competing and some complementary, have been forwarded for the observed decline. These hypotheses include changes in farming practices, larger average farm sizes, maximum basal area silviculture practices, increased use of chemicals to control insect and plant pests, changes in fire regimes, increased predator populations, and general habitat degradation (Klimstra 1982, Brennan 1991). Changing land-use patterns may be a major factor for the decline of bobwhite quail throughout its geographical range (Brennan 1991) and may interact with other factors that negatively impact northern bobwhite populations. For example, changing land use patterns, such as those listed above, not only directly decrease available habitat and reduce habitat quality but also may decrease the quality of remaining habitat by increasing predator populations (Allen et al. 1995).

In the southeastern United States, quality bobwhite quail habitat and effective habitat management have historically existed (Brennan 1991) and still occur over extensive areas, even though large-scale and extensive landscape transformations also have occurred. However, even where land-use patterns and northern bobwhite quail habitat management have remained little changed for decades (i.e., many quail plantations), northern bobwhite quail populations are declining.

Another factor possibly contributing to the rapid decline of northern bobwhite quail in the southeastern United States is the introduction and spread of the red imported fire ant (*Solenopsis invicta* Buren) (Allen et al. 1995). The red imported fire ant was introduced into the United States through the port of Mobile, Alabama, in the early 1930s (Vinson & Sorensen 1986, Callcott & Collins 1996). It has spread throughout the southeastern United States causing a wide array of problems to humans, livestock, and native biota (Vinson & Sorensen 1986, Callcott & Collins 1996, Allen et al. 1998, Wojcik et al. 1999). Its negative impacts on numerous invertebrates (Porter & Savignano 1990) and vertebrates (Allen et al. 1994, 1998) have been well documented.

Brennan (1991, 1993) suggested that fire ants have little effect on bobwhite quail populations and were unlikely to cause their decline, citing the lack of data at that time (Brennan 1993). However, more recent large-scale experimental manipulations (Allen et al. 1995) have documented population-level impacts on bobwhite quail by polygynous fire ants and a negative correlation between the number of years that a county had been infested with red imported fire ants and trends in northern bobwhite quail abundance in Texas. Several studies have since documented negative impacts by fire ants on northern bobwhite quail. Impacts may include ant predation on chicks, the effect of nonlethal envenomization (reduced weight gain and reduced survival), irritation affecting feeding and resting behavior, and competition for insect food resources (Allen et al. 1993, 1995; Giuliano et al. 1996, Pederson et al. 1996).

The effects of red imported fire ant infestations on bobwhite quail have been studied recently only in Texas, where the ant is mainly polygynous (i.e., has multiple queens). Mound densities and overall populations are much greater in the polygynous form than in the monogynous (single queen) form (Porter et al. 1991). Red imported fire ants in the southeast are predominately monogynous (Vinson & Sorensen 1986). Therefore, we wished to determine if monogynous red imported fire ant infestations are related to bobwhite quail population trends in

the southeastern United States, as was documented for polygynous fire ants and bobwhite quail in Texas (Allen et al. 1995). Our objective was to determine if there was a relationship between time since invasion with monogynous red imported fire ants and abundance trends in northern bobwhite quail populations in the southeastern United States.

Materials and Methods

To determine trends in northern bobwhite quail abundance, we used the long-term data sets available from Christmas bird counts (published in the journal *American Birds*). Northern bobwhite abundance data were collected for Florida, Georgia, and South Carolina. Christmas bird counts are standardized 1-day counts of all birds detected in a 24-km diameter circle around the center of a count site (Bock and Root 1981). Data were standardized by converting to an index of abundance that accounted for observer effort and the number of observers, by dividing total bobwhite quail observed during Christmas bird counts by observer hours (number of observers × party hours). Although Christmas bird counts lack precision in estimating abundance, especially of rare species, they lend themselves well to large-scale analyses and the detection of population trends (Bock & Root 1981). We used data only for count sites that had at least 5 yr of pre- and post-fire ant invasion and bobwhite quail data available (Allen et al. 1995). For Florida, because of the large number of Christmas bird count sites, we collected data for count sites in the first half of the alphabet, before exclusion of sites with insufficient data. This balanced the number of replicate count sites in each state and prevented the southeastern analysis from being unduly weighted by trends in Florida. The year of initial fire ant invasion for each county (year 1 postinvasion) was considered to be the year the counties were quarantined by the U.S. Department of Agriculture (Callcott & Collins 1996). These dates varied widely by count site.

In our first analysis, we simply determined average northern bobwhite quail abundance for each count site before and after fire ant infestation, and compared those averages with paired *t* tests (t) or, if the data failed to meet assumptions of normality and equal variance, with nonparametric Wilcoxon Signed Ranks Tests (Steel and Torrie 1980). We performed these tests for the regional data and also for the individual states.

We conducted regression analyses on bobwhite quail abundance and year pre-invasion, and abundance and year postinvasion regionally and for each state (Allen et al. 1995). We averaged bobwhite quail abundance across count sites for each year prior to or after invasion (such that year 1 postinvasion for each site was the year that that site was quarantined, regardless of calendar year). A minimum of five count sites was used to determine average abundance for each state and for the regional analysis (Allen et al. 1995).

Results

Florida and Georgia each provided six usable Christmas bird count sites, and South Carolina seven. Thus, regional analyses were based on 19 sites (Fig. 1). For our regression analyses Georgia, Florida, and South Carolina provided 11, 10, and 7 yr of data prior to fire ant invasion and 8, 17, and 11 yr of usable data postin-

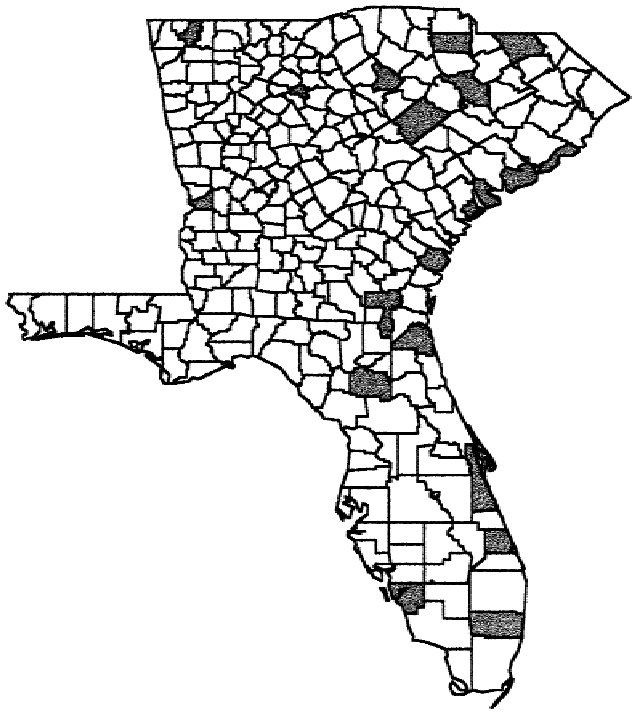

Fig. 1. Counties in Florida, Georgia, and South Carolina providing adequate Christmas Bird Count data over a sufficient time span for analysis of the relationship between northern bobwhite quail abundance trends and county-level infestation by the red imported fire ant.

vasion, respectively. Pre- and postinvasion *t* test or Wilcoxon comparisons used as much as 38 yr of data because average abundance across years within a site was not constrained by the rule requiring data for at least five sites, as were the regression analyses (Table 1). Earliest and latest invasion dates for the sites that met our criteria for use were 1961 and 1988 for Georgia, 1958 and 1976 for Florida, and 1958 and 1991 for South Carolina. Because of this broad range of invasion dates, and the comparison of northern bobwhite quail population trends pre- and postinvasion, population trends due only to landscape change were likely to have been averaged out and have minimal impacts on our results.

Regionally, northern bobwhite quail were more abundant prior (0.067 ± 0.018) to fire ant invasion than after invasion (0.019 ± 0.006, $Z = -3.746$, df = 18, $P < 0.001$). In Florida and South Carolina, bobwhite were more abundant prior to invasion (0.038 ± 0.007 and 0.070 ± 0.035 quail per observer hour, respectively) than post-invasion (0.006 ± 0.001, $Z = -2.226$, df = 5, $P = 0.026$ and 0.024 ± 0.009, $Z = -2.117$, df = 6, $P = 0.034$, respectively). In Georgia, however, there was no difference between bobwhite quail abundance prior to invasion (0.091 ± 0.042) and after invasion (0.025 ± 0.015; $t = 1.49$, df = 5, $P = 0.15$).

Regionally, there was no significant population trend for northern bobwhite

Table 1. Average northern bobwhite abundance (± SEM) estimated using Christmas bird counts in 19 counties in the southeastern United States before and after red imported fire ant infestation. Sample size (years of data) indicated in parentheses.

County	Year of infestation	Average abundance[a] Before infestation	Average abundance[a] After infestation
Aiken, SC	1972	0.267 ± 0.092 (16)	0.033 ± 0.010 (26)
Beaufort, SC	1970	0.013 ± 0.004 (11)	0.009 ± 0.002 (26)
Charleston, SC	1958	0.007 ± 0.003 (11)	0.008 ± 0.002 (38)
Chester, SC	1987	0.075 ± 0.037 (7)	0.072 ± 0.051 (10)
Chesterfield, SC	1987	0.017 ± 0.011 (7)	0.016 ± 0.008 (11)
Greenwood, SC	1991	0.013 ± 0.007 (6)	0.005 ± 0.002 (7)
Richland, SC	1970	0.095 ± 0.027 (21)	0.023 ± 0.006 (28)
Charlton, GA	1971	0.047 ± 0.009 (11)	0.010 ± 0.003 (20)
Murray, GA	1993	0.031 ± 0.018 (12)	0.000 ± 0.000 (5)
Chattahoochee, GA	1961	0.152 ± 0.055 (9)	0.097 ± 0.017 (23)
Clarke, GA	1977	0.275 ± 0.113 (11)	0.015 ± 0.004 (21)
McIntosh, GA	1971	0.015 ± 0.012 (12)	0.000 ± 0.000 (23)
Whitfield, GA	1988	0.028 ± 0.012 (12)	0.027 ± 0.012 (10)
Alachua, FL	1969	0.054 ± 0.013 (11)	0.010 ± 0.002 (23)
Brevard, FL	1969	0.033 ± 0.016 (18)	0.004 ± 0.001 (23)
Broward, FL	1976	0.019 ± 0.004 (18)	0.002 ± 0.001 (16)
Duval, FL	1958	0.016 ± 0.007 (10)	0.005 ± 0.001 (34)
Lee, FL	1973	0.052 ± 0.023 (17)	0.008 ± 0.002 (19)
St. Lucie, FL	1976	0.053 ± 0.016 (18)	0.009 ± 0.002 (14)
Average abundance		0.067 ± 0.018	0.019 ± 0.006

[a]Average abundance in birds × observer hour.$^{-1}$

prior to fire ant invasion ($r^2 = 0.24$, df = 9, $P = 0.13$; Fig. 2). This was also true for analyses at the state level (Florida $r^2 = 0.16$, df = 8, $P = 0.25$; Georgia $r^2 = 0.18$, df = 9, $P = 0.20$; South Carolina $r^2 = 0.07$, df = 5, $P = 0.57$). Postinvasion, northern bobwhite populations significantly declined in Florida ($r^2 = 0.71$, df = 15, $P < 0.01$) and South Carolina ($r^2 = 0.50$, df = 9, $P = 0.01$), but there was no significant trend in Georgia ($r^2 = 0.23$, df = 8, $P = 0.22$). Across the three states, northern bobwhite population trends declined ($r^2 = 0.76$, df = 15, $P < 0.001$; Fig. 3) following invasion by red imported fire ants, and the number of years that a county had been infested by fire ants explained 75% of the yearly variation in northern bobwhite abundance postinvasion.

Discussion

Northern bobwhite quail abundance was nearly four times greater across a broad band of the southeastern United States before fire ants invaded. Bobwhite quail populations exhibited no significant trend in abundance prior to fire ant

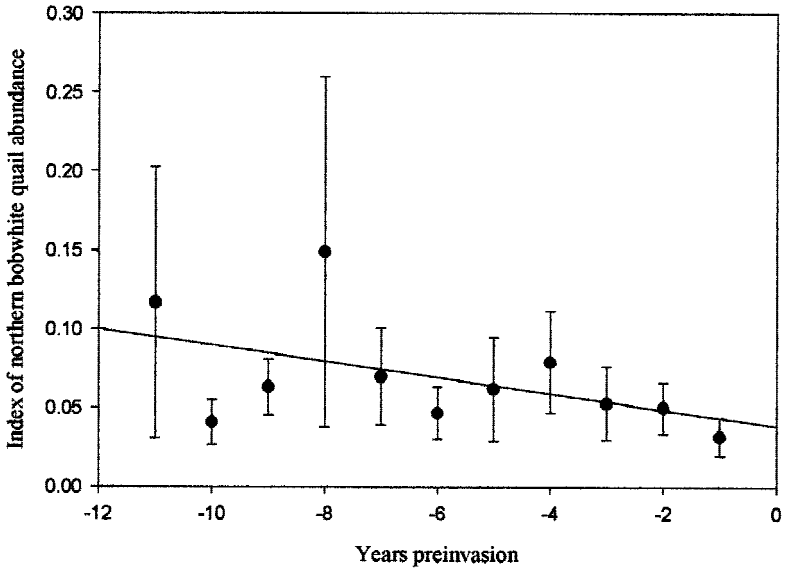

Fig. 2. Regional (Florida, Georgia, and South Carolina) northern bobwhite quail abundance (bobwhite × observer hours^{-1}) trend across 19 count sites before red imported fire ant infestation. Error bars represent ±1 SEM.

invasion, but declined significantly after invasion. Across the region, the number of years fire ants have been present in a county explained 75% of the year-to-year variance in northern bobwhite abundance. If landscape changes alone were driving the decline in northern bobwhite abundance, we would expect declines both before and after invasion by fire ants because fire ant infestation dates at the county level varied by > 30 yr across the region. Also, the counties providing data varied widely in both their present level of development and the level of development at the time of fire ant invasion.

Negative, population-level impacts by fire ants on northern bobwhite quail have been documented in areas of polygyny (Allen et al. 1995). Polygynous fire ants are characterized by extremely high mound densities that result from a failure of the nest-mate recognition system. Polygyny is rare in South America, where *S. invicta* is native, and uncommon in the United States except in Texas, where >50% of infestations are polygynous (Porter et al. 1991). In Florida, the rate of polygyny is about 15% (Porter 1992), and in other southeastern states polygyny is rare. The relationship between time of invasion at the county-level and decline in bobwhite quail also has been documented for Texas (Allen et al. 1995). The relationship between decline and years of infestation is as strong in the eastern United States, with predominately monogynous fire ants, as it was in Texas with polygyne ants (Allen et al. 1995), even though monogynous fire ant denstities are normally about 10% those of the polygynous form.

Early investigations of fire ant impacts on northern bobwhite quail (Stoddard 1931, Johnson 1961, Simpson 1972) emphasized direct predation of hatching chicks during the pipping process. More recent investigations have recognized

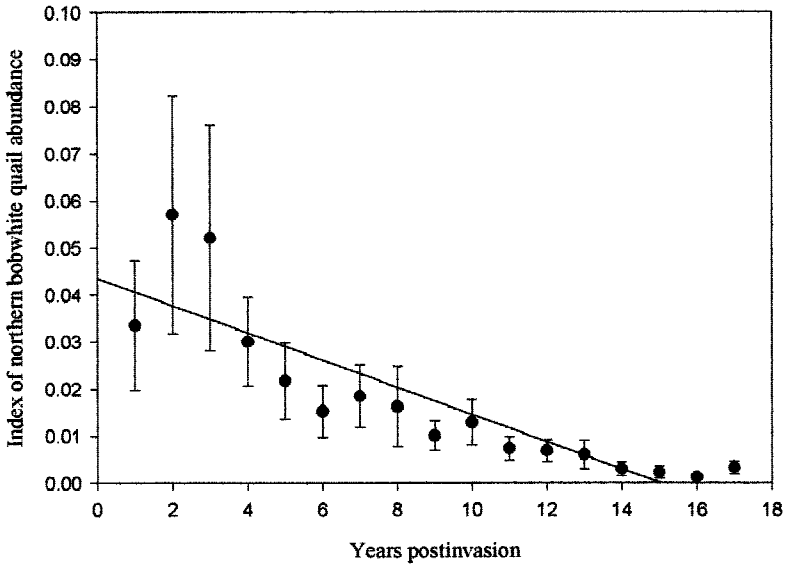

Fig. 3. Regional (Florida, Georgia, and South Carolina) northern bobwhite quail abundance (bobwhite × observer hours^{-1}) trend across 19 count sites after red imported fire ant infestation. Error bars represent ±1 SEM.

that direct predation may play only a minor role in impacts on vertebrates. Reduced weight gain and survival (Giuliano et al. 1996), altered activity patterns (Pederson et al. 1996), altered foraging patterns (Holtcamp et al. 1997), and a reduction in available insects (Porter & Savignano 1990) for reproductive hens and young chicks may have indirect effects on bobwhite quail populations.

Our analysis, further documenting the negative effects of the red imported fire ant on northern bobwhite quail, suggests that monogynous fire ants have an impact similar to that of the polygynous form. We note, however, that although most of the southeastern United States remains free of polygynous fire ants, the southeast has been infested longer than Texas. We do not suggest that fire ants are the sole driving force of the northern bobwhite's decline. Rather, we suggest that this analysis and past manipulations and experiments provide further evidence that fire ants significantly adversely affect northern bobwhite quail. Furthermore, we suggest that landscape-level changes that decrease the quality of northern bobwhite quail habitat also promote invasions (Allen et al. 1999), including that by nonnative fire ants into uninfested areas and population increases in habitats already invaded. The invasion of the southeastern United States by fire ants will continue into the next millennium as their population densities continue to increase (Wojcik 1994) and as new areas are colonized.

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Potential of Reduced-Waxbloom Oilseed *Brassica* for Insect Pest Resistance[1]

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ABSTRACT Insect pest and predator populations were field-sampled on three lines of *Brassica napus* L. and *B. rapa* L. with reduced crystalline surface waxes (waxblooms) and two *B. napus* with normal waxblooms. Seasonal per plant densities of the cabbage aphid, *Brevicoryne brassicae* L., were higher on normal waxbloom lines (range 6.0–8.2) than on reduced waxbloom lines (range 2.6–4.3) ($P < 0.002$). Two of the lines (closely related *B. napus* lines differing in waxbloom) were tested in a second year, and per plant densities of aphid colonies again were higher on the normal waxbloom line (15.2) than on the reduced waxbloom line (3.8) ($P < 0.001$). Flea beetle (*Phyllotreta cruciferae*) densities tended to be higher on reduced waxbloom lines, significantly so in the first season ($P < 0.002$). Densities of coccinellid adults and larvae were higher on the reduced waxbloom *B. napus* line only in the second year ($P < 0.005$). In the laboratory, attachment by adults of the dominant coccinellid, *Hippodamia convergens* Guérin-Méneville, was greater to leaf surfaces of the reduced waxbloom plants ($P < 0.0001$). Detrimental effects of genetically reduced waxbloom on plant growth and susceptibility to flea beetles would probably offset any advantages due to lower aphid populations on these plants. More moderate reductions in waxbloom produced by applications of the herbicide, S-ethyl dipropylthiocarbamate were not associated with any differences in insect populations in the field over two years.

KEY WORDS Plant surface waxes, host plant resistance, *Brevicoryne brassicae, Phyllotreta cruciferae, Hippodamia convergens, Brassica napus,* S-ethyl dipropylthiocarbamate

Worldwide demand for edible oils has encouraged expansion of oilseed *Brassica* production from cool temperate zones, where they are established and successful crops, to warmer climates. Greater insect damage threatens the viability of oilseed *Brassica* production in these warmer climates, including potential production areas in the Pacific Northwestern USA (Homan & McCaffrey 1993, Buntin & Raymer 1994, Buntin et al. 1995). Important pests of oilseed *Brassica* in cool and warm temperate climates include the cabbage aphid, *Brevicoryne brassicae* L., the turnip aphid, *Lipaphis erysimi* (Kaltenbach), the diamondback moth, *Plutella xylostella (L.),* the cabbage seedpod weevil, *Ceutorhynchus assimilis* (Paykull), and flea beetles, *Phyllotreta* spp. (Lamb 1989). Management of these

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insects can be complicated and expensive and may be constrained further in the USA by anticipated restrictions on pesticide use mandated by the Food Quality Protection Act of 1996.

Given these problems and regulatory constraints, alternatives to insecticides, including host plant resistance, for pest management in oilseed *Brassica* are necessary. Potential sources of resistance to some important insect pests of these crops include genes that reduce crystalline epicuticular waxes or waxblooms on the surface of the plant. Cultivated *Brassica* spp. typically have prominent waxblooms. Reduced waxbloom varieties of vegetable crop *Brassica oleracea* L. are resistant to aphids, whiteflies, lepidopteran pests, and thrips (Anstey & Moore 1954, Thompson 1963, Way & Murdie 1965, Superak 1976, Dickson & Eckenrode 1980, Lin et al. 1983, Shelton et al. 1983, Stoner 1990, Eigenbrode et al. 1991, Elsey & Farnham 1994). Reduced waxblooms also have been associated with reduced oviposition by cabbage seed pod weevil (Harmon & McCaffrey, University of Idaho, personal communication).

The potential of reduced waxbloom-based resistance to aphids and Lepidoptera in oilseed *B. napus* and *B. rapa* has been explored, but has not shown much promise (Lamb et al. 1993, Ramachandran et al. 1998). Lamb et al. (1993) found no evidence for antibiosis to turnip aphid in reduced waxbloom varieties of 3 oilseed *Brassica* species. Ramachandran (1998) reported that reduced waxbloom *B. napus* had no effect on feeding and survival of diamondback moth larvae. However, these studies were conducted in greenhouses or in growth chambers. Previous work has shown that insect resistance in reduced waxbloom *B. oleracea* is expressed more strongly in the field than in controlled environments (Eigenbrode 1996). This dependence of reduced waxbloom-based resistance on field conditions has been attributed to increased water stress-induced changes in defenses (Cole & Riggall 1992) and to increased effectiveness of generalist predators on reduced wax plants (Eigenbrode et al. 1995, Eigenbrode 1996). This latter effect has been linked to increased attachment by predators to plant surfaces with reduced waxbloom (Eigenbrode et al. 1999). Whatever the mechanism, evaluating the potential of reduced waxbloom oilseed *Brassica* for conferring insect resistance will require field trials. Reduced waxbloom *Brassica* are also more susceptible to flea beetles (Stoner 1990, Bodnaryk 1992a;b, Stoner 1992), a factor that must be considered along with potential resistance to aphids in the field in order to optimize crop protection.

To determine the effect of waxbloom variation in oilseed *Brassica* on natural insect pest populations in the field, we monitored insects on field-grown *Brassica napus* L. and *Brassica rapa* L., with genetically reduced waxblooms and on normal waxbloom controls. We also monitored natural populations of insects on a *B. napus* variety with waxblooms moderately reduced by a herbicide application. Each type of experiment was conducted for 2 field seasons.

To assess the possibility that reduced waxbloom on oilseed *Brassica* increases predation by causing increased aggregation of predators to the plants, aphidophagous insects were monitored in the field trials. In addition, to gauge the potential for increased effectiveness of predators on the reduced waxbloom, attachment by a principal coccinellid predator to the leaf surfaces of *Brassica* with genetically different waxblooms was measured in the laboratory. Waxbloom characteristics on the test plants were quantified by scanning electron microscopy.

Materials and Methods

***B. napus* and *B. rapa* with genetically-reduced waxbloom.** In 1998, insect infestations were monitored in the field on 6 oilseed *Brassica* accessions varying in waxbloom. One reduced waxbloom *Brassica napus* (PI 470055), and 2 reduced waxbloom *B. rapa*, (PI 469895, and PI 470064) were obtained from the Northern Central Regional Plant Introduction Station of the USDA, Ames Iowa. The spontaneous variant of the *B. napus* canola variety 'Bingo' (Calgene, Inc., Davis, California) with reduced waxbloom (and hereafter referred to as 'Bingo R') was obtained from G. D. Buntin and P. L. Raymer, University of Georgia. Normal waxbloom controls were standard 'Bingo' ('Bingo N'), obtained commercially and the normal waxbloom *B. napus* variety 'Sunrise', obtained from J. Brown (University of Idaho). Plants from each variety were started in seedling trays on 8 May, grown for 3 wk at 12:12 L:D[h], 25 ± 5°C, and then placed outside for 5 d to harden-off. Seedlings were transplanted into the field at the University of Idaho Plant Science Farm, near Moscow ID, on 3 June. Plants were set out in 3 m-long double-row plots of 30 plants each, spaced approximately 20 cm between plants within rows. There were 5 replicate plots of each line arranged in a randomized complete block (RCB) design.

On a weekly basis, from 25 June to 23 July, 10 plants were randomly selected within each plot and sampled for insects. The insects sampled were cabbage aphids, crucifer flea beetles, *Phyllotreta crucifera* (Goeze), diamondback moth larvae, adults and larvae of *Coccinella* spp. and *Hippodamia convergens* Guérin-Méneville. Sampling involved carefully inspecting each individual plant and counting all insects. Cabbage aphid densities were also estimated by counting aphid colonies covering more than 2 cm of stem in each plot. Seasonal per-plant densities of these insects were compared with ANOVA using mean counts within each plot as observations and testing the effect of line using the date × line interaction as the error term. Linear contrasts were used to test for differences between all reduced waxbloom and all normal waxbloom entries, and to compare insect populations on Bingo N and Bingo R.

In 1999, only the sister lines Bingo N and Bingo R were compared, and were seeded directly into the field on 20 June in a completely randomized design with 5 replicate, 3-row plots of each line, spaced approximately 15 cm within, and 20 cm between the rows. Each plot contained approximately 75 plants. The plots were sampled weekly from 1 July to 5 August by inspecting ten randomly selected plants per plot, for insects and aphid colonies, following the procedures used to sample insects in 1998. These plots were harvested on 1 September with a research combine. The seed were dried and weighed and converted to per-plant yield on the basis of stand counts for each plot.

Plant waxes on all the *Brassica* lines tested were compared by growing 4 plants of each in the greenhouse in 30-cm diam. pots until they were 8 weeks old. Then, two leaves (4^{th} node) were removed from each plant and prepared for scanning electron microscopy (SEM) as described below.

Attachment to leaf surfaces of each of the genetic *Brassica* lines by *H. convergens* adults was measured following the methods of Eigenbrode et al. (1999). Briefly, the insects were placed on a 1-cm^2 piece of leaf, affixed to an accelerating turntable. The centrifugal force at which the insect is dislodged is then calculated

from the mass of the insect, the revolutions per second, and the radius of its trajectory on the turntable.

***B. napus* with artificially reduced waxbloom.** The herbicide S-ethyl dipropylthiocarbamate (EPTC) can be used to reduce surface wax loads and wax crystal densities of *Brassica* and other waxy dicots. This method has been used to study the ecological effects of plant waxes of *Brassica* and *Pisum* (Flore and Bukovac 1976, Klingauf et al. 1978, Reed 1979, Eigenbrode and Shelton 1992, Eigenbrode et al. 1993). Field-grown *B. oleracea* var. *capitata* (cabbage) treated with moderate concentrations of this herbicide, have reduced waxbloom and are resistant to caterpillars, but sustain no herbicide injury (Eigenbrode et al. 1993). In preliminary trials, we determined that *B. napus* at the pre-budding stage would express a glossy wax phenotype without other signs of damage when treated with EPTC (formulation Eptam 7E, Zeneca Ag. Products, Surrey, United Kingdom) at up to 13 Kg AI/Ha. During two years, we established replicate field plots of the rapeseed variety 'Sterling' at the Plant Science Farm at the University of Idaho. During each year we treated the plots at 5 rates of EPTC (0, 1.8, 3.6, 7.2 and 14.4 Kg EPTC/Ha) in aqueous solution using a backpack sprayer just before the development of flowering shoots. The experimental designs differed in each year; in 1997 the zero EPTC control was included as a subplot within each of the treated plots; in 1998, the zero EPTC control was treated as a separate replicated treatment. In each year, plots were arranged in a RCB design with 5 replications. In 1997, the experiment was bordered with untreated Sterling and in 1998 with *S. alba.*

Insects were sampled on the EPTC-treated plants and controls by beating foliage over a 5-gallon plastic bucket and counting all insects captured in the bucket. Each sample consisted of the insects dislodged from a single location in the plot by 4 swipes with a clipboard; 4 samples were taken randomly in each plot on each sample date. The insects sampled in this manner were the same as those sampled visually on *Brassica* with genetically different surface waxes. Cabbage aphid densities were also estimated by counting all aphid colonies covering more than 2 cm of stem in each plot. Insect samples were taken weekly from 14 July to 11 August, in 1997, and from 7 July to 29 July, in 1998. On 25 July, 1997 and 8 July, 1998, 2 leaf samples from each plot were taken for SEM. Plots were combine-harvested on 2 September in both years, and the seed was weighed after drying at 35°C for 48 hrs. In 1997, twenty 5-gram seed samples were obtained from each plot to determine oil yield/g following the methods of Hammond (1991) and Howard & Duane (1991).

The effect of EPTC dose on insect populations throughout the season during both years was examined by analysis of variance with 3 factors: EPTC dose, block, and sample week (date). The effect of EPTC dose on insect populations was examined using dose × date as the error term. The average counts for bucket samples within each plot were used as observations for all insects; for aphid colonies there was a single estimate for each plot. Yields were compared with a two-factor ANOVA, with block and dose as factors. For all the 1997 analyses, data from control halves of plots within each block were used as covariates to compare the 4 EPTC treatments. For the 1998 analysis, the replicated control (0 EPTC) was included as a treatment in the ANOVA.

Plant wax crystal densities. Leaf samples were prepared for SEM by attaching them to aluminum stubs, lyophilizing them on the stubs at –20°C for 4 d,

and then at room temperature for 4 d. The samples were coated with gold and images prepared as described in Eigenbrode et al. (1999). Wax crystal densities were measured by counting all discrete crystals on 2 randomly selected fields of the SEM on each of the leaf samples (4 leaves from each dose from EPTC experiments and 4 leaves from each *Brassica* accession). Only adaxial surfaces were examined. The mean density for each leaf was used as an observation for ANOVA to compare crystal densities among treatments. Representative images of the genetically different *Brassica* lines were selected for presentation.

Results

***B. napus* and *B. rapa* plants with genetically reduced waxbloom.** In 1998, based on visual inspection and SEMs, waxbloom of the genetic variants of *B. napus* and *B. rapa* were distinct (Fig. 1). PI 470055, PI 469895, PI 470064 and Bingo R had greatly reduced wax crystal morphology compared to the 2 normal-wax *B. napus* entries. Among the reduced wax types, crystalline waxes were reduced least on Bingo R. In the field trial, plants of PI 470055 were extremely feeble in the field trial and only reached a mean biomass approximately 1/4 the mean biomass of other lines, so insect populations on this entry were not included in this report. Among the rest of the lines, aphid colonies per plant were

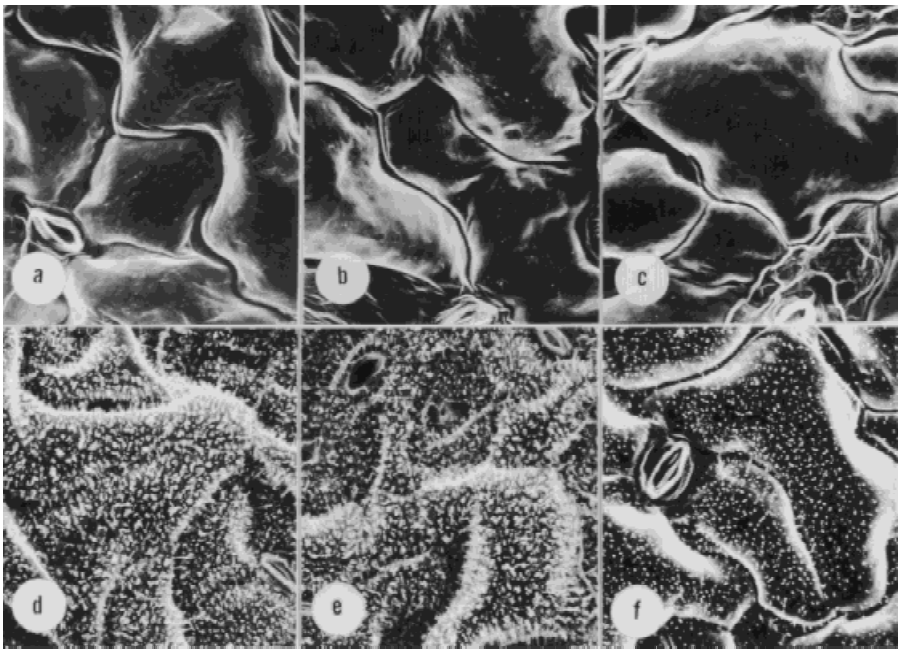

Fig. 1. SEM of the leaf surfaces of the 6 lines of *Brassica* tested. a) *B. napus* PI 470055, b) *B. rapa* PI 469895, c) *B. rapa* PI 470064 , d) *B. napus* Sunrise, e) *B. napus* Bingo N, f) *B. napus* Bingo R. Scale: the diagonal dimension of each panel = 140 μm.

significantly lower and flea beetles per plant were significantly higher on reduced waxbloom lines than on the normal-waxbloom lines (Table 1). Based on linear contrasts, Bingo R had significantly fewer aphids than Bingo N, but the 2 lines did not have significantly different flea beetle densities (Table 1). No other insect population differences were significant, nor were trends apparent, except that diamondback moth larvae tended to be higher on Bingo R than on Bingo N (contrast significant at $P \leq 0.05$, but overall ANOVA not significant, $P = 0.1406$).

Above-ground biomass did not differ consistently between normal waxbloom and reduced waxbloom *Brassica,* but tended to be lower for Bingo R than on Bingo N ($P = 0.0657$) (Table 1). Due to the late establishment of the plants, none set seed, so yield data were not obtained.

In 1999, seasonal mean numbers of aphid colonies per plot and aphids per plant were significantly lower on Bingo R than on Bingo N (Table 2). Seasonal mean coccinellids and flea beetles per plant were significantly higher on Bingo R than on Bingo N. Yields (calculated on a per-plant basis) were significantly higher on Bingo R than on Bingo N (Table 2).

Attachment force produced by adult *H. convergens* varied several-fold among the lines and was greater to the reduced-wax accessions than to the normal-wax plants in the test (Table 3).

***B. napus* with artificially reduced waxbloom.** Applications of EPTC reduced surface waxbloom on Sterling in 1997 ($F = 3.69$; df = 3, 11; $P = 0.0464$) and 1998 ($F = 25.87$; df = 4,12; $P = 0.0001$). However, densities of leaf wax crystals decreased with EPTC dose only in 1998 (range 85–24 wax crystals/ 100 μm^2). There were no significant effects of EPTC dose on either pest insects (cabbage aphids, diamondback moth larvae, flea beetles), or predatory insects (coccinellids), in either year.

Yields were not affected within either year by EPTC dose (1997, $F = 2.61$; df = 3, 11; $P = 0.1043$, 1998 $F = .005$; df = 4,14; $P = 0.699$). Oilseed content (measured in 1997 only) was not affected by EPTC application (mean for all treatments = 36.5% vs. controls = 35.4%; contrast $F = 2.93$; df = 1; $P = 0.1825$).

Discussion

Over the 2 field seasons, cabbage aphid populations were lower on oilseed *Brassica* with genetically reduced waxblooms. In both years aphid densities were lower on reduced waxbloom Bingo R than on the normal waxbloom variety from which it is derived, Bingo N. In contrast, flea beetle densities were consistently higher on *Brassica* with genetically reduced waxbloom. These trends in aphid and flea beetle populations on reduced vs. normal waxbloom *B. napus* in the field are consistent with previous records of these insect species on reduced waxbloom *B. oleracea* (Way & Murdie 1965, Superak 1976, Stoner 1990, Bodnaryk 1992b, Stoner 1992). No other herbivores were consistently affected by waxbloom variation on oilseed *Brassica* in our experiments. The lack of response to waxblooms by diamondback moth larvae populations is at odds with previous reports of reduced densities and damage by this insect on reduced waxbloom *B. oleracea* in the field (Dickson & Eckenrode 1975, 1980, Dickson et al. 1990, Stoner 1990, 1992). However, populations of diamondback moth were low in both years, with seasonal means reaching a high of ~ 0.9 larvae per plant, and may not have provided an adequate test.

Table 1. Seasonal means for naturally occurring insect populations sampled on five oilseed *Brassica* genotypes, and above-ground biomass per plant of each line in 1998.

Line	Species	Wax type[a]	Aphid colonies per plant	Coccinellids per plant	Flea beetles per plant	Diamondback moth larvae and pupae per plant	Wax crystals /100μm^2	Above-ground biomass (g dw) per plant
PI 469895	*B. rapa*	R	4.3 ± 1.1bc	0.9 ± 0.3	54 ± 11a	0.9 ± 0.3	8.7 ± 2.8c	155 ± 31ab
PI 470064	*B. rapa*	R	3.9 ± 0.5bc	0.6 ± 0.1	47 ± 8a	0.6 ± 0.1	8.7 ± 3.1c	240 ± 68a
Bingo R	*B. napus*	R	2.6 ± 0.6c	0.9 ± 0.2	26 ± 7b	0.9 ± 0.2	20.1 ± 1.2b	105 ± 34bc
Bingo N	*B. napus*	N	8.2 ± 0.6a	0.6 ± 0.3	10 ± 3b	0.6 ± 0.3	44.5 ± 3.5a	198 ± 17ab
Sunrise	*B. napus*	N	6.0 ± 1.2ab	0.7 ± 0.2	10 ± 2b	0.7 ± 0.2	43.9 ± 3.0a	173 ± 8ab
F			6.95	0.39	10.69	2.02	40.47	2.34
df			4, 16	4, 16	4, 16	4, 16	4, 15	4, 16
P			0.0019	0.8111	0.0002	0.1406	0.0001	0.1060
P value for contrasts								
Wax: reduced vs normal			0.0002	0.4816	0.0001	0.9585	0.0001	0.5460
Bingo R vs Bingo N			0.0002	0.3969	0.0803	0.0459	0.0001	0.0657

Seasonal means were calculated by determining the seasonal mean for each replication (block) and then determining means and standard errors of these means (n = 5). ANOVA was conducted as a split plot in time repeated measures with block x line as the error term for all effects. Means were separated with LSMEANS procedure. [a]R = reduced waxbloom; N = normal waxbloom.

Table 2. Seasonal means for naturally occurring insect populations and yield (g/plant) on Bingo R (reduced waxbloom) and Bingo N (normal waxbloom) in 1999.

Line	Aphid colonies per plot	Coccinellids per plant	Flea beetles per plant	Diamondback moth larvae and pupae per plant	Yield (g/plant)
Bingo R	3.8 ± 0.2	1.5 ± 0.2	56 ± 4	0.5 ± 0.04	0.74 ± 0.10
Bingo N	15.2 ± 1.4	0.7 ± 0.1	35 ± 4	0.7 ± 0.15	0.36 ± 0.05
P value for *t* test	0.001	0.005	0.2301	0.0046	0.0098

Seasonal means were calculated by determining the seasonal mean for each replication and then determining means and standard errors of these means (n = 5).

Reduced waxbloom plants provided better attachment by the predominant coccinellid, *H. convergens,* than did the normal waxbloom *Brassica* plants. In laboratory experiments, better attachment to plants is correlated with increased efficiency of individual predators (Eigenbrode et al. 1999). In the field, coccinellid numbers tended to be higher on reduced waxbloom Bingo R than on Bingo N, significantly so in 1999. This suggests that lower aphid populations on reduced waxbloom plants may have been due, in part, to an increase in individual predator efficacy on reduced waxbloom plants, as well as a tendency toward increased predator aggregation.

Insect populations, yield, and oilseed quality were not affected by the more moderate reduction of waxbloom of Sterling achieved by artificial alteration with EPTC (25 to 65 crystals per 100 μm^2 vs. 9 to 20 crystals per 100 μm^2 on genetically reduced waxes). Based on the results of our trials with genetically and artificially reduced waxbloom, at least a 2-fold reduction in waxbloom seems to be

Table 3. Attachment force by adult *H. convergens* to leaves of *Brassica* accessions.

Line	Attachment force (mN) produced by *H. convergens*
PI 470055	0.029 ± 0.001a
PI 469895	0.037 ± 0.002b
PI 470064	0.028 ± 0.001b
Bingo R	0.015 ± 0.001c
Bingo N	0.002 ± 0.000d
Sunrise	0.002 ± 0.000d
F	10.309
df	19.3
P	0.0001

necessary to affect insect population levels. Unfortunately, an explicit test of this hypothesis using EPTC is apparently not feasible. Higher doses of EPTC in our pilot studies produced phytotoxicity evidenced by necrotic patches on the treated leaves.

Although wax crystal densities lower than 25 per 100 μm^2 , as occurred on the reduced-waxbloom *Brassica* lines, may reduce cabbage aphid densities, the benefits in improved aphid control may be offset by liabilities. The most obvious liability is the associated greater susceptibility of reduced waxbloom plants to flea beetles. In production areas such as the northern Great Plains, where flea beetles are a severe pest of seedling spring canola (Weiss et al. 1990), reduction in wax crystals would clearly be an undesirable trait. However in growing areas where aphids are the key pest of canola, such as the southeastern United States (Buntin & Raymer 1994), an increase in flea beetle damage may be acceptable. Another possible liability is that yield potential may be lower in reduced waxbloom oilseed *Brassica*. In 1998, *Brassica* with genetically reduced waxbloom tended to have lower plant biomass. Although reduced waxbloom Bingo R outyielded Bingo N in 1999, yields for both lines were low, probably as a result of high insect densities in this unprotected trial. Further testing is necessary to determine how Bingo R and Bingo N might perform under standard planting densities (approx. 3 × those used in our experiments) and with some pest control. Thus, our overall conclusion is that waxbloom variation in oilseed *Brassica* may offer some potential for insect resistance, but that that potential is likely to be limited to a few canola production areas.

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Pyridaben Baseline Assays for Adult Female European Red Mite (Acari: Tetranychidae) and Eggs[1]

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ABSTRACT Bioassays of *Panonychus ulmi* (Koch) (Acari: Tetranychidae) with the miticide/insecticide pyridaben indicate this product is toxic to both eggs and motile stages. For contact ovicidal tests, adult female *P. ulmi* were allowed to lay eggs on untreated peach leaf disks. Mites were removed at 18 h then the leaf disks with eggs were dipped for 5 s in serial dilutions of pyridaben or water as a control. Treated leaf disks with eggs were then placed on wet cotton and held in a growth chamber to allow eggs to hatch. For residual ovicidal assays, adult female *P. ulmi* were allowed to oviposit either on water-treated peach leaf disks or leaf disks treated with serial dilutions of pyridaben for 18 h before removal of the mites. Adult mortality was assessed when the mites were removed from the residual ovicidal assays. Concentration-response line parameters for treated eggs from the contact ovicide assays were: LC_{50}s (2.4–3.3 ppm), LC_{90}s (7.2–8.7 ppm), slopes (2.3–2.9). Line parameters from the residual ovicidal assay were: LC_{50} (32 ppm), LC_{90} (130 ppm), slope (2.1). Adult female *P. ulmi* placed on treated surfaces were more sensitive to pyridaben than eggs. Concentration-response line parameters for the residual adult *P. ulmi* assays were: LC_{50}s (1.1–1.5 ppm), LC_{90}s (2.6–3.9 ppm), slopes (2.8–3.3). Because pyridaben is toxic to several lifestages of *P. ulmi*, use patterns should be part of an overall miticide resistance management program.

KEY WORDS baseline assays, miticide, ovicide, *Panonychus ulmi*, pyridaben, resistance management

Pyridaben is a miticide/insecticide that received full Federal registrations in 1997 for apples, pears, and almonds in the USA. Full registrations for stone fruit were received in 2000. Pyridaben is a mitochondrial electron transport inhibitor (METI) in the chemical family pyridazinone (Hollingsworth et al. 1994). Marketing information associated with pyridaben indicates that it kills by contact action and not through systemic, translaminar, or vapor action. Pyridaben is reported by some authors to have no ovicidal activity (Anonymous 1996) although others have demonstrated that pyridaben is ovicidal to certain phytophagous mite species (Kimura & Kushita 1994).

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Pyridaben provides good control of *Panonychus ulmi* (Koch) (Reissig et al. 1997, Hull 1997). Because of its effectiveness and the scarcity of good alternative summer acaricides, pyridaben will likely be used extensively in eastern US fruit orchards. Therefore, it is important to collect baseline susceptibility information for pyridaben before it is widely used. Knowledge gained from such tests is valuable for monitoring changes in target-pest susceptibility over time and for developing recommendations to delay the onset of resistance (Welty et al. 1989, Riedl et al. 1990, Campos et al. 1995).

The overall objective of this study was to evaluate susceptibility of adult female *P. ulmi* and their eggs to pyridaben. Contact and residual ovicidal assays were used to clarify whether pyridaben kills *P. ulmi* eggs. Residual adult assays were also conducted. Concentration-response line parameters from the above 3 types of assays can be used for future resistance monitoring studies.

Materials and Methods

Adult *P. ulmi* females were collected from 'Loring' or 'Jersey Dawn' peach trees maintained at the Rutgers Agricultural Research and Extension Center, Upper Deerfield Township, New Jersey. All assays were conducted on 'Ernie's Choice' peach leaf disks. Leaves for test-substrate disks were collected from unsprayed trees and then brought to the laboratory. Leaf disks (21 mm diam.) were cut from the leaves using a cork borer and placed bottom-side up on de-ionized-water-soaked cotton in petri dishes. Bioassays were conducted in a growth chamber set at 25°C, 70 ± 5%RH, and a photoperiod of 16:8 [L:D] h. *P. ulmi* eggs were considered dead if they did not hatch within 10 d after treatment. Adult mortality also was assessed in some of the residual ovicidal tests. Adult female *P. ulmi* were considered dead 18 h after treatment if they failed to move one body length after being prodded with a brush or had run off the leaf disk and were trapped in the wet cotton.

Contact ovicide test. Three to five leaf disks per concentration were used for each assay. Twenty adult *P. ulmi* females were transferred to each single leaf disk using a fine camel-hair brush and allowed to oviposit. After 18 h, females were removed from disks with an aspirator. Eggs were counted on each disk. Leaf disks were then arranged in groups of 3–5 on moistened cotton in petri dishes to provide approximately equal egg totals per petri dish, then assigned to a treatment. Concentrations of pyridaben (Pyramite 60 WP, BASF Corporation, Research Triangle Park, North Carolina) ranging from 0.5 to 8 ppm were prepared by serial dilution. The spreader Triton AG-987 (Rohm and Haas Company, Philadelphia, Pennsylvania) was added at 0.5 ml/liter to each concentration to improve wetting. Water with spreader served as the control. Leaf disks with eggs <24 h old were dipped in the concentrations for 5 s with a gentle back-and-forth motion. Treated leaf disks were air-dried for ~ 1 h then placed in a growth chamber. This test was repeated 3 times.

Residual adulticide and ovicide test. Two separate tests were conducted to determine if pyridaben exhibited residual activity against *P. ulmi*. For all residual ovicide and adulticide tests, leaf disks were treated before adult *P. ulmi* females were transferred to the disks.

In the first series of tests, residual activity of pyridaben against both adults and eggs of *P. ulmi* was determined. Leaf disks were dipped for 5 s in serial

dilutions of pyridaben ranging from 0.5 to 8 ppm containing Triton AG-98 (0.5 ml/liter). Disks dipped in water with spreader served as the control. After leaf disks were air-dried (~1 h), 20 adult *P. ulmi* females were transferred to each disk with a brush and allowed to oviposit for ~18 h in a growth chamber. Adult *P. ulmi* female mortality was assessed after 18 h as they were removed from the disks with a brush. Eggs on each disk were then counted. The disks were then returned to the growth chamber and the eggs were allowed to hatch. This test was repeated three times.

The second test included higher concentrations of pyridaben to investigate residual ovicidal effects against *P. ulmi* because the concentrations tested in the first residual assays were not high enough to generate concentration-response lines for eggs laid on peach leaf disks treated with pyridaben. The residual ovicide assay was conducted as above except that 30–40 adult *P. ulmi* females were transferred to treated peach leaf disks and adult mortality was not evaluated. Serial dilutions ranged from 20-100 ppm.

Data analysis. Egg and adult mortality data were analyzed by probit analysis with POLO (Russell et al. 1977) to estimate LC_{50} and LC_{90} values, coefficients of concentration-response lines, as well as the likelihood ratio test for parallelism.

Results

Contact ovicide test. Pyridaben is ovicidal when applied directly to freshly laid *P. ulmi* summer eggs (Table 1). The LC_{50} values ranged between 2.4–3.3 ppm and the LC_{90} values were between 7.2–8.7 ppm. The slopes of the concentration-response lines ranged from 2.3–2.9. Probit analysis test of equality revealed that the concentration-response lines from the 3 repetitions were different ($X^2 = 14.8$; df = 4; $P = 0.01$) although the 95% CL of the LC_{50}s and LC_{90}s from the 3 contact ovicide tests overlapped (Table 1). Control mortality (unhatched eggs) was 6.2, 17.2, and 8.7 % for repetitions 1–3, respectively.

Residual adulticide and ovicide test. Results from residual assays indicate that adult *P. ulmi* females are extremely sensitive to pyridaben. The LC_{50} values for the 3 repetitions were all $\leqslant$ 1.5 ppm and the slopes of the concentration-response lines were relatively steep (Table 2). A probit analysis test of equality revealed that the concentration-response lines were not the same ($X^2 = 18.3$; df = 4; $P = 0.001$). The likelihood ratio test for parallelism indicated that the slopes were parallel ($X^2 = 1.09$; df = 2; $P = 0.581$). However, the 95% CL of the LC_{50} and LC_{90} values from the 3 residual adulticide assays overlapped. Control mortality for the 3 repetitions of the residual adult assays was 8.1, 5.5, and 5.4%, respectively. Residual ovicide concentration-response lines could not be determined for this set of assays because mortality was too low at the range of concentrations tested. Average egg mortality was 4.9% for the control while the highest concentration of pyridaben tested (8 ppm) only prevented 25.5% of the eggs from hatching.

Concentration-response line parameters were generated for *P. ulmi* eggs oviposited on treated peach leaf disks when tested with higher concentrations of pyridaben (20–100 ppm). The estimated LC_{50} was 31.9 (95% CL: 24.6–41.4 ppm), the estimated LC_{90} was 129.9 (95% CL: 86.9-263.9 ppm), and the slope ± SE of the concentration-response line was 2.10 ± 0.15 ($n = 1164$) ($X^2 = 13.7$; df = 5).

Table 1. Concentration-response line parameters for pyridaben-treated *P. ulmi* eggs using contact assay.

Repetition	Eggs tested, *n*	Slope ± SEM	LC_{50}, ppm (95% CL)	LC_{90}, ppm (95% CL)	χ^2 (df)
1	1788	2.7 ± 0.2	2.6 (2.1–3.1)	7.9 (6.3–10.9)	59.9 (5)
2	1824	2.3 ± 0.2	2.4 (1.3–3.4)	8.7 (5.9–19.5)	39.5 (5)
3	1192	2.9 ± 0.3	3.3 (2.3–4.4)	7.2 (5.6–12.6)	13.1 (6)

Discussion

All life stages of *P. ulmi* tested were susceptible to pyridaben. Baseline ovicidal assays confirm results from Kimura and Kushita (1994) that pyridaben is ovicidal against freshly laid *P. ulmi* summer eggs. Based upon results presented here, adult female *P. ulmi* were the most sensitive lifestage tested followed by eggs treated topically with pyridaben in the contact assay. *P. ulmi* eggs laid on pyridaben-treated peach leaf disks were also killed but at considerably higher concentrations.

Statistical variation was observed between repetitions in both the contact ovicidal and adult residual assays. These small variations can be attributed to normal variation frequently observed in testing living organisms. All of the concentration-response line parameters among the repetitions of a given assay were within a 1.5-fold difference indicating a high degree of precision for these assays.

Previously, Riedl et al. (1990) determined that the LC_{50} and LC_{90} values for clofentezine-treated summer eggs of *P. ulmi* eggs laid on apple leaves were approximately 12.5 and 64 ppm, respectively. Their study used a different test substrate (apple) and environmental holding conditions than the study reported here; thus, direct comparisons of efficacy cannot be made regarding clofentezine and pyridaben. However, results presented here indicate that summer eggs of *P. ulmi* may be more sensitive to pyridaben than clofentezine and further research is warranted to determine this.

Pyridaben is toxic to both female *P. ulmi* and eggs; thus, resistance may occur in either or both of these life stages. Pyridaben resistance has already developed in other orchard mites including *Tetranychus urticae* (Koch) (Acari: Tetranychidae) (Funayama and Takahashi 1995) and *P. citri* McGregor (Acari: Tetrany-

Table 2. Concentration-response line parameters for adult *P. ulmi* females exposed to pyridaben using a residual assay.

Repetition	Adults tested, *n*	Slope ± SEM	LC_{50}, ppm (95% CL)	LC_{90}, ppm (95% CL)	χ^2 (df)
1	501	3.3 ± 0.4	1.1 (0.8–1.3)	2.6 (2.2–3.5)	26.9 (18)
2	500	2.8 ± 0.3	1.2 (0.9–1.5)	3.5 (2.6–5.9)	44.9 (18)
3	600	3.1 ± 0.3	1.5 (1.2–1.8)	3.9 (3.2–5.2)	42.1 (23)

chidae) (Etoh et al. 1996). Therefore, use of this product should include a resistance management scheme that prevents short-term repeated use. The baseline data presented here can be used as part of a resistance management program to evaluate changes in susceptibility of *P. ulmi* to pyridaben.

Currently, BASF Corp.'s fruit label for apples and pears in the USA restricts pyridaben to two applications for a total maximum amount of 748.4 g of product (1.0 lb a.i.) per season on fruit crops with a minimum 30-day treatment interval. The label also indicates that growers should alternate products having different modes of action. A more conservative approach would prevent growers from using back-to-back applications of pyridaben or other 'METI' products.

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NOTE

Comparison of Tobacco Variety CU 263 Versus a Standard Tobacco Variety Against Tobacco Budworm Under Field Conditions in South Carolina[1]

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The tobacco budworm, *Heliothis virescens* (F.), is a major insect pest of flue-cured tobacco (*Nicotiana tabacum* L.). In South Carolina, it is usually the most important insect pest on tobacco. In 1995, Clemson University's Pee Dee Research and Education Center released 'CU 263', the first insect-resistant tobacco cultivar available in the United States. In replicated small-plot tests, this cultivar exhibited moderate resistance to the tobacco budworm (Johnson 1996). Budworms will still feed on CU 263; however, their life cycle and larval development are slowed compared with conventional cultivars (Johnson 1996). Although CU 263 was tested in the Flue-cured Tobacco Regional Small Plot Test in 1992 and 1994, and in the Regional Farm Test in 1994 (Flue-Cured Tobacco Variety Evaluation Committee 1992, 1994), it had not been tested under large-scale grower conditions. The purpose of this experiment was to compare CU 263 against standard tobacco cultivars under large field grower conditions.

This test was conducted in 1996 in two different locations in Horry County, South Carolina. The first location, Tyler farm, was 4 km south and 2 km east of Allsbrook, South Carolina (33.993°N, 78.971°W). Part of the field (1.26 ha) was planted in CU 263 and the rest (2.62 ha) was planted in the standard 'K 149'. The tobacco was transplanted on 22 April 1996 without the use of any transplant-water insecticides. Both cultivars were grown using identical, standard agronomic practices except for foliar insecticide treatments, which were made on the basis of weekly scouting. Insecticides were applied when 10% of the plants were infested with budworms. The tobacco was topped between 2 and 9 July, after which tobacco budworms were no longer a factor. The tobacco was harvested after the 13 August observations.

The second location was on Willoughby farm, about 24 km west of Loris (34.137°N, 79.171°W). Approximately 0.4 ha of CU 263 was planted on each end of the field, with approximately 0.8 ha of 'K 326' planted in between. The tobacco was transplanted on 18 April 1996 without the use of any transplant-water insecticides. The insect control practices, topping, and harvesting were the same as at the first location, except that only part of the field could be harvested due to damage from hurricane Fran.

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Table 1. Tobacco budworm infestation levels (percentage of plants infested) on tobacco cultivar CU 263 versus a standard tobacco cultivar (Horry County, South Carolina, 1996).

	Tyler		Willoughby	
Date	CU 263	K 149	CU 263	K 326
May 8	0	0	—	—
16	0	2	0	0
23	0	0	4	2
29	4	2	0	12*
Jun 5	2	0	2	8
12	8	12*	0	0
18	34*	14*	32*	8*
25	6	4	6	24*
Jul 2	2	4	6	0
Total Sprays for Budworm	1	2	1	3

*Sprayed with insecticide on this date.

Results of the weekly scouting are shown in Table 1. The CU 263 and K 149 were sprayed one and two times, respectively, for tobacco budworm on the Tyler farm, based on South Carolina's economic threshold level of 10% infestation. The CU 263 and K 326 were sprayed one and three times, respectively, on the Willoughby farm. It should be noted that on 18 June the K 326 on the Willoughby farm was treated even though the infestation level was at 8%. The decision to treat was made based on the fact that budworm populations at that time were extremely high throughout the state and were rising rapidly. At each location, CU 263 exhibited overall lower budworm pressure and damage. Yields for the two cultivars on the Tyler farm were 2,565 kg/ha and 3,237 kg/ha, respectively. Due to non-replication of the test, this difference could not be subjected to statistical analysis.

In his registration of CU 263, Johnson (1996) noted that budworm damage is reduced by approximately 50% compared with NC 2326 or NC 95. He also noted that CU 263 is susceptible to damage by the tobacco aphid (*Myzus nicotianae* Blackman) and the tobacco hornworm [(*Manduca sexta* (L.)]. Clearly, in this test, infestation levels and damage by the tobacco budworm were not as significant on CU 263 (as determined by the number of insecticide applications required) as on either K 149 or K 326. Although budworms were present, they were not generally as numerous, and damage was not as great, based on visual observations. This is consistent with the findings of Johnson (1996). The tobacco aphid was not a major factor in either of these tests. Late-season hornworm infestations, however, did cause some damage and each field required one additional insecticide application for hornworms. There were not any differences by cultivar. The reason for the yield differences on the Tyler farm cannot be readily explained. There were no apparent differences except for the amount of budworm damage, and that was lower in CU 263.

In conclusion, CU 263 resulted in fewer tobacco budworms and less budworm damage than the standard cultivars with which it was compared. However, in the one test in which yield could be compared, CU 263 yielded lower than K 149. The test could not be repeated in 1997 due to the small amount of tobacco that was planted using CU 263.

Acknowledgment

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Sex Pheromone of Yellow Scale, *Aonidiella citrina* (Homoptera: Diaspididae): Evaluation as an IPM Tactic[1]

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ABSTRACT The effects of dose and field longevity of lures treated with synthetic female yellow scale sex pheromone ((5*E*)-6-isopropyl-3,9-dimethyl-5,8-decadienyl acetate) were evaluated for monitoring flight activity of male yellow scale, *Aonidiella citrina* (Coquillett). Pheromone doses of 1–200 μg per lure attracted large numbers of males. Lower doses (1–5 μg per lure) generally attracted fewer males, but trap counts unequivocally showed the beginnings and peaks in male flight activity at both low and high population densities. Furthermore, the low-dose pheromone traps that collected fewer individuals were easier and less time-consuming to assess, and so the lower doses are recommended for monitoring phenology and population densities. In field longevity tests, pheromone lures continued to attract sufficient numbers of male scale to follow population trends for up to 4 months. Insecticide applications suppressed the number of male scale captured on pheromone cards. A 1989 pheromone trap survey of yellow scale in Tulare County, California demonstrated that yellow scale was distributed throughout the citrus growing region of that county, and was especially heavy in the area between Porterville and Terra Bella.

KEY WORDS Homoptera, Diaspididae, *Aonidiella citrina*, yellow scale, sex pheromone, phenology, integrated pest management

Yellow scale, *Aonidiella citrina* (Coquillett), can be a significant pest of citrus in the San Joaquin Valley of California (Pehrson et al. 1991, Grafton-Cardwell et al. 1998). Heavy densities of yellow scale cosmetically damage the fruit and directly damage leaves resulting in twig dieback (DeBach et al. 1978). Yellow scale was the primary armored scale pest of San Joaquin Valley citrus until the closely related California red scale, *A. aurantii* (Maskell), was introduced to this region in the early 1900s (DeBach et al. 1978). Eradication of California red scale was attempted in some regions of the San Joaquin Valley from 1939 until 1967, using

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chemicals such as hydrogen cyanide and, later, parathion (Ervin et al. 1986). This intensive application of insecticides suppressed populations of both scale species for many years. Furthermore, California red scale appeared to competitively displace yellow scale in mixed infestations (Hendrickson 1974, DeBach et al. 1978). When eradication efforts ended in 1967, California red scale was the primary armored scale pest in the majority of scale-infested citrus orchards in the San Joaquin Valley.

Since the early years of the California red scale eradication program, San Joaquin Valley growers have depended upon organophosphate and carbamate insecticides for control of armored scale as well as other pests of citrus. Intensive use of these two insecticide classes over a period of four decades has resulted in insecticide resistance in many scale populations (Grafton-Cardwell & Vehrs 1995). In orchards where both species of armored scale have developed resistance to pesticides, yellow scale frequently dominates, because it develops a higher level or frequency of resistance than California red scale (Grafton-Cardwell & Vehrs 1995). Thus, yellow scale is increasing in importance as a pest due to resistance. When growers stop using organosphosphate and carbamate insecticides, yellow scale populations are suppressed by parasitoids, especially *Comperiella bifasciata* Howard (Hendrickson 1974), and California red scale dominates. Yellow scale is the larger of the two species and supports a greater number or size of parasitoids. It occurs primarily on citrus leaves and fruit, whereas California red scale is distributed throughout the tree. Parasitoids are less effective in finding and parasitizing the scale on the interior wood (Carroll & Luck 1984). It is important that growers know which armored scale species dominates an orchard because yellow scale is more likely to develop resistance to insecticides and is more amenable to biological control.

Where yellow scale is present, the scale population is usually composed of a mixture of the two species, which are difficult even for experts to distinguish accurately. Yellow scale, generally is slightly more yellow than California red scale, causes a characteristic chlorotic streak on leaves, and infests twigs only when the population reaches very high densities (Grafton-Cardwell et al. 1999). The species are most accurately differentiated by two internal sclerotized structures on the ventral side of the pygidium of the 3rd instar nongravid females (McKenzie 1938). This identification process is not useful for field assessments of populations because it requires a compound microscope, technical expertise, and experience in differentiating the two species.

It may be possible to dramatically simplify the problem of species identification in potentially mixed populations by use of species-specific pheromone traps. Sex pheromones of California red scale and yellow scale differ considerably in chemical structure and there is no cross attraction between the two species (Moreno et al. 1972a,b). California red scale pheromone has been used for many years in integrated pest management (IPM) programs for California citrus (Moreno & Kennett 1985, Pehrson et al. 1991). Pheromone-baited traps are used to detect scale outbreaks that warrant pesticide treatments (Moreno and Kennett 1985, Pehrson et al. 1991), to time pesticide applications to target 1st instar scale crawler activity (Hoffmann & Kennett 1985, Kennett & Hoffman 1985, Walker et al. 1990), and to time releases of *Aphytis melinus* DeBach to coincide with their preferred 3rd instar female scale (Forster et al. 1995). However, pheromones have

not been included in IPM programs for yellow scale, because until recently, it has been a relatively minor pest of citrus.

Yellow scale pheromone was identified almost two decades ago as (5E, 6S)-6-isopropyl-3,9-dimethyl-5,8-decadienyl acetate (Gieselmann et al. 1979) and various syntheses were developed (Mori & Kuwahara 1982, Alvarez et al. 1988). However, its biological activity has been tested only once: Suguro et al. (1981) reported that doses of 10 and 100 μg of pheromone attracted male yellow scale, but used no controls and did not analyze the data statistically. Because of the lack of information on applications of yellow scale pheromone and the increasing importance of yellow scale as a key pest of California citrus, we conducted a more thorough evaluation of the potential role for yellow scale pheromone-baited traps in citrus pest management. Our specific objectives were to determine: 1) the optimum dose for yellow scale pheromone lures, 2) the longevity of pheromone lures under field conditions, 3) the utility of pheromone-baited traps in monitoring the flight phenology of male yellow scale, and 4) the distribution of yellow scale in Tulare County, Calif.

Materials and Methods

Racemic yellow scale pheromone used in all experiments was synthesized as described by Millar (1989). Traps consisted of 7.6 × 12.7 cm cards coated with Stickem Special to which we attached at the top of the trap a pheromone-treated rubber septa using a paper clip (Pehrson et al. 1991).

Dose response experiments. In the first dose experiment, equivalent volume heptane solutions of 25, 50, 100,and 200 μg pheromone were loaded onto 11 mm red rubber septa (#1780J07, Thomas Scientific, Philadelphia, Pennsylvania). Septa for blank trap controls were treated with 0.05 ml of heptane alone. Loaded lures for each dose were stored separately in glass jars at -20°C until used. Traps were placed in a 5 × 5 Latin square design, 5 replications of the 4 doses and the control, in a Valencia orange orchard in Tulare County, California. Trap cards were hung from branches at a height of approx. 1.5 m in the north quadrant of a tree, with a trap being placed on every second tree. Trap cards were changed weekly and the same lures were used throughout the duration of the experiment (2 June through 6 July 1989).

Because of the large number of male scale collected for an extended period with all pheromone doses in the first dose trial, a second trial was carried out using lower doses of pheromone. Septa were loaded with 1, 5, 25, and 50 μg doses of pheromone in heptane, or heptane alone (control lures), and placed two trees apart. All doses were present in each of four quadrants (four replicates) of four citrus orchards in Tulare County, California. Lures were changed every 4 wk and cards were changed weekly from 23 March through 11 November 1993. The mean number of males per card per wk was calculated for each orchard.

Lure longevity experiment. At the end of the 1989 dose trial described above, the lowest and highest dose lures (25 and 200 μg, respectively) were incorporated into an experiment examining the longevity of the two doses of pheromone lures under field conditions. Field-aged lures (aged 2 June–6 July 1989) and fresh lures were set out with sticky traps in a randomized block design (4 treatments and 5 replications). The fresh lures were changed monthy (8 August and 5 September) whereas the 30-d old field-aged lures were used for the duration of the

trial (6 July–4 October). Male scale collected on trap cards were counted and cards were changed approximately once a week, rotating each trap one position within a block at each count to control for positional effects.

Correlation of pheromone trap catches to scale phenology. To monitor scale phenology during 1994, we captured male scales in twelve orchards near the cities of Ivanhoe, Woodlake, Porterville, and Terra Bella, California at weekly intervals. We used 4 traps with lures loaded with 1 μg doses of yellow scale pheromone, 2 traps with lures loaded with 120 μg doses of California red scale pheromone, and 2 control traps with heptane-treated septa. Yellow scale, California red scale, and control traps were separated from one another by 3 trees and the replicates were separated by 10 or more trees. Lures were changed monthly and trap cards were replaced weekly. The total number of scale per trap was recorded.

Degree days (DD) for each of the scale generations were estimated using the basic model of average daily temperature minus the lower developmental threshold (Wilson and Barnett 1983). Temperature data for the period of January through November of 1993 and 1994 were downloaded from the California Irrigation Management Information System in Lindsay, California. The biofix was the first appearance of male scale on 8 March 1993 and 18 March 1994.

To monitor scale phenology, we collected 20 one-year-old twigs during the periods of 20–30 June, 28–29 July, and 18–19 August 1994, and 20 scale-infested fruit in August 1994 from the trees surrounding those containing the pheromone traps. We identified to species all 3rd instar nongravid female scale on plant samples using the methods of McKenzie (1938).

Survey for Yellow Sale Distribution

During 1989, we monitored the distribution of yellow scale in Tulare County with the assistance of farm and pest control advisors. Traps baited with septa loaded with 100 μg of yellow scale pheromone were hung for 4–6 wk in citrus groves during the September-October flight period. One trap was placed in each citrus orchard and total male scale per trap was recorded.

Statistical Analysis. In all dose and field longevity trials, male scale counts were $\log_{10}$ transformed to minimize the correlation between the mean and the variance of the data. The transformed data were analyzed by the SAS Repeated Measures Analysis of Variance procedure (SAS Institute 1988) with the goal of testing differences in between-treatment means. The REGWF means comparison procedure (SAS Institute 1988) was used to identify statistically different means ($P = 0.05$). The ANOVA assumption of homogeneity of variances among treatments was tested with Hartley's F-max test (Milliken and Johnson 1984). F-max tests for both studies were not significant at the $P = 0.05$ level, indicating that the log transformation was successful in equalizing variances between treatments.

Results and Discussion

Dose response experiments. In the first dose trial, traps baited with 25 to 200 μg doses of pheromone caught substantially more insects ($F = 11.0$; df = 1,18; $P = 0.004$) than solvent-treated controls during the entire course of the

experiment (Fig. 1). Captures in pheromone-baited traps were often large, with 20,000–30,000 male scale caught on a single trap card during a one wk trapping period, demonstrating the high biological activity of the synthetic pheromone. In contrast, control traps caught 2–3 orders of magnitude fewer scale (tens to a few hundred scale). There was a general trend towards increasing trap catch of male scale with increasing dose and significant differences between capture rates of the 25 μg and 200 μg doses on 22 June and 6 July (Fig. 1). Even the lowest dose (25 μg) attracted large numbers of insects (>2,500 per week), indicating that lures within this range of doses were effective for at least 5 weeks. Pheromone lures for California red scale are replaced every 4–6 weeks, which is generally the activity period of the males (Pehrson et al. 1991). Our data suggest that yellow scale lures could be replaced on the same time interval, or even less frequently.

Synthetic yellow scale pheromone was highly attractive at the lower doses as well, with even the 1 μg dose attracting many thousands of males per card per week in orchards B and C (Fig. 2). The higher doses resulted in peak catches of 27,000–80,000 scale per card per week in sites with high yellow scale populations (sites A-C) and catches of up to 400 scale per card for a low density population (site D). Trap cards with heptane-treated controls captured increasing numbers of yellow scale males as densities in the orchards increased (up to 22, 495, and 140 male scale per week in sites A-C, respectively), but trap catches were 10–100 fold fewer males than traps baited with even the lowest dose of pheromone (1 μg). In addition, heptane-treated control traps were not reliable in defining the initiation or the peak of male emergence. In the low density scale population Site D, the heptane-treated control traps collected no male scale.

In all four locations, traps with doses of 25 or 50 μg pheromone caught similar numbers of male scale. Traps with doses of 1 or 5 μg pheromone generally caught fewer scale, but nevertheless, clearly showed the peaks and valleys of male yellow scale flight activity. In the San Joaquin Valley, there are usually four flights of male California red scale per year (Fig. 2). The first, second, and fourth flights are generally well-defined and are used for pest management decisions. For practical purposes, the lower doses of pheromone (1 or 5 μg) would be most appropriate for monitoring because they clearly delineate emergence of male scale, but reduce the cost of the pheromone per lure and the total number of scale counted. Traps with more than 20,000 male scale per card are time-consuming to process and difficult to accurately assess.

Lure longevity. At both the high (200 μg) and low (25 μg) dose rates, traps baited with fresh lures captured significantly more scale than those with field-aged lures on most dates throughout the 4 mo sampling period (Fig. 3). Differences between new and field-aged lures appeared to be most pronounced during periods of low population density (August to mid-September). At the end of the season, the lures that had aged for 4 mo were still catching considerable numbers of insects (> 100), and at the low dose level (25 μg) there was no statistical difference between old and new lures (Fig. 3). At the high dose level (200 μg), differences between treatments were significant, but old lures were still effective in monitoring phenology of male scales (Fig. 3). These data indicate an unusually long field life of yellow scale lures, suggesting that the typical grower practice of replacing lures in pheromone baited traps at approximately monthly intervals (Pehrson et al. 1991), or just prior to the estimated onset of male emergence, should provide consistent trap captures for monitoring purposes.

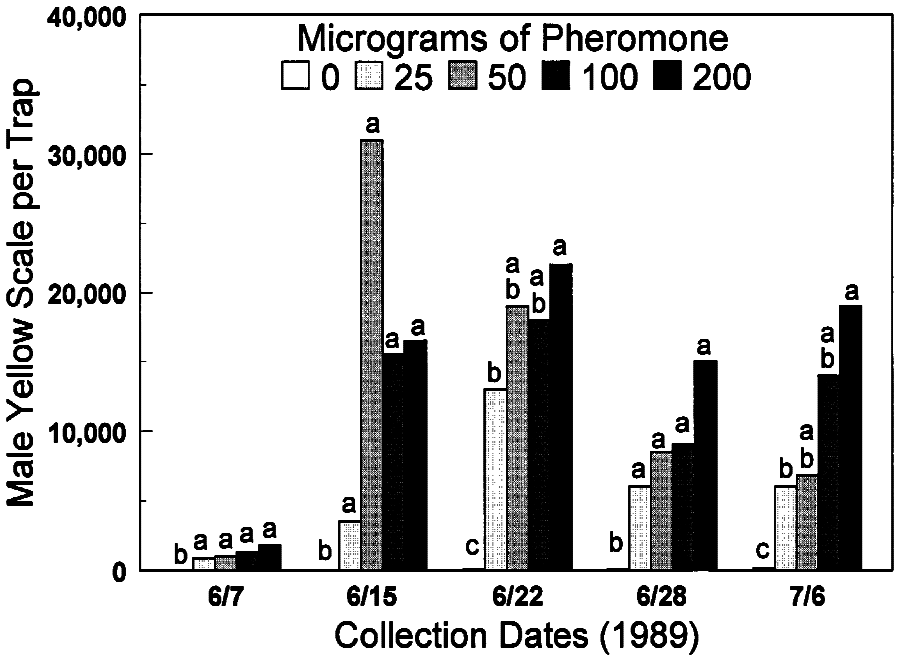

Fig. 1. Trap catches of male yellow scale on sticky cards baited with 0 or 25–200 μg of synthetic female yellow scale pheromone in 1989. Different letters above bars represent significantly different means for each sample date (REGW F-Test, $P < 0.05$).

Correlation of trap captures with male scale phenology. Initiation of 2nd, 3rd, and 4th male scale emergence for California red scale occurs at intervals of 636 DD (°C) [1145 DD (°F)] after the biofix of first male capture using a lower developmental threshold of 11.5°C (52.7°F) (Yu & Luck 1988). The lower developmental threshold for yellow scale has not yet been experimentally determined. Figure 4 shows the weekly male scale counts in 4 of 12 of the orchards sampled in 1994. Sites 1 and 3 had an abundance of both scale species, site 6 was dominated by yellow scale, and site 11 was dominated by California red scale. Initiation and peak emergence of males was similar for yellow scale and California red scale, suggesting that the two species have a similar lower developmental threshold and a similar growth rate. Using the California red scale lower developmental threshold and the first capture of male yellow scale as the biofix (8 March 1993 and 18 March 1994), the beginning of the 2nd, 3rd, and 4th male yellow scale flights (Fig. 2 and Fig. 4) can be estimated. Figure 2 shows that the beginning of the 2nd and 4th flights in orchards A-C and the 4th flight in orchard D closely matched the predicted dates of flight initiation during the weeks of 13 June and 4 September 1993 using this procedure. Figure 4 shows that, the beginning of the 2nd, 3rd and 4th flights in another set of four orchards in 1994 closely matched the predicted dates of 13 June, 23 July, and 31 August. The data suggest that pheromone traps provide a

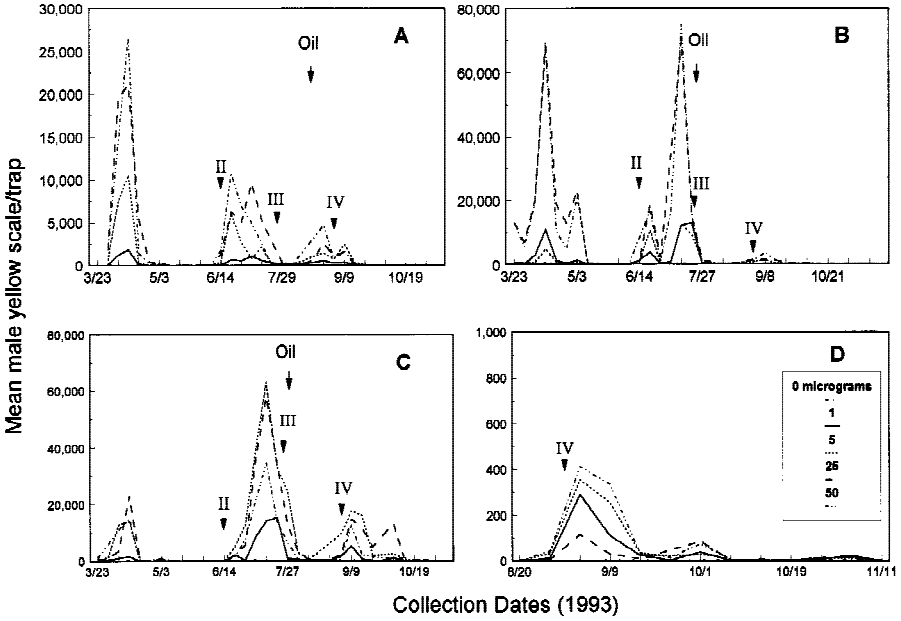

Fig. 2. Weekly captures of male yellow scale on traps baited with 1–50 μg of pheromone in four San Joaquin Valley citrus orchards (A, B, C, and D) in 1993. Roman numerals indicate the calculated initiation of the second, third, and fourth male flights based on degree days.

more reliable indicator of male emergence than degree days alone. In several cases, degree-day estimates of the initiation of emergence lagged behind observed increases in trap catches.

Accurate estimates of the initiation of each male flight are important, because male emergence is used as a biofix to predict major phenological events such as the 1st or 2nd generation of crawler emergence. Broad spectrum organophosphate insecticides are most effective if timed for peak crawler emergence (Walker et al. 1990) and degree day unit calculations can help improve the timing of these sprays. The first or second male emergence is also used as a biofix to estimate the beginning of the 4th male flight which is used by growers and their pest control advisors to estimate the severity of California red scale infestations in citrus orchards (Moreno & Kennett 1985, Pehrson et al. 1991). If more than 1,000 male scale are collected on a trap during the fourth flight, then an insecticide treatment is often recommended the following spring or summer. Frequently, the initiation or peak of the 2nd, 3rd or 4th flight is obscured because citrus growers use narrow range oil sprays or organophosphate insecticides to control armored scale or citricola scale and these sprays suppress male scale collections on trap cards (Fig. 2 and 4). In addition, the 3rd flight is often low in density and overlaps with the 4th flight at this time of the season. If any of these male flights are obscured, the grower can use initiation of the first male flight as the biofix to predict all subsequent events.

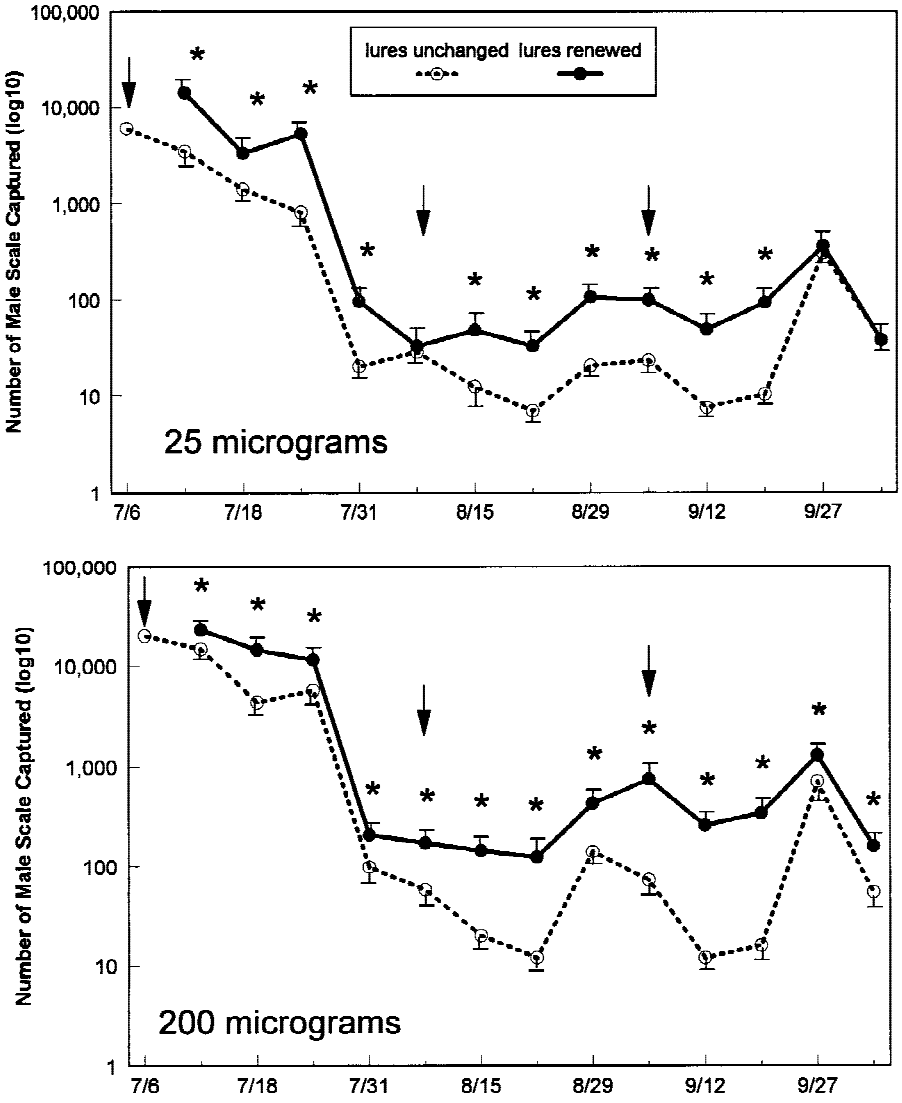

Fig. 3. Trap captures ($\log_{10}$ scale) of male yellow scale on sticky cards baited with 25 or 200 μg of synthetic yellow scale pheromone with and without monthly replacement of lures. Asterisks indicate significantly different means within dates and arrows indicate lure change dates.

Pheromone traps may provide information about the relative densities of the two scale species. In site 6, the population consisted of a high proportion of yellow scale relative to red scale. In site 11, the population had a higher proportion of California red scale. The two species were both abundant in sites 3 and 1.

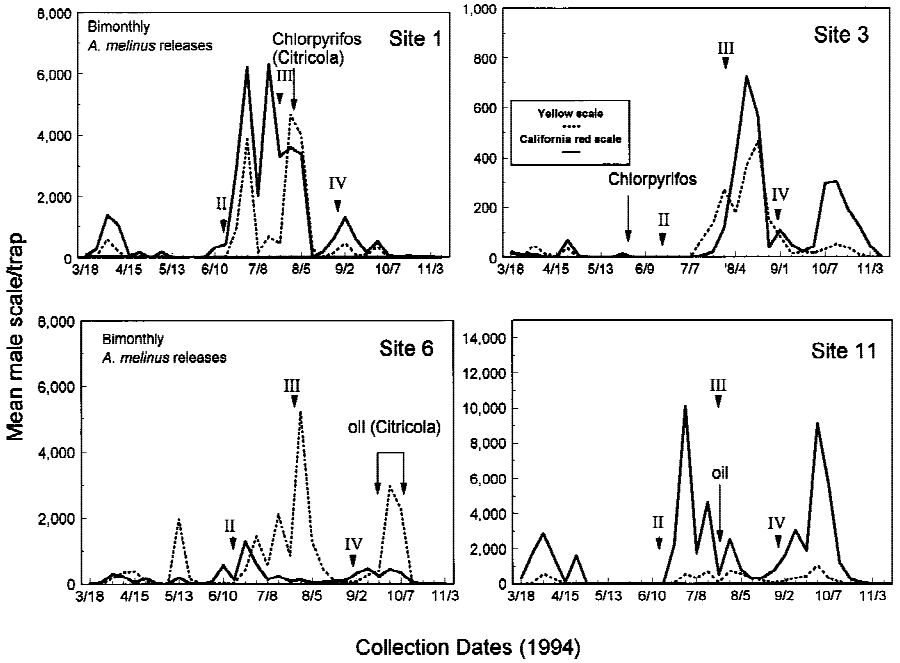

Fig. 4. Weekly captures of male scale on traps baited with 1 μg yellow scale or 120 μg California red scale pheromone in 4 San Joaquin Valley citrus orchards in 1994. Roman numerals indicate the estimated initiation of the second, third, and fourth male flights based on degree day units.

In 1994, pheromone cards in all 12 citrus orchards captured males of their respective species (Table 1) even though 3rd instar nongravid females were not detected on plant samples from two orchards. These data verify that the pheromone traps are effective in detecting the two armored scale species. Site 12 had such low densities of scale that none could be found infesting leaves or twigs, yet small numbers of males were caught in the pheromone traps, demonstrating the extraordinary sensitivity of males of the two scale species to their pheromones.

The proportions of yellow scale in the scale populations that were identified from scale-infested twig and fruit samples ranged from 0 to 86% (Table 1). In some cases, the proportion of females identified as yellow scale roughly reflected the proportion of yellow scale males captured (Sites 1-2, 4-5, and 7-12) and in other cases it did not (Sites 3 and 6). The lack of correlation ($r^2 = 0.20, y = 0.35x + 22.36$) could be due to the inaccuracy of measuring the relative densities of female scale because yellow scale generally do not infest twigs, while California red scale readily infests all plant parts. In addition, pest management practices often result in an uneven distribution of scale species within trees; since we sampled only the lower third of the outside of the tree, our samples may have been biased towards one species. Finally, the majority of citrus growers in this study were practicing a "soft" pesticide program that utilized releases of *A. melinus* and preservation of *C. comperiella*. Both of these parasitoids exhibit a high preference

Table 1. Densities of male scale collected on yellow scale (1 μg) and California red scale (120 μg) pheromone traps and 3rd instar females collected from plant samples in 12 San Joaquin Valley citrus orchards during 1994.

	Total male scale captured on pheromone traps during March-November 1994			3rd Instar females identified from plant samples		Maximum male scale/ trap card/week	
Orchard Nearest city	Yellow scale	California red scale	% yellow scale males	Total number identified	% yellow scale females	Yellow scale	California red scale
Site 1 Ivanhoe	17,200	35,404	32.7	21	28.6	4,661	6,300
Site 2 Ivanhoe	251	1,360	15.6	44	11.4	55	588
Site 3 Woodlake	2,060	3,229	38.9	21	85.7	465	725
Site 4 Porterville	34,053	77,210	30.6	34	73.5	7,093	16,239
Site 5 Porterville	4,573	19,234	19.2	60	6.7	1,715	2,560
Site 6 Porterville	21,564	6,162	77.8	23	4.3	5,231	1,283
Site 7 Terra Bella	28,945	12,365	70.1	15	73.3	5,275	2,915
Site 8 Terra Bella	30,949	15,553	66.6	52	71.2	9,351	3,019
Site 9 Terra Bella	8,129	129,301	5.9	44	0.0	1,633	24,586
Site 10 Terra Bella	7,815	41,548	15.8	64	26.6	1,844	8,079
Site 11 Terra Bella	6,699	54,558	10.9	38	15.8	1,039	10,081
Site 12 Terra Bella	3,185	557	85.1	0	0.0	690	126

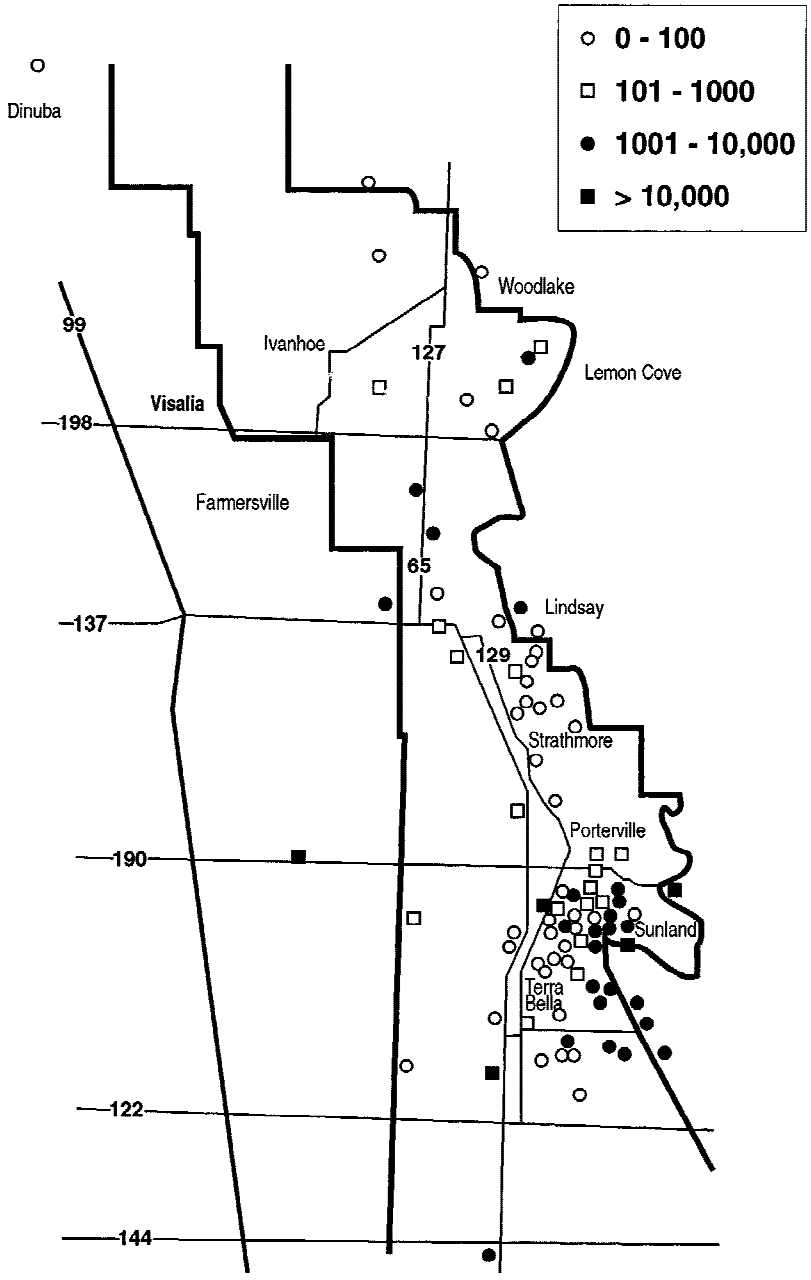

Fig. 5. Distribution of yellow scale in Tulare county, California in 1989. The major citrus-growing region is outlined with a bold line. Squares and circles correspond to the number of male yellow scale per trap caught at each location.

for 3rd instar female scale (Forster et al. 1995), the stage most reliable for identification of the scale to species, and so there were few unparasitized individuals of this stage available for identification.

Table 1 shows that the maximum number of scale caught on a trap during any week of the 1994 field season varied from site to site. However, the maximum number of scale collected was 9,351 using lures with 1 μg yellow scale pheromone and 24,586 scales using lures with 120 μg California red scale pheromone. Both of these maximum densities are in the range that can be reliably counted.

Survey for Yellow Scale Distribution

The distribution of yellow scale throughout the citrus-growing regions of Tulare County, California (as determined with pheromone traps baited with 100 μg/lure) varied widely from less than 100 male scale/trap to an estimated tens or even hundreds of thousands of scale/trap (Fig. 5). Occasionally, the sticky trap surface was completely covered with a layer of males several insects deep, making it impossible to count accurately. No region was completely free of yellow scale. The area between Porterville and Terra Bella had the highest densities of yellow scale. In the most heavily trapped area around Terra Bella, orchards with very high yellow scale populations were often adjacent to orchards with very low populations, suggesting that pest management practices used in different orchards strongly influenced population densities of the scale.

In summary, our results show that traps baited with yellow scale pheromone can be useful tools for making management decisions in California citrus orchards. The pheromone clearly has high biological activity at remarkably low doses, and pheromone lures remain attractive for extended periods under field conditions. Thus, traps set out to monitor flight phenology and time insecticide sprays require, at most, rebaiting before each emergence of males from March to November. The low dose and extended longevity of pheromone lures will encourage commercial development of the pheromone; with 5 or 1 μg doses, 1 gram of synthetic pheromone will provide 200,000 or 1 million lures, respectively! In addition, we have shown that the racemic form of the pheromone has strong biological activity, even though the insect only produces one of the two enantiomers. This will simplify and lower the cost of pheromone synthesis.

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Impact of Early-season Pesticide Treatments for Control of *Lygus lineolaris* (Hemiptera: Miridae) on Predators of *Helicoverpa zea* (Lepidoptera: Noctuidae) in Cotton[1]

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ABSTRACT The impact of early-season pesticide applications on beneficial arthropods necessary for control of mid- and late-season bollworms, *Helicoverpa zea* (Boddie), was determined in 1994 and 1995 by monitoring densities of arachnids, insidious flower bugs, and big-eyed bugs following treatment of plots with early-season pesticides. Densities of these arthropods decreased 1 d after treatment in most plots. At 3 and 7 d after treatment densities began to increase. Densities of arachnids in 1994 did not regain pre-treatment levels by 14 d after treatment. Arachnid densities in 1995, in select plots, exceeded pre-treatment levels 14 d after treatment. In 1994 and 1995, the densities of insidious flower bugs at 14 d after treatment surpassed pre-treatment densities. Densities of big-eyed bugs 14 d after treatment in 1994 and 1995, did not regain pre-treatment levels.

KEY WORDS Cotton, tarnished plant bug, bollworm, predators, insecticides, resurgence

In 1994, *Lygus* spp. ranked third among pests of cotton in the U.S., accounting for 1.16% of the total yield losses of 6.03% and $66 million in economic losses. Losses to *Lygus* spp. ranked behind the bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* F., complex (1.80%) and the boll weevil, *Anthonomus grandis* Boheman, (1.75%) (Williams 1995). Control of *Lygus* spp. is primarily accomplished through the use of organophosphate insecticides during the early growing season (Snodgrass & Elzen 1995).

Cotton plants are most susceptible to damage by the tarnished plant bug, *Lygus lineolaris* Palisot de Beauvois, early in the season, from the 4- to 6-leaf stage through the small or pinhead square stage. Uncontrolled movements of adult *L. lineolaris* from secondary hosts that occur during cotton bloom could significantly reduce the number of blooms produced through the growing season, therefore reducing crop yields (Scales & Furr 1968).

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The dependence on pesticides to control early-season cotton pests such as *L. lineolaris* leads to increases in pests and in the selection pressure for pest resistance (Scott et al. 1987, DeBach & Rosen 1991). Organophosphate resistance in cotton pests has resulted in the increased use of pyrethroids and the insecticidal bacterium, *Bacillus thuringiensis* (Berliner); late-season pests, such as the bollworm, and the tobacco budworm, can often be managed with these compounds. The use of organophosphates for management of early-season pests reduces the selection pressure and development of resistance to pyrethroids in pests that are present early in the season but increase to cause damage late in the season (Snodgrass & Elzen 1995).

Because synthetic chemical pesticides do not discriminate between pests and natural enemies, their application can be detrimental to populations of both. Resurgence of key pests and/or outbreaks of secondary pests can result from the elimination of natural enemies by pesticides (Bartlett 1966, Scott et al. 1987, DeBach & Rosen 1991, Johnson 1995). Beneficial organisms, along with pests, are killed and show sublethal effects from contact with insecticides (Croft 1990, England et al. 1997). Sterling et al. (1989) report that, in the San Joaquin Valley of California, organophosphate treatments for *Lygus* spp. had severe impacts on beneficial arthropod populations that aided in keeping *Heliothis* spp., the beet armyworm, *Spodoptera exigua* (Hübner), and the cabbage looper, *Trichoplusia ni* (Hübner), at acceptable levels.

Big-eyed bugs, insidious flower bugs, and arachnids are important for control of *H. zea* late in the season. These beneficial arthropods are present early in the season when treatments for *L. lineolaris* are applied. Utilization of selective insecticides may prove advantageous in controlling early-season pests, such as *L. lineolaris*, while allowing increases in beneficial arthropod numbers. The objective of this study was to determine the response of these beneficial arthropod populations to early-season cotton insecticides, applied for management of *L. lineolaris*.

Materials and Methods

Treatment application. Plots were arranged in a randomized complete block design with four blocks and seven treatments at the Milan Experiment Station (MES), Milan, Tennessee, in the summer of 1994 and 1995. Treatments (insecticides and rates) were: acephate 90S at 1.02 kg (AI) /ha, azinphosmethyl 2EC at 0.28 kg (AI) /ha, dicrotophos 8EC at 0.45 kg (AI) /ha, malathion 5EC at 1.42 kg (AI) /ha, methyl parathion 4EC at 0.28 kg (AI) /ha, oxamyl C-LV at 0.20 kg (AI) /ha and the untreated control. Plots were 16 rows wide and 30.5 m long. Treatments were applied on July 5, 1994, and June 26, 1995, using an IH 660 (Case IH, Racine, Wisconsin) highboy with a 4-row boom at 5 km/hr. The boom, equipped with three hollowcone TXVS 4 nozzles (Delavan, Lexington, Tennessee) per row with one nozzle over the row and two nozzles on drops, delivered 14.29 liters/min at 276 kPa.

Sampling arthropods. Beneficial arthropods were sampled using a 38-cm dia sweep net. On each sampling date, 50 sweeps per 30.5 m were collected from each of two rows per treatment. Samples were collected immediately before treatment, and 1, 3, 7 and 14 d after treatment. Border rows were left between treatments, and on each collection date samples were taken from rows not previously

sampled. Prior to treatment, samples were collected from rows 4 and 13; 1 d after treatment from rows 5 and 12; 3 d after treatment from rows 6 and 11; 7 d after treatment from rows 7 and 10; and, 14 d after treatment from rows 8 and 9. After sweeping, the net was quickly and carefully inverted, and the sample emptied into a cardboard container (3.7 liter), which held ethyl acetate-moistened paper towels and a collection label (i.e., test number, date, block, and treatment).

Processing samples. Samples were taken to the laboratory, where each sample was emptied into a dissecting pan. Plant material was removed and the contents of the pan were transferred through a funnel into a 20-ml scintillation vial containing the appropriate label. The interior of the funnel was rinsed into the vial with 70% ethyl alcohol until the vial was filled. Each vial was later emptied into a petri dish, and beneficial arthropods (arachnids, big-eyed bugs and insidious flower bugs) were counted. Data were log transformed and analyzed using General Linear Models (SAS 1989). Means were separated using least squares means pair-wise comparisons ($P \leq 0.05$).

Results

In 1994, arachnid densities were not significantly different among treatments on any of the post-treatment sampling dates (Table 1). Densities of arachnids in treated plots followed the same trend as those in untreated plots.

Arachnid densities in 1995 were lower than densities in 1994. After treatment, arachnid densities were significantly different at 7 and 14 d after treatment (Table 2). Seven days after treatment, methyl parathion-treated plots had significantly greater densities than other plots. The low residual effects of methyl parathion would possibly allow re-population sooner than in other treated plots. However, at 14 d after treatment, densities of arachnids in methyl parathion-treated plots were not significantly different from any other plot. Among insecticide treatments, densities of arachnids were not significantly different. Densities returned to pre-treatment densities, showing that reestablishment of the population in treated areas is feasible.

Overall numbers of big-eyed bugs collected during 1994 were low. More big-eyed bugs were collected in 1995 than in 1994. No significant differences among treatments were found 1, 3 and 7 d post-treatment (Table 3). Fourteen days post-treatment, densities of big-eyed bugs were significantly different in treated plots; however, none of the treatments had densities significantly different from densities in untreated plots. Significant differences in big-eyed bug densities were found 1 and 3 d after treatment (Table 4).

In 1994, densities of insidious flower bugs in treated plots followed the same trend as in untreated plots (Table 5). No significant differences were found among treatments for each sampling date until 14 d post-treatment. On this date, acephate-, dicrotophos-, and oxamyl-treated plots had significantly lower densities of insidious flower bugs than did the untreated, malathion-, and methyl parathion-treated plots. A reduction in prey densities due to the systemic nature of acephate, dicrotophos, and oxamyl, as well as insidious flower bug feeding on the plant, may have caused the decrease in densities of insidious flower bugs within these plots.

In 1995, average densities in the untreated plots dropped after the remaining plots were treated (Table 6). However, an increase in the average number of

Table 1. Impact of selected insecticides on densities of arachnids per 50 sweep-net samples (Milan Experiment Station, Milan, Tennessee, 1994).

	Sampling date				
Treatment	Pre-treatment July 5	1 DAT[a] July 6	3 DAT July 8	7 DAT July 12	14 DAT July 19
Untreated	2.13 ± 0.8b[b]	1.75 ± 0.5a	0.38 ± 0.2a	1.75 ± 0.7a	1.50 ± 0.4a
Acephate	2.63 ± 0.9ab	1.00 ± 0.3a	0.13 ± 0.1a	0.88 ± 0.4a	1.00 ± 0.4a
Azinphosmethyl	2.50 ± 0.5ab	0.88 ± 0.3a	0.00 ± 0.0a	0.75 ± 0.3a	1.00 ± 0.3a
Dicrotophos	3.00 ± 0.9ab	1.13 ± 0.3a	0.13 ± 0.1a	2.50 ± 0.6a	1.25 ± 0.6a
Malathion	2.13 ± 0.3ab	1.13 ± 0.3a	0.25 ± 0.2a	1.63 ± 0.4a	1.00 ± 0.5a
Methyl Parathion	3.63 ± 0.7a	1.88 ± 0.9a	0.00 ± 0.0a	1.63 ± 0.5a	1.63 ± 0.5a
Oxamyl	4.13 ± 0.9a	0.75 ± 0.3a	0.50 ± 0.3a	1.63 ± 0.7a	1.50 ± 0.4a

[a]DAT = days after treatment.
[b]Means (± SE) within a column followed by the same letter are not significantly different ($P > 0.05$). The letters following are from Least Square Means Pairwise Comparison Tests of the log transformed data.

insidious flower bugs was noted 1 d after treatment for the dicrotophos- and methyl parathion-treated plots, with these plots containing significantly greater densities of insidious flower bugs than the other plots. No significant differences were found 3 or 7 d after treatment. Malathion- and methyl parathion-treated plots had significantly greater densities of insidious flower bugs than all plots except the dicrotophos-treated plot at 14 d post treatment. The trend of these results at 14 d after treatment was similar between the 1994 and 1995 seasons.

Discussion

Table 2. Impact of selected insecticides on densities of arachnids per 50 sweep-net samples (Milan Experiment Station, Milan, Tennessee, 1995).

	Sampling date				
Treatment	Pre-treatment June 26	1 DAT[a] June 27	3 DAT June 29	7 DAT July 3	14 DAT July 11
Untreated	0.13 ± 0.1ab[b]	0.13 ± 0.1a	0.00 ± 0.0a	0.00 ± 0.0a	0.63 ± 0.4a
Acephate	0.13 ± 0.1ab	0.13 ± 0.1a	0.00 ± 0.0a	0.13 ± 0.3a	0.25 ± 0.2ab
Azinphosmethyl	0.00 ± 0.0a	0.13 ± 0.1a	0.00 ± 0.0a	0.25 ± 0.2a	0.25 ± 0.2ab
Dicrotophos	0.13 ± 0.1ab	0.00 ± 0.0a	0.13 ± 0.1a	0.25 ± 0.3a	0.25 ± 0.2ab
Malathion	0.63 ± 0.4b	0.25 ± 0.2a	0.00 ± 0.0a	0.38 ± 0.2a	0.50 ± 0.3a
Methyl Parathion	0.00 ± 0.0a	0.13 ± 0.1a	0.00 ± 0.0a	1.00 ± 0.3b	0.13 ± 0.1ab
Oxamyl	0.13 ± 0.1ab	0.00 ± 0.0a	0.38 ± 0.3a	0.13 ± 0.1a	0.00 ± 0.0a

[a]DAT = days after treatment.
[b]Means (± SE) within a column followed by the same letter are not significantly different ($P > 0.05$). The letters following are from Least Square Means Pairwise Comparison Tests of the log transformed data.

Table 3. Impact of selected insecticides on densities of big-eyed bugs per 50 sweep-net samples (Milan Experiment Station, Milan, Tennessee, 1994).

	Sampling date				
Treatment	Pre-treatment July 5	1 DAT[a] July 6	3 DAT July 8	7 DAT July 12	14 DAT July 19
Untreated	0.88 ± 0.3b[b]	0.25 ± 0.2a	0.00 ± 0.0a	0.50 ± 0.3a	0.38 ± 0.2ab
Acephate	1.13 ± 0.1a	0.25 ± 0.2a	0.00 ± 0.0a	0.13 ± 0.1a	0.75 ± 0.3ab
Azinphosmethyl	1.38 ± 0.4a	0.25 ± 0.2a	0.00 ± 0.0a	0.13 ± 0.1a	1.00 ± 0.3a
Dicrotophos	1.00 ± 0.3ab	0.25 ± 0.2a	0.00 ± 0.0a	0.38 ± 0.3a	0.13 ± 0.1b
Malathion	0.88 ± 0.5ab	0.50 ± 0.2a	0.00 ± 0.0a	0.13 ± 0.1a	0.25 ± 0.2b
Methyl Parathion	1.00 ± 0.5ab	0.00 ± 0.0a	0.00 ± 0.0a	0.50 ± 0.3a	0.50 ± 0.4ab
Oxamyl	1.25 ± 0.5ab	0.25 ± 0.2a	0.13 ± 0.1a	0.25 ± 0.2a	0.13 ± 0.1b

[a]DAT = days after treatment.
[b]Means (± SE) within a column followed by the same letter are not significantly different ($P > 0.05$). The letters following are from Least Square Means Pairwise Comparison Tests of the log transformed data.

Densities of beneficial arthropods in these studies were low, but were comparable to those cited by others (Gaines 1955, Dinkins et al. 1970, Roach 1980) considering that sweep-net samples underestimate numbers of both adult and immature insects (Byerly et al. 1978). These studies were conducted in isolated fields surrounded by woodlands as these predators were immigrating into and colonizing the fields. Early-season establishment of these generalist predators is dependent on a number of hosts including eggs and small caterpillars of bollworm, tobacco budworm, loopers and armyworms, whiteflies, plant bugs, aphids, mites and thrips. Big-eyed bugs also feed occasionally on plant sap (Knutson &

Table 4. Impact of selected insecticides on densities of big-eyed bugs per 50 sweep-net samples (Milan Experiment Station, Milan, Tennessee, 1995).

	Sampling date				
Treatment	Pre-treatment June 26	1 DAT[a] June 27	3 DAT June 29	7 DAT July 3	14 DAT July 11
Untreated	1.75 ± 0.7abc[b]	0.25 ± 0.3b	0.75 ± 0.3a	0.13 ± 0.1a	0.75 ± 0.4a
Acephate	1.75 ± 0.5ab	0.13 ± 0.1b	0.00 ± 0.0b	0.00 ± 0.0a	0.50 ± 0.2a
Azinphosmethyl	0.50 ± 0.3d	1.00 ± 0.3a	0.00 ± 0.0a	0.25 ± 0.2a	0.75 ± 0.3a
Dicrotophos	1.13 ± 0.6bcd	0.00 ± 0.0b	0.00 ± 0.0a	0.38 ± 0.3a	0.63 ± 0.4a
Malathion	1.63 ± 0.3abc	0.25 ± 0.2ab	0.00 ± 0.0a	0.50 ± 0.3a	1.00 ± 0.5a
Methyl Parathion	2.38 ± 0.6a	0.25 ± 0.2ab	0.13 ± 0.1ab	0.13 ± 0.1a	0.88 ± 0.4a
Oxamyl	1.25 ± 0.6ab	0.00 ± 0.0b	0.25 ± 0.2ab	0.25 ± 0.2a	1.25 ± 0.5a

[a]DAT = days after treatment.
[b]Means (± SE) within a column followed by the same letter are not significantly different ($P > 0.05$). The letters following are from Least Square Means Pairwise Comparison Tests of the log transformed data.

Table 5. Impact of selected insecticides on densities of insidious flower bugs per 50 sweep-net samples (Milan Experiment Station, Milan, Tennessee, 1994).

	Sampling date				
Treatment	Pre-treatment July 5	1 DAT[a] July 6	3 DAT July 8	7 DAT July 12	14 DAT July 19
Untreated	0.13 ± 0.1a[b]	0.25 ± 0.2a	0.00 ± 0.0a	0.38 ± 0.3a	1.75 ± 0.3a
Acephate	0.75 ± 0.4a	0.25 ± 0.2a	0.13 ± 0.1a	0.13 ± 0.1a	0.88 ± 0.3b
Azinphosmethyl	0.25 ± 0.2a	0.25 ± 0.2a	0.00 ± 0.0a	0.00 ± 0.0a	1.38 ± 0.5ab
Dicrotophos	0.50 ± 0.4a	0.25 ± 0.2a	0.13 ± 0.1a	0.00 ± 0.0a	0.75 ± 0.4b
Malathion	0.63 ± 0.4a	0.00 ± 0.0a	0.25 ± 0.3a	0.25 ± 0.2a	2.63 ± 0.8a
Methyl Parathion	0.25 ± 0.2a	0.13 ± 0.1a	0.13 ± 0.1a	0.13 ± 0.1a	2.38 ± 0.8a
Oxamyl	0.25 ± 0.3a	0.63 ± 0.4a	0.13 ± 0.1a	0.25 ± 0.3a	1.00 ± 0.5b

[a]DAT = days after treatment.
[b]Means (± SE) within a column followed by the same letter are not significantly different ($P > 0.05$). The letters following are from Least Square Means Pairwise Comparison Tests of the log transformed data.

Ruberson 1996). Plots adjacent to the study area in 1994 were infested with cotton aphid at 0.96 aphids/leaf (Lentz, unpublished data), but the study areas in both years were not infested with economic levels of bollworms until August. The immediate impact of these early-season insecticide applications to these highly-mobile beneficials was a reduction in overall numbers, even in the untreated plots. Beneficial arthropod numbers generally did not differ 14 d after treatment, except those of the big-eyed bug in 1994 which were reduced in the systemic dicrotophos- and oxamyl-treated plots. Ridgway (1969) reported that predator numbers were reduced 75% with the systemic aldicarb treatment, but less than

Table 6. Impact of selected insecticides on densities of insidious flower bugs per 50 sweep-net samples (Milan Experiment Station, Milan, Tennessee, 1995).

	Sampling date				
Treatment	Pre-treatment June 26	1 DAT[a] June 27	3 DAT June 29	7 DAT July 3	14 DAT July 11
Untreated	0.50 ± 0.3a[b]	0.00 ± 0.0b	0.00 ± 0.0a	0.00 ± 0.0a	0.63 ± 0.3bc
Acephate	0.00 ± 0.0b	0.00 ± 0.0b	0.00 ± 0.0a	0.00 ± 0.0a	0.13 ± 0.1d
Azinphosmethyl	0.13 ± 0.1b	0.00 ± 0.0b	0.00 ± 0.0a	0.13 ± 0.1a	0.63 ± 0.2bc
Dicrotophos	0.00 ± 0.0b	0.13 ± 0.1a	0.00 ± 0.0a	0.00 ± 0.0a	0.88 ± 0.2ab
Malathion	0.13 ± 0.1b	0.00 ± 0.0b	0.00 ± 0.0a	0.00 ± 0.0a	1.38 ± 0.3a
Methyl Parathion	0.13 ± 0.1b	0.38 ± 0.2a	0.00 ± 0.0a	0.00 ± 0.0a	1.25 ± 0.3a
Oxamyl	0.00 ± 0.0b	0.00 ± 0.0b	0.00 ± 0.0a	0.00 ± 0.0a	0.50 ± 0.2c

[a]DAT = days after treatment.
[b]Means (± SE) within a column followed by the same letter are not significantly different ($P > 0.05$). The letters following are from Least Square Means Pairwise Comparison Tests of the log transformed data.

20% with the foliar non-systemic azinphosmethyl treatment. Ables et al. (1983) reported that predator populations usually returned to near their original levels in ca. 14 d if no further applications were made.

This study illustrates that pesticide selectivity can have an effect on densities of natural enemies as was noted by other researchers (Sterling et al. 1989, Young et al. 1997, Parker 1999, and Ruberson & Tillman 1999). Overall, densities of arachnids, insidious flower bugs, and big-eyed bugs decreased 1 d after treatment and began to show increases 3 and 7 d after treatment. The densities of insidious flower bugs at 14 d after treatment in 1994 and 1995 exceeded pre-treatment densities. However, densities of big-eyed bugs 14 d after treatment in 1994 and 1995 did not regain pre-treatment levels.

The effect of a pesticide on an arthropod depends on the characteristics of that arthropod, i.e., habitat, life cycle, feeding behavior, and mating behavior (Croft 1990, Stark & Wennergren 1995). The responses of beneficials may be correlated to changes in prey densities resulting from treatment applications. Pesticides that are not harmful to generalist predators, such as insidious flower bugs and big-eyed bugs, may eliminate prey from cotton fields and cause these predators to move to a more suitable location (Croft 1990). This emigration may encourage a resurgence of primary or secondary pests.

Studies concerning the effects of insecticide treatments on beneficial arthropods are important in establishing IPM guidelines. Due to pesticide selectivity, a treatment that may be harmless to, or enhance beneficial populations, can be used. When determining the pesticides to be used, the biology of the natural enemy must be known, as well as the effects of environmental conditions on the natural enemy, the available prey, and the treatment to be applied.

Early-season management of pests in cotton is dependent upon the use of insecticides. If early-season insecticides deplete beneficial arthropod populations and restrict repopulation by these arthropods, late-season pests, such as the bollworm, are given a greater potential to survive and cause damage and loss within cotton fields.

This study shows that some pesticides may not pose a great threat to populations of beneficial arthropods in a cotton agroecosystem; however, studies support that populations of beneficial arthropods are affected in numerous ways by pesticides (Studebaker & Kring 1999). By knowing the pest situation and the natural enemies effective against that pest, the selection of appropriate pesticides can be made. By using selective insecticides, early-season insect control can be accomplished without affecting the densities of beneficial arthropods that aid in late-season pest control.

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Numbers of Rice Water Weevil Larvae (Coleoptera: Curculionidae) and Rice Plant Growth in Relation to Adult Infestation Levels and Broadleaf Herbicide Applications[1]

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ABSTRACT We conducted experiments to determine the numbers of rice water weevil (RWW, *Lissorhoptrus oryzophilus* Kuschel) larvae and the growth of rice (*Oryza sativa* L.) plants resulting from combinations of adult-RWW-infestation levels and broadleaf herbicide (bensulfuron or MCPA [2-methyl-4-chlorophenoxyacetic acid]) applications. In the greenhouse, bensulfuron (applied either before or after adult RWW were added to rice plants) did not affect the number of immature RWW or rice plant growth. In field plots, herbicides did not affect the numbers of immature RWW produced from various adult-RWW-infestation levels in either 1989 or 1990, but affected various measures of plant growth. In both 1989 and 1990, adult-RWW-infestation levels affected the number of immature RWW in plots and affected all (1989) or most (1990) rice growth characteristics. In 1990, rice plant growth measurements and yield generally declined sharply between 0 and 12 adult RWW added, but they declined gradually at higher infestation levels. Interaction between herbicides and adult-RWW-infestation level was not significant in either year, suggesting largely independent action of herbicides and RWW on plant growth and no moderating effect of herbicides on numbers of immature RWW. Our results underscore that RWW and broadleaf weeds are each important pests of rice in California, but the potential interaction from their management on immature RWW and corresponding rice plant growth appears to be of little relevance.

KEY WORDS rice, *Lissorhoptrus oryzophilus*, broadleaf herbicides, bensulfuron, 2-methyl-4-chlorophenoxyacetic acid

Invertebrate pests and weeds can seriously limit production of rice, *Oryza sativa* L., in California (Bayer et al. 1983, Grigarick 1984, Hill et al. 1994). The rice water weevil, (RWW) *Lissorhoptrus oryzophilus* Kuschel, is the major insect

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pest of rice in California. Only parthenogenic females are known in California (Grigarick 1984). Adults colonize paddies in the spring and deposit eggs in the leaf sheath of rice plants (Grigarick 1984). Maximum oviposition occurs within 2 weeks after rice emerges through the water (AAG, pers. obs.). Upon hatching, first instars mine the leaf sheath and then drop to the roots, where subsequent instars feed and may inflict economic damage (Grigarick & Beards 1965).

Broadleaf weeds are also major pests in California rice paddies, as they reduce the yield of rice plants through competition for light and nutrients (Bayer et al. 1983). Herbicides and proper water management are key components in broadleaf weed control in California (Hill et al. 1994, Williams et al. 1990). Bensulfuron and 2-methyl-4-chlorophenoxyacetic acid (MCPA) are two of the more commonly used herbicides for broadleaf weed control. Bensulfuron is optimally applied to seedling rice about 10 d after planting (DAP), whereas MCPA is optimally applied during tillering, or about 25 to 40 DAP (Bayer et al. 1983).

Management of broadleaf weeds in rice paddies can help prevent damage from insect pests such as the aster leafhopper (*Macrosteles fascifrons* Stål) and the western yellowstriped armyworm (*Spodoptera praefica* (Grote)) (Grigarick 1984, Way et al. 1984). Populations of these pests can build up early in the season on broadleaf weeds within paddies and then move to rice plants in July and August.

Similarly, in previous field research, we found that research plots receiving early season, broadleaf-weed control with bensulfuron had lower numbers of immature RWW and less rice plant damage (AAG, MJO and LSH, pers. obs.). We hypothesized that treating rice plots with bensulfuron may result in fewer RWW larvae (some direct or indirect effect of the herbicide on RWW); that rice can tolerate RWW infestation better when treated with herbicide; or that both effects occur. We report here on one greenhouse and two field experiments designed to test these hypotheses.

Materials and Methods

Greenhouse experiment. The greenhouse experiment was designed to test the effect of bensulfuron on numbers of immature RWW and rice plant growth when applied either before or after adult RWW were added to rice plants. The experiment was a randomized complete block design with three herbicide treatments (no bensulfuron applied, applied before adult RWW added, or applied after adult RWW removed) replicated in four blocks.

Rice, cv. 'M202', was planted (8 seeds per plastic container, 11-cm diam. × 14.3-cm tall) on April 17, 1989 in containers filled to 50% capacity with Stockton adobe clay and fertilized with 0.5 g ammonium sulfate 7 d before seeding. Containers were filled with water before seeding to simulate paddy conditions, and a water level of about 5 cm was maintained throughout the experiment. Plants were thinned on April 29 to 2 seedlings per container based on uniformity of seedling size.

Two field-collected RWW adults were caged within each container on May 5 and removed May 7. Bensulfuron was applied to four containers on May 4 and to another set of four containers on May 9 by a miniature aerosol sprayer at the recommended field-equivalent rate of 73 ml AI ha^{-1}. A third set of containers received no bensulfuron application.

Treatments were evaluated on June 2 by washing the soil from the roots of

each plant in 0.84-mm mesh screen cylinders (23-cm diameter). Immature RWW (larvae and pupae) were collected from the roots of the plants and in the screens. The number of immature RWW recovered per container was recorded. Rice plants were then measured for wet mass, shoot length, root length, the number of leaves and tillers, and the wet and dry mass of the roots. Immature RWW per container and rice plant growth data were analyzed separately with a one-way analysis of variance that included a blocking factor (PROC ANOVA, SAS Institute 1988).

Field studies. These studies were designed to test for the effects of herbicides, adult RWW infestation, and their interaction in a field environment on number of immature RWW and rice plant growth. Studies were conducted in 1989 and 1990 at the Rice Research Station near Biggs, CA. Rice, cv. 'M202', was seeded by airplane in twelve adjoining, flooded basins (4.6 × 61.0 m), each having independent water inflow and exit. Basins received commercial fertilizers 24 to 25 days before seeding. Water level in the basins was maintained at a depth of about 10 cm until a few weeks before harvest. Plots were treated 14 DAP each year with copper sulfate (11.2 kg AI ha $^{-1}$) to control algae and with molinate (Ordram™, 4.5 kg AI ha $^{-1}$) to control grassy weeds.

The experiment was set up each year as a split-plot design. A basin represented one main plot, and each main plot received one of three herbicide treatments (none, bensulfuron, or MCPA). A group of three adjacent basins that included all herbicide treatments represented one replicate block, with four blocks per experiment. Herbicide treatments were randomized within each block and applied with a CO_2-pressured backpack sprayer having a 4.1-m boom width. Bensulfuron (Londax™) was applied 9 or 15 DAP in 1989 and 1990, respectively, at a rate of 73 ml AI ha $^{-1}$. MCPA 4EC was applied 35 DAP in 1989 at a rate of 0.9 liters AI ha $^{-1}$ and 40 DAP in 1990 at 1.2 liters AI ha $^{-1}$.

Subplots were established by placing two (1989) or four (1990) 0.73-m^2 metal rings about 4 m from one end of each basin (main plot). Subplot treatments consisted of infesting various densities of field-collected adult RWW for subsequent oviposition in rice plants. In 1989, we added 0 or 50 RWW per ring 22 DAP. In 1990, we added 0, 12, 25, or 50 RWW per ring 20 DAP. Subplot treatments were randomized within each basin. Rice plants were thinned to 70 seedlings per ring just before adding adult RWW. Carbofuran (1.12 kg AI ha^{-1}, Furadan 5G™) was applied before seeding to the 0-RWW rings each year and also 22 DAP in 1989 to eliminate naturally occurring infestations of RWW.

To determine the effect of treatments on the number of immature RWW and rice plant growth parameters, we removed 10 cores of soil (10 by 10 cm) from each ring 4 weeks after infestation. Cores typically contained 2 rice plants and were washed free of soil in 0.84-mm mesh screen cylinders (23-cm diameter). Immature RWW were collected from the roots of the plants and in the screens. The number of immature RWW recovered per container was recorded. Rice plants were taken to the laboratory and measured for shoot and root length, wet mass of the entire plant, number of leaves and tillers, and the wet and dry mass of the roots. Rice plants remaining within each ring were hand-harvested at maturity in October each year. Grain was dried at 20°C for 3 weeks before weighing to determine yield.

Variances were calculated for the numbers of immature RWW and for plant growth data within the main plot and subplot treatments. Variances for the main plot and subplot treatments were tested for homogeneity using Cochran's *F* test

(Beyer 1968). For treatments in which a null hypothesis of homogeneity was rejected, data were square root- or log-transformed so that the variances were homogeneous. Data were then analyzed using an ANOVA for split-plot design (PROC ANOVA, SAS Institute 1988). Herbicide treatment effects were tested using the main plot mean square error (MSE), and adult-RWW-infestation level effects were tested by using the subplot MSE (Little & Hills 1978). Main plot means of plant growth characteristics or yield were separated by calculating a least significant difference (LSD) among herbicide treatments (Little & Hills 1978).

Results

Greenhouse experiment. Applications of bensulfuron did not affect the number of immature RWW recovered or any plant growth parameters (Tables 1 and 2). These results showed that bensulfuron applications made before adding or after removing adult RWW are absent did not affect their ability to oviposit, did not affect the survivorship of immature RWW, or both. Failure of bensulfuron treatments to affect rice plant growth was expected as weeds were absent in the culture pots.

Field studies. *1989*. The number of immature RWW in main plots did not differ significantly among herbicide treatments (Table 1). However, herbicide treatments did affect plant height, root length, the number of leaves, number of tillers, and yield of rice plants. Plants were taller in bensulfuron-treated plots and shorter in MCPA plots than in untreated plots (Table 3). Roots were shorter in MCPA-treated plots than in untreated or bensulfuron-treated plots. Rice plants in bensulfuron plots had more leaves and tillers and yielded more than those in the MCPA or untreated plots. Rice plants in MCPA plots yielded more than those in untreated plots.

As expected, adult RWW-infested subplots had more immature RWW than non-infested ones (Tables 1 and 3). As a consequence, most rice plant growth characteristics were significantly reduced in RWW-infested plots (Table 3). Interaction between herbicide and adult RWW treatments was not significant for any of the variables (Table 1), suggesting that herbicide and adult RWW treatments independently affected rice plant growth, and that the herbicides did not modify the number of immature RWW resulting from adult RWW infestations.

1990. The number of immature RWW did not differ among herbicide treatments (Table 1). Herbicide treatments affected the number of leaves but not other measured growth characteristics of rice plants (Tables 1 and 4). MCPA plots had fewer leaves than untreated or bensulfuron plots.

Rice plant growth characteristics were reduced in infested plots, with reductions generally more severe at higher adult-RWW-infestation levels (Table 4). Rice plant growth measurements and yield generally declined sharply between 0 and 12 adult RWW added, but declined gradually at higher infestation levels. The decline in the number of immature RWW was paralleled by a decline in root mass at higher adult-RWW-infestation levels (Table 4). Apparently, plants with less root mass supported fewer immature RWW, regardless of adult-RWW-infestation levels. The interaction between herbicide and RWW treatments was not significant for any of the variables measured (Table 1), suggesting independent action the herbicide and adult RWW treatments as in 1989.

Table 1. *F* values from ANOVAs for the number of immature rice water weevils (RWW) and various rice plant growth characteristics.

Experiment	Factor (df)	No. immature RWW[a]	Wet mass of 10 plants	Plant height	Root length	No. leaves	No. tillers per plant	Wet mass of root	Dry mass of root	Dry mass of grain (yield)
Greenhouse[b]	Bensulfuron (2, 6)	1.79	0.56	1.47	1.52	1.87	0.83	1.59	0.40	—[c]
	Block (3, 6)	2.12	2.24	1.86	0.13	0.78	0.02	1.35	0.27	—[c]
Field 1989[d]	Herbicide (2, 6)	1.48	4.36	31.31*	33.73*	7.40*	9.14*	1.72	1.89	9.99*
	Adult RWW (1, 9)	596.35*	18.50*	81.15*	30.59*	21.47*	14.52*	3.38	2.28	38.96*
	Herbicide X Adult RWW (2, 9)	0.51	0.02	0.95	0.24	0.31	0.26	0.19	0.45	2.47
Field 1990[d]	Herbicide (2, 6)	4.48	0.02	0.24	1.05	6.98*	0.07	0.33	0.46	5.03
	Adult RWW (3, 27)	220.93*	96.87*	187.66*	55.55*	98.01*	101.13*	24.92*	20.23*	77.26*
	Herbicide X Adult RWW (6, 27)	1.05	1.69	0.96	0.57	0.85	2.02	2.37	2.03	1.62

*F value significant ($P < 0.05$).

[a]Number of immature RWW per two plants. RWW field experiment data were transformed: 1989, $x' = \log (x + 1)$; 1990, $x' = (x + 0.5)^{1/2}$.

[b]Randomized complete block design and two-way analysis of variance (ANOVA).

[c]Not measured.

[d]ANOVA performed for a split plot design with herbicide treatments randomized within blocks and RWW treatments randomized within herbicide plots.

Table 2. Means of the number of immature rice water weevils (RWW) and various rice plant growth characteristics, greenhouse experiment, Davis, 1989.

Bensulfuron application	Immature RWW per container	Wet mass of 10 plants (g)[a]	Plant height (cm)	Root height (cm)	No. leaves	Tillers per plant	Root wet mass (g)	Root dry mass (g)
None	11.3NS	3.6NS	27.3NS	6.8NS	4.8NS	1.5NS	0.88NS	0.13NS
Before adult RWW added	6.7	3.2	25.7	5.5	4.7	1.3	0.80	0.13
After adult RWW removed	12.8	3.4	27.6	5.9	3.8	1.1	1.05	0.10

[a]One plant measured per container (replicate).
NS = means within a column do not differ significantly.

Table 3. Means for the number of immature rice water weevils (RWW) and various rice plant growth characteristics from a field plot experiment near Biggs, 1989.

Treatment	Immature RWW per 10 cores[a]	Wet mass of 10 plants (g)[b]	Plant height (cm)	Root height (cm)	No. leaves	Tillers per plant	Root wet mass (g)	Root dry mass (g)	Dry mass of grain (kg, yield)[c]
Herbicide									
None	50.4NS	161.0NS	48.2b	13.6a	19.4b	4.8b	42.9NS	6.5NS	0.43c
Bensulfuron	42.3	191.4	51.7a	13.1a	23.6a	5.7a	52.6	7.6	0.59a
MCPA	50.3	166.5	44.9c	9.2b	18.4b	4.7b	55.8	8.6	0.48b
RWW adults ring^{-1}									
0	0.6a	206.5a	52.0a	13.9a	23.5a	5.7a	55.6NS	8.1NS	0.58a
50	94.7b	139.4b	44.5b	10.0b	17.4b	4.4b	46.7	7.1	0.42b

[a]Two plants per soil core.
[b]One plant measured per core.
[c]Per 0.73 m^2 ring.
Means within columns and treatment groups followed by a different letter are significantly different according to Fisher's LSD studentized range test ($P < 0.05$); NS = not significant.

Table 4. Means for the number of immature rice water weevils (RWW) and various rice plant growth characteristics for a field plot experiment near Biggs, 1990.

Treatment	Immature RWW per 10 cores[a]	Wet mass of 10 plants (g)[b]	Plant height (cm)	Root length (cm)	No. leaves	Tillers per plant	Root wet mass (g)	Root dry mass (g)	Dry mass of grain (g, yield)[c]
Herbicide									
None	59.9NS	80.3NS	39.8NS	9.9NS	11.3 a	2.8NS	21.1NS	3.0NS	290.1NS
Bensulfuron	44.6	79.4	39.1	8.6	11.0a	2.7	18.5	2.7	371.4
MCPA	48.8	81.1	38.7	8.4	9.4b	2.7	21.8	3.1	274.8
RWW adults ring^{-1}									
0	0.3a	187.2a	55.4a	15.1a	21.5a	5.1a	35.5a	5.0a	673.8a
12	79.8c	67.4b	37.7b	8.8b	9.3b	2.6b	22.2b	3.1b	300.6b
25	66.8bc	41.6bc	33.3c	7.0c	6.5c	1.8c	14.5c	2.3bc	183.6c
50	57.6b	24.9c	30.5d	5.0d	4.9d	1.5c	8.8c	1.4c	90.3d

Means within columns and treatment groups followed by a different letter differ significantly according to Fisher's LSD test ($P < 0.05$); NS = not significant.
[a]Two plants per soil core.
[b]One plant measured per core.
[c]Per 0.73 m^2 ring.

Discussion

Herbicides had a greater effect on plant growth characteristics in the field in 1989 than 1990. We expected this, as weed pressure in field plots was considerably greater in 1989 than 1990, although we did not quantify the difference in weeds between years. Thus, the effect of herbicides in protecting rice plants from weed competition was more pronounced in 1989. The predominant weed species each year were *Heteranthera linosa* (ducksalad) and *Scirpus* spp. (bulrush), along with relatively light infestations of *Sagittaria* spp. (arrowhead).

The highest infestation level of RWW (50 adults per ring) appeared to be more damaging to rice plants in 1990 than in 1989, and this difference corresponded with a greater production of immature RWW in 1989 than 1990 when 50 adult RWW were placed in the ring (Tables 3 and 4). Undetermined factors may have been responsible for differences in the severity of damage between years, although we note the parallel declines in the number of immature RWW and rice root mass with progressively greater adult-RWW-infestation levels seen in 1990 (Table 4). Apparently, plants with less root mass supported fewer immature RWW, regardless of adult-RWW-infestation levels, and despite randomization, smaller plants may have been assigned to higher adult-RWW-infestation levels. Sampling of RWW larvae and plant growth at earlier plant growth stages would have allowed us to determine if this trend actually occurred, and earlier sampling may be applicable to similar studies in the future to measure separately rates of oviposition and survivorship of immatures.

We failed to find a moderating effect of herbicides on numbers of immature RWW produced by adult RWW (i.e., no interaction). We are also unaware of any studies showing that rice herbicides are toxic or repellent to RWW. The potential for a herbicide to directly affect RWW depends on the timing of applications that bring herbicide and RWW into contact. Timing of herbicide applications (before or after placement of adult RWW) did not make a difference in the outcome of our experiments. MCPA was applied after peak infestation of adult RWW in the field plots, and adult RWW would not have come into contact with the sprays or residues of MCPA. Bensulfuron was generally applied before adult RWW were added, but in one of the greenhouse treatments, bensulfuron was applied after RWW had oviposited and were removed. The timing of bensulfuron applications a few days before RWW infestation, as in three of our treatments, simulates typical field conditions. Slight deviations in the timing of these factors (e.g., delayed bensulfuron application, earlier colonization of rice fields by RWW, or both) can occur in the field and may be more likely to bring adult RWW into contact with bensulfuron. The effect of such deviations on RWW infestation levels and the subsequent damage to rice plants is unknown but may be of interest for future experiments.

Finally, we had thought that rice plants might tolerate RWW infestation better in plots treated with herbicides (particularly bensulfuron), as these reduce the additional stress of weed competition. However, we failed to find significant interaction between herbicide treatments and adult-RWW-infestation levels when we measured various rice plant growth characteristics. Thus, it appears that herbicides and RWW infestations independently influence rice plant growth and yield, given the experimental restrictions noted above. Our results underscore that RWW and broadleaf weeds are each important pests of rice in California

(Bayer et al. 1983, Grigarick 1984). Respective management of each by use of insecticides and herbicides is beneficial, but the potential interaction of these management tactics on immature RWW and corresponding rice plant growth appears to be of little relevance.

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Averting-Cost Measures of the Benefits to South Carolina Households of Red Imported Fire Ant Control[1]

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ABSTRACT A telephone survey of South Carolina households was conducted between November 1998 and January 1999 to gather information on the households' experiences with the red imported fire ant, *Solenopsis invicta* Buren. Fifty-four percent of the 809 respondents reported cash expenditures and/or household time for control and remediation. The estimated mean of annual cash expenditures for this control and remediation is $80.37 per household. When household time used in control and remediation is valued at the minimum wage of $5.15 per hour, the red imported fire ant is estimated to cost an average of $100.93 per household in cash expenditures and opportunity costs of household time.

KEY WORDS Hymenoptera: Formicidae, *Solenopsis invicta*, red imported fire ant, household expenditures

The red imported fire ant (RIFA), *Solenopsis invicta* Buren (Hymenoptera: Formicidae), is believed to have been introduced by accident to Mobile, Alabama, in the 1930s via ship ballast from South America. The RIFA has since spread throughout the South and is now established in Texas, Oklahoma, Arkansas, Louisiana, Mississippi, Tennessee, Alabama, Florida, Georgia, and North and South Carolina, according to information obtained through the National Agricultural Pest Information System (http://www.ceris.purdue.edu/napis/pests/ifa/imap/ifaall.html). Households in these states are impacted adversely by RIFA in several ways. First, RIFA poses a health threat, as it is aggressive and has a venomous sting. Based on a survey of South Carolina physicians, Caldwell et al. (1999) estimated 660,000 cases of RIFA stings in the state in 1998, a rate of 33.8 per 10,000 population. Of these, an estimated 33,000 individuals sought medical treatment, and there were two reported deaths. To avoid RIFA stings, household members might have to forgo outdoor leisure activities (e.g., gardening, playing in the yard). Second, RIFA causes property damage in and around residences. Tunneling activity by RIFA can undermine the foundations of hardscape (e.g., sidewalks and driveways) and buildings. Also, RIFA readily infests electrical devices (e.g., water pumps and kitchen appliances) and can damage their motors and

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switches. Households incur costs in treating and preventing RIFA infestations and in repairing RIFA damage in and around their residences.

Households benefit from government-sponsored research, extension, and regulatory programs to control RIFA and to prevent its spread to new areas. Because these government programs incur costs, the potential benefits of RIFA control must be weighed against program costs. Several previous studies have provided estimates of the household benefits and/or cost savings from improved fire ant control. Lemke & Kissam (1989) surveyed individuals who visited booths providing RIFA-related information at various South Carolina public gatherings. When the 430 respondents were asked how much they were willing to pay per year for RIFA control, 4% indicated $0, 22% indicated $1.00 to $10.00, 57% indicated $10.01 to $30.00, and 17% indicated more than $30.00. Individuals with RIFA-related problems would be more likely to visit the information booths than individuals without such problems; thus, the results might inflate the extent of RIFA-related problems in South Carolina. Diffie et al. (1991) surveyed visitors to a RIFA information booth at an agricultural exposition in Georgia and employees of an agricultural experiment station in southern Georgia. Based on the pooled sample data (320 respondents), the mean household RIFA-related expenditure was $35.26 per year. As with the South Carolina survey, the visitors to the RIFA information booth might not constitute a random sample of the general population. Thompson & Jones (1994) and Thompson et al. (1995) surveyed households in three counties in southern Arkansas, using Cooperative Extension Service mailing lists. Based on a sample of 325 households, they estimated mean annual RIFA-related costs of $87.10 for urban households, defined as households with 0.40 hectares (one acre) of land or less; and $298.00 for rural households, defined as households with more than 0.40 hectares (one acre) of land, with the costs for the latter households including RIFA damage to crops and livestock. Whether the households included in the Arkansas survey constitute a random sample is unknown. These households might be on the mailing lists because of previous contacts with extension personnel concerning RIFA problems. Salin et al. (2000) conducted an area-frame survey of households in five metropolitan areas in Texas. The mean annual household RIFA-related expenditure was $151.

The purpose of this research is to estimate the potential benefits that would accrue to South Carolina households from RIFA control. A random sample of South Carolina households was surveyed by telephone to determine the households' experiences with RIFA and the expenses they incurred for RIFA control and remediation. To the authors' knowledge, this is the first statewide-survey based on random sampling techniques to determine households' experiences with RIFA. Information from the survey is used to estimate a lower bound on the benefits of RIFA control to South Carolina households.

Materials and Methods

From the household perspective, RIFA can be thought of as a form of environmental pollution. Approaches used to assess the benefits of environmental improvements (e.g., improved air or water quality) can be adapted to measure the benefits of RIFA control. Contingent valuation is one such approach (Mitchell & Carson 1989). People are asked how much they would be willing to pay for a specified environmental improvement if they were able to purchase the improve-

ment in a market. Summing the individuals' willingness to pay responses gives an estimate of the aggregate benefits of the environmental improvement.

An alternative is the "averting cost" or "defensive expenditures" approach, which provides an indirect estimate of willingness to pay by measuring the individuals' expenditures to avert or defend against the pollution effects. The household production model provides the theoretical underpinnings for the averting-cost approach. Households are assumed to produce goods for consumption, using purchased goods, household inputs (e.g., time and technology), and other items such as personal environmental quality. If an input such as environmental quality is degraded (e.g., by a RIFA infestation), more of other inputs (e.g., RIFA insecticides, professional pest control services, and household members' time) must be used to produce ultimate consumption goods.

The "averting costs" or "defensive expenditures" provide a lower bound on the willingness to pay for the environmental improvement under certain conditions (Abdalla et al. 1992, Bartik 1988, Laughland et al. 1993; 1996). These conditions are the following: (a) households incur no significant adjustment costs in reducing the level of investment in defensive measures; (b) households do not derive any direct utility from averting behavior; (c) there is no joint production, so that averting measures are not used as inputs in the production of other consumption goods; and (d) government programs can provide the environmental improvement. Households do invest in equipment such as insecticide sprayers and spreaders to control RIFA, but this equipment represents an inconsequential portion of the assets of most households. Household members would not be expected to obtain satisfaction from RIFA control and remediation activities. Observed RIFA-related averting measures appear to be specific to the RIFA problem, so joint production should not be present. Finally, it seems plausible that government programs are capable of mitigating RIFA-related problems. Thus, the conditions under which the averting-cost approach can be used to derive a lower bound for willingness to pay for an environmental improvement appear to be met for the RIFA problem, and the use of averting-cost measures of the benefits of RIFA control is defensible on theoretical grounds.

A random sample of South Carolina households was contacted by telephone between November 1998 and January 1999, using the Computer-Assisted Telephone Interview Laboratory, Department of Sociology, Clemson University. The survey relied on an instrument used by Salin et al. (2000) in their survey of Texas households. The authors modified the instrument to meet the objectives of the current research. The 35-question instrument solicited information on households' experiences with RIFA, their expenditures on RIFA control and remediation, and their socio-economic characteristics. Participants were asked to list the cash expenses they incur in an average year for RIFA control and remediation. The expenditures were grouped into the following categories:

- treatment expenses including materials (e.g., insecticide mound treatments, baits, and home remedies), equipment (e.g., sprayers, spreaders, and injection equipment), supplies (protective clothing, gloves, and repellent sprays), and professional services (e.g., lawn maintenance and pest control companies), labeled TREAT;
- repair expenses for electrical equipment (e.g., well pumps, appliances, air conditioners, and televisions), labeled ELECT;

- other repair expenses (e.g., gardens, landscape, sidewalks, and driveways), labeled OTHER;
- medical treatment expenses, labeled MED; and
- veterinary treatment expenses for household pets, labeled VET.

Some survey respondents indicated that they had incurred cash expenses in RIFA control and remediation, but were not able to estimate dollar amounts. In order to provide a conservative estimate of RIFA-related cash expenses, the missing cash expense amounts were set to zero. The respondents also were asked to estimate the household time spent treating RIFA and repairing RIFA damage, labeled HOURS. Some respondents indicated that household time was spent in RIFA control and remediation, but were unable to estimate the hours spent in those activities. The variable HOURS was set equal to zero for these households.

Two measures of RIFA-related averting costs were calculated for each household. The first measure ignores the opportunity cost of household time spent in RIFA control and remediation and was computed as the sum of the cash expenditures:

$$AC1_i = TREAT_i + REPAIR_i + OTHER_i + MED_i + VET_i,$$

where i indexes households. The second measure includes the opportunity cost of household time valued at the current minimum wage of $5.15 per hour, and was computed as:

$$AC2_i = AC1_i + TIME_i,$$

where $TIME_i = 5.15*HOURS_i$.

A double-hurdle model was used to explain households' RIFA-related expenditures, measured alternatively as either AC1 or AC2. Almost one-half of the sample values of AC1 and AC2 equal $0. Application of ordinary least squares (OLS) in estimation of a model explaining AC1 or AC2 would be inappropriate because the OLS estimators are biased regardless of whether all values or only positive values of the dependent variable (AC1 or AC2) are used in estimation (Kennedy 1998). As discussed by Greene (1997), the double-hurdle model overcomes this problem. The double-hurdle model consists of two equations: a decision equation to explain whether the household makes RIFA-related expenditures, estimated as a probit model; and an equation to explain the level of RIFA-related expenditures for households that decide to make those expenditures, estimated as a truncated regression model. Estimation was carried out using the maximum likelihood procedures of LIMDEP (Greene 1998).

Results and Discussion

Out of a random sample of 861 household representatives contacted, 809 (94%) agreed to participate in the survey.[4] At least two residents from each county took part in the survey. Charleston County had the largest number of respondents with 60, followed by Greenville and Richland with 56 respondents each. As discussed in more detail below, the spatial distribution of the sampled households

[4]The 861 households contacted represented approximately 60% of the total telephone calls. The other numbers called resulted in no answer, a busy signal, an answering machine, or information that the telephone number was no longer in service.

corresponds to the spatial distribution of the state's households. Selected characteristics of the respondent households follow:

- 61.1% of the respondents were female,
- 46.7% of the respondents indicated the head of the household was male,
- 68.7% of the respondents classified themselves as married,
- the mean age of the household head was 53.3 years (95% confidence interval for the mean = CI = (mean) ± 1.1 years).
- the mean number of household members was 2.8 (CI = ± 0.1 members),
- 81.3% of the respondents classified themselves as white, 17.2% as black, and 1.5% as other,
- 18.0% of the respondents reported an annual household income of less than $20,000 (low income), 44.9% reported an annual household income from $20,000 to $50,000 (middle income), and 37.1% reported an annual household income greater than $50,000 (high income),
- the highest level of educational attainment of the household head was: high school diploma or less, 35.1%; some college or technical school, 19.2%; college or technical school diploma, 32.8% and; graduate or professional degree, 12.8%,
- 67.8% of the respondents said they were native South Carolinians,
- 84.8% of the respondent households owned their residences,
- 91.9% of the respondents lived in detached dwellings (houses or mobile homes), 3.4% lived in apartment buildings, and 3.4% lived in townhouses or condominiums, and
- 6.1% of the respondent households had no residential lots, 18.0% had residential lots of less than ½ acre (0.20 ha), 37.2% had residential lots between ½ and 1 acre (0.20 and 0.40 ha), and 38.7% had residential lots larger than 1 acre (0.40 ha).

Based on data for the South Carolina population from the 1990 Census (U. S. Bureau of the Census 1992), middle and high income, white and married households who live in their own homes are over-represented in our sample. On average, heads of the respondent households are older than household heads in the South Carolina population, and have a higher level of educational attainment than adults in the population.[5] However, these characteristics are typical for telephone survey research (Babbie 1992). Women are more likely to answer the phone at home and more likely to participate in surveys than are men. Whites tend to be more comfortable than other races in participating in telephone survey research. Also, participation rates in telephone surveys tend to be higher for individuals with higher levels of education and income.

Respondents were asked whether RIFA was now a problem in their residences or yards. Thirty-two percent considered RIFA somewhat of a problem and 14%

[5]Using the Consumer Price Index for all urban consumers in the South region (available at http://stats.bls.gov/) to express the income distribution data from the 1990 Census in terms of 1998 dollars, the state's household income distribution is low income, 28%; middle income, 40%; and high income, 32%. Whites and blacks constituted 73% and 26%, respectively, of the South Carolina population in 1990. In 1990, 46% of the state's households classified themselves as married, and 70% of the households lived in their own homes. Among the households represented in the survey, 32% had heads 44 years of age or younger, and 65% had heads with some education beyond high school. In 1990, 49% of the state's households heads were 44 years of age or younger, and only 45% of the state's population 18 years of age or older had some education beyond high school.

considered RIFA a serious problem. When asked whether RIFA had been a problem in their residences or yards in the past, 43% indicated RIFA had been somewhat of a problem and 16% indicated RIFA had been a serious problem. The percentage of respondents indicating that RIFA is a serious problem is lower in this survey than in the survey of Lemke & Kissam (1989), where 87% indicated that RIFA was a serious problem.

Table 1 provides summary statistics for the averting cost-measures by the South Carolina regions shown in Figure 1.[6] There is a close correspondence between the regional distribution of the sample households and the estimated regional distribution of households for 1996 from the 1990 Census (U. S. Bureau of the Census 1992). The North West region is the only region in the state in which less than 50% of the sampled households had expenses associated with RIFA control and remediation. RIFA has spread to the North West region only in the last few years, so these results are not surprising. Among the other regions, the West Central had the highest percentage (71.2%) of sampled households reporting cash expenses due to RIFA. Statewide, 53.9% (CI = ± 3.4%) of the sampled households reported cash expenses and/or household time for RIFA control and remediation. This is a lower value than the 85% of the Georgia households reporting attempts at RIFA control in the Diffie et al. (1991) survey. The estimated mean annual direct cash expenditures for South Carolina, $80.37 per household, falls between the estimates of $35.26 per household for Georgia (Diffie et al. 1991) and $87.10 per household for Arkansas (Thompson et al. 1995).

Mean RIFA-related expenditures (among households reporting such expenditures) do not differ significantly across the five regions, regardless of whether those expenditures are measured by AC1 (calculated $F_{5, 411} = 0.45$, $P = 0.82$) or AC2 (calculated $F_{5, 427} = 0.33$, $P = 0.89$). For each region, the mean of treatment expenses is higher, and the mean of veterinary expenses is lower, than the means of the other cash expense categories. The mean treatment expense is significantly different (at the 5% level) from the means of the other expense categories in the North West, North Central, and West Central regions. However, the mean veterinary expense is not significantly different (at the 5% level) from the second lowest mean expense in any region. The ordering of mean cash expenses from highest to lowest for the state is as follows: treatment expense, other repair expense, medical expense, electrical repair expense, and veterinary expense. At the state level, there are significant differences (at the 5% level) between the mean treatment expense and the means of the other expense categories, and between the mean of the other repair expense category and the mean veterinary expense.

For the state, the mean household time devoted to RIFA-related activities is 0.29 hours (CI = ± 0.33 hours) for households with no RIFA-related cash expenses and 7.44 hours (CI = ± 2.18 hours) for households with cash expenses due to RIFA. For households with cash expenses due to RIFA, those in the North West region had the highest mean time used in RIFA-related activities, 10.35 hours (CI = ± 8.62 hours), and those in the North Central region the lowest, 4.48 hours (CI = ± 4.57 hours).

[6]The regions correspond to the crop reporting districts of the South Carolina Agricultural Statistics Service (1998).

Table 1. Summary statistics for South Carolina household annual expenditures on red imported fire ant control and remediation, by region.

Item[a]	Region							
	North West	North Central	West Central	Central	Southern	Eastern	Not reported	State
Number of sample households	211	83	59	150	115	176	15	809
Percentage of households in the sample	26.1	10.3	7.3	18.5	14.2	21.8	1.9	100.0
Percentage of households in the state, 1996 (estimated)[b]	27.8	7.8	7.8	18.5	15.1	23.0	—	100.0
Percentage of sampled households with:								
AC1 = $0, TIME = $0	70.6	38.6	25.4	36.0	40.3	34.8	80.0	46.1
AC1 = $0, TIME > $0	0.5	2.4	3.4	1.3	2.3	4.3	0.0	2.0
AC1 > $0, TIME = $0	13.7	33.7	27.1	31.3	25.0	32.2	6.7	25.0
AC1 > $0, TIME > $0	15.2	25.3	44.1	31.3	32.4	28.7	13.3	26.9
Mean values ($/year/household)								
for sampled households with AC1 = $0								
TIME = AC2	0.01	0.09	2.24	0.32	5.58	2.04	0.00	1.48
for sampled households with AC1 > $0								
TREAT	65.98	82.86	75.71	78.54	95.67	81.77	46.33	81.37
ELECT	4.79	16.82	6.31	7.12	8.71	48.29	0.00	15.02
OTHER	21.43	30.27	12.79	34.41	54.04	35.64	66.67	35.04
MED	20.84	9.80	29.29	40.55	21.81	4.83	0.00	22.22
VET	0.66	1.94	0.95	2.81	0.53	0.01	0.00	1.17
AC1	113.69	141.67	125.05	163.44	180.76	170.54	113.00	154.83
95% confidence interval = mean AC1±	54.49	58.69	51.32	78.58	71.25	98.13	201.00	31.25
TIME	53.28	23.10	46.22	29.99	47.37	30.30	3.43	38.23
AC2	166.97	164.77	171.27	193.43	228.13	200.84	116.43	193.06
95% confidence interval = mean AC2±	88.83	72.22	85.23	87.47	78.43	106.40	202.24	36.31
for all sampled households								
TREAT	19.08	48.92	53.90	49.22	54.90	49.77	9.27	42.24
ELECT	1.39	9.93	4.49	4.46	5.00	29.39	0.00	7.80
OTHER	6.19	17.87	9.10	21.57	31.01	21.70	13.33	18.19
MED	6.02	5.78	20.85	25.41	12.52	2.94	0.00	11.54
VET	0.19	1.14	0.68	1.76	0.30	0.01	0.00	0.61
AC1	32.87	83.64	89.02	102.42	103.73	103.81	22.60	80.37
95% confidence interval = mean AC1±	16.95	37.41	39.03	50.55	42.72	61.13	30.95	17.05
TIME	15.41	13.67	33.54	18.92	29.56	19.23	0.69	20.55
AC2	48.28	97.31	122.56	121.34	133.29	123.05	23.29	100.93
95% confidence interval = mean AC2±	27.19	45.61	63.23	56.50	47.74	66.62	31.67	19.96

[a]Variables are defined as follows: TREAT is treatment cash expense, ELECT is electrical repair cash expense, OTHER is all other repair cash expense, MED is medical cash expense, VET is veterinary cash expense, AC1 is total cash expense, TIME is the opportunity cost of household time, and AC2 is total expense (i.e., the sum of AC1 and TIME).

[b]Regional population estimates are from the U.S. Bureau of the Census (1992).

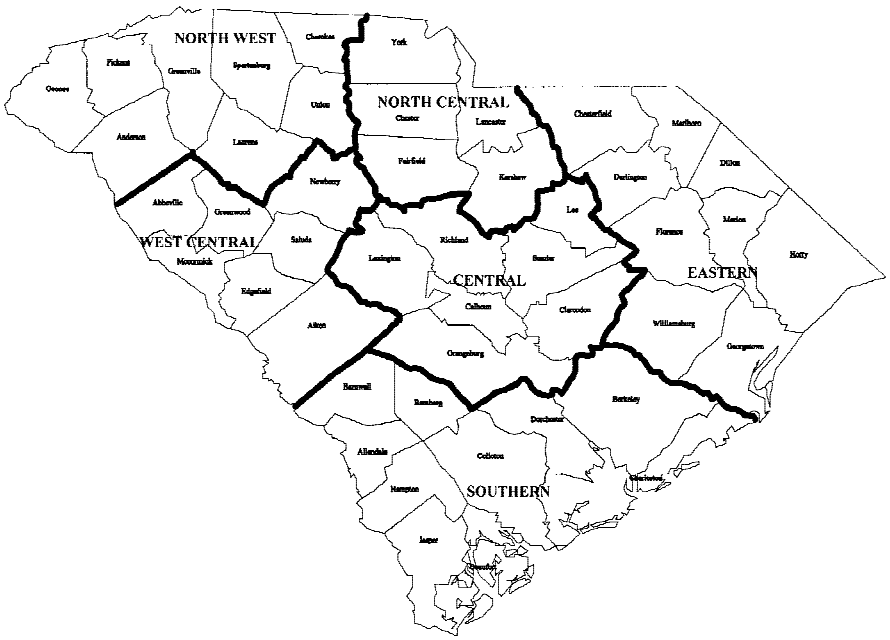

Fig. 1. South Carolina regions used in this survey. Taken from the South Carolina Agricultural Statistics Service (1998).

The lower and upper 95% confidence limits for the mean annual expenditures for RIFA control and remediation by South Carolina households are $63.33 and $97.43 when those expenditures are measured by AC1, and $80.97 and $120.89 when those expenditures are measured by AC2. According to the U. S. Bureau of the Census (1998), there were an estimated 1.376 million households in South Carolina in 1996 (the latest year for which population estimates are reported). The point estimates of the annual expenditures for RIFA control and remediation by South Carolina's household sector are $110.59 million when measured by AC1 and $138.88 million when measured by AC2. The lower and upper 95% confidence limits for annual expenditures for RIFA control and remediation by South Carolina's household sector are $87.14 million and $134.06 million when expenditures are measured by AC1, and $111.42 million and $166.35 million when expenditures are measured by AC2.

The expenditures summarized in Table 1 are conditioned only on household location. A double-hurdle model was estimated to determine whether other household characteristics such as income, education, and marital status affect RIFA-related expenditures. Absent any previous research dealing with the determinants of RIFA-related household expenditures, alternative specifications of the probit and truncated regression models were estimated using the following measures of household characteristics as explanatory variables: age of the household head; number of household members; and dummy variables for household annual income (less than $20,000, $20,000 to $50,000, or greater than $50,000), educational attainment of the household head (high school or less, or at least some

college or technical school), gender of the household head (male or female), race of the household head (white or non-white), residence location (North West, North Central, West Central, Central, Eastern, or Southern region), residence ownership (owned or rented), residence type (detached or other), and residence lot size (none, less than 0.20 ha, 0.20 to 0.40 ha, greater than 0.40 ha). The double-hurdle model was estimated with RIFA-related expenditures defined alternatively as AC1 and AC2. The two continuous explanatory variables (age of household head and number of household members) and most of the dummy explanatory variables had coefficients that were not significant at the 5% level and were dropped from the model.

Table 2 provides the probit estimation results. Because of missing information on one or more of the independent variables, only 683 observations were used in estimation. Looking first at the results for cash expenses, the estimated constant of the probit model applies to white households with residential lots located outside the North West region. The predicted probability that such a household will have cash expenses for RIFA control and remediation (i.e., AC1 > $0) is $\Phi(0.6202) = 0.7324$, where Φ is the standard normal cumulative distribution function. The predicted probability is reduced if the household lives in the North West region, has no residence lot, and/or is non-white. If each of these characteristics applies, the predicted probability that the household will have RIFA-related cash expenses is $\Phi(0.6202–1.0409*1–1.4893*1 –0.4774*1) = \Phi(–2.3873) = 0.0085$. According to the 1990 Census (U. S. Bureau of the Census 1992), the proportion of non-white South Carolina households is 0.2666 and the estimated proportion of households in the North West region for 1996 is 0.2775. Based on these values and the assumption that the sample proportion of households with no residence lot, 0.0613, is reflective of all SC households, the predicted probability that the "average" South Carolina household will have RIFA-related cash expenses during a typical year is $\Phi(0.6202–1.0409*0.2775–1.4893*0.0613–0.4774*0.2666) = \Phi(0.1128) = 0.5449$. This is close to the proportion of the sampled households with values of AC1 > $0, 0.519, from Table 1.

Turning to the results for all expenditures, the predicted probability that a white household with a residence lot located outside the North West region will have positive total expenditures for RIFA control and remediation (i.e., AC2 > $0) is $\Phi(0.6869) = 0.7539$. The lowest predicted probability is for a non-white household in the North West region with no residence lot, $\Phi(0.6869–1.0908*1–1.5497*1 –0.4864*1) = \Phi(–2.4400) = 0.0073$. Based on the "average" values of the independent variables, the predicted probability of AC2 > $0 for the "average" South Carolina household is $\Phi(0.6869–1.0908*0.2775–1.5497* 0.0613–0.4864*0.2666) = \Phi(0.1595) = 0.5634$. This value is only slightly higher than the proportion of the sampled households with values of AC2 > $0, 0.539, from Table 1.

Estimation of the truncated regression models failed to identify any household characteristics that explain the level of RIFA-related expenditures among households that have those expenditures. In particular, there is no evidence that household income affects the level of AC1 or AC2 when either AC1 or AC2 is positive. These results are consistent with Abdalla et al. (1992) and Laughland et al. (1996), who found that the level of household income did not affect the level of households' averting expenditures in response to contamination of their drinking water. However, Jakus (1994) found that income had a positive effect on households' expenditures on gypsy moth control. The gypsy moth degrades the aes-

Table 2. Results for probit models explaining South Carolina household decisions to incur expenditures for red imported fire ant control and remediation.

	Decision variable[a]	
Explanatory Variable[b]	= 1 if AC1 > $0, 0 otherwise	= 1 if AC2 > $0, 0 otherwise
constant	0.6202***[c]	0.7869***
	(0.0679)[d]	(0.0690)
northwest	−1.0409***	−1.0908***
	(0.1153)	(0.1157)
nolot	−1.4893***	−1.5497***
	(0.2692)	(0.2699)
race	−0.4774***	−0.4864***
	(0.1338)	(0.1344)
sample size	683	683
chi-square[e]	129.2***	139.7***
count R-square[f]	0.70	0.72

[a]AC1 is total cash expense ($/year/household) and AC2 is total cash expense plus the opportunity cost of household time ($/year/household).
[b]Explanatory variables are as follows: northwest = 1 if the household is located in the North West region, 0 otherwise; nolot = 1 if the household residence does not have a lot, 0 otherwise; and race = 1 if the household is non-white, 0 otherwise.
[c]***denotes significance at the one percent level.
[d]Numbers in parentheses are standard errors.
[e]Calculated test statistic for the null hypothesis that each of the model coefficients, exclusive of the intercept, equals zero.
[f]Number of correct model predictions divided by the sample size.-

thetic quality of landscapes and thus might lower property values, but unlike drinking water pollution and RIFA infestations, does not pose a health threat. Because expenditures to control RIFA constitute a relatively small proportion of income for most households, the inelastic response of those expenditures to income is understandable given the health risks and potential property damage due to RIFA. Based on the truncated regression results, the "best" estimates of AC1 and AC2 for households with RIFA-related expenditures are the corresponding sample means of these variables, $154.83 per year and $193.06 per year, respectively.

These results indicate that the household sector of South Carolina would realize substantial benefits from research, extension, and regulatory programs that are effective in controlling RIFA. These potential benefits should be considered by the relevant policy makers in making their funding decisions for RIFA programs.

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Screening for Plant Resistance to Oriental Armyworm, *Mythimna separata* (Lepidoptera: Noctuidae) in Pearl Millet, *Pennisetum glaucum*[1]

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ABSTRACT Pearl millet, *Pennisetum glaucum* (R.Br.) L., is an important crop in the semiarid tropics of Asia and Africa. It is damaged by several insects of which the Oriental armyworm, *Mythimna separata* (Walker), is an important pest in Asia, and also in Australia. Chemical control is not possible for resource-poor farmers in the semiarid tropics, thus host plant resistance assumes a central role in pest management. We evaluated a set of 40 pearl millet landraces under choice and no-choice conditions to identify lines with resistance to this insect. Genotypes IP 6577, PIB 228, IP 6069, IP 6251, and IP 5836 were significantly less damaged than the susceptible check IP 3072. These lines can be used to develop cultivars with less susceptibility to *M. separata*. Most of the lines showing resistance to the Oriental armyworm originated from West Africa. Hybrids based on the male-sterile line 111A were less susceptible under field conditions than those based on 5141A and 5054A, suggesting that there are possibilities for reducing armyworm damage through development and use of cultivars with resistance to this pest.

KEY WORDS Oriental armyworm, *Mythimna separata*, pearl millet, host plant resistance, screening techniques, *Pennisetum glaucum*

Pearl millet, *Pennisetum glaucum* (R.Br.) L., is an important crop in the semiarid tropics of Asia and Africa. It is damaged by more than 500 insect species of which white grubs (*Holotrichia consanguinea* Blanchard), shoot fly (*Atherigona approximata* Malloch), stem borers [*Chilo partellus* (Swinhoe) and *Coniesta ignefusalis* Hampson], armyworms [*Mythimna separata* (Walker), *Spodoptera exempta* Walker, and *S. frugiperda* (J.E. Smith)], pearl millet midge (*Geromyia penniseti* Felt), head caterpillars [*Heliocheilus albipunctella* de Joanis, *Helicoverpa armigera* (Hubner), *Eublemma* spp.], and blister beetles (*Mylabris* spp., *Cylindothorax* spp., *Psalydolytta* spp., *Pachnoda* sp.) are most damaging worldwide (Jotwani & Butani 1978, Sharma & Davies 1988, Sharma & Youm 1999).

The Oriental armyworm, *Mythimna* (=*Pseudaletia*) *separata* (Walker) (Lepidoptera: Noctuidae) is a pest on cereals between 45°N to 45°S latitude and 60°E

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to beyond 170°W longitude (Sharma & Davies 1983). Before the 1950s, it was of minor importance in India. Since then, it has periodically caused serious damage in India to millet, sorghum [*Sorghum bicolor* Moench (L.)], rice (*Oryza sativa* L.), maize (*Zea mays* L.), and wheat (*Triticum aestivum* L.). Probable causes leading to this change have been the increase in the area under irrigation and consequent changes in farming systems, including the introduction of high yielding varieties, heavy fertilizer use, and continuous cultivation.

Heavy outbreaks of *M.* separata also have occurred in Bangladesh, China, Japan, Australia, and New Zealand. In outbreak situations, there may be large-to-complete loss of the crop over extensive areas. Yield losses are influenced mainly by the stage at which the damage occurs and the gregarious behavior of the larvae. Although it is a polyphagous pest that feeds on a number of host plants, efforts were made to see whether genotypic differences exist in pearl millet germplasm for resistance to leaf damage by *M. separata*.

Materials and Methods

Greenhouse experiments were conducted at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. Adult moths were reared from field-collected larvae in 30-cm^3 cages at ambient temperature (27 ± 2°C) and relative humidity (65 ± 5%). The adults were provided with 10% honey solution in a cotton swab. Females laid eggs on dry sorghum leaves or glycine paper placed at the bottom of the cage. Larvae were reared on sorghum leaves in 1-liter plastic jars. Neonate or third instars raised under laboratory conditions were used for infesting the test entries.

In the first experiment, a set of 40 pearl millet genotypes, originating from different geographical regions, was evaluated using three kinds of containers: large pots (30 cm in diameter), large metal trays (60 × 30 cm), and small metal trays (15 × 15 cm). The genotypes were planted in a randomized complete block design with three replications. The potting mixture consisted of red-laterite soil (alfisols) and farmyard manure (2:1). The pots and trays were kept inside a greenhouse having four coolers (30 ± 5°C, 65-85% RH) and were watered daily. There were 15 seedlings in each row (replication) and there were seven rows in each tray. Fifteen days after seedling emergence, the plants were infested with five first instars per plant. The material was evaluated for leaf damage 3 d after infestation when the differences between the susceptible and resistant genotypes were maximal. Leaf damage was evaluated on a 1–5 scale (1 = <10% leaf area consumed, 2 = 11–25%, 3 = 26–40%, 4 = 41–60%, and 5 >60% leaf area consumed).

In the second experiment, a set of 17 variably susceptible lines was selected from the first experiment and tested again with small trays, which gave more consistent results across replications than trials with large pots and large metal trays. The experiment was repeated twice. The test entries were infested with five first instars per plant and the leaf damage was evaluated on a 1–5 scale as described previously.

In the third experiment, a set of 10 lines was infested with a single third instar per plant. In 24 h, most of the leaves were consumed by the larva, and it was decided to evaluate the test material for recovery resistance 5 d after terminating

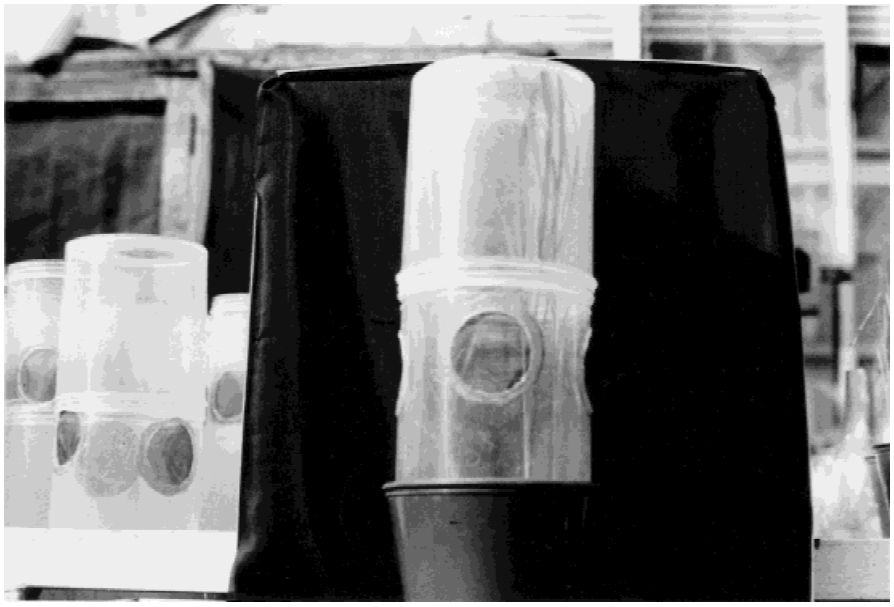

Fig. 1. Cage used to confine *M. separata* larvae to pearl millet seedlings for resistance screening.

the experiment. A 1–5 scale was used: 1 = plants showing >5 cm growth but able to recover following damage by the armyworm larvae, 2 = plants showing 4 or 5 cm growth after insect damage, 3 = plants showing 2 or 3 cm growth, 4 = plants showing 1 or 2 cm growth, and 5 = plants showing no recovery following damage by the armyworm larvae.

Larval behavior was one of the factors that led to variation in leaf damage across replications in the metal trays and large pots. The larvae tended to congregate in the middle of the tray and in the space in between the trays or under the tray during the daytime. During the night, the larvae started feeding from one corner of the tray and this led to variation in leaf damage across replications. To overcome this difficulty, we decided to screen the cultivars under no-choice conditions. A set of 11 armyworm-resistant and -susceptible genotypes was selected based on their performance in preliminary experiments. The entries were grown in small plastic pots (15 cm in diameter). Fifteen days after germination, three pairs of pots (replicates) were selected from the 10 pots raised for each genotype. Five plants were retained in each pot and covered with a cage. The confinement cage consisted of a plastic jar (30 cm in length and 15 cm in diameter) having four wire-mesh screened openings (4 cm in diameter) on the sides, and one on the top. This technique (Fig. 1) confined the armyworm larvae to the test plants, and there was no moisture accumulation inside the cage. Five third instars (held for 4 h under 100% RH) from the laboratory culture were released in each pot. Twenty-four hours after infestation, the larvae were taken out and the plants were evaluated visually for leaf damage on a 1–5 scale as described previously. In addition, the leaf area and dry weight of the infested and uninfested plants were recorded

Table 1. Relative susceptibility of 40 pearl millet genotypes to first instars of *M. separata* under greenhouse conditions (ICRISAT Center, 1980 rainy season).

Genotype	Collection no. of landrace	Origin	Damage rating[a]		
			Large pots	Large metal trays	Small metal trays
IP 3122	SAR 116	India	4.3cd	5.0e	5.0f
IP 3166	SAR 170	India	4.0cd	4.7d	4.3def
IP 3274	SAR 289	India	4.0cd	5.0e	5.0f
IP 3474	SAR 699	India	3.7bcd	5.0e	3.3bcd
IP 3553	SAR 807	India	4.3cd	3.0ab	3.3bcd
IP 3859	SAR 1254	India	4.0cd	4.3cde	4.7ef
IP 3874	SAR 1270	India	1.0a	4.7de	3.7cd
IP 4382	SAR 1805	India	4.7cd	4.3cde	4.0de
IP 7038	SAR 2236	India	4.0cd	4.3cde	2.7ab
IP 7156	SAR 2363	India	4.0cd	4.7de	3.7cd
IP 6115	P 3	Cameroon	3.3bc	4.0bcde	4.0de
IP 6069	P 184	CAR	2.3ab	2.7a	4.3def
IP 6251	P 213	Mali	3.3bc	2.7a	3.3bcd
IP 6385	P 387	Mali	3.3bc	3.3a	3.3bcd
IP 5836	P 1430	Senegal	3.0bc	2.7a	2.7ab
IP 5890	P 1495	Senegal	3.3bc	3.3abc	5.0f
IP 5314	P 2584	Niger	3.3bc	5.0e	4.3def
IP 5335	P 2612	Niger	4.7a	4.3cde	4.3def
IP 5366	P 2645	Niger	3.7bcd	3.0a	4.3def
IP 8182	IP 406B	India	3.3bc	4.0bcde	5.0f
IP 6893	ACC 154	Kenya	3.0bc	2.7a	5.0f
IP 5301	Ex-Bornu	Niger	4.3cd	4.7de	4.3def
IP 5692	45-324	Nigeria	3.3bc	5.0c	4.7ef
IP 6577	Souga local	Burkina Faso	3.0bc	3.0a	3.0bc
J 2002-2-4	NA	India	4.3cd	4.3cde	4.0de
IP 17939	700112	Nigeria	2.3ab	4.3cde	4.0de
IP 7848	J 104	India	2.7bc	4.7de	4.0de
NEP 11-5603	NA	Lebanon	4.3cd	4.7de	4.7f
IP 4905	NA	Lebanon	4.3cd	5.0c	2.0a
IC 17530	NA	India	3.7bcd	4.3cde	2.7ab
PIB 228	NA	India	3.0bc	3.7ac	3.0bcd
GAM 75 C-Bulk	NA	Senegal	3.7bcd	4.0bcde	4.0dc
Bit 33	NA	NA	3.3bc	4.3cde	3.7cd
Controls					
IP 3072 (S)	SAR 57	India	5.0d	5.0e	5.0f
WC-C 75 (CC)	NA	India	3.7bcd	4.3cde	3.7cd

Table 1. Continued.

Genotype	Collection no. of landrace	Origin	Damage rating[a] Large pots	Large metal trays	Small metal trays
MBH 110 (CH)	NA	India	4.0cd	5.0e	5.0f
BJ 104 (CH)	NA	India	3.0bc	4.3ce	3.7c
ICH 105 (EH)	NA	India	5.0d	5.0e	3.7cd
ICMS 7703 (CC)	NA	India	3.3bc	4.0bcde	3.3bcd
ICH 118 (EH)	NA	India	3.0bc	4.7de	3.7c
SE (±)			3.52	4.18	3.95
LSD at 5%			1.56	1.11	0.78
CV (%)			15.6	9.3	7.1

CAR, Central African Republic; NA, not available; S, susceptible check; CC, commercial cultivar; CH, commercial hybrid; EH, experimental hybrid.

[a]1 = Damage rating (1 = <10% leaf area consumed and 5 = >60% leaf area consumed). Numbers followed by the same letter in a column are not significantly different at $P < 0.05$ (LSD test).

by using a leaf area meter (Licor Model 3100) immediately after terminating the experiment. The plants were then dried in an oven at 80°C for 3 d, and the dry weight of infested and uninfested plants was recorded on a Mettler balance. The percentage of leaf area and dry weight consumed by the larvae were compared with those of the control plants.

In the fourth experiment, a pearl millet hybrid trial in the field also was evaluated visually for resistance or susceptibility to the armyworm under field conditions. There were 36 genotypes planted in a randomized complete block design. Of the 35 experimental hybrids tested in this trial, 14 hybrids were based on 5151A male-sterile line, 10 on 5054A male-sterile line, and nine on 111A male-sterile line. A commercial variety (WC-C75) and two hybrids (ICH 226 and BJ 104) were included as checks. There were three replications, and each plot was four rows, 4 m in length. The rows were spaced at 75 cm and the plants were thinned to one plant every 10 cm. At panicle emergence, the trial was evaluated for leaf damage by *M. separata* on the 1–5 scale.

Results and Discussion

In the first experiment, of the 40 genotypes screened using large pots, 15 were significantly less susceptible (damage rating <3.3) than IP 3072 (dr 5) (Table 1). Of these, IP 3874, IP 6069, IP 17939, IP 7848, PIB 228, Bit 33, ICMS 7703, ICH 118, and BJ 104 suffered the least damage, and there was no significant difference in leaf damage between these genotypes. When using the large metal trays, 10 genotypes appeared resistant to armyworm, whereas 11 genotypes were resistant in small-tray tests. IP 3072 was susceptible (dr 5), whereas IP 6251, IP 6385, IP 5836, IP 6577, and PIB 228 were resistant in all the three tests in this experiment. GAM 75-C-bulk and WC-C 75 did not differ significantly from IP 3072 when grown in the large trays. The remaining lines were susceptible to the

Table 2. Relative susceptibility of 17 pearl millet genotypes to first instars of *M. separata* in small metal trays under multi-choice conditions (ICRISAT Center, 1981 rainy season).

Genotype	Damage rating[a]		Recovery[b] resistance score
	Exp. 1	Exp. 2	
IP 3122	4.0b	3.8ab	NA
IP 3274	3.0ab	4.0b	NA
IP 3874	3.0ab	3.2a	3.8b
IP 7038	3.0ab	3.6ab	NA
IP 6069	3.0ab	2.6a	2.8a
IP 6251	3.0ab	3.2a	NA
IP 5836	3.0ab	3.0a	3.0a
IP 6577	3.0ab	3.0a	2.8a
IP 17939	2.0a	2.6a	2.8a
IC 17530	3.0ab	3.4ab	NA
PIB 228	2.0a	2.6a	2.8a
Bit 33	3.0ab	3.8ab	NA
Controls			
IP 3072 (S)	4.0b	4.6b	5.0c
WC-C 75 (CC)	3.0ab	3.6ab	2.5a
BJ 104 (CH)	4.0b	4.0b	3.8b
MBH 110 (CH)	4.0b	3.8ab	4.0b
ICH 118 (EH)	4.0b	3.8ab	NA
Mean	3.20	3.45	3.33
SE (±)	0.4	0.4	0.3
LSD at 5%	1.2	1.2	0.7
CV (%)	12.6	11.6	9.0

NA, not available; S, susceptible check; CC, commercial cultivar; CH, commercial hybrid; EH, experimental hybrid.

[a]Damage rating (1 = <10% leaf area consumed, and 5 = >60% leaf area consumed).

[b]Recovery resistance (1 = good plant growth after leaf feeding by the larvae and 5 = poor plant growth after leaf feeding by the *M. separata* larvae. Numbers followed by the same letter in a column are not significantly different at $P < 0.05$ (LSD test).

armyworms. The coefficient of variation was greatest in large pots (15.3%), followed by large trays (9.3%) and small trays (7.1%), respectively.

In the second experiment with small trays, IP 3874, IP 6069, IP 6251, IP 5836, IP 6577, IP 17939, and PIB 228 were less damaged than IP 3072, IP 3122, MBH 110, BJ 104, and ICH 118 (Table 2). Among the resistant genotypes, IP 6069 (except in small trays), IP 6251, IP 6577, and PIB 228 also showed resistance to armyworm larvae in the three tests in the first experiment. Among the genotypes less damaged by armyworm larvae, WC-C75, IP 6069, IP 5836, IP 6577, IP 17939, and PIB 228 showed good recovery at 5 d after the experiment was terminated.

In the third experiment, there were significant differences in genotypic susceptibility to third instars of *M. separata* in terms of leaf area consumed, weight

Table 3. Leaf area and dry weight consumption by the third instars of *M. separata* (in 24 h) on 11 pearl millet genotypes under no-choice conditions (ICRISAT Center, 1982 rainy season).

Genotype	Leaf area consumed, %	Dry weight consumed, %	Damage rating[a]
IP 6577	35.1a	24.7a	1.8a
PIP 228	70.2bcde	37.8abc	2.5ab
IP 4905	68.4bcde	51.5bcd	2.7ab
IP 6069	80.8cdef	54.9bcd	3.3bc
IP 17939	67.5bcd	34.9ab	2.3a
SAD 4031	88.0def	53.3bcd	4.7d
IP 5459	88.1def	56.1bcd	4.0cd
IP 13070	59.5abc	54.2bcd	2.2a
Controls			
IP 3072 (S)	99.0f	65.7d	5.0d
BJ 104 (CH)	94.3ef	58.7cd	5.0d
MBH 110 (CH)	44.4ab	21.9a	2.3a
Mean	72.30	46.70	3.25
SE (±)	8.98	7.49	0.35
LSD at 5%	26.33	21.96	1.04
CV (%)	12.4	16.0	10.8

S, susceptible check; CH, commercial hybrid.
[a]Damage rating (1 = <10% leaf area consumed and 5 = >60% leaf consumed). Numbers followed by the same letter in a column are not significantly different at $P < 0.05$ (LSD test).

of food consumed, and leaf damage rating under no-choice cage conditions (Table 3). Correlation analysis showed a significant relationship between damage rating and leaf area consumed ($r = 0.85$), and between damage rating and leaf dry weight consumed ($r = 0.95$). The leaf area and dry weight of leaves consumed also were highly correlated ($r = 0.87$), indicating that visual leaf damage ratings are logical indicators of resistance to the Oriental armyworm. The genotypic reactions were similar across replications. In this experiment, the genotypes IP 6577, IP 17939, PIB 228, MBH 110, and IP 13070 (Fig. 2) were only moderately damaged by the armyworm, whereas IP 7032, BJ 104, IP 5459, and SAD 4031 were highly susceptible (completely defoliated).

In the fourth experiment, significant differences among the hybrids also were recorded for *M. separata* damage under field conditions (Table 4). However, the damage tended to be greater in the middle of the field and lower in the border plots. Hybrids based on 111A male-sterile line were less damaged (mean damage rating = 2.6) than those based on 5141A (mean damage rating = 3.4) and 5054A (mean damage rating = 3.5). These observations, as well as those on *Myllocerus* sp. leaf damage in the hybrid trials (H.C.S. unpublished data), indicated that hybrids based on the 111A male-sterile line are less susceptible to Oriental armyworm damage than those based on other male-sterile lines. The hybrids 5141A × (23 D_2B × SD_2 × EB 1052-2-1-1-1), 111A × (P1B-228 × 3/4EB 108-2-2-3, 111A ×

Fig. 2. *M. separata* damage in 24 h to the susceptible pearl millet genotype IP 3072 (left), commercial cultivar WC-C 75 (center), and resistant genotype IP 6577 (right).

(P1B-228 × 3/4 S 68-28-1) P5, 5141A × J 803-3-1, 111A × 7229, 111A × 2989-109-1-1, and 111A × GAM 75-948-2 were significantly less susceptible than 5141A × 7137 (white).

Several workers have reported the existence of varietal resistance to *M. separata* in sorghum, wheat, rice, maize, and pearl millet under field conditions (Leuck et al. 1968, 1977; Verma & Khurana 1971; Kalode et al. 1971; Wiseman & Gourley 1982; Sharma 1987; Thakur & Sharma 1990; Kishore 1991). Under field conditions, the extent of damage is influenced by 1) the amount of hiding space available to the larvae; 2) plant characteristics (height, tillering, and leaf canopy); and 3) the position or location of the cultivar in the field. Insect damage tends to be greater in the center of the field and in areas with surface mulch or any other plant material lying on the ground. The dwarf genotypes having more tillers suffer heavier damage than the tall and less tillering genotypes. In addition, the genotypic differences in resistance are less apparent with an increase in insect pressure and exposure time. Thus, we must look for genotypic resistance under controlled conditions. The resistance parameters should include leaf damage, larval survival and rate of development, and fecundity. All cases of field resistance should be tested for these parameters under controlled conditions.

In our study, variation in leaf damage observed under free-choice conditions in the large trays was minimized to some extent in the small trays when the material also was tested under free-choice conditions. However, the no-choice assay with the plastic jar cages and recording data both on leaf feeding and amount of food consumed provided the most useful information on genotypic susceptibility to

Table 4. Relative susceptibility of some pearl millet hybrids to *M. separata* under field conditions (ICRISAT Center, 1980 rainy season).

Hybrid[a]	Damage rating[b]
5141A × ($23D_2B$ × SD_2 × EB 1052-2-1-1-1)	2.0a
5054A × J1043 × 3/4 HK 9-8-1). 3	3.3bcd
5054A × (700112 × 3/4 S-G-14-9)	3.7bcd
111A × (P1B-228 × 3/4 EB 108-2-2-3)	2.3ab
5054A × (P1B-228 × 3/4 S 68-S1-4) 3	3.0bcd
5141A × (MC 125 × Serere 33-4-1) 6	2.7abc
111A × (P1B-228 × 3/4 S 68-28-1) P5	2.3ab
5141A × J 803-3-1	2.3ab
111A × J 2249-3	2.3ab
5141A × J 1653-1-1	3.0bcd
111A × J 1532	2.7abc
111A × 7229	2.3ab
5054A × (50HB × 3/4 EB 4-1-1)	3.0bcd
111A × 2989-109-1-1	2.7ab
111A × GAM 75-948-2	2.3ab
Controls	
WC-C 75 (CC)	2.3ab
BJ 104 (CH)	2.7abc
5141A × 7137 (White)-(S)	4.7cd
Mean	3.10
SE (±)	0.90
LSD at 5%	1.70
CV (%)	29.40

CC, commercial cultivar; CH, commercial hybrid; S, susceptible check.
[a]There are 36 test entries in the trial of which data are shown for hybrids with less susceptibility to the armyworm, and three checks.
[b]Damage rating (1 = <10% leaf area consumed and 5 = >60% leaf area consumed).
Numbers followed by the same letter in a column are not significantly different at $P < 0.05$ (LSD test).

M. separata. There was good correlation among the reactions of the resistant and susceptible lines across screening techniques. Hence, these lines can be used in pearl millet improvement programs to develop cultivars with less susceptibility to the Oriental armyworm.

Although the Oriental armyworm is a polyphagous pest on a number of plant species, there are some indications of a feeding preference for certain hosts. The extent of damage also differs on different genotypes in cereals. However, the extent of damage depends upon the insect population and exposure time. Instances of field resistance need to be verified under no-choice conditions. Pearl millet genotypes IP 6577, IP 6069, IP 6251, and IP 5836 (which originated from West Africa), and PIB 228 (an improved cultivar from Punjab, India) were less susceptible to the armyworm and can be used as sources of resistance to the

Oriental armyworm. Resistance could be further verified by screening under controlled conditions and planting the resistant lines in the field.

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Integration of Chlorfenapyr into a Management Program for the German Cockroach (Dictyoptera: Blattellidae)[1]

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ABSTRACT Chlorfenapyr, a new active ingredient discovered by American Cyanamid Company was found to have excellent insecticidal activities against field populations of German cockroaches. Trap catch reduction in cockroach infested apartments treated with the suspended concentrate (SC) and wettable powder (WP) formulations of chlorfenapyr was between 52.4 and 80.5% and 64.6 and 82.3%, respectively, after 8 wks of post-treatment sampling. The level of control recorded for these formulations was not significantly different compared to that of cypermethrin, (Demon® emulsifiable concentrate (EC)), a commercially available cockroach control insecticide. Trap catch reduction was between 53.6 and 71.8% for Demon® EC over the same period.

In a laboratory bioassay designed to test the residual toxicity of the two formulations of chlorfenapyr for up to 180d post-treatment, the WP formulation was found to have significantly higher activities than the SC formulation against cockroaches exposed on treated surfaces. We recorded 100% mortality for cockroaches exposed on WP treated surfaces after 180d compared to 62.5% for cockroaches exposed on SC treated surfaces.

Chlorfenapyr has been reported to be active on insects' respiratory system. The potential for its use in German cockroach resistance management program is discussed.

KEY WORDS *Blattella germanica*, Blattellidae, chlorfenapyr, residual toxicity, resistance management, formulation

The German cockroach, *Blattella germanica* (L.), has become the most serious urban pest in homes, commercial kitchens and food handling facilities (Cornwell 1976). Aside from being a nuisance pest in human dwellings, epidemiological studies have also shown that sensitization to allergens produced by this species is an important risk factor for paroxysmal allergic disorder of the respiratory system (Kang et al. 1979, Roseintreich et al. 1997). The cost of controlling this pest runs into millions of dollars every year (Douce and McPherson 1989). Complicating control effort for the German cockroach is the development of resistance to all the major classes of insecticides. Resistance to the organophosphates (Milio et al. 1987), carbamates (Cochran 1989) and pyrethroid insecticides (Valles and Yu

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1996) have been documented. Thus, there is need for a continuous search for new active ingredients with either a different mode of action or with activities at target sites other than the central nervous system.

Chlorfenapyr (AC 303,630) is a novel insecticide that targets the insects' respiratory system (Kuhn et al. 1993), but has minimal effects on the nervous system (Black et al. 1994). It interferes with energy production in the mitochondria by uncoupling the oxidative phosphorylation process (Addor et al. 1992). Chlorfenapyr is effective against many economic insect pests, such as tobacco budworm (Pimprale et al. 1997), hornfly (Sheppard and Joyce 1998), and pavement ants (Smitley et al. 1998). There is no published information on the biological activities of this compound against the German cockroach. Laboratory bioassays were thus conducted to evaluate the residual toxicity of two formulations of this compound to the German cockroach. In addition, field efficacy of the compound was evaluated against cockroach populations in a multi-family apartment complex.

Materials and Methods

Residual toxicity bioassay. A laboratory-reared insecticide susceptible strain of *B. germanica*, the 'Johnson Wax' (JWAX) strain, was used to evaluate residual toxicity of the suspended concentrate (SC) and wettable powder (WP) formulations of chlorfenapyr on a masonite surface. This culture of *B. germanica* has been maintained at Purdue University, West Lafayette, Indiana since 1984 on a standard laboratory diet of Wayne Rodent Blox (Continental Grain, Chicago, Illinois) at 26 ± 1°C, 70% RH and 12:12 [L:D] h photoperiod. The JWAX strain was isolated from a field-collected population before the introduction of synthetic organic insecticides (Koehler and Patterson 1980). The chlorfenapyr formulations were applied onto masonite panels (15.24 × 15.24 cm) with a spray tower apparatus (Spraying System Tee-Jet SS8001E, Dayton Electric Manufacturing Company, Chicago, Illinois) with a flat fan stainless steel nozzle. The spraying equipment was calibrated to deliver 25mg/m^2 of formulated materials at a rate of 1 gal/1000 sq. ft horizontal surface at a constant pressure of 60 psi. Treated panels were aged under laboratory conditions for 180 d. Test insects were exposed on treated panels at 1, 30, 90, and 180 d post-treatment. At each aging period, ten adult cockroaches (5 of each sex) were confined on treated panels for 60 s using a 12.7 cm Plexiglas® ring, which was lightly greased with a petrolatum-mineral oil mixture to prevent escape. After exposure, test insects were transferred into a clean glass jar, provisioned with moistened dental wicks, and observed for mortality. Mortality data was collected at 1 (acute toxicity) and 5 d (chronic toxicity) after exposure. Cockroaches exposed on untreated masonite panels served as the control. Treatments were replicated four times.

Field evaluation. The relative efficacy of chlorfenapyr SC and WP formulations was compared to that of cypermethrin (Demon® EC) to control field populations of German cockroaches. The study was conducted from 19 May to 15 July, 1997, in multifamily housing communities (Munsyana Homes) operated by the Muncie Housing Authority, Muncie, Indiana. Faulty construction, poor sanitation, and absence of effective pest management programs favored high cockroach population densities in these apartments.

At the beginning of the study, the housing authority was advised to terminate all insecticide applications in the apartments 4 to 6 wk prior to our pre-treatment sampling program. Cockroach sticky traps (Agrisense® Lo-Line, Palo Alto, California) were used to estimate cockroach population densities in each apartment, and to measure the impact of treatments on populations. Cockroaches were sampled at the cabinetry below and above the kitchen sink, around the stove, and the refrigerator, the utility room (area around water heater and furnace), and in the bathroom around the toilet seat. One trap was placed in each of these areas overnight and collected the next day. Trap catches were recorded as number of adult males, adult females, gravid females, large nymphs (instars 4–6), and small nymphs (instars 1–3). A minimum of 24 total cockroaches on the six traps was required for an apartment to be selected for our study.

After pre-treatment sampling, a total of 6, 9, and 8 apartments respectively were established for the chlorfenapyr SC, WP and cypermethrin EC treatments, respectively. Treatments, which were randomly assigned, were applied from a 3.79-l (1 gal) B & G compressed air sprayers (B&G Equipment Co., Plumbsteadville, PA), fitted with a crack-n-crevice injector tip. Treatments were applied into cracks and crevices throughout the apartments, but with greater concentration in the general areas where sampling traps were placed. A maximum of 1 l of formulated product was applied in each apartment. Post-treatment population samplings were conducted at 1, 2, 4, and 8 wk using the same materials and methods described above.

Statistical analyses. In all cases, the experimental design was completely randomized. Residual toxicity of the two chlorfenapyr formulations were compared with analysis of variance by aging period (SAS Institute 1996). Analysis of variance was also used to compare pre-treatment counts in the field evaluation studies, and this revealed no significant differences among apartments grouped by treatments ($F = 1.31$, $df = 2$, $P = 0.2797$). As a result, treatment efficacy was determined by calculating percent reduction in cockroach population density for each experimental unit at each sampling interval using the formula: % reduction $= (T_i - T_o)/ T_i * 100$, where T_i and T_o are pre-and post-treatment trap counts, respectively. Percent reduction was transformed by arcsine $\sqrt{p}$, where p is percentage expressed as proportion. Transformed data were then analyzed with the SAS GLM procedures (SAS Institute 1996). Means were separated by Tukeys studentized range test at $\alpha = 0.05$ (SAS Institute 1996).

Results and Discussion

Residual toxicity. The WP formulation had better residual toxicity than the SC formulation at the 1and 5 d post-exposure periods (Table 1). Cockroach mortality was consistently higher at the 5 d than at the 1d post-exposure period indicating the chronic toxicity characteristic of chlorfenapyr (Table 1). In terms of residual activity, 100% mortality was recorded for cockroaches exposed to the WP treated surfaces after 180d of aging compared to 62.2% for the SC formulation. These results indicate that chlorfenapyr WP is a better formulation than the SC on a masonite surface and, under similar conditions, up to 6 months of residual activities can be expected against German cockroaches when applied onto this surface.

Field efficacy. Efficacy of the treatments as determined by the average reduction in trap catches of cockroaches are shown in Table 2. There were no

Table 1. Residual toxicity of chlorfenapyr formulations applied onto a masonite surface and aged up to 180 d against a laboratory-reared insecticide susceptible strain of the German cockroach.

	% Post-exposure mortality (Mean ± SE)							
	1d	5d[a]	1d	5d[a]	1d	5d[a]	1d	5d[a]
Treatment	Aging 1d		Aging 30d		Aging 90d		Aging 180d	
Chlorfenapyr SC	30.0 ± 4.1b	87.5 ± 2.5a	27.5 ± 2.5b	82.5 ± 2.5b	17.5 ± 2.5b	75.0 ± 5.0a	7.5 ± 4.8b	62.5 ± 2.5a
Chlorfenapyr WP	77.5 ± 4.8a	100.0 ± 0.0a	72.5 ± 2.5a	100.0 ± 0.0a	67.5 ± 4.8a	100.0 ± 0.0a	57.5 ± 12.5a	100.0 ± 0.0a
Untreated control	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b

Means in columns followed by the same letter are not significantly different ($\alpha = 0.05$, Tukey test).
[a]Cumulative mortality at 5 days.

Table 2. Trap catch reduction of field populations of German cockroaches exposed to two formulations of chlorfenapyr compared to Demon® EC in a multi-family apartment complex.

Treatment	% Active ingredient	Quantity of formulated product per gal of water	Pre-treatment counts (Mean ± SE)	% Post-treatment reduction in trap catches (Mean ± SE)			
				Wk 1	Wk 2	Wk 4	Wk 8
Chlorfenapyr 2SC	25.0	75.5 ml	29.3 ± 6.2a	52.4 ± 16.8a	68.9 ± 8.9a	80.5 ± 7.3a	78.3 ± 4.7a
Chlorfenapyr 50WP	22.1	85.6 g	56.2 ± 11.3a	64.6 ± 5.7a	74.9 ± 3.6a	68.1 ± 3.5a	82.3 ± 1.9a
Demon® EC	25.3	30.0 ml	64.4 ± 20.7a	53.6 ± 10.1a	60.8 ± 6.1a	56.3 ± 6.9b	71.8 ± 4.8a
F			1.31	2.09	1.39	4.49	1.87
p			0.2797	0.1504	0.2730	0.0245	0.1797

Means within columns followed by the same letter are not significantly different ($\alpha = 0.05$, Tukey test).
Post-treatment *F* and *p* values were genterated from analysis of transformed data (see text).

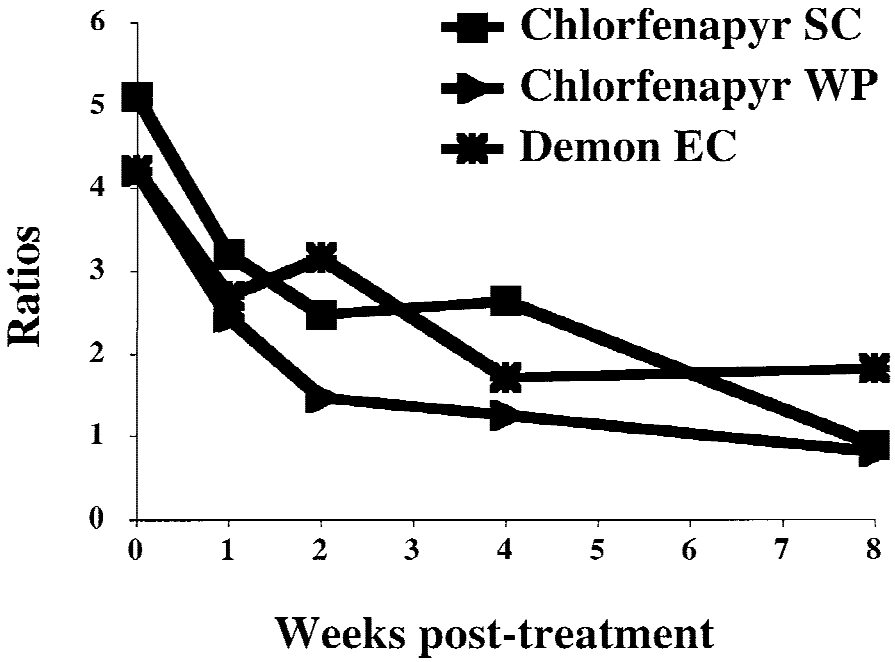

Fig. 1. Changes in total nymph: adult ratios over time for field populations of German cockroaches exposed to Chlorfenapyr SC, WP and Demon® EC treatments in a multi-family housing complex.

significant differences in the post-treatment population reduction recorded for thetwo chlorfenapyr formulations (Table 2). We did not also detect any significant differences between population reduction recorded for the chlorfenapyr formulations compared to the standard treatment, cypermethrin (Demon® EC), except at the 4-week post-treatment census where the average population reduction was significantly lower for Demon® EC (Table 2). When tests were terminated after the 8-week post treatment census, the chlorfenapyr formulations gave satisfactory reduction in cockroach populations in treated apartments. Percent population reduction was between 58.0 and 74.7% and 59.3 and 81.5% for the SC and WP formulations, respectively. Trap catch reduction for the cypermethrin EC was between 52.5 and 72.4%.

Unlike the residual toxicity bioassay, we did not detect any significant differences in the performance of the SC and WP formulations of chlorfenapyr in treated apartments. This might be because these formulations have been reported to have long term residual activities on most of the commonly found substrates in human dwellings. The substrates we encountered in the treated apartments were plywood, in kitchen cabinets, and vinyl tiles found below the stove, refrigerator, utility area and area around the toilet. The WP and SC formulations of most insecticides have been reported to have long term residual activities on these surfaces (Scirocchi & D'erma 1979).

Changes in the age structure of treated populations as determined by trap catches are shown in Figure 1. This ratio is a useful tool for determining the biological activities of a compound on various stages of exposed populations. Changes in population structure were determined by computing the mean nymph:adult ratios by treatment. Nymph:adult ratios decreased over time for all treatments (Figure 1), indicating that chlorfenapyr has broad-spectrum nondiscriminating activities on adult and nymphal German cockroaches. This trend in post-treatment nymph:adult ratios recorded for all the treatments in this study is similar to that reported by Scharf et al. (1997) and Kaakeh et al. (1997).

Our results indicate that chlorfenapyr has excellent biological activities against field populations of German cockroaches. Our laboratory bioassays also showed that chlorfenapyr WP formulation has excellent residual activities for up to 6 m on a masonite surface. In addition, we believe that chlorfenapyr will be an excellent compound for German cockroach resistance management program. The innate ability of chlorfenapyr to control insect populations that are resistant to other classes of insecticides was reported by Pimprale et al. (1997) and Sheppard & Joyce (1998). Based on these reports, we believe this product will also be effective against pyrethroid resistant populations of the German cockroach, since it is active on sites other than the insect central nervous system.

Acknowledgement

Mention of product names is not intended to promote or criticize their use. This project was partly funded by the American Cyanamid Company. We thank Byron Reid, Susan Burkart and Chuck Klein of American Cyanamid for their invaluable assistance. We appreciate the help of Rick Foster and Linda Mason of the Department of Entomology, Purdue University, for reviewing an earlier draft of this manuscript. This is journal paper No 16256 of the Agricultural Research Programs of Purdue University, West Lafayette, Indiana.

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Flight Performance of Some Nitidulid Beetles (Coleoptera) Using a Computer-Monitored Flight Mill[1]

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ABSTRACT Computer-monitored flight mills were used to record the flight performance of five species of nitidulids: *Stelidota geminata* (Say), *S. Octomaculata* (Say), *S. ferruginea* (Reitter), *Carpophilus hemipterus* (L.), and *C. Lugubris* Murray. Both *Carpophilus* species flew extensively, whereas the *Stelidota* species' flight was insufficient for analysis, and *S. octomaculata* never flew. Comparisons between the two *Carpophilus* species and between the sexes of these species were made based on five measures of flight performance: the number of flight bouts, laps per bout, average time per bout, time spent flying per bout, and average speed. Although there were small but significant differences documented between both sex and species for laps per bout, time spent flying and average time per bout, the most significant differences were the number of flight bouts and time of day that the beetles were flown. The computer monitored flight mill used in this study was the same as that of Taylor et al. (1992). Minor modifications were made to accommodate these small beetles, some of which were stronger fliers than the leafhoppers previously investigated. A revised circuit diagram is included.

KEY WORDS *Carpophilus* spp., flight endurance, flight speed, *Stelidota* spp.

The North American Nitidulidae is composed of 35 genera (Connell 1957), with members of the genera *Carpophilus* and *Stelidota* among the most numerous. More than 30 species of *Carpophilus* are widely distributed throughout the United States (Williams et al. 1983). Of the ≈50 species of *Stelidota* worldwide only three species occur north of Mexico (Weiss & Williams 1980). Numerous articles have been published on the general biology and hosts of nitidulid beetles (Williams et al. 1983). However, little is known concerning the flight behavior of these beetles and the role it plays in their dispersal, migration, and host location. Blackmer & Phelan (1991) investigated free flight behavior of *Carpophilus hemipterus* (L.) and *C. lugubris* Murray in a vertical wind tunnel and found that initial flight behavior was phototactic in response. After a period of vertical flight the phototactic response declined, resulting in reduced vertical flight and increased horizontal displacement. They concluded that initial flight represented dispersal

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flight and latter flight was host finding flight. In further studies Blackmer & Phelan (1993) demonstrated that species differ in their locomotive response to host volatiles. Some species take flight more readily than others, while some respond only by walking toward host odors. Field studies (Alm et. al. 1989, Peng & Williams 1991, Williams et al. 1993) have demonstrated that species respond differently to host odors and pheromones under field conditions. Factors, such as trap design, trap height, and habitat, influence the number of beetles captured. *C. hemipterus*, *C. lugubris* and *Glischrochilus quadrisignatus* (Say) responded better to aerial traps than to traps placed on the ground, whereas *Carpophilus freemani* Dobson indicated no clear preference between aerial or ground traps. On the other hand, *Stelidota geminata* (Say) has shown a 10 fold preference to ground traps over aerial traps, while its close relative *S. octomaculata* (Say) has only been captured in ground traps (Peng & Williams 1991). The lack of vertical or horizontal flight by *S. octomaculata* was demonstrated under laboratory conditions (Blackmer & Phelan 1993). Beetles would not fly to host odors, but would walk to the odors. It is believed that this species may have lost its ability to fly due to its adaptation to a specific niche; it has become a specialist depending exclusively on acorns (Galford et al. 1991).

This study was initiated to look at a new means of studying flight behavior utilizing computer-monitored flight mills. We report on experiments comparing several flight performance parameters of 5 common species of sap beetle: *S. geminata, S. octomaculata, S. ferruginea* (Reitter)*, C. lugubris,* and *C. hemipterus*. Voucher specimens from the cultures are lodged with the Entomology Museum, Thorne Hall, OARDC, Wooster, Ohio.

Material and Methods

All species used in the experiment were maintained in cultures at the Ohio Agricultural Research and Development Center located in Wooster, Ohio. *S. geminata*, *S. octomaculata*, and *C. hemipterus* were originally collected near Wooster, Ohio from traps containing various fruits and nuts. The original collection of *S. ferruginea* was from near Sarasota, Florida. *C. lugubris* was acquired from a culture maintained at the USDA National Center for Agricultural Utilization Research in Peoria, Illinois. At the time of these experiments the cultures here were not more than 1 year old.

The cultures were maintained at 23–26°C with a 16:8 [L:D] photophase. Both male and female beetles were kept in clear containers (18 × 18 × 25 cm) capped with screened lids. 50-100 beetles were placed in a container with a prune-based diet along with 10 squares (5 cm^2) of seed germination paper (Seedburo Equipment Company, 1022 W. Jackson, Chicago, Illinois). The squares of germination paper were soaked in water and stacked upon each other, providing an oviposition site for female beetles between the sheets.

Adult beetles were removed from the cultures, sexed, and attached to the arm of a flight mill originally designed to record the flight of leafhoppers (Homoptera: Auchenorrhyncha) (Taylor et al. 1992) using a computer capable of monitoring 16 such mills. Details of the construction and use of the 16-channel flight recorder are given by Taylor et al. (1992). To accommodate the faster flying nitidulids, only eight channels were used simultaneously and some minor modifications were made to the electronics (see the Appendix for details).

To test flight performance, a beetle was first cooled on an ice pack, which reduced its activity with minimal modification to behavior. Beetles were never left on the ice pack longer than 5 min. A small drop of clear nail polish was placed on the short end of a #0 insect pin with a 90° bend at 1/5 of its length. The beetle was then placed under a dissecting microscope allowing for accurate attachment of the pin to the pronotum. After attachment, the long end of the pin was inserted into one end of the flight mill arm which consisted of a 100-ml capillary tube with a 1 × 1 cm square of aluminum foil glued to the other end. Another insect pin was attached perpendicularly at the capillary tube's center of gravity. This pin forms a low friction magnetic mount by hanging from the pole of a very strong magnet. To minimize interference by drafts, the flight mill was placed inside a clear plastic cylindrical windscreen. Recording commenced as soon as the flight mill arm was suspended from the magnet. While an insect was flying the aluminum foil square interrupted the light beam every revolution and the computer recorded the time (to the nearest 0.01 sec) elapsed. The elapsed time from the start of the run was recorded every revolution by the attached computer. By examining each flight mill 12 times per s, the flight activity of eight insects was monitored simultaneously.

At the end of each run, the length of time per lap was calculated by taking the difference of the beginning and ending times of each lap. From these data, flight bouts (series of consecutive laps under a maximum time per lap criterion for each species) were determined for each beetle. From preliminary experiments, the criterion for a true lap was determined to be one $\leq$3.50 s for *C. hemipterus*, and $\leq$2.00 s for *C. lugubris*. This was to insure that laps resulting from coasting were not included in further calculations. An average time per lap was calculated for each individual flight bout by dividing the total flight time for the bout by the number of revolutions of the bout. Because each revolution describes a circle of 47 cm the average flight speed could also be calculated. Comparisons of the number of flight bouts, average number of laps per bout, average time per lap, and average speed were made for both males and females of each species using a two-way analysis-of-variance.

Six to 15-d old beetles were used in each run, with equal numbers of both sexes of each species. Runs lasting for 6 h were made twice a day, beginning at 9:00 am or 4:00 p.m. The room temperature was maintained at 23–26°C, the humidity was 40–46%, and the photophase was 16:8 [L:D]h. Different insects were flown on the mill (eight per run) until 12 individuals of both sexes of each species had made 2000 or more revolutions or 32 individuals had been tested. Runs of <2000 revolutions were discarded as they provided insufficient data for analysis.

Results

Neither *S. geminata* nor *S. ferruginea* flew sufficiently long to permit analysis (no individual flew >500 revolutions) and *S. octomaculata* was never observed to fly (0 revolutions for all 32 individuals tested). Occasionally, *S. octomaculata* would open its elytra and extend its wings in an uncoordinated fashion; however, this action generated no forward movement.

Both *C. hemipterus* and *C. lugubris* showed much greater flight activity than the *Stelidota* species. Eighty-five percent (12 of 14) of *C. hemipterus* and 90% (12 of 13) of *C. lugubris* tested flew >2000 revolutions within the 6 h test period. Table

1 shows the means (± SEM) of flight bouts, laps per bout, and average time per lap for both sexes of each species. Maxima for flight bouts, laps per bout, and average time per lap are listed for each sex of *C. hemipterus* and *C. lugubris* in Table 2.

Ignoring species, sex, and time of day, analysis of variance showed no difference in the number of bouts between mills ($F = 1.41$, df = 7,88, $P > 0.20$) or between dates ($F = 1.27$, df = 5,24, $P < 0.20$). Consequently, we have ignored when or on which mill flights were run, and concentrated the analysis on differences in flight performance attributable to species, sex, and time of day. Tables 3–5 show the results of two-way analyses of variance (ANOVA) for flight bouts, revolutions per bout, time flying per bout, and average speed for sex, species, and time of day. There was a significant difference between number of flight bouts of the two *Carpophilus* species ($F = 9.89$, df = 1,205, $P < 0.001$,). Specifically, *C. lugubris* had more flight bouts per 6 hour period than *C. hemipterus.* In terms of number of bouts, no significant difference was recorded between sexes ($F = 1.97$, df = 1,205, $P > 0.10$) or the interaction between sex and species ($F = 0.41$, df = 1,205, $P > 0.25$). A significant difference between species was recorded in the total time in flight (equivalent to the total number of revolutions) ($F = 3.99$, df = 1,383, $P < 0.05$). Both species flew significantly longer in the evening runs than the morning runs ($F = 4.97$, df = 1,383, $P < 0.05$). A significant difference in total time of flight was also obtained in the interaction of sex and time of day ($F = 3.55$, df = 1,383, $P < 0.05$). Although *C. hemipterus* flew longer than *C. lugubris*, when it was flying, *C. lugubris* flew significantly faster than *C. hemipterus* ($F = 142.0$, df = 1,383, $P < 0.001$). Also, average flight speeds differed significantly between sex ($F = 12.5$, df = 1,383, $P < 0.01$) and interestingly, the average speed differed significantly between morning and evening runs ($F = 107.2$, df = 1,383, $P < 0.001$). Significant interactions between species and sex ($F = 6.8$, df = 1,383, $P < 0.01$) and between species and time of day ($F = 8.7$, df = 1,383, $P < 0.001$) were also obtained for average flight speed, whereas the interaction between sex and time of day was not significant ($F = 1.1$, df = 1,383, $P > 0.25$).

Discussion

The number of flights of the *Stelidota* species was inadequate for comparison with the *Carpophilus* species. A possible reason for inactivity in flight for the *Stelidota* species is their extreme sensitivity to dehydration. Often at the end of a 6-hour flight period many of the beetles were moribund. We have found that *Stelidota* spp. are not able to survive as well as *Carpophilus* spp. in a low humidity environment. These large differences in flight between the genera are supported by a recent trapping study. Different species of nitidulids were trapped using both aerial and ground traps. Ninety four percent of *C. lugubris* captured were caught in aerial traps while only 10% of *S. geminata* were caught in aerial traps (Williams et al. 1993).

Studies have shown a correlation between flight and food source. Using *C. hemipterus*, Blackmer & Phelan (1991) found that beetles reared on a water-only diet initiated flight one day earlier than those beetles reared on artificial diet. Differences between flight among beetles may in fact be linked to their habitat and availability of food sources. Darlington (1970) suggested that species with highly unstable habitats and food sources would have the greatest need for dispersal, and with stabilization of its environment and food resource a loss in

Table 1. Average flight performance of *Carpophilus hemipterus* and *C. lugubris* on the flight mill.

Species and sex	Mean number of flight bouts (±SEM)	Mean number of laps/bout (±SEM)	Mean time per lap (s ± SEM)
C. hemipterus			
female	2.62 ± 0.92	2070 ± 62	1.46 ± 0.02
male	3.61 ± 0.92	2045 ± 58	1.70 ± 0.02
combined	2.98 ± 0.97	2061 ± 63	1.55 ± 0.02
C. lugubris			
female	8.53 ± 1.10	1557 ± 47	1.20 ± 0.02
male	5.78 ± 1.02	1275 ± 52	1.18 ± 0.01
combined	7.31 ± 1.07	1532 ± 45	1.16 ± 0.01

dispersal power can occur. We have never observed *S. octomaculata* to fly either under laboratory conditions or in the field. Thus, *S. octomaculata* may have lost the ability to fly as a result of the location, long-term stability, and abundance of acorns, its principal food source.

Although *S. geminata* and *S. ferruginea* can be induced to fly in vertical and horizontal wind tunnels neither species flew when placed in the flight mills. This is unusual, because most insects will open their wings and attempt to fly when their tarsi lose contact with a surface. Their smaller size may have made it more difficult for them to initiate flight in the mills, although this also seems unlikely because much weaker fliers like leafhoppers can initiate and sustain long flights on these mills (Taylor et al. 1992).

The overall flight performance of the two species of *Carpophilus* was similar. Small but significant differences between the two species and between the sexes were observed in laps per bout, time flown, and average speed. The large difference in number of bouts between the species suggest that *C. lugubris* flies more

Table 2. Average maximum flight statistics for male and female *Carpophilus hemipterus* and *C. lugubris*.

Species and sex	Max. number of flight bouts (±SEM)	Max. number of laps/bout (±SEM)	Max. time per lap (s ± SEM)
C. hemipterus			
female	8.0 ± 2.9	17,306 ± 3,007	3.09 ± 0.91
male	13.4 ± 3.3	24,993 ± 4,167	1.70 ± 0.65
C. lugubris			
female	41.3 ± 4.5	27,988 ± 3,785	1.20 ± 0.55
male	20.9 ± 3.6	25,729 ± 2,256	1.18 ± 0.56

Table 3. Analysis of the number of flight bouts by male and female *Carpophilus lugubris* and *C. hemipterus*.

	df	SS[1]	MS[2]	*F*	*P*[3]
Species	1	217	217	8.89	***
Sex	1	48	48	1.97	
Species* sex	1	10	10	0.41	
Residual error	205	5011	24.4		
Total	208	5286	25.4		

[1]SS = sums of squares; [2] MS = mean squares; [3]*** signifies a probability of $P < 0.001$.

often then *C. hemipterus*. However, once flight was initiated little difference was found between the two species.

There was no significant difference between the sexes of the *Carpophilus* species in either the number of bouts flown or the time spent flying. A study of the flight behavior of *C. hemipterus* found no differences in flight propensity between males and females (Blackmer & Phelan 1991). A difference in flight in relation to time of day (morning versus evening) is frequently observed because takeoff is usually in response to a combination of light and temperature. Evening flights occur more frequently because evening temperatures are usually higher than morning with the same light intensity. Blackmer & Phelan (1991) found that *C. hemipterus* was most likely to fly at dusk, consistent with this model. However, in our laboratory flight trials morning and evening temperatures were the same, and clearly adequate to initiate flight. Similarly the light intensity was the same at both times and adequate for flight. Thus, our data indicating greater flight propensity in the evening are surprising and suggests a third variable governing flight activity. One possibility may be a greater urgency to locate a source of food as the day passes.

Table 4. Analysis of the amount of time spent flying (minutes square root transformed) by male and female *Carpophilus lugubris* and *C. hemipterus*.

	df	SS[1]	MS[2]	*F*	*P*[3]
Species	1	73	73	3.99	*
Sex	1	0	0	0	
Species* sex	1	7	7	0.38	
AM/PM	1	91	91	4.97	*
Species* AM/PM	1	8	8	0.44	
Sex* AM/PM	1	65	65	3.55	*
Residual	383	7004	18.3		
Total	389	7248	18.6		

[1]SS = sums of squares; [2]MS = mean squares; [3]* signifies a probability of $P < 0.05$.

Table 5. Analysis of the average speed of flight (cm/sec square root transformed) by male and female *Carpophilus lugubris* and *C. hemipterus*.

	df	SS[1]	MS[2]	*F*	*P*[3]
Species	1	2.840	2.840	142.0	***
Sex	1	0.250	0.250	12.5	***
AM/PM	1	2.144	2.144	107.2	***
Sex* AM/PM	1	0.021	0.021	1.1	
Species* sex	1	0.136	0.136	6.8	**
Species* AM/PM	1	0.174	0.174	8.7	***
Residual	383	7.750	0.020		
Total	389	12.91	0.033		

[1]SS = sums of squares; [2]MS = mean squares; [3]*** signifies a probability of $P < 0.001$; **$P < 0.01$.

Due to the numerous extraneous factors involved with the flight mill experiment, it is impossible to directly link observed flight behavior with flight behavior in nature. Although a fairly accurate measurement of distance flown in a fixed time period can be calculated from average speed and time flown, it can not be correlated with distance flown under natural conditions. However, the approach taken in this study allows for comparisons between groups (species and sex), permitting relative differences in performance to be estimated.

Acknowledgments

We are grateful to R. J. Bartelt (USDA National Center for Agricultural Utilization Research, Peoria, Illinois) for specimens of *Carpophilus lugubris* to start our culture. We acknowledge and appreciate the assistance of Dan Fickle and Sean Ellis (Ohio Agricultural Research and Development Center). Salaries and research support were provided by State and Federal funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University. This is Journal Article No. 051-99.

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Appendix

The interface between the flight mills and the computer was originally designed to operate with comparatively weak flying leafhoppers (Taylor et al 1992). In the original specification, the monitor sampled 16 mills about 4 times per s,

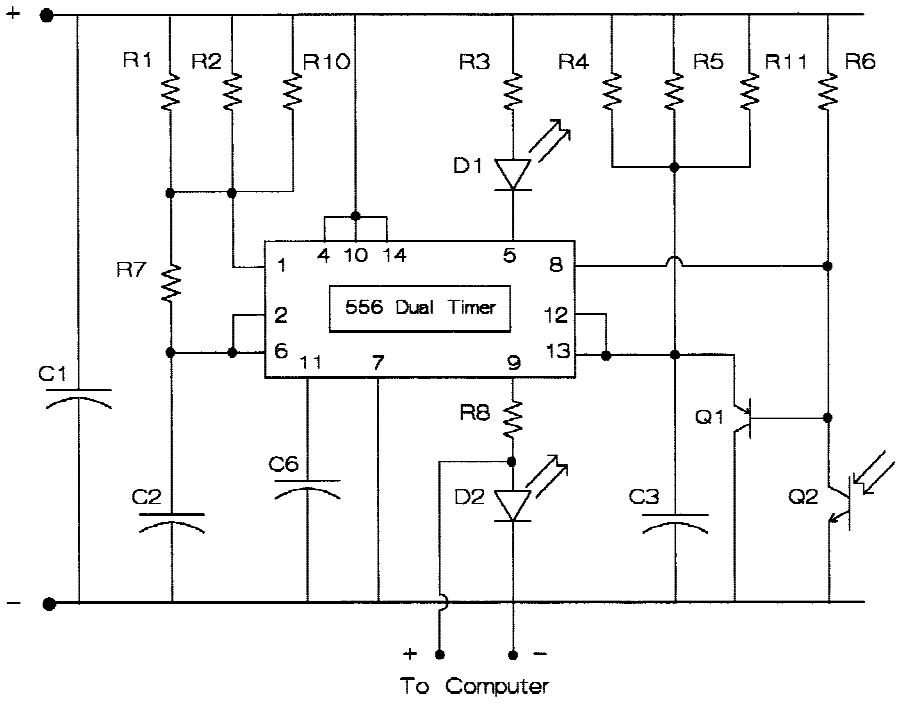

Fig. 1.

Table 1A. Parts list for revised computer-monitored flight mill

Symbol	Quantity	Description
R1	16	47 KΩ 0.25 watt resistor
R5	32	pair of 47 KΩ 0.25 watt trim resistors in parallel
R2	16	4.7 KΩ & 10 KΩ 0.25 watt trim resistors in parallel
R3, R8, R9	48	220 Ω 0.25 watt resistor
R4	16	2.2 MΩ 0.25 watt resistor
R6	16	220 KΩ 0.25 watt resistor
R7	16	1 KΩ 0.25 watt resistor
R10	**16**	**4.7 KΩ 0.25 watt trim resistor**
R11	**16**	**47 KΩ 0.25 watt trim resistor**
C1	16	0.1 μF 25-V ceramic capacitor
C2	16	4.7 μF 35-V capacitor
C3	16	1.0 μF 50-V capacitor
C4	1	4700 μF 35-V capacitor
C5	1	250 μF 25-V capacitor
C6	**16**	**0.01 μF 0.25-V ceramic capacitor**
D1	16	Infrared light-emitting diode
D2	16	Red light-emitting diode
Q1	16	2N3906 transistor
Q2	16	Infrared phototransistor
556 Dual TImer	16	556 dual timer integrated circuit

which was quite adequate to record every revolution of *Dalbulus maidis* DeLong and Wolcott. The nitidulids fly more than twice as fast as the leafhoppers. To compensate for this, the number of mills used simultaneously was reduced to 8, and modifications were made to the monitor to compensate for the faster flight speeds of the *Carpophilus* spp. Fig. 1A shows the revised circuit diagram of the monitor with a revised parts list with the changes required marked in bold on Table 1A. They were: 1, to decrease the resistance at R1/R2 by adding a 4.7K resistor (R10), 2, decreasing the resistance at R4/R5 with a 47K resistor (R11), and 3, inserting a 0.01 μf capacitor between terminal 11 of the 556 dual timer IC and the negative terminal (C6). The affect of these changes was to double the frequency of sampling by the monitor. Combined with halving the number of mills in use, this effectively increased the sampling rate by a factor of 4, sufficient to sample the faster beetles with a high degree of accuracy.

NOTE

Efficacy of Emamectin Benzoate at Controlling Diamondback Moth (Lepidoptera: Plutellidae) on Collard in South Carolina[1]

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KEY WORDS *Plutella xylostella*, diamondback moth, emamectin benzoate, *Bacillus thuringiensis*.

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is the most destructive insect pest of crucifers and it is estimated that it costs $1 billion annually to control this pest worldwide (Talekar & Shelton 1993). The diamondback moth probably originated in the Mediterranean area (Hardy 1938) and was first found in the United States in 1854 (Fitch 1855). The diamondback moth emerged as the most important pest of collard, *Brassica oleracea* ssp. *acephala* de Condolle, in Lexington County, South Carolina, in the 1980s. Initially, broad-spectrum insecticides were used to control diamondback moth, but this control method resulted in the reduction of beneficial arthropods. Growers then adopted *Bacillus thuringiensis* Berliner (Bt) products for control of diamondback moth and to conserve beneficials. However, since 1994, collard growers have reported a reduction in the efficacy of Bt products used to control diamondback moth larvae, particularly in July through September, even though they increased both the rate and frequency of applications. Field failures occurred with both the *kurstaki* and *aizawai* subspecies of *B. thuringiensis*. This situation has resulted in severe crop losses, and in some cases, crop abandonment.

Because of selection pressure resulting from intensive pesticide use to control diamondback moth, insecticide resistance has been documented for all synthetic chemical classes registered in the United States (Lasota et al. 1996). Field failures and resistance of DBM to *B. thuringiensis* in Hawaii have been documented (Tabashnik et al. 1990). Because of resistance, there is an urgent need for chemicals with different modes of action and which do not select for cross-resistance to conventional insecticides. New insecticides that circumvent the mechanisms of resistance in diamondback moth are important not only for insect control but also

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Prod. #173-05

for managing insecticide resistance. The availability of new chemistry insecticides with different modes of action in an insecticide rotation program should reduce selection for resistance to any one insecticide and prolong the usefulness of all products. The experimental product Proclaim 5SG is a new, semi-synthetic avermectin insecticide that contains the active ingredient emamectin benzoate, derived from the fermentation product of avermectin B_1 (abamectin). Proclaim, developed by Novartis Crop Protection, Inc., is considered active against a broad range of lepidopteran larvae of various crops (Anonymous 1997). Our paper presents results concerning the efficacy of Proclaim 5SG in controlling diamondback moth on collard in Lexington County, South Carolina.

Field trials on collard were conducted at Walter Rawl & Sons Farm in Lexington County between October and November 1997. 'Top-bunch' collard seedlings were transplanted on plots 1.8m in width (two 0.9m rows) and 4.5m in length with a spacing of 30 cm between plants in the row. Treatments were replicated four times in a randomized complete block design. Treatments were applied with a CO_2 pressurized backpack sprayer operating at 4.2 kg/cm^2 and delivering 893 liters/ha through a 3-nozzle boom equipped with TX 10 hollow cone tips. Treatments evaluated were Proclaim applied at 0.0084 kg (AI)/ha and *B. thuringiensis* subspecies *kurstaki* (MVP®II, Mycogen Corp., San Diego, California) at 1.01 kg (AI)/ha. All treatments were applied with a spreader-sticker, Triton (Rohm & Haas Co., Philadelphia, Pennsylvania), at 2 ml per 3 liters of spray. Treatments were applied on 10, 17, 24, and 31 October and on 8 November. There were also untreated check plots. Standard cultural practices were used for fertilization and weed control.

The number of diamondback moth larvae and pupae were counted on five randomly selected plants per plot on 10 (pre-treatment), 13, 20, and 27 October and on 3, and 11 November. The economic threshold is one larva or pupa per plant in South Carolina. Data were subjected to analysis of variance and treatment means were separated by the least significant difference (LSD) test (Pesticide Research Manager 5.0, 1997). The diamondback moth population increased from 3.5 larvae and pupae per five plants in the untreated check plots at the beginning of the trial and averaged 17.8 larvae and pupae per five plants for the remainder of the trial (Table 1). Larval numbers in *B. thuringiensis* subspecies *kurstaki* treatments were not significantly different from the control and this material was ineffective in controlling the diamondback moth population. Proclaim reduced the diamondback moth population below the economic threshold 10 d after its first application, and thereafter effectively controlled the diamondback moth population.

Results indicate that *B. thuringiensis* subspecies *kurstaki* was ineffective in controlling the diamondback moth population that was later confirmed to be resistant to *B. thuringiensis* (Khan 1998), whereas Proclaim was effective. Proclaim, with its unique mode of action, long residual activity in target crops, low rates, and safety to most beneficial arthropods (Anonymous 1997) may be a useful tool in an insecticide rotation program for controlling diamondback moth and managing insecticide resistance.

Acknowledgment

We thank Walter Rawl & Sons Farm for use of their collard field. We are grateful to Gerald Carner and Gloria McCutcheon of Clemson University for reviewing the manuscript.

Table 1. Effect of insecticides on the number of diamondback moth larvae and pupae per five collard plants (±SE) 3 d after treatment in Lexington County, South Carolina, 1997.

Treatment[a]	Rate[b]	10 Oct.[c]	13 Oct.	20 Oct.	27 Oct.	3 Nov.	11 Nov.
Proclaim 5 SG	0.0084	10.3 ± 6.1a	1.0 ± 0.4b	0.0b	0.3 ± 0.2b	0.0b	0.0b
Bt *kurstaki*	1.01	6.8 ± 1.9a	24.8 ± 3.9a	11.5 ± 3.1a	20.3 ± 2.8a	19.5 ± 2.2	17.5 ± 5.4a
Untreated Check	—	3.5 ± 1.3a	20.3 ± 2.3a	18.5 ± 3.6a	15.5 ± 3.6a	15.8 ± 2.4a	18.8 ± 6.2a

Means in a column followed by the same letter do not significantly differ (P = 0.05, LSD).
[a]Plots treated on 10, 17, 24, 31 Oct and 8 Nov.
[b]Kg [AI]/ha.
[c]Pre-treatment count.

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Behavioral Responses to Heat in Artificial Galleries by the Western Drywood Termite (Isoptera: Kalotermitidae)[1]

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ABSTRACT We quantified the response of the western drywood termite, *Incisitermes minor* (Hagen), to heat within two galleries routed into a wood cross. Nymphs were placed in the gallery of the right arm and after 2 h, the gallery temperature was raised >40°C. Trials also were conducted with the addition of alates or soldiers to groups of nymphs. Termites moved only as far as necessary to avoid temperatures >40°C and never relocated to areas furthest from the highest gallery temperatures. Termites tended to occupy the central, cross-shaped portion of the gallery. Mean temperature preference values for nymphs only, nymphs with alates, and nymphs with soldiers were 29.5°, 31.9°, and 28.9°C, respectively, indicating that the presence of other castes had no effect on termite thermal preference. The corresponding mean highest gallery temperatures were 42.8°, 44.2°, and 42.8°C and the mean lowest temperatures were 25.8°C. The presence of alates or soldiers had no effect on the distribution of termites. A calculated mean termite walking speed of 1.41 cm/s suggests that *I. minor* can respond and move quickly away from increasing gallery temperatures. Trials also were conducted with the termites introduced into the left arm of the cross. Results of choice tests conducted with termites permitted to move either upward or downward in the vertical gallery, or right or left in horizontal galleries, indicate a possible turn bias in *I. minor*.

KEY WORDS *Incisitermes minor,* Isoptera, Kalotermitidae, behavior, heat, thermotolerance, turn bias

Colonies of the western drywood termite, *Incisitermes minor* (Hagen), live entirely within sound, dry wood. Drywood termite colonies may escape the harmful effects of high temperatures by retreating deep into their galleries where, presumably, the combination of the insulating properties of wood (Harvey 1934; Pence 1956; Williams 1976; Rust et al. 1979) and the modification of the microenvironment by the termites (Kofoid 1934) enhances their survival. However, there is no published data on what the actual gallery conditions are like or whether drywood termites can modify their microenvironment.

Tolerance to extreme temperatures has been well documented for *I. minor*. The critical thermal minimum (CT_{min}) and maximum (CT_{max}) (the temperatures at which irreversible torpor occurs in an insect after exposure to slowly decreasing or increasing temperatures) for *I. minor* are –21.3°C (Rust et al. 1997) and 52°C

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(Rust et al. 1979), respectively. Ebeling (1994) reported 265- and 33-min exposures to 46° and 49°C were required for complete kill of *I. minor* nymphs. Infestations by *I. minor* are often found in attics, where temperatures frequently surpass the CT_{max} (52–57°C in some rafters; Rust et al., unpublished data). How do these colonies survive exposure to temperatures above the CT_{max}? Rust & Reierson (1998) state that *I. minor* colonies survive either by remaining in portions of the wood protected from extreme temperatures or by temporarily moving away to cooler parts of the wood. Direct observations of behavior in response to heat under natural conditions are not possible because of the wood-inhabiting nature of *I. minor* and the questionable accuracy of devices used to locate termites within wood.

Rust et al. (1979) found colonies of *Incisitermes fruticavus* Rust in the basal portion of jojoba plants, *Simmondsia chinensis* (Link) Schneid., where the gallery temperatures were 4° to 5°C cooler than the ambient air. Galleries were constructed to allow escape from exceedingly high temperatures in the peripheral branches to the basal areas. Likewise, *I. minor* nymphs and larvae restrict their activity toward the central portions of wood as conditions become hotter and dryer (Rust et al. 1979). Cabrera & Rust (1996) observed *I. minor* nymphs avoiding temperatures >45°C and aggregating in the cooler areas of a heated temperature gradient.

Movement of drywood termites within wood also has been inferred from data generated with an acoustic emissions detector (Scheffrahn et al. 1993) and from observations of termite response to temperature or relative humidity under laboratory conditions (Sen-Sarma & Chatterjee 1966; Agarval 1978; Minnick et al. 1973; Steward 1981, 1982; Smith & Rust 1994; Cabrera & Rust 1996).

In this study, the behavior and movement of *I. minor* nymphs, alates, and soldiers in response to heat was observed in a specially constructed apparatus containing simulated galleries. Our main objectives were to identify behaviors that are used by *I. minor* to survive the hot and xeric conditions that may be encountered in natural infestations. We also experimentally tested the hypothesis that *I. minor* colonies move within the wood that they infest to avoid potentially lethal temperatures. In addition, we wanted to determine how far and how quickly *I. minor* nymphs traveled within a gallery in response to elevated temperatures, whether the presence of alates or soldiers affected nymphal behavior, at which temperatures nymphs settle at after being displaced by high temperature, and how they behave in vertical galleries.

Materials and Methods

Termites. Termites were obtained from infested logs, branches, and lumber collected in Riverside County, California, and extracted and maintained as described by Cabrera & Rust (1999). The termites were left undisturbed from 1 to 2 wk before they were used in experiments and workers used in each trial came from the same container. For each trial, the source container from which the workers were obtained was randomly chosen so that workers for each of five replicates per treatment did not all come from the same colony.

Apparatus. Galleries (248 cm × 1 cm × 0.5 cm) were routed into the surface of one side of two construction-grade Douglas fir nominal two-by-four studs (cross-sectional dimensions of 3.8 cm × 8.7 cm) and a cross-shaped gallery (1 cm × 0.5

cm) was routed into the surface of an 8.7 cm × 8.0 cm × 3.0 cm Douglas fir block. The studs were cut into four 120-cm-long sections and were attached with four metal elbow braces to the block, resulting in a large cross (Fig. 1). A 120 cm × 5.3 cm flat, metal light strap was attached to the backside of the cross to provide added support to the horizontal arms, especially when the cross was in a standing position. Rope tied to screw eyes positioned at the ends of the horizontal arms and the top arm was used to anchor the cross and provide additional support. Four 1-mm-wide grooves, spaced ≈39 cm apart, oriented perpendicularly and extending to the gallery, were routed into each arm. The location of the grooves divided the entire gallery into 12 equal-sized linear zones plus one smaller cross-shaped zone in the center. Each arm was covered with two 9 cm × 61.5 cm pieces of Plexiglas and the center block with one 8.7 cm × 8.0 cm piece and attached with nylon screws. Each piece was covered lengthwise down the center by 1.5-cm-wide strips of red gelatin filter (No. 29; Eastman Kodak Co., Rochester, New York) to filter all visible light, except for wavelengths of ≈600 nm (*I. minor* nymphs have an aversion to fluorescent or incandescent light [Cabrera & Rust 1996]), from the galleries.

Thermocouples (PT-6; Physitemp Instruments Inc., Clifton, New Jersey) were inserted into each of the 16 perpendicular grooves so that the tips were flush with the gallery. Another thermocouple was inserted into a 1-mm hole drilled through the center of the Plexiglas covering the center block and positioned so that the tip was above the gallery to avoid interfering with the termites. To record temperatures from the center of the wood, thermocouples were inserted into each of four 1-mm holes drilled into the center of the wood and parallel to the perpendicular grooves. Thermocouples were connected to one of two 12-channel scanning digital thermometers (Model 92800-00, Benchtop 115V; Cole Parmer Instrument Co., Chicago, Illinois) and temperatures were recorded on two dot-matrix printers.

Heat was provided by four Cal-Cord heating coils (Glas-Col Company, Terre Haute, Indiana) attached to the backside of the right arm and center block with insulated staples. The wood was covered with aluminum foil and foil adhesive tape to prevent scorching and the coils were likewise covered to retain heat and facilitate the heating of the arm. Heat was controlled by four input controllers (Type 45500; Barnstead/Thermolyne, Dubuque, Iowa). Trials with no heat applied to the right arm served as the controls.

Pretrial preparation. Twenty-five *I. minor* nymphs, placed in a plastic petri dish cover (10 cm in diameter) lined with a filter paper disk, were chilled at 4.4°C for a minimum of 10 min to slow down their activity. Each nymph was marked (DecoColor Pen; Uchida of America Corp., Carson, California) with a distinct pattern of black, white, or brown spots on the abdomen so that each individual could be identified and their behaviors observed during each trial. Preliminary work revealed that marking the termites was not harmful. Marked nymphs were held in the dark at 4.4°C for an additional 10 min to minimize contact between the nymphs, thereby reducing their chance of sticking to each other as the ink dried. The nymphs were kept overnight in the dark at ambient room conditions (≈23°–26°C, 40–50% RH) in a covered petri dish provisioned with damp filter paper.

Standard trials. The cross was suspended in a horizontal position to facilitate the addition of termites into the gallery. Marked nymphs were immobilized by placing them in an aluminum weighing pan inside an ice bath and then trans-

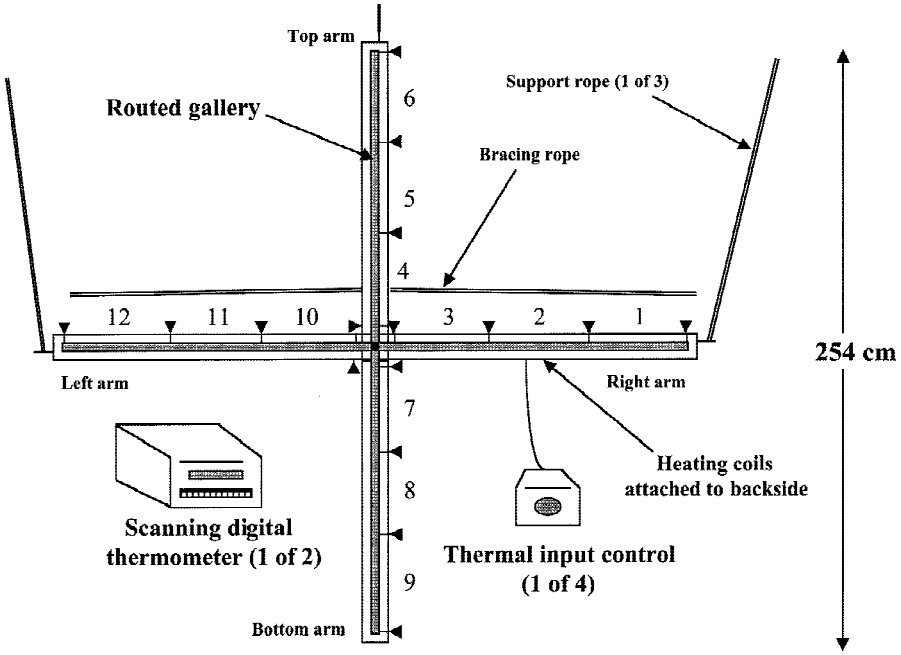

Fig. 1. Artificial gallery system used for studying movement of *I. minor* within wood in response to heat. Numbers correspond to the zones in the cross; arrows indicate insertion points of thermocouples and lines perpendicular to the gallery represent grooves through which they reached the gallery. The scanning thermometer and thermal input control are not drawn to scale; thermocouples are not shown to preserve clarity of diagram.

ferred to the gallery in zone 1. The Plexiglas cover was replaced over the gallery and the cross was returned to a standing position. The termites were allowed to acclimate for 2 h, with full access to the entire gallery, after which the heating coils were turned on so that the gallery temperature in zones 1–3 reached a minimum of 40°C. The arm was heated slowly (e.g., 25.0° to 45.4°C in ≈2 h) to minimize scorching of the wood and to prevent the termites from becoming trapped and killed if a large portion of the gallery reached lethal temperatures too quickly. After 6 h, heating was discontinued and the right-hand gallery returned to ambient temperature within 2 h. Each trial lasted 10 h and was performed under ambient room conditions.

At the end of each trial, the Plexiglas covers and termites were removed and the gallery and covers were washed with 85% ethanol to remove any semiochemicals (Cabrera & Rust 1996) and allowed to dry completely before the covers were reattached. The cross was rotated 90° counterclockwise around its vertical axis after every third trial to eliminate any structural effects that could affect the behavior and distributions of the termites. The heating coils were removed from the arm that was now vertical and were reattached to the right arm so it could be heated.

Counts. Hourly counts of termites within each zone were made and the temperatures recorded at the end of each hour during the 10-h trial. A mean number of nymphs per zone was determined to obtain a mean distribution of termites within the gallery for each trial for hours 3–8 (the period that the arm was heated) and hour 8 (the time at which heat had been applied for 6 h).

Effect of caste composition. Trials were conducted with 25 nymphs plus the addition of three soldiers or five alates to determine if the presence of these castes affected the behaviors and distributions of nymphs. Soldiers were marked on the abdomen with a black, white, or brown dot and alates were marked on the uppermost wing with one to five white dots for identification during the trials. Three caste treatments (nymphs, nymphs with three soldiers, and nymphs with five alates) were tested at two temperatures (heated and ambient).

Effect of location of introduction. Additional trials were conducted to determine if the distribution of termites would differ significantly if the initial introduction of termites was made in a vertical rather than a horizontal gallery. Twenty-five unmarked nymphs were introduced into zone 6 or zone 9 and tested as described previously. No heat was applied during these trials.

Up/down choice tests. Observations of the termites during standard trials indicated that few termites ventured into the lower vertical arm of the gallery and aggregations were never found in zones 7–9. Choice tests were conducted to determine if *I. minor* nymphs were more likely to move up a vertical gallery rather than down. Plugging the left-hand gallery of the center block with two Plexiglas bars blocked access to zones 10–12 in the left arm. Twenty-five unmarked nymphs were introduced into the horizontal arm, allowed to acclimate for 2 h and the arm heated as in the standard trials. The number of termites in the upper and lower arms (zones 4–6 and 7–9, respectively) was counted at the end of each hour. The cross was rotated 90° counterclockwise around its vertical axis after the first and third of five trials and the coils were relocated so that the horizontal arm could be heated. These choice tests were repeated with the exception that the nymphs were introduced into the left arm and access to the right arm was blocked.

Left/right choice tests. The choice tests were repeated with the exception that the cross was laid flat to eliminate the effects of gravity on the termites. Termites were introduced into the arm perpendicular to the left and right galleries. The number of termites in the left or right galleries was counted every hour for 10 h. The cross was rotated 90° clockwise and the termites introduced into a different arm after the first and third trials.

Termite movement. Movement of individual nymphs was tracked sporadically for 5 min of a given hour between hours 3 and 8 during the standard trials. Individual termites were identified and tracked to determine how many of the termites moved distances >25 cm after they had vacated zone 1, 2, or 3 due to elevated gallery temperatures. Individuals reaching or moving past designated points in the gallery during one counting session that were observed again at a later time were called repeats. To determine if movement was restricted to specific individuals or if certain castes moved more than others, the percentage of repeats and the total number counted that were nymphs, alates, or soldiers were calculated.

Four marks spaced 5 cm apart were drawn on the red filter over zone 3 and any termite that traveled a minimum of 10 cm from one mark to another without

stopping was timed with a stopwatch. A mean walking rate (centimeters per second) was calculated from these measurements.

Mean temperature preference value. A mean temperature preference value (mTPV) (Steward 1981; Smith & Rust 1994; Cabrera & Rust 1996) was calculated for the interval between 3 and 8 h. Comparison of these values also could indicate whether heat had a significant effect on termite behavior and distributions. The mTPV is the average temperature where the termites are likely to be found and is calculated: $\Sigma(a_I \cdot b_i)/n$ where a = the average number of termites per zone, b = the mean temperature of each zone, i = 1 to 13, and n = the mean total number of termites counted each hour. Termites that died during each trial were not counted. An mTPV that was significantly higher than the average lowest gallery temperature would indicate that the termites settled at warmer temperatures, whereas an mTPV that was not significantly different from the lowest gallery temperature would indicate that termites are seeking out the coolest temperatures in the gallery.

Data analyses. All trials were replicated five times. The order in which the experimental combinations for the standard trials were performed was randomly assigned. The mean distribution of termites for each experimental combination for the standard trials was compared with its corresponding control for both time periods with a Kolmogorov goodness of fit test (Conover 1971). This test is used to compare a sample with an expected distribution. Distributions with nymphs only, nymphs with soldiers, and nymphs with alates were compared with a Kolmogorov-Smirnov two-sample test (Conover 1971) for both heated and control trials. This test is used to compare two sample distributions with each other. Distributions of termites from the location of introduction trials were compared with the standard, nymph-only control trial distributions with a Kolmogorov-Smirnov two-sample test. Chi-square analyses were used to determine if there was a tendency for the termites to move either up or down in the vertical choice tests or left or right in the horizontal choice test. In addition, an *a posteriori* chi-square analysis was applied to the total number of termites in the upper and lower arms for pooled heated trials and pooled controls as a further check for a tendency for termites to move upwards in the gallery.

Comparisons between the mTPV for each experimental combination and the corresponding lowest gallery temperature were made with paired *t* tests. To determine if the presence of alates or soldiers influenced nymphal behavior, primarily by affecting their temperature preference and where they settled, i.e., at what temperature termites are found, the mTPVs among the three different caste treatments of the heated trials were analyzed by analysis of variance (ANOVA).

Results

Distribution. The distributions of nymphs, nymphs with soldiers and nymphs with alates were significantly different in heated trials between hours 3 and 8 from their respective controls ($D_{0.05} = 0.1540$, $D_{obs} = 0.17$, 0.26, and 0.18, respectively). As the temperatures within zone 1–3 increased to >40°C, the termites moved towards the center of the cross, whereas in the unheated controls termites remained in these zones or continued to venture into them (Tables 1 and 2). Distributions did not differ in both heated and control trials for nymphs versus nymphs with alates ($D_{0.05} = 0.46$, $D_{obs} = 0.23$ and 0.23), nymphs versus nymphs

Table 1. Mean number of termites[a] (±SD) within each zone of the gallery when heat is applied to the right arm of the cross from hours 3 to 8.

Group composition[c]	Zone[b]												
	Right arm			Top arm				Bottom arm			Left arm		
	1	2	3	4	5	6	Center	7	8	9	10	11	12
25n	0.3	3.7	2.7	0.9	0.2	1.9	6.9	0.3	0.1	0.2	3.3	0.1	2.1
	± 1.2	± 6.8	± 4.4	± 1.4	± 0.4	± 4.6	± 6.3	± 0.7	± 0.2	± 0.4	± 4.5	± 0.4	± 4.6
	42.0°	**39.1°**	**32.8°**			**26.5°**	**26.5°**			**24.7°**			**26.3°**
25n, 5a	1.2	3.2	8.3	1.2	1.1	0.5	7.2	0.5	0.1	0.1	0.1	0	0.0
	± 3.9	± 5.9	± 8.2	± 0.4	± 2.2	± 0.8	± 6.1	± 0.8	± 0.2	± 0.3	± 2.8		± 0.2
	43.1°	**39.8°**	**33.1°**			**26.2°**	**26.0°**			**25.0°**			**26.1°**
25n, 3s	0.2	1.0	4.0	3.6	0.2	0.0	12.8	0.6	0.1	0	1.9	0	0
	± 0.7	± 2.0	± 3.7	± 4.8	± 1.0	± 0.2	± 4.3	± 1.1	± 0.4		± 2.4		
	42.6°	**39.2°**	**32.4°**			**26.3°**	**26.9°**			**25.3°**			**26.3°**

[a]Means of 6 h × 5 replicates = 30 counts.
[b]Bold numbers beneath zones 1–3, 6, center, 9, and 12 correspond to average zone temperature (°C) between hours 3 and 8.
[c]n, nymphs; a, alates; and s, soldiers.

Table 2. Mean number of termites[a] (±SD) within each zone of the gallery with no heat applied to the right arm of the cross from hours 3 to 8.

	Zone[b]												
	Right arm			Top arm				Bottom arm			Left arm		
Group composition[c]	1	2	3	4	5	6	Center	7	8	9	10	11	12
25n	4.6	1.6	4.8	2.1	0.1	0	9.1	0.3	0	0.3	0.5	0.0	0.0
	± 8.4	± 1.6	± 4.9	± 3.7	± 0.4		± 7.4	± 0.8		± 0.5	± 0.8	± 0.02	± 0.2
	25.6°	**25.7°**	**25.6°**			**26.8°**	**25.2°**			**25.2°**			**26.4°**
25n, 5a	4.3	1.3	4.0	0.9	0.4	0.2	7.0	0.4	0	0.2	4.8	0.6	0.8
	± 6.6	± 1.9	± 4.2	± 1.2	± 0.6	± 0.6	± 6.3	± 1.0		± 0.7	± 7.0	± 1.2	± 1.7
	25.4°	**25.4°**	**25.3°**			**26.5°**	**26.0°**			**25.1°**			**26.0°**
25n, 3s	1.9	0.6	10.1	2.3	0.2	0.1	6.4	0.0	0	0	3.3	0	0.1
	± 3.3	± 0.9	± 9.1	± 3.9	± 0.6	± 0.3	± 6.4	± 0.2			± 6.5		± 0.3
	25.1°	**25.1°**	**25.0°**			**25.9°**	**25.0°**			**24.8°**			**25.8°**

[a]Means of 6 h × 5 replicates = 30 counts.
[b]Bold numbers beneath zones 1–3, 6, center, 9, and 12 correspond to average zone temperature (°C) between hours 3 and 8.
[c]n, nymphs; a, alates; and s, soldiers.

with soldiers (D_{obs} = 0.23 and 0.08), and nymphs with alates versus nymphs with soldiers (D_{obs} = 0.15 and 0.31). Likewise, the distributions of nymphs when introduced into either zone 6 or 9 were not different from the standard controls or each other ($D_{0.05}$ = 0.46, D_{obs} = 0.23, 0.31, and 0.23, respectively) demonstrating that the place of introduction had no effect on where the termites distributed themselves within the gallery.

Comparisons of the distributions of termites at hour 8, at which time the right arm had been heated for 6 h, revealed differences between the heated trials and their respective controls for nymphs only or nymphs with soldiers ($D_{0.05}$ = 0.17, D_{obs} = 0.26, and 0.32, respectively) but not for nymphs with alates (D_{obs} = 0.13). Comparisons of distributions between the different caste combinations within heated trials were not significantly different for nymphs versus nymphs with alates ($D_{0.05}$ = 0.46, D_{obs} = 0.31), nymphs versus nymphs with soldiers (D_{obs} = 0.31), and nymphs with alates versus nymphs with soldiers (D_{obs} = 0.08). Distributions also were not significantly different within the controls for nymphs versus nymphs with alates (D_{obs} = 0.23), nymphs versus nymphs with soldiers (D_{obs} = 0.15), and nymphs with alates versus nymphs with soldiers (D_{obs} = 0.31).

Movement and speed. The speed (mean ± SD) of *I. minor* nymphs (n = 42) that moved a minimum of 10 cm between the tick marks within the gallery in zone 3 was 1.4 ± 0.66 cm/s (range: 0.48–3.34 cm/s). *I. minor* nymphs could theoretically traverse the entire length of the gallery in just <3 min. However, this speed may be biased because it is based only on those individuals that traveled a minimum of 10 cm and these termites walked faster than those that moved <10 cm.

A total of 138 nymphs, six soldiers, and one alate were observed moving distances >25 cm after the termites had been forced out of the right arm by high temperatures and relocated to another area. Of these, 50 nymphs (36%) were repeats, i.e., observed walking again at a later hour, of which 19 were seen at three different times and two observed at four other hours.

Choice tests. In vertical choice tests where the nymphs were introduced into the right arm, 69% of the termites moved into the upper vertical gallery (χ^2 = 39.8, df = 1, α = 0.05). However, when introduced into the left arm, 80% of the termites moved down into the lower vertical gallery (χ^2 = 145.8, df = 1, α = 0.05). In the horizontal choice tests they had an even stronger tendency for one side with 88% of the termites ending up on the right hand side (χ^2 = 216.2, df = 1, α = 0.05). *A posteriori* chi-square analysis revealed that 81.7% of the termites in pooled heated trials and 82.6% in the pooled controls moved into the upper vertical gallery of the apparatus. This gallery is oriented to the right-hand side relative to the horizontal gallery where the termites were introduced (χ^2 = 249.0 and 141.8, respectively, df = 1, α = 0.05).

Mean temperature preference value. The average gallery temperatures among the three caste combinations were not significantly different within control (F = 0.37, df = 2, 14, P = 0.70, n = 5) and heated trials (F = 0.13, df = 2, 15; P = 0.88, n = 5). Thus, we could make valid comparisons of mTPVs between heated trials. The mTPVs for hours 3–8 were 29.5 ± 3.60°, 31.9 ± 3.09°, and 28.9 ± 0.95°C for nymphs, nymphs with alates, and nymphs with soldiers, respectively, and were not significantly different from each other (F = 1.59; df = 2, 14; P = 0.24). The corresponding average gallery temperatures for this time period were 28.6 ± 0.30°, 28.8 ± 0.29°, and 28.9 ± 0.12°C. The t tests revealed no differences

between mTPVs and the corresponding average gallery temperatures for nymphs ($P = 0.59$; df = 4, 4), alates ($P = 0.09$; df = 4, 4), and soldiers ($P = 0.98$; df = 4, 4). The mTPVs for hours 3-8 also were compared with the average temperatures of zones 6, 9, and 12, which usually had the lowest temperatures, to see if the termites selected the coolest portions of the gallery. The paired *t* tests showed that the mTPVs for nymphs with alates or soldiers were higher than the average coolest temperatures in the gallery ($P = 0.007$; df = 4, 4, and $P = 0.0004$; df = 4, 4) but were not different when only nymphs were present ($P = 0.08$; df = 4, 4). The mean gallery end temperatures were 25.8 ± 0.69, 25.8 ± 0.40, and 25.8 ± 0.72°C for heated trials with nymphs only, nymphs with alates, and nymphs with soldiers, respectively.

Discussion

The extremely large difference between the CT_{max} and CT_{min} attests to the adaptation of *I. minor* to both its geographic range and the drywood nesting habit. One major factor that allows *I. minor* to survive high temperatures is that wood is a poor conductor of heat. As the wood temperatures slowly increased during the heated trials, the termites followed the gradually developing gradient until they reached a tolerable temperature, which usually was only several centimeters away and was often very warm (up to 40°C). For example, during one trial we found that the temperature was 61.8°C at the beginning of zone 2, but only 30.7°C at the beginning of zone 3. Thus, within a distance of only 39 cm, there was a temperature differential of 31°C. Consequently, termites did not have to move very far to escape from lethal temperatures. Aggregations of termites were often found at temperatures between 30° and 35°C and some termites were even found in areas between 35° and 40°C (Table 1). Smith & Rust (1993) found that oxygen consumption was significantly lower in *I. minor* at 35°C than it was in the western subterranean termite, *Reticulitermes hesperus* Banks (0.40 versus 0.79 μl O_2/mg body wt/h), and did not follow the expected Q_{10} (Eckert 1988), thus reflecting *I. minor's* adaptation to tolerate exposure to higher temperatures. The presence of alates or soldiers (at least in the numbers used in this study) did not significantly influence the distributions of the termite groups within heated and control trials. It is interesting that at hour 8 the distribution of nymphs and five alates during heated trials was not significantly different from the respective control trials. This is explained by the presence of an unusually large number of termites (11 and 25) in zone 3 at hour 8 in two of the trials. In the other trials, and with the other caste compositions, there were many fewer termites in zone three. Additionally, the low thermal conductivity of the wood made it difficult at times for the entire length of the gallery within zone 3 to attain the desired temperature of at least 40°C during most trials. In these two instances, the temperature at one end of zone 3 was ≈44°C, whereas the temperature at the opposite end of the zone was 27.5°C. Thus, tolerable temperatures were present within zone three that allowed the termites to remain there.

The combination of low thermal conductivity of the wood, a high thermotolerance (Rust et al. 1979; Forbes & Ebeling 1987; Lewis & Haverty 1996) and a relatively low cuticular permeability that reduces water loss (Collins 1969) allows *I. minor* to remain at warmer temperatures. Although a few nymphs left zone 1 during the 2-h acclimation period and when it started to warm up, there was very

little movement within zones 1-3 until temperatures reached 40°–45°C. Rapid long distance movement of groups of termites were not frequently observed, and few termites ever moved to and remained at the ends of the gallery (Tables 1 and 2).

Other factors besides thermal and desiccation tolerance might contribute to survival of *I. minor* at high gallery temperatures. Acclimatization to high temperatures can develop as daily mean temperatures gradually increase during the months preceding the summer (Meganasa 1964, Bursell 1974). Mitchell et al. (1993) found a linear relationship between acclimation temperature and CT_{max} in the harvester termite, *Hodotermes mossambicus* (Hagen). However, Scheffrahn et al. (1997) found that an acclimation temperature of 35°C for 2 weeks did not confer thermotolerance to *Cryptotermes brevis* (Walker). Perhaps, in this case, the acclimation temperature was not high enough. Lethal effects of temperature can occur indirectly by killing the symbiotic protozoa in the termite hindgut (Williams 1977; Collins 1991). Meganasa (1964) found that the thermal death point of the protozoa of the drywood termite, *Marginitermes hubbardi* Banks, was 2° to 3°C lower than those of the host nymphs. In the current study, *I. minor* nymphs generally vacated galleries when the temperatures were within ≈10°C of the CT_{max}, thereby protecting themselves and gut symbionts from the lethal effects of high temperature.

Western drywood termites have shown the ability, in both this and a previous study (Cabrera & Rust 1996), to detect temperature differentials and discriminate between lethal and non-lethal temperatures. This behavior must be mediated by temperature or heat receptors, which have yet to be identified in any termite species.

Another adaptive behavior that may allow *I. minor* to tolerate warmer gallery temperatures is aggregation. It is thought that aggregation of drywood termites in arid environments reduces cuticular water loss because they have less exposed cuticular surface area (Pence 1956; Collins 1969; Williams 1977). Yoder et al. (1992) stated that aggregation lowers metabolic rates and reduces the surface-to-volume ratio resulting in increased water conservation. Thus, net transpiration rates for isolated fungus beetles, *Stenotarsus rotundus* Arrow, were higher than for groups of 250 beetles. Yoder and Grojean (1997) found that water loss in groups of male or female Madagascar hissing cockroaches, *Gromphadorina portentosa* (Schaum), was reduced in comparison to isolated individuals. Yoder & Smith (1997) determined that net water loss was lowest in groups of 20 adult female convergent lady beetles, *Hippodamia convergens* (Guerin-Meneville), greatest in isolated beetles and intermediate in groups of five or ten. Glass et al. (1998) suggested that the relative humidity within clusters of the house dust mite, *Dermataphagoides farinae* (Hughes), was actually higher than the relative humidity outside of the cluster. Cabrera (unpublished data) found that water loss was greater for isolated *I. minor* workers than for workers in groups when held under desiccating conditions. Additionally, a higher local relative humidity established by the nymphs within aggregations may further act to decrease cuticular water loss. Thus, a reduction in water loss is one of the benefits of aggregation but may not be the absolute reason for it. A device or method for measuring microenvironmental relative humidity would be useful in determining the relative humidity within an aggregation or termite gallery.

Aggregations of termites were frequently seen during both the heated and control trials. Thus, aggregation occurs even in the absence of heat, reflecting the

social and thigmotactic nature of *I. minor*. Aggregation also may be mediated by pheromones. Labial gland secretions, deposited at sites where termites gnaw on wood, function as an aggregation pheromone in some termite species and can induce aggregation even in the absence of food (Kaib 1999). Tactile cues might also facilitate aggregation. Perhaps a coarser, uneven wood surface resulting from gnawing and feeding is more attractive to termites than a smooth surface. Choice tests between smooth and rough areas on a subtrate could be used to determine whether aggregation or feeding initiation sites are influenced by texture. Carbon dioxide, released from termite aggregations in the nest center, also may be used as an orientation cue for locating the colony by termites in peripheral areas of a complex gallery system (Kaib et al. 1993).

Termites had a tendency to occupy the central cross-shaped portion of the gallery (Tables 1 and 2) that provided more space than in the linear portions of the gallery. This allowed for more contact between a greater number of individuals within the aggregation.

In addition to aggregating, Collins (1969) and Cabrera (unpublished data) also have seen individuals being used as a source of water by other termites in groups subjected to extremely xeric conditions. The trophallactic exchange of moisture between colony members is thought to enhance survival of the colony during periods of water stress (Collins 1969, Mishra and Singh 1979).

The termites had a strong tendency to move to the gallery located to the right of the center of the cross, resulting in their moving upward into the vertical gallery when introduced into the right side, downward into the vertical gallery when introduced into the left side and to the right when the galleries were horizontal, indicating the possibility of a turn bias in *I. minor*. This innate behavior has been documented in two ant species, *Formica pallidefulva* Latreille and *Crematogaster cerasi* (Fitch) by Jander (1990) and was found to optimize searching on plants by these two species. The adaptiveness of a turn bias in drywood termites is not readily apparent. Specific bioassays that eliminate all other factors affecting direction of movement are needed to prove unequivocally that a turn bias exists in *I. minor* and to speculate on its possible advantages. Orientation to magnetic fields also could be a possibility as the position of the cross remained the same relative to magnetic north as it was rotated after the first and third trial for each test. Evidence for the use of orientation to magnetic fields by the harvester termite, *Trinervitermes geminatus* (Wasmann), was demonstrated by Rickli & Leuthold (1988).

The purpose of conducting trials with the inclusion of alates or soldiers was to determine if they influenced group behaviors or distributions. The results indicate that neither alates nor soldiers had a significant effect on group behavior, at least in these ratios and for this time period. Termites also did not vacate heated areas as a cohesive group indicating that there is individual variation in thermotolerance or response to temperature.

Observations of marked termites revealed that, for the most part, a few termites were actively walking at a given time, whereas most were aggregated in one to several groups. Because observations were made sporadically and, moreover, only for 5 min at a time, the number of termites actually moving was probably much greater. Frequent movement of a few individuals may be one method for monitoring and obtaining information on gallery conditions in other areas.

The walking speed of 1.41 cm/s indicates that *I. minor* can move quickly through their galleries in response to heat and move to cooler areas. Thus, the insulative properties of the wood plus movement from temperatures 10°C below the CT_{max} allows *I. minor* to escape high lethal temperatures. This information, along with the already known physiological, behavioral and morphological adaptations, provides a greater understanding of how *I. minor* can survive hot and xeric conditions within wood. It also provides supporting evidence that *I. minor* colonies disperse within wood in response to elevated gallery temperatures.

Acknowledgment

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Surface and Subsurface Application of *Beauveria bassiana* for Controlling Mole Crickets (Orthoptera: Gryllotalpidae) in Golf Courses[1]

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ABSTRACT Three commercial formulations of *Beauveria bassiana* (Balsamo) Vuillemin were tested for controlling the southern mole cricket, *Scapteriscus borellii* Giglio-Tos (formally as *S. acletus* Rehn & Hebard), and the tawny mole cricket, *S. vicinus* Scudder, on bermudagrass, *Cynodon dactylon* (L.) Pers, fairways in 1997, 1998, and 1999. Mole cricket damage ratings from *B. bassiana*-treated plots were not significantly different from the untreated control in all surface applications, although damage ratings in the plots treated with *B. bassiana* were numerically lower than the untreated control in most situations. Imidacloprid, bifenthrin, or deltamethrin did not show better mole cricket control than *B. bassiana.* No synergistic effect was observed in this study between imidacloprid and *B. bassiana* for mole cricket control. Although subsurface application of *B. bassiana* did not provide significantly better mole cricket control compared with the corresponding surface application, it appeared that certain formulations of *B. bassiana* were more effective with subsurface application. One formulation (Biological Insecticide 7695 SCK) provided significantly better mole cricket control with subsurface application compared with the untreated check.

KEY WORDS *Scapteriscus borellii, Scapteriscus vicinus,* turfgrass, subsurface, *Beauveria bassiana*

Mole crickets cause severe turfgrass damage in the southeastern United States and many other parts of the world (Ulagaraj 1975, Brandenburg 1997). Controlling mole crickets is difficult because they live in the soil and are less likely to contact insecticides directly than foliar-living insects. Many abiotic and biotic factors, such as soil moisture, percentage of organic matter, soil type, temperature, weather conditions, and insect developmental stage can cause significant variation in the performance of control agents. Chemical insecticides are frequently used to control mole crickets. Insecticide application rates for control of these soil-dwelling pests are usually much higher than those used for controlling foliar insect pests. Repeated applications during the season are often necessary to achieve acceptable control. Higher insecticide application rates and the proximity

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of infested turf areas to residential or chemically sensitive natural habitats have caused increasing concerns about the potentially adverse impacts of insecticide application to humans and the environment.

The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin has been used to control turfgrass insect pests with various success (Gardner et al. 1977, Gardner & Noblet 1978, Ramoska 1983, Ramoska & Todd 1985, Krueger et al. 1991, 1992, Heller & Walker 1997, 1999, Shetlar et al. 1999, Brandenburg & Royals 1999, Baxendale et al. 1999, Power et al. 1999, Swier et al. 1999a,b). There are two brief reports on using *B. bassiana* to control mole crickets in southeast of the United States (Hertl & Brandenburg 1998, Harris 1999). Here we report the results of our 3-yr field study in using *B. bassiana* to control nymphs of southern mole cricket, *Scapteriscus borellii* Giglio-Tos, and tawny mole cricket, *S. vicinus* Scudder, in North Carolina.

Materials and Methods

Five field tests were conducted on a bermudagrass, *Cynodon dactylon* (L.) Pers., fairway at the Fox Squirrel Country Club in Brunswick County, North Carolina, in 1997, 1998, and 1999. The test plots were laid out in a completely randomized design with four replications for each treatment. The fairway irrigation system was used to apply 0.6 cm of water prior to treatment (preirrigation), and 1.3 cm of water after treatment (postirrigation). Twenty soil cores (10 cm in depth) were taken with a standard soil sampler (Lesco Inc., Rocky River, Ohio) from the surrounding nontreated areas after treatment. Soil cores were placed into sealed plastic bags and sent to the North Carolina Department of Agriculture and Consumer Services for analysis of soil pH, soil organic matter, and soil type.

1997 tests. Two field studies were conducted with two formulations of *B. bassiana.* Plots were established in an area with relatively uniform mole cricket damage.

Test 1. B. bassiana spores (BotaniGard ES, Mycotech Corp., Butte, Montana), imidacloprid (Merit 75WP, 0.5 G, respectively, Bayer Corp, Kansas City, Missouri), and ethoprop (Mocap 10 G, Rhone-Poulenc Ag Co., Research Triangle Park, North Carolina) were tested for efficacy against newly hatched mole cricket nymphs. Plots were 8.3 × 8.3 m. Applications were made on two separate dates because imidacloprid had to be applied earlier to achieve maximum mole cricket control. Imidacloprid was applied on 27 June with an EZ Hand Spreader (Republic Tool Manufacturing Corp., Carlsbad, California). Soil was wet and soil temperature (10 cm in depth) at the time of application was 29°C. Approximately 3 cm rain occurred shortly after application. *B. bassiana* and ethoprop treatments were applied late in the afternoon on 13 August. *B. bassiana* treatments were applied with a John Deere (Deere & Co., Moline, Illinois) Turfgator mounted research sprayer (R&D Sprayers Inc., Opelousas, Los Angeles) delivering ≈252 l/ha. The ethoprop treatment was applied with an EZ Hand Spreader. Soil temperature (measured at 10 cm deep throughout this study) at the time of application was 30°C. Soil at the test site was classified as Leon fine sand with a pH of 6.0 and 0.86% humic matter. Soapy water flush sampling (Short & Koehler 1979) adjacent to the test site indicated that southern mole crickets dominated the fairway.

Test 2. B. bassiana spores (Naturalis-T; Troy Biosciences Corp., Phoenix, Arizona), imidacloprid, and ethoprop were tested for efficacy against late-instar mole crickets late in the season. Imidacloprid and *B. bassiana* were tested alone or in combination to evaluate the potential synergistic effect previously reported for the control of other insect pests (Boucia et al. 1996, Quintela, 1996; Quintela & McCoy 1997). Field plots were 6.7 × 6.7 m (smaller than that in Test 2 due to the space limitation). All treatments were applied late in the afternoon on 17 September by using a CO_2-powered backpack sprayer delivering 280 l/ha. Ethoprop was applied to the appropriate plots with an EZ Hand Spreader. Soil temperature at the time of application was 28°C. A second application of *B. bassiana* was made to the *B. bassiana* split-treatment plots with a backpack sprayer in the late afternoon on 23 September. Soil temperature at the time of application was 27°C. Soil at the test site was classified as a Kureb fine sand with a pH of 5.8 and 0.41% humic matter. Soapy water flush sampling indicated that the fairway population was ≈83% southern and 17% tawny mole crickets.

No precounting of mole cricket damage (surface activity) was made in this test because the plots were established in an area with relatively uniform mole cricket damage. Surface activity of mole crickets was evaluated weekly after application with the damage grid evaluation method of Cobb & Mack (1989). A one square-meter grid divided into nine subsections was randomly placed in each plot. A damage rating (0–9) was given based on the occurrence of fresh mole cricket damage in the nine subgrids of the frame where 0 indicates no damage and 9 indicates severe damage (damage observed in each of the nine subgrids). Five ratings were made in each plot on each sampling date.

1998 tests. Two field studies were conducted with the same formulations of *B. bassiana* as in the 1997 field tests. Field plot size was 5 × 5 m due to space consideration. All treatments were applied with the Turfgator.

Test 1. B. bassiana spores (Naturalis-T), imidacloprid, and bifenthrin (Talstar GC Flowable; FMC Corp., Philadelphia, Pennsylvania) were used in this test. No combination treatment of imidacloprid and *B. bassiana* was included. The imidacloprid application was made on 12 June as a preventive treatment applied prior to mole cricket nymphal hatch. Air (measured at 1.5 m height throughout this study) and soil temperatures at the time of application were 29°C and 27°C, respectively. The soil was wet at the time of application. Bifenthrin and the first *B. bassiana* applications were applied on 22 July as a peak hatch treatment for the control of small nymphs. Two *B. bassiana* treatments were applied at the same rate (spores per hectare), but treatment one was applied at an application volume of 407 l/ha, whereas treatment two was applied at the application volume of 1629 l/ha. Soil temperature at the time of application was 30°C. The second *B. bassiana* treatments were applied on 5 August with the same application rate and volumes as specified above. Treatments were applied under overcast skies late in the day. Air and soil temperatures were 26°C. Soil at the test site was classified as Mandarin fine sand with a pH of 6.0 and 0.56% humic matter. Soapy water flush sampling indicated that the fairway population was composed of 94% southern and 6% tawny mole crickets.

Test 2. B. bassiana spores (BotaniGard ES), imidacloprid, and bifenthrin were used in this test. The combination treatment of imidacloprid and *B. bassiana* was tested for synergistic effects. Imidacloprid was applied on 12 June prior to mole cricket nymphal hatch. Air and soil temperatures at the time of application were

29°C and 27°C, respectively. The soil was wet at the time of application. All other treatments (targeting small nymphs as a peak hatch application) were applied late in the afternoon on 22 July. Air and soil temperatures at the time of application were 29°C and 30°C, respectively. Soil at the test site was classified as Mandarin fine sand with a pH of 6.0 and 0.56% humic matter. Soapy water flush fairway sampling results indicated that the population was comprised of ≈94% southern and 6% tawny mole cricket nymphs. Mole cricket damage was rated on 5 and 19 August with the damage grid evaluation method described earlier.

No precounting of mole cricket damage (surface activity) was made in this test for the same reason as the 1997 test. Two damage ratings after treatment were made with the same method mentioned earlier.

1999 test. This study compared surface and subsurface application of three *B. bassiana* formulations for mole cricket control. The study was conducted at Meadowlands Golf Links in Brunswick County, North Carolina. A complete randomized block design was used. Each block (3.7 × 48 m) consisted of four plots (3.7 × 12 m). A Toro Subsurface Liquid Injector (The Toro Co., Bloomington, Minnesota) was used for subsurface application. Equipment slicing blades were spaced at 7.6-cm intervals to cut slits in the turf and followed by stainless steel nozzles that inject the insecticide solution to a depth of 1.3 cm below the soil surface. Equipment ground speed was 9.7 km/h, delivering an application volume of 1,400 l/ha under a pressure of 11 kg/cm^2. The surface application was applied using a backpacked hand-held sprayer powered by CO_2 and application volume was 284 l/ha. Deltamethrin (DeltaGard G, AgrEvo USA Company, Montvale, New Jersey) was applied with a Scotts (The Scotts Co., Marysville, Ohio) hand spreader as the standard insecticide for comparison. Three *B. bassiana* products, Naturalis-T, BotaniGard ES, and Biological Insecticide 7695 SCK (BI7S) (JABB of the Carolinas, Inc., Pine Level, North Carolina) were used. The application was made on 7 October. The soil was wet during the experimental period. Air and soil temperatures at the time of application were 26.7°C and 21.1°C. As soapy water flush sampling indicated the mole cricket population was composed of 99% tawny mole crickets.

The preapplication mole cricket damage rating was made. Two posttreatment surface damage ratings were conducted with technique describing earlier.

Data analysis. All mole cricket damage ratings were transformed (square root of X + 0.5) prior to conducting ANOVA (SAS Institute 1990). A Tukey's studentized range test was used to test differences between treatments with a 5% level of significance for all tests. Data with preratings (1999 study) were subjected to covariance analysis (GLM Procedures, SAS 1990). Untransformed damage rating means are reported in Tables.

Results

1997 tests. *B. bassiana* (BotaniGard ES), imidacloprid, or ethoprop alone or in combinations of *B. bassiana* and imidacloprid did not provide significantly better mole cricket control compared with the untreated check on August 20, 27, and September 10 in test 1 (Table 1). Ethoprop, *B. bassiana* (6.7×10^{13} spores/ha) plus imidacloprid 75 WP (448 g/ha) provided better mole cricket control than that of imidacloprid 0.5 G (336 g/ha) alone on August 27. Ethoprop, B. bassiana (6.7×10^{13} spores/ha) plus imidacloprid 75 WP (448 g/ha), and *B. bassiana* (1.7×10^{13}

Table 1. Efficacy of *B. bassiana* (BotaniGard ES) and insecticides alone and in combination against mole cricket nymphs in a golf course fairway, test 1, 1997.

Treatment	Rate (AI)/ha	Appli. day	Average mole cricket damage ratings[a,b]			
			20 August[c]	27 August	3 September	10 September[c]
B. bassiana	6.7×10^{12} spores	13 August	0.3	0.5ab	1.3abc	2.5
B. bassiana + imidacloprid (Merit 0.5 G)	1.7×10^{13} spores + 336 g	13 August + 27 June	0.1	0.6ab	1.1ab	1.4
B. bassiana + imidacloprid (Merit 0.5 G)	6.7×10^{13} spores + 336 g	13 August + 27 June	0.9	1.1ab	1.5abc	2.6
B. bassiana + imidacloprid (Merit 75 WP)[d]	6.7×10^{13} spores + 448 g	13 August + 27 June	0.3	0.3a	0.8ab	0.4
Imidacloprid (Merit 0.5 G)	336 g	27 June	1.1	1.8b	2.3bc	2.4
Ethoprop (Mocap 10 G)	11,200 g	13 August	0.1	0.3a	0.2a	0.8
Untreated			1.2	1.1ab	3.1c	2.3

[a]Mole cricket damage ratings (0 to 9), 0 = no damage, 9 = severe damage.
[b]Means followed by the same letter in each column are not significantly different (Tukey's Studentized Range Test, $\propto = 0.05$).
[c]Means in this column are not significantly different (Tukey's Studentized Range Test, $\propto = 0.05$).
[d]A tank mix of these materials.

spores/ha) plus imidacloprid (336 g/ha) 0.5 G, provided significantly better mole cricket control than the untreated check on 3 September. *B. bassiana* plus imidacloprid did not provide a better mole cricket control than that of either imidacloprid or *B. bassiana* (at a lower rate) alone.

Neither *B. bassiana* (Naturalis-T) alone nor in combination with imidacloprid provided significant mole cricket control compared with the untreated check, although mole cricket damage ratings in the treated plots were always lower than those in the untreated in test 2 (Table 2). There was no significant difference in damage ratings between *B. bassiana* under different rates, application date, or in combination with imidacloprid.

1998 tests. There was no significant difference in mole cricket damage ratings between any of treatments on August 5 in test 1 (Table 3). Analysis of damage ratings from 19 August showed that imidacloprid only provided significantly better mole cricket control than the untreated check. However, the damage rating in the imidacloprid treatment was not significantly different from those observed in either *B. bassiana* or bifenthrin treatments.

The combination of *B. bassiana* (BotaniGard-ES) and imidacloprid did not provide better mole cricket control than either *B. bassiana* or imidacloprid alone in test 2 (Table 4). *B. bassiana,* or imidacloprid alone, or in combination had lower mole cricket damage ratings than the untreated check. However, differences between these treatments and the untreated check were not statistically different.

1999 test. Subsurface applications of *B. bassiana* did not provide significantly better mole cricket control than those of corresponding surface applications (Table 5). However, subsurface applications resulted in a subtle improvement of the efficacy of two formulations, B17S and Naturalis-T, when comparing the changes in mole cricket damage ratings from 0 day after treatment (precounting) to 17 days after treatment. Surface application of deltamethrin and subsurface application of B17S provided significant control of mole crickets at 17 days after treatment.

Discussion

Results from this study indicate that although the numeric values of mole cricket damage ratings from the application of *B. bassiana* were often lower than those in the untreated check, the reduction in mole cricket damage from *B. bassiana* applications was not significantly different ($\propto = 0.05$) from the untreated check. However, Mole cricket damage ratings from *B. bassiana* applications were also seldom different from those of imidacloprid, bifenthrin, or deltamethrin. Mole cricket infestations in the five tests ranged from mild to severe. *B. bassiana* treatments failed to provide statistically significant control under different pest pressures.

Previous studies suggested that there was a synergistic relationship between *B. bassiana* and select insecticides for control of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Anderson et al. 1989), and other insect pests (Benz 1971). Recent studies indicated that imidacloprid enhanced the efficacy of *B. bassiana* for controlling the citrus root weevil, *Diaprepes abbreviatus* (L.) (Quintela 1996, Quintela & McCoy 1997), and the eastern subterranean termite, *Reticulitermes flavipes* (Koller) (Boucias et al. 1996). Results from this study did not demonstrate a synergistic effect between *B. bassiana* and imidacloprid for control of mole crickets.

Table 2. Efficacy of *B. bassiana* (Naturalis-T) and insecticides alone and in combination against mole cricket nymphs in a golf course fairway, test 2, 1997.

Treatment	Rate (AI)/ha	Appli. day	Average mole cricket damage ratings[a,b]		
			23 September	1 October[c]	8 October
B. bassiana	7.3×10^{10} spores	17 September	6.2ab	6.8	5.35b
B. bassiana	2.2×10^{11} spores	17 September	5.7ab	5.7	4.85ab
B. bassiana + *B. bassiana*	7.3×10^{10} spores + 7.3×10^{10} spores	17 September + 23 September	6.2ab	7.0	5.35b
B. bassiana + imidacloprid (Merit 75 WP)[d]	7.3×10^{10} spores + 112 g	17 September	4.0ab	4.5	3.85ab
Imidacloprid (Merit 75 WP)	336 g	17 September	4.2ab	3.9	3.60ab
Ethoprop (Mocap 10 G)	11,200 g	17 September	3.7a	4.0	2.55a
Untreated			7.2b	7.2	5.80b

[a]Mole cricket damage rate ranged from 0 to 9, 0 = no damage, 9 = severe damage.
[b]Means followed by the same letter in each column are not significantly different (Tukey's Studentized Range Test, $\propto = 0.05$).
[c]Means in the column are not significantly different (Tukey's Studentized Range Test, $\propto = 0.05$).
[d]A tank mix of these materials.

Table 3. Comparison of efficacy of *B. bassiana* (Naturalis-T), imidacloprid, and bifenthrin for control of mole cricket nymphs in a golf course fairway, test 1, 1998.

Treatment	Rate (AI)/ha	Application day	Avg. mole cricket damage rating[a]	
			5 August[b]	19 August[c]
B. bassiana, application volume = 4071/ha	7.3×10^{10} spores + 7.3×10^{10} spores	22 July + 5 August	1.2	2.9ab
B. bassiana, application volume = 16291/ha	7.3×10^{10} spores + 7.3×10^{10} spores	22 July + 5 August	2.6	4.1ab
Imidacloprid (Merit 75 WP)	448 g	12 June	0.8	1.8a
Bifenthrin (Talstar GC Flowable)	120 g	22 July	2.3	4.6ab
Untreated			3.6	6.0b

[a]Mole cricket damage ratings (0 to 9), 0 = no damage, 9 = severe damage.
[b]Means in the column are not significantly different (Tukey's Studentized Range Test, $\propto = 0.05$).
[c]Means followed by the same letter in the column are not significantly different (Tukey's Studentized Range Test, $\propto = 0.05$).

Table 4. Efficacy of *B. bassiana* (BotaniGard ES) and insecticides alone and in combination against mole cricket nymphs in a golf course fairway, test 2, 1998.

Treatment	Rate (AI)/ha	Application day	Average mole cricket damage rating[a,b]	
			5 August	19 August
B. bassiana	6.7×10^{13} spores	22 July	2.4	4.5
B. bassiana + Imidacloprid (Merit 0.5 G)	1.7×10^{13} spores + 448 g	22 July + 12 June	1.4	2.9
B. bassiana + Imidacloprid (Merit 0.5 G)	6.7×10^{13} spores + 448 g	22 July + 12 June	1.2	1.9
B. bassiana + Imidacloprid (Merit 75 WP)	6.7×10^{13} spores + 448 g	22 July + 12 June	2.1	3.1
Imidacloprid (Merit 0.5 G)	448 g	12 June	0.8	1.8
Bifenthrin	120 g	22 July	2.3	4.6
Untreated			3.6	6.0

[a]Mole cricket damage ratings (0 to 9), 0 = no damage, 9 = severe damage.
[b]Means in each column are not significantly different (Tukey's Studentized Range Test, $\alpha = 0.05$).

Many factors might have contributed to the outcomes of this study. Mole crickets may be able to avoid *B. bassiana* spores applied to the soil surface by remaining deep in the soil profile (Villani et al. 1999). Mole crickets nymphs, especially southern mole crickets, are very active in the summer (as indicated by surface tunneling) compared with most other soil-dwelling insects. A study by Quintela (1996) suggested that soil insect larvae actively moving within a soil substrate can remove attached fungal spores from their cuticle by abrasion with the soil substrate. Therefore, it may be possible that the tunneling activity of mole cricket nymphs might remove a substantial number of spores from their body, thereby reducing their rate of infection with this pathogen.

Storey & Gardner (1986) found that the method of application can substantially affect the efficacy of *B. bassiana* in the field. More than 90% of *B. bassiana* spores applied with the conventional application method were restricted to the upper surfaces of soil when soils were not saturated with water. Wraight & Roberts (1987) therefore suggested using mechanical injection of spore preparations into the soil at a desired depth to overcome this problem. However, results of the 1999 test indicated that subsurface application did not improve the efficacy *B. bassiana* significantly. The mole crickets in the test were medium to large nymphs. This might reduce the efficacy of *B. bassiana* because small nymphs are most susceptible to control agents (Brandenburg 1997). *B. bassiana* in the other four tests of this study was applied with conventional ground application with

Table 5. Comparison of surface and subsurface application of three products of *B. bassiana* for control of large nymphs of tawny mole cricket, 1999.

Treatment	Application method	Rate (AI)/ha	Mole cricket damage ratings[a]		
			0 DAT[b]	17 DAT[c]	31 DAT[b]
B. bassiana, Naturalis-T	Surface	2.2×10^{11} spores	0.9	1.4ab	1.1
B. bassiana, Naturalis-T	Subsurface	2.2×10^{11} spores	2.1	2.0ab	1.5
B. bassiana, BI7S	Surface	2.2×10^{11} spores	1.7	1.7ab	1.4
B. bassiana, BI7S	Subsurface	2.2×10^{11} spores	1.1	0.8b	0.9
B. bassiana, BotaniGard ES	Surface	2.2×10^{11} spores	2.1	2.5ab	1.8
B. bassiana, BotaniGard ES	Subsurface	2.2×10^{11} spores	1.3	1.4ab	0.8
Deltamethrin, G	Surface	148 g	1.5	1.1b	0.9
Untreated			0.6	1.5a	0.5

[a]Mole cricket damage ratings (0 to 9), 0 = no damage, 9 = severe damage.
[b]Means in the column are not significantly different ($\propto$ = 0.05, 0 DAT(Day After Treatment: Tukey's Studentized Range Test, 17 and 31DAT: covariance analysis, GLM Procedure, SAS Institute 1990).
[c]Means followed by the same letter in the column are not significantly different ($\propto$ = 0.05, 0 DAT: Tukey's Studentized Range Test, 17 and 31 DAT: covariance analysis, GLM Procedure, SAS Institute 1990).

≈1.3 cm water of post-treatment irrigation. Theoretically, post-treatment irrigation can move spores deeper into the soil profile. However, we did not determine how deep the spores were carried by the irrigation water, or how soil texture, moisture, and related factors affect spore movement. A heavy rain (3 cm) occurred shortly after application in test 1 in 1997. The rainfall did not appear to enhance mole cricket control with *B. bassiana* in the test (Table 1).

Temperature plays a critical role in the performance of *B. bassiana* (Inglis et al. 1997, Hywell-Jones & Gillespie 1990, Studdert & Kaya 1990a,b). The mean optimum temperature for mycopathogens survival is normally between 20°C to 25°C, and maximum is ≈35°C. The four tests in 1997 and 1998 were conducted in the summer. Soil temperatures at the time of application ranged from 26°C to 30°C and the applications were made in late afternoon. Soil surface temperature at noon often exceeded 35°C during the summer. If most of the spores remained on the soil surface as Storey & Gardner (1986) reported, temperature may have had a substantial impact on the outcome of the tests. The 1999 test was conducted in the fall and high temperatures were not a concern.

Relative humidity is another important factor affecting the efficacy of *B. bassiana.* High relative humidity is generally thought to be necessary for the survival and germination of spores. However, relative humidity should not be a major concern when applying *B. bassiana* for soil insect pest control because relative humidity in the soil environment is usually 99%, even when the soil moisture is low enough to cause the permanent wilting in plants (Griffin 1963). Soil on golf courses might be moister than that in agricultural fields in the summer because golf courses irrigate turfgrass regularly. Therefore, relative humidity was not believed to be a factor in this study. Walstad et al. (1969) suggested that the application rate was more critical to the control efficacy than relative humidity. Results in this study indicated that higher application rates did not result in better mole cricket control, suggesting that the application rate may not have been a major factor affecting control in this study.

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Enhanced Activity of *Beauveria bassiana* to Red Imported Fire Ant Workers (Hymenoptera: Formicidae) Infected with *Thelohania solenopsae*[1]

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ABSTRACT A range of concentrations of *Beauveria bassiana* (Balsamo) Vuillemin conidia was tested against red imported fire ant, *Solenopsis invicta* Buren, workers from colonies infected with the microsporidian, *Thelohania solenopsae* Knell, Allen, and Hazard, and on workers from healthy colonies. Median lethal concentrations (LC_{50}) of *B. bassiana* in healthy and microsporidian-infected colonies showed that fire ants from infected colonies were more susceptible to *B. bassiana* infection than ants from healthy colonies. The LC_{50} of *B. bassiana* in microsporidian-infected workers was 4.5X less than that of healthy colonies. Mortality of ants from *T. solenopsae*-infected colonies that were treated with 9.66×10^6 *B. bassiana* colony forming units per cm^2 was subadditive compared to either pathogen alone.

KEY WORDS *Beauveria bassiana*, Hyphomycetes, Moniliales, *Thelohania solenopsae*, Microsporida, Thelohaniidae, *Solenopsis invicta*

The red imported fire ant, *Solenopsis invicta* Buren, is an exotic pest that has proliferated primarily because of the rarity of natural enemies in the southern United States (Jouvenaz 1983, Porter et al. 1997). *Thelohania solenopsae* Knell, Allen, and Hazard (Microsporida: Thelohaniidae) is a common pathogen of fire ants in South America (Knell et al. 1977) and was recently discovered in red imported fire ants collected near Gainesville, FL (Williams et al. 1998). The mode of infection of *T. solenopsae* is not fully understood, but it is thought to be transovarial from queen to offspring. Williams & Oi (1998) found that infected *S. invicta* colonies produced less brood, smaller worker populations, and decreased queen weight compared with uninfected colonies.

Beauveria bassiana (Balsamo) Vuillemin (Hyphomycetes: Moniliales) is an entomopathogenic fungus that infects many different insect species (Cantwell et al. 1986, Marcandier & Khachatourians 1987, Steinkraus et al. 1990, Brinkman et al. 1997a,b) including the red imported fire ant (Stimac et al. 1993). *Beauveria bassiana* infects primarily through host cuticle and compatibility with *T. solenopsae* is not known. The objective of the research reported herein was to determine the interaction resulting from exposure of *T. solenopsae*-infected workers to

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B. bassiana. If high levels of mortality can be achieved by combining the two pathogens, it may provide another tool for red imported fire ant IPM.

Materials and Methods

Red imported fire ants used in these assays were from colonies collected from a pasture located 8 km northwest of Griffin, Georgia. Colonies infected with *T. solenopsae* were originally obtained from the USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, Florida. The colonies used in the experiments were tested to confirm presence or absence of *T. solenopsae* infection by using procedures described by Williams et al. (1998). Random samples of 15–20 workers were taken from each of the colonies and were stained with Giemsa stain (LabChem Inc., Pittsburgh, Pennsylvania). Fifty-three percent of workers from *T. solenopsae*-infected colonies possessed octet stage spores. No signs of *T. solenopsae* infection were found in workers from local colonies.

Test arenas were prepared by burning a 5-mm diam hole in the bottom of clear 35-mL plastic cups and adding dental plaster to about 25% of total cup volume. Fluon (Northern Products Inc., Woonsocket, Rhode Island) was applied to the inside walls to prevent escape of workers. Ten ants were placed in each cup. Diluted honey was provided as a food source, and cups were placed on a wet foam pad to maintain moisture on dental plaster within cups.

Beauveria bassiana formulated as BotaniGard ES (Mycotech Corp., Butte, Montana) was pipetted onto the dental plaster in a range of treatment concentrations. Twelve treatments containing from 0 (untreated control) to 3.86×10^8 colony forming units (CFU) per cm^2 were tested on workers from healthy colonies; five concentrations containing from 0 to 1.9×10^8 CFU per cm^2 were tested on workers from *T. solenopsae*-infected colonies. Mortality was checked daily for 10 d, and treatments were replicated 8–10 times in a randomized complete block design. During the bioassays, temperature in the laboratory ranged from 26 to 29°C. Data were analyzed using the probit analysis procedure of the Statistical Analysis System (SAS Institute 1985) to obtain estimates of lethal concentrations and associated parameters.

In separate similar tests, workers from healthy colonies and *T. solenopsae*-infected colonies were either treated with water or 9.66×10^6 *B. bassiana* CFU per cm^2. Treatments were replicated 19–20 times in a randomized complete block design. Data were analyzed by analysis of variance using the general linear models procedure of SAS; means were separated by the least significant differences test (LSD, $\alpha = 0.05$).

Results and Discussion

Probit analysis of the concentration-mortality response of fire ant workers from healthy colonies 10 d after exposure to *B. bassiana* yielded an LC_{50} of 11.0×10^6 CFU per cm^2. This value was 4.5X greater than the LC_{50} of 2.4×10^6 CFU per cm^2 for *B. bassiana* against fire ants from *T. solenopsae*-infected colonies (Table 1). Following correction for control mortality (Abbott 1925), cumulative mortality of workers from *T. solenopsae*-infected colonies not treated with *B. bassiana* was 48.4% at 10 d (Figure 1 shows non-corrected mortality for the treatments). This mortality level was slightly lower than the *T. solenopsae* infec-

Table 1. Responses of red imported fire ant workers from two types of colonies (colonies infected with *Thelohania solenopsae* and colonies not infected with *T. solenopsae*) to *Beauveria bassiana* exposure for 10 d.

Colony	n[a]	LC_{50} (95% CL)[b]	Slope ± SE	χ^2	$P > \chi^2$
Infected	500	2,440,801 ($6.25 \times 10^5 - 1.21 \times 10^7$)	0.217 ± 0.045	23.308	0.0001
Uninfected	1040	10,984,086 ($6.79 \times 10^6 - 1.91 \times 10^7$)	0.943 ± 0.112	71.401	0.0001

[a]Total number of workers tested (10 ants per cup).
[b]Number of colony forming units per cm^2.

tion rate in colonies. At 10 d, cumulative mortality of fire ants from healthy colonies that were treated with *B. bassiana* was 57.5%, but this was not significantly ($P > 0.05$) different from mortality for untreated workers from *T. solenopsae*-infected colonies. At 4 d after treatment, mortality of workers from *T. solenopsae*-infected colonies that were also treated with *B. bassiana* was about 30% higher than for workers from *T. solenopsae*-infected colonies not treated with the fungus. This trend continued until the end of the experiment at 10 d when cu-

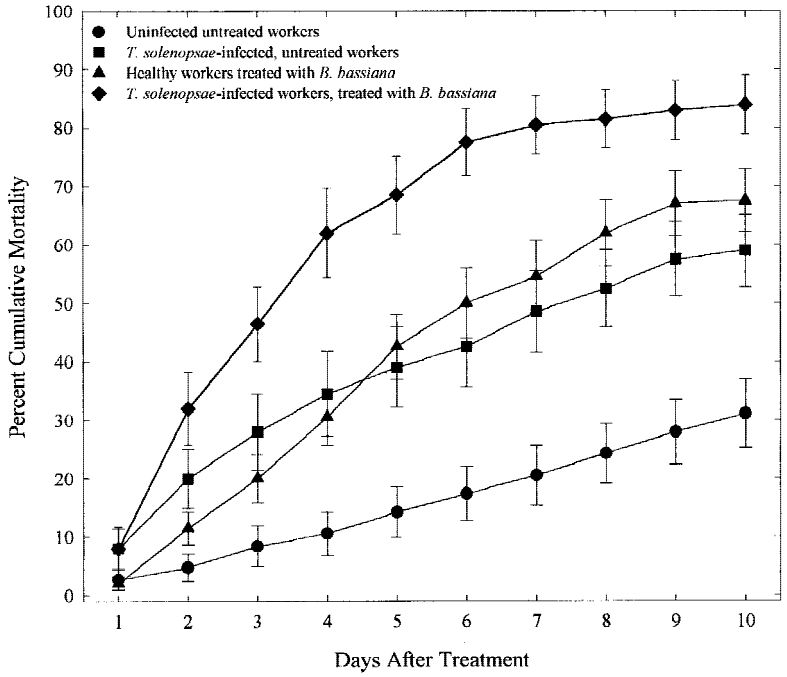

Fig. 1. *Beauveria bassiana* induced cumulative mortality (SEM indicated by bars) of red imported fire ant workers infected and not infected with the microsporidian *Thelohania solenopsae*. Workers were exposed to 9.66×10^6 colony forming units of *B. bassiana* per cm^2.

mulative mortality was significantly ($F = 47.86$; df = 2,9; $P = 0.0001$) different from that of untreated workers from *T. solenopsae*-infected colonies and from healthy colonies that were treated with *B. bassiana*. Although the resultant mortality for workers exposed to *T. solenopsae* and *B. bassiana* was subadditive (Cossentine and Lewis 1984), it was relatively high at 78.8%. According to Cossentine and Lewis (1984), mortality is subadditive when it is less than the sum of the effects of the two pathogens, but greater than the effect of either component alone. Fuxa (1979) found compatibility between the microsporidian *Vairimorpha necatrix* (Kramer) and the fungus *Nomuraea rileyi* (Farlow) Sampson in corn earworms, *Helicoverpa zea* (Boddie). These two pathogens have different modes of entry into the host, thus, additive mortality was attributed to the cooperative action of the two pathogens following initial infection by the agent (Fuxa 1979). This may at least partially explain the subadditive interaction of *B. bassiana* and *T. solenopsae* in red imported fire ant workers.

Numerous studies have been conducted with *B. bassiana* and insects, but there are very few published works that describe the effects of *T. solenopsae* on fire ants or the interactions of *T. solenopsae* with other pathogens. It is known that *T. solenopsae* weakens fire ant queens so that they produce less brood and die sooner than uninfected queens (Williams & Oi 1998). As a stress factor, *T. solenopsae* infection may also increase the susceptibility of red imported fire ants to other pathogens and control agents. Currently, efforts are being made to increase the incidence of *T. solenopsae* in North American red imported fire ants. As *T. solenopsae* becomes more widespread, additional studies of the interactions with *B. bassiana* will be needed to determine the effects of the two pathogens in field populations of fire ants.

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NOTE

Effect of Carbon Dioxide Anesthesia on Imiprothrin Toxicity in German Cockroach (Blattodea: Blattellidae)[1]

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The use of carbon dioxide (CO_2) as an anesthetic agent for insects is a convenient and widespread practice. Although normally considered harmless, exposure to CO_2 may have side effects that contribute to misinterpretations of experimental data. Brooks (1965) and Tanaka (1985) found that repeated exposures to CO_2 affected growth and adult size of the German cockroach, *Blattella germanica* (L.). Brooks (1965) noted that multiple 3-min exposures retarded growth rate, reduced adult weight, and decreased fecundity of *B. germanica*. Tanaka (1985), working with multiple 5-min exposures to CO_2, found that the nymphal stages of *B. germanica* were prolonged. Freckleton & Wahlsten (1968) reported that CO_2 administered to *Periplaneta americana* (L.) immediately after a training session interfered with retention of a learned task when the insects were tested 24 h later.

Valles & Koehler (1994) studied the influence of CO_2 anesthetization on chlorpyrifos toxicity in the German cockroach. They found that CO_2 administered for up to 1 h did not increase chlorpyrifos toxicity, but that multiple CO_2-induced knockdowns did increase toxicity. They concluded that CO_2 used once for <15 min, or fewer than five times of short duration (≈15 s) did not increase chlorpyrifos toxicity in the adult male German cockroach. The objective of this study was to determine the effect of varying the lengths of CO_2 exposure on the adult German cockroach's susceptibility to the knockdown agent imiprothrin.

Insecticide-susceptible *B. germanica* (Heal strain) were reared on water and commercial dog food under a photoperiod of 14:10 (L:D) h at a constant temperature of 26°C and 50% RH. Routine rearing conditions required that CO_2 be administered twice, at 1 and 4 wk after egg emergence to separate sexes and stage the colony developmentally. The exposure to CO_2 for each of these procedures lasted ≈2 min.

Experimental insects were collected by removing a harborage from stock colony containers and placing it into a knockdown chamber (30.0 × 30.0 × 10.0 cm). The knockdown chamber, containing a perforated floor for distribution of

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CO_2, was connected to an in-house gas source calibrated (1,000 mL/min flow rate) to completely saturate the chamber. The period of anesthesia (1, 5, 30, or 60 min) started when the harborage was placed in the knockdown chamber. The anesthetized cockroaches were rapidly sorted with forceps. Only males, 7 wk after hatching, were used in these experiments as this is the standard age used in all Johnson SC German cockroach tests. Care was taken to ensure that the cockroaches were of a similar size but due to time constraints imposed by anesthesia, weights were not taken. Cockroaches were exposed to CO_2 for 1, 5, 30 or 60 min. After CO_2 exposure, the insects were transferred to grease-lined clear plastic cups (8.4 cm diam × 7.9 cm high) (Tri-State Plastics, Dixon, KY) for recovery. The grease used consisted of one-half petroleum jelly and one-half mineral oil.

After recovering from CO_2 anesthesia for 4 hours, cockroaches were transferred from the plastic cups to greased Lucite ring (12.1 cm diam × 5.1 cm high) (Tri-State Plastics, Dixon, KY) arenas with 16-gauge mesh steel screen welded to the bottom to minimize residual exposure of the active ingredient. Each Lucite ring arena, containing the adult males was then placed into a spray chamber where 0.025% imiprothrin [(2,5-dioxo-3-(2-propynyl))-1-imidazolidinyl methyl 2,2-dimethyl-1-propenyl) cyclopropanecarboxylate] was applied for 0.5 s from a distance of 45.7 cm. A propellent canister with inert ingredients was used to apply the imiprothrin. The cockroaches were observed and the knockdown times recorded. Knockdown time was defined as the point when noticeable muscular activity ceased. The time from insecticide application to the knockdown of half the replicate was recorded. Ten males were used in each replication and each exposure and recovery time combination consisted of 10 replications. Knockdown times were statistically analyzed with ANOVA and the Student-Newman-Keuls test at the $P = 0.05$ level of significance (SAS Institute 1996).

The effects of CO_2 on cockroach movement were rapid. Within 2 to 4 s, walking became erratic and then ceased. The insects underwent loss of muscle control leading to involuntary leg convulsions and loss of balance. By 10 s, all muscular activity had ceased and the majority of the insects were resting on their dorsal side. After transferring cockroaches to fresh air, those exposed to CO_2 for <5 min exhibited a rapid and complete recovery within 5 min. During this recovery time, a short period of involuntary muscle movement preceded the return to the upright position. Cockroaches exposed to CO_2 for >30 min took between 30 and 50 min to fully recover normal walking behavior. During the recovery period, while the insect was still resting on its dorsal surface, considerable leg motion, from involuntary twitching to walking movement, was observed. Long exposure (60 min) to CO_2 sometimes resulted in insects that did not fully recover within 1 h. In these cases, the entire replicate (10 insects) containing these animals was discarded and replaced with a new replicate.

Imiprothrin knockdown times were influenced by the duration of CO_2 anesthesia ($F_{\alpha_{(1),\ 3,\ 9}} = 64.27$, df = 3, $P < 0.05$). A 60-min CO_2 exposure resulted in a significantly faster knockdown time (11.40 ± 1.26 s) than the shorter CO_2 exposure periods (13.87 ± 1.22 s). No significant differences in knockdown times were found among the 1-, 5-, and 30-min exposures.

The data on both CO_2 exposure and recovery time should prove useful to those working with CO_2 regularly as a German cockroach anesthetic. These results indicate exposing cockroaches to CO_2 in excess of 30 min risks altering knockdown results. These data reflect specific conditions with regards to the species

studied, anaesthetizing techniques, and the active ingredients used. But the data underscore the importance of establishing strict controls when testing rapid knockdown agents.

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Seasonal Activity and Bait Preferences of the Argentine Ant (Hymenoptera: Formicidae)[1]

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ABSTRACT Foraging by workers of Argentine ants, *Linepithema humile* (Mayr), in California was seasonal, with peak activity occurring from June to October. Workers foraged throughout the other cooler months, albeit at a much reduced level. The number of foragers was directly associated with early morning and late afternoon temperature. As temperature at 1600 hours increased, the number of foragers increased. Sucrose and honey water and solid foods containing insect protein were the most preferred food throughout the year. During the summer months, 26 to 60% (wt.) of the food foraged by workers was protein whereas this dropped to 16 to 40% during the winter. When given a choice between carbohydrates and protein bait-bases throughout the study, *L. humile* foraged considerably more carbohydrate. Presuming that foraging choice reflects the nutritional needs within the colony, these data suggest colony requirements for protein may be greater than initially suspected. Optimal baiting programs might include protein baits in the early summer and liquid sucrose baits in summer and early fall.

KEY WORDS Argentine ant, *Linepithema humile*, *Iridomyrmex humilis*, choice tests, baiting, foraging activity, seasonal activity

The Argentine ant, *Linepithema humile* (Mayr) (previously *Iridomyrmex humilis*, Shattuck 1992), is a polygynous and polydomous tramp species now widely established between the 30° and 60° latitudes in the northern and southern hemispheres (Majer 1994). *L. humile* adversely affects biological control in agriculture (DeBach et al. 1951, Flanders 1958, Prins et al. 1990) and natural faunal and flora communities (De Kock 1990, De Kock & Giliomee 1989, Reimer 1994, Ward 1987), and invades homes and other structures (Hadlington & Gerozisis 1985, Knight & Rust 1990, Prins et al. 1990, Smith 1965).

Perimeter or barrier sprays or baits are typically applied around structures to provide control (Forschler & Evans 1994, Knight & Rust 1991, Rust & Knight 1990, Rust et al. 1996). Sprays typically provide control <60 days because the sprays are directed against workers and heavy irrigation, dense ground cover, exposure to direct sunlight, and alkaline nature of stucco and concrete, and extremely high temperatures decrease the performance of barriers (Rust et al.

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1996). Toxic baits can be taken to the colony by foraging ants and therefore, eliminate the need for all ants to contact chemical barriers or broadcast applications of insecticides. However, for ant baits to be effective the toxicant must have a delayed action over a wide range of concentrations and the bait must have an attractive and acceptable food attractant (Stringer et al. 1964). Baker et al. (1985) found that *L. humile* preferred 25% sucrose water over other foods tested even when ants had been satiated with sucrose water for 2 days. Similarly, Krushelnycky & Reimer (1998a) reported that 25% sucrose water and a protein based granular bait made from ground silkworm, *Bombyx mori* (L.), pupae were readily taken. Baits containing ground silkworm pupae and hydramethylnon were readily taken *by L. humile* (Knight & Rust 1991, Krushelnycky and Reimer 1998b)

The objective of this study was to determine the bait preferences and seasonal foraging activity of *L. humile,* with special attention being given to foraging on different carbohydrates and protein bait bases.

Materials and Methods

Study site. Food preference studies with multiple colonies of *L. humile* were conducted in a citrus grove on the UC Riverside campus. The grove had never been sprayed with insecticide. This ensured the presence of homopterous insects and an abundance of honeydew for the ants. Large colonies of *L. humile* living at the bases of trees actively tended honeydew-producing insects in the trees. Most of the trees were foraged upon by at least several distinct foraging columns of ants. Based on the report of ants in this same grove by Markin (1968), we presumed that the soil surface foraging territory for each colony of *L. humile* was no more than 36 m^2. Workers labeled with food containing $Phosphorus^{32}$ were dispersed between colonies within an area of about 3,000 m^2 in 3 days. Consequently, ant colonies at 5 trees about 18 m (60 ft) from each other were selected to help insure that different ant colonies were being sampled. Additionally, a buffer zone of at least one row of trees (6 m) was maintained between study colonies and none of the colonies were irrigated by the same drip line. Because the grove was flood-irrigated every other week, bait preference studies were made on alternate weeks to minimize disturbance of irrigation on the ants.

Foraging activity. Foraging activity of the ants was determined by counting ants. The number of ants moving up and down the tree trunk was determined by counting the number of ants crossing an unmarked point on the trunk about 24 cm from the ground during 1 min at ≈0900 and 1600 h. Each trail on the tree was counted and summed to provide a total per tree with as many as five separate trails on some trees. Temperature and relative humidity at the base of the tree and in the shade at each count time was measured with a sling psychrometer (Bacharach, Inc., Pittsburgh, Pennsylvania).

Choice tests. Solid and liquid foods provided by The Clorox Service Co. (Pleasanton, California) were given to ants in covered choice feeding arenas placed at the base of each of the five test trees. The arenas, made from aluminum cake pans (20 cm diameter by 4 cm high), had four holes (8 mm diameter) punched through the sides above the bottom of the pan. The holes were placed 90° apart. Pieces of glass tubing (7 cm long by 7 mm outer diameter) were inserted through

the holes so that the end of the tube was flush with the outer edge of the pan. The tubes were glued to the floor of the pan (silicone sealant, Dow Corning Corp., Midland, Michigan). Ten hexagonal plastic weighing pans (2.5 cm by 1 cm high) cemented to the floor of the pan in a circular pattern equidistant from one another served to hold weighed amounts of food material in individual weigh pans. Ants entered and left the arena via the glass tubing. By entering near the center, the ants then could choose or reject foods being presented.

The ten liquid and solid baits formulated without toxicants were tested using the above method: MaxForce without toxicant, a granular silkworm protein-based bait (43/57% protein/carbohydrate, P1); high protein, doughy bait (100/0% protein/carbohydrate, P2); insect protein granules, a granular bait containing ground silkworm pupae (43/57% protein/carbohydrate, P3); fish protein bait, a doughy bait consisting of fishnmeal in a gel matrix (35/65% protein/carbohydrate, P4); honey granules, formulated from Domino Qwik-Flo Honey (0/100% protein/carbohydrate, C1): honey doughy bait (0/100% protein/carbohydrate, C2); 20% sucrose water (0/100% protein/carbohydrate, S1); 20% sucrose water plus emulsifiers (0/100% protein/carbohydrate, S2); 20% honey water (0/100% protein/carbohydrate, S3); and 20% honey water plus emulsifiers (0/100% protein/carbohydrate, S4).

Each food was weighed in individual plastic weighing pans. Pans of food were randomly assigned to one of 10 positions in the arena. The arena was covered with transparent mylar (26 cm diameter), a piece of plywood (30 by 30 by 1 cm) covered with aluminum foil was placed over the arena to reflect sunlight, and a brick was placed on the plywood to prevent wind or animals from disturbing the arena. A similarly configured pan placed outside the laboratory, where ants were not present, served as a control to account for changes of weight attributed to drying or absorption. Control pans were placed out for each test. To insure an adequate supply of food as ant foraging increased, the amount of food in each pan was increased from 0.5 g (5 May 1994 to 23 June 1994) to 1.0 g (24 June 1994 to 11 October 1994) to 2.0 g (25 October 1994). After 25 October, the amount of food placed in each dish was reduced to 0.5 g.

Loaded arenas were placed at the bases of the trees at ≈0900 h. The arenas were checked at 1600 h and the number of empty pans was recorded. The arenas were returned to the laboratory at 24 h where the pans of food were weighed and the amount removed was calculated. The choice tests were repeated every two weeks.

To compensate for changes in moisture for any given food substance, the ratio of weight gained or lost in the control arena during the test period was multiplied by the initial weight of that food placed in the choice arena. The amount of food foraged by the ants in the choice tests was subtracted from these compensated data.

In instances when there was exceptionally heavy foraging, it was occasionally necessary to compensate for foods that were completely removed. If all food was consumed when the arenas were checked at 8 h, then the weight (mg) of that particular food was multiplied by 3. For food removed between 8 and 24 h, the amount of food removed was multiplied by 1.5.

To determine the amount of carbohydrate or protein foraged, the amount of each food foraged was multiplied by its percent composition of protein or carbohydrate listed in Table 1.

Table 1. Feeding preferences (ranks) of Argentine ants of various baits.

Date	P1	P2	P3	P4	C1	C2	S1	S2	S3	S4[a]	Total[b] *Ranks*
5/5/94	140.0	184.0	31.0	35.0	10.5	6.5	142.0	77.0	52.0	39.5	717.5
5/23	158.0	189.0	102.0	93.0	144.5	48.5	156.0	94.0	151.0	100.0	1236.0
6/6	218.0	227.0	97.0	106.5	164.5	54.0	223.0	130.5	199.0	124.0	1543.5
6/23	253.0	285.0	171.0	157.0	246.0	108.0	269.0	74.0	258.0	126.0	1947.0
7/5	254.0	280.0	129.0	168.0	206.5	84.0	267.0	188.0	281.0	176.0	2033.5
7/18	251.5	287.0	137.0	75.0	62.5	42.5	275.0	177.0	214.0	161.0	1682.5
8/1	244.0	290.0	159.0	149.0	120.0	76.0	271.0	135.5	255.0	136.0	1835.5
8/15	284.0	274.0	236.0	237.5	265.0	225.0	266.0	205.0	264.0	220.5	2477.0
8/29	286.0	289.0	170.0	166.0	190.5	110.0	213.0	178.0	240.0	152.0	1994.5
9/14	288.0	270.0	109.0	169.0	144.5	98.5	279.0	118.0	215.0	125.0	1816.0
9/27	277.0	249.0	210.0	186.0	119.0	117.0	273.0	150.0	262.0	155.0	1998.0
10/10	263.0	259.5	167.0	138.0	193.0	80.5	201.0	174.0	243.0	190.5	1909.5
10/25	230.0	209.0	36.0	57.0	18.0	13.5	232.0	162.0	88.0	127.0	1172.5
11/7	90.0	146.0	27.0	6.5	10.5	45.0	183.0	164.5	3.0	103.0	778.5
11/21	15.5	56.0	3.0	8.0	3.0	3.0	68.0	3.0	72.0	10.5	242.0

Table 1. Continued.

12/6	31.0	41.0	10.5	18.0	13.5	15.5	115.0	37.0	28.5	22.5	332.5
12/19	71.0	104.0	60.0	68.0	54.0	62.5	181.0	123.0	132.0	106.5	962.0
1/18/95	31.0	24.0	34.0	18.0	25.0	28.5	26.0	22.5	21.0	33.0	263.0
1/30	70.0	82.0	42.5	51.0	50.0	39.5	197.0	130.5	182.0	121.5	966.0
2/16	78.0	128.5	95.5	64.0	133.0	65.5	234.0	89.0	231.0	187.0	1305.5
2/27	113.0	111.0	54.0	44.0	91.0	38.0	229.0	79.0	220.5	160.0	1139.5
3/14	141.0	116.0	48.5	46.0	47.0	20.0	211.0	83.0	139.0	61.0	912.5
3/27	92.0	172.0	73.0	65.5	68.0	58.5	179.0	173.0	192.0	163.0	1236.0
4/12	224.0	245.0	143.0	134.0	233.0	101.0	278.0	148.0	268.0	208.0	1982.0
4/24	195.0	242.0	112.0	121.5	212.0	87.0	272.0	185.0	250.0	204.0	1880.5
5/11	200.0	206.5	95.5	85.0	147.0	58.5	228.0	114.0	216.0	153.5	1504.0
5/22	175.0	222.0	105.0	80.5	153.5	86.0	276.0	98.5	238.0	196.0	1630.5
6/5	248.0	247.0	217.0	202.0	259.5	194.0	282.0	226.0	239.0	219.0	2333.5
6/22	261.0	283.0	198.0	203.0	256.5	180.0	251.5	235.0	257.0	241.0	2366.0
Total Ranks	5182.0	5718.5	3072.5	2952.0	3641.0	2146.5	6277.5	3774.0	5411.0	4022.0	42,197

[a]P1 = MaxForce® bait without toxicant; P2 = high protein, doughy bait; P3 = insect protein granules; P4 = fish protein bait; C1 = honey granules; C2 = honey doughy bait; S1 = 20% sucrose water; S2 = 20% sucrose water + emulsifiers; S3 = 20% honey water; S4 = 20% honey water + emulsifiers.
[b]Maximum rank = 290.

Statistical analyses. The average milligrams of each food foraged was divided by the number of counted ants ascending or descending the tree per minute at 1600 h to help compensate for seasonal foraging intensities at all dates. These corrected values were ranked from low to high. Differences in food acceptance and seasonal differences were analyzed with a Mann Whitney U-Test and a nonparametric multiple comparison by STP (Conover 1971, Sokal & Rohlf 1969).

The association between ant foraging and temperature and relative humidity and the ant foraging activity was determined with a Kendall coefficient of rank correlation (Sokal & Rohlf 1969).

Results

Ant foraging activity. Fig. 1A shows that the seasonal pattern of the number of worker *L. humile* foraging in citrus trees was directly associated with afternoon temperatures ($\tau = 0.6475$, $t_s = 4.738$, $P < 0.001$), with maximum foraging activity from August to October. Most trees had up to 5 different columns of ants trailing on the trunk. There was no association between ant traffic and afternoon relative humidity. Foraging activity was also directly associated with morning temperatures ($\tau = 0.3651$, $t_s = 2.727$, $P = 0.006$), the maximum foraging occurring July 5 and August 15 (Fig. 1B, Table 1). There was no association between ant traffic and relative humidity. Ants foraged throughout the winter - an average number of 22 to 96 ants/min in the trees. Because of large variation, the apparent difference in the number of workers foraging in the morning versus the afternoon between July and September could not be separated statistically (Fig. 1). However, the large variation may have been due to significant declines in foraging whenever ant trails were exposed to sunlight, extremely high temperature, or rainfall.

Bait preferences. Sucrose (S1) and honey solutions (S3) and silkworm pupae protein-base granular food (P1) were readily accepted throughout the year and consistently had the highest total feeding ranks (Table 1). They were taken in greater quantities than were other proteins (P4) and carbohydrate (C2) (Table 2). The protein (P2), carbohydrate (C1), sucrose + emulsifier (S2), and honey + emulsifier (S4) were foraged upon at approximately equal rates. The carbohydrate solid food (C2) was the least preferred. Addition of emulsifiers, oils or preservatives significantly decreased the attractiveness of 20% sucrose and 20% honey water.

Lowest levels of foraging occurred between 7 November 1994 and 16 February (Table 1). This corresponds to an observed decline in ant trailing in trees in November (Fig. 1A,B).

As expected, the total amount of protein and carbohydrate foraged by the ants was directly related to the number of ants foraging in the trees. As the ant population increased in the summer, the amount of food taken from choice arenas totaled nearly 16 g per colony. During the summer months the percent protein of the total amount of food presented foraged from the arenas ranged from 26–60% whereas it ranged from 16–40% in the winter (Fig. 2).

Discussion

Argentine ants showed a distinct seasonal pattern of foraging similar to that reported by Markin (1970b) and Krushelnycky and Reimer (1998a). Peak num-

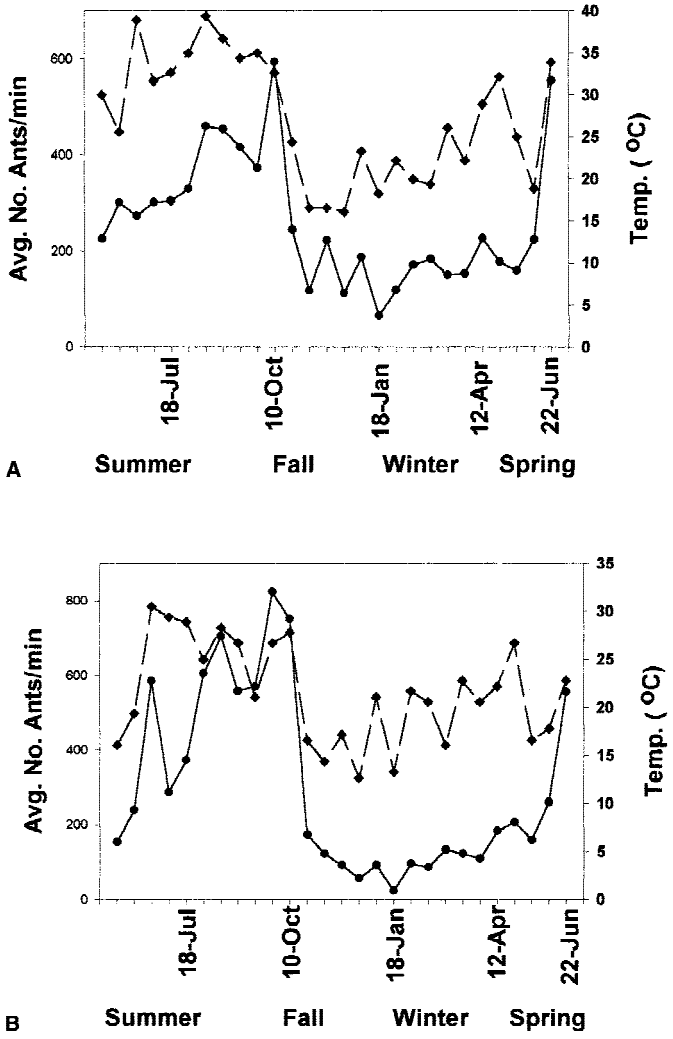

Fig. 1. A. Average seasonal foraging activity of workers from afternoon counts from five trees. B. Average seasonal foraging activity of workers from morning counts from five trees. Solid lines and circles = ant count; dashed line and diamonds = temperature.

bers of foragers occurred in late September and mid-October. However, contrary to Markin's observation (1970b) that significant numbers of foragers persisted through mid-December, we observed a dramatic decline by mid-November. The decline of workers apparently corresponds to the absence of worker pupae in the colony (Markin 1970c). On several occasions in late October and early November, we found large windrows of dead Argentine ants that correspond to the decline in foraging we observed. It is possible that mild winters permitted *L. humile* to begin

Table 2. Non-parametric comparison by STP of Wilcoxon-Mann-Whitney statistic (U) of the feeding preferences of *L. humile*.

	P1	P2	P3	P4	C1	C2	S1	S2	S3
P1[a]									
P2	474								
P3	621	**677**[b]							
P4	**628**	**684**	446						
C1	570	609	475.5	448.5					
C2	**691.5**	**738**	563.5	523	**732.5**				
S1	449.5	452	**682**	**744.5**	**685.5**	**783**			
S2	568	**629**	513.5	536	458	607	**663**		
S3	422	471	534.5	**693**	590.5	**705**	515	**664.5**	
S4	551	613	595.5	550.5	469	**665.5**	**696**	482	578

[a]P1 = MaxForce® bait without toxicant; P2 = high protein, doughy bait; P3 = insect protein granules; P4 = fish protein bait; C1 = honey granules; C2 = honey doughy bait; S1 = 20% sucrose water; S2 = 20% sucrose water + emulsifiers; S3 = 20% honey water; S4 = 20% honey water + emulsifiers.
[b]Comparisons in bold are significantly different at $P < 0.05$ ($U_{0.05(10,29)} = 624$, Sokal and Rohlf 1969).

producing brood earlier in the spring than in those years that Markin studied ants in the same grove.

Markin (1970b) reported a relationship between mean daily temperatures and ant traffic in the trees, with optimum foraging occurring between 15 and 30°C. Foraging activity decreased when temperatures on tree trunks exceeded 30°C. Our ant traffic counts are similar to those reported by Markin (1970b) with about

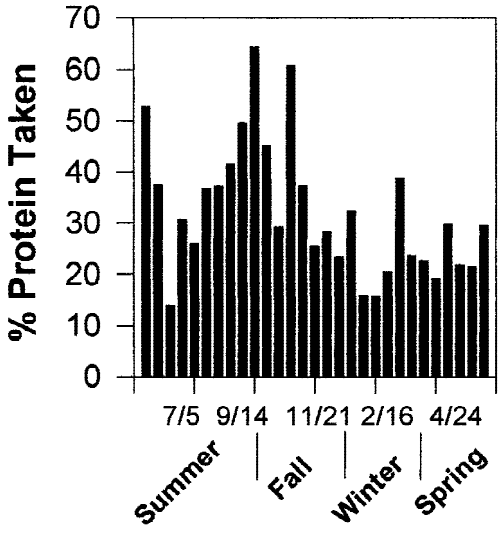

Fig. 2. The percent of protein foraged from the total amount of food taken by *L. humile*.

200–300 ants/min in February and 700–800 ants/min in October. Both Markin (1970b) and our study showed no relationship between relative humidity and foraging activity. However, Tremper (1976) reported that *L. humile* lost 2–3 times more water than did other native ant species in California, suggesting that their cuticle is more permeable to water. This could explain Markin's (1970b) observation that during the hot, dry months of August, September and October workers frequently formed trails to water. Possibly, workers compensate water loss by foraging free water.

Even though certain preferred foods such as sucrose water, honey, and granulated silkworm pupae were readily foraged throughout the year by *L. humile*, the percent of protein foraged in December, January, and February was low compared with June, July and August. Sorensen et al. (1985) found that foraging red imported fire ants, *Solenopsis invicta* Buren, respond to the nutritional needs of nestmates. Howard and Tschninkel (1981) found that sugars were passed to workers of *S. invicta*, amino acids were distributed throughout colony, but preferentially to growing larvae, and oil was distributed equally among workers and larvae. Foods containing casein hydrolysate were passed to *S. invicta* queens. Markin (1970a) found that $Phosphorus^{32}$ in a protein solution was readily fed to larvae whereas $Phosphorus^{32}$ in a sugar solution was primarily retained by workers. Queens were fed a considerable amount of protein within 24 h and the amount of sugar increased gradually over several days. Males and workers exchanged comparable amounts of sugar and protein. In laboratory tests, Baker et al. (1985) found that as little as 0.3% enzymatic casein hydrolysate in 25% sucrose water decreased consumption by *L. humile*. Egg white was the only proteinaceous substance that did not reduce feeding. Markin (1970c) found that during December, January and February that *L. humile* workers comprised up to 88% of the colony biomass and eggs and larvae <13%. In June, July and August, workers comprised only 50–52% and eggs and larvae increased to 14–23%. The demand of protein by *L. humile* appears to be related to the number of brood being produced in the summer.

Liquids such as sucrose and honey water are highly preferred by *L. humile*. Approximately 50% of all *L. humile* workers had crops containing honeydew whereas only 0.4% had prey (Markin 1970b). Greater than 99% of the material carried to the nest by *L. humile* workers foraging citrus trees was nectar or honeydew produced by the citrus mealybug, *Planococcus citri* (Risso). The number of ants returning to the nest with insects, spiders or pillbugs ranged from <1 ant/1,000 in November to 9 ants/1,000 in May, the yearly average being 3.7 ants/1,000. Markin (1970b) suggested that in addition to sugar, the colony depends on honeydew for amino acids. However, our feeding studies indicate that *L. humile* readily forage solid protein sources when available. Interestingly, Keller et al. (1989) reported excellent fecundity of laboratory queens of *L. humile* and colony maintenance on 8% sucrose water and a solid diet mixture consisting of eggs, mealworms and hashed beef meat. Our experience also indicates that thriving colonies require substantial amounts of protein, thriving only when we give them protein such as housefly pupae or live German cockroaches. *L. humile* is an opportunistic forager and is likely to forage upon virtually any available suitable protein in the field. For example, Dreistadat et al. (1986) reported that *L. humile* foraged 98% of the eggs of the lacewing *Chrysoperla carnea* (Stephens) released in

a biological control program. Cat flea eggs and larvae were readily foraged by *L. humile* (Silverman & Appel 1984).

Food preference and foraging activity of *L. humile* may reflect the nutritional needs of the colony throughout the year. Even though sucrose solutions may provide an energy-rich food and are highly preferred, *L. humile* has a high demand for protein, especially during colony growth phase that occurs in the spring and summer. Markin (1970b) found that the highest rates of worker *L. humile* with prey was from February to May, concluding that this protein is incorporated into developing sexual larvae that are abundant in the nest. High rates of worker *L. humile* with prey are also consistent with large numbers of worker larvae from May to August. Laboratory colonies of *L. humile* prefer 25% sucrose water or honey water to brown granulated sugar or protein-rich solid foods and the carbohydrate solutions are competitive to naturally occurring honeydew in the field (Baker et al. 1985). Little is known about bait preferences of field colonies of *L. humile*, but our rearing experience and observations suggest that colonies of *L. humile* may require substantial amounts of protein for optimal growth and development.

The changes in feeding preference do explain our field observations that *L. humile* workers refuse insect protein and carbohydrate baits such as MaxForce in the late summer. The seasonal food preferences and colony needs of *L. humile* suggest that a program incorporating protein baits in the early summer and liquid sucrose baits in the summer and early fall might be highly effective.

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NOTE

First Report of the Western Bean Cutworm, *Richia albicosta* (Smith) (Lepidoptera: Noctuidae), in Minnesota Corn[1]

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The western bean cutworm, *Richia albicosta* (Smith) (Lepidoptera: Noctuidae), formerly *Loxagrotis albicosta* (Smith), is a pest of bean, *Phaseolus vulgaris* L. and corn, *Zea mays* L. in the west-central U.S. (Douglass et al. 1957, Blickenstaff 1979, Blickenstaff & Jolley 1982). To date, the western bean cutworm has been observed in Arizona, Idaho, Kansas, Nebraska, Iowa, Utah, Colorado, New Mexico, Texas, South Dakota, Wyoming, and Oklahoma (Appel et al. 1993; Keaster 1999). However, in some states the cutworm is only rarely observed. For example, in Iowa, where *R. albicosta* is not typically reported, corn fields in the northwestern portion of the state were heavily infested in September, 2000 (Rice 2000).

Although *R. albicosta* has been described as polyphagous (Douglass et al. 1955), Blickenstaff and Jolley (1982) conducted extensive feeding trials and concluded that the only suitable hosts were corn and bean. Deleterious effects of the cutworm on corn include the formation of misshapen ears and subsequent yield loss (Hagen 1962; Appel et al. 1993). Additionally, feeding damage aids in the introduction of disease pathogens and other insect pests (Hagen 1962). In this paper we document the incidence of several late-season infestations of *R. albicosta* in experimental sweet corn plots in southern Minnesota during 1999.

Research was conducted in 1999 at five University of Minnesota Agricultural Research and Outreach Centers in Minnesota. Sentinel plots of transgenic and non-transgenic sweet corn hybrids were planted as part of a larger study to monitor the frequency of surviving late-instar European corn borer, *Ostrinia nubilalis* (Hübner), and corn earworm, *Helicoverpa zea* (Boddie) (Bolin et al. 1998; Venette et al. 2000). The transgenic sweet corn hybrid, 'GH-0937', provided by Novartis Seeds Inc., Nampa, Idaho, expressed a protein toxin Cry1Ab derived from the bacterium *Bacillus thuringiensis* var. *kurstaki* (i.e., Bt corn), using 'Event BT11' (Lynch et al. 1999; Burkness et al. 2001). Plots were planted at

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Becker (25 Jun), Lamberton (16 Jun), Morris (20 Jun), Rosemount (9 and 15 Jun), and Waseca (16 Jun). Plots of 'GH-0937' and its non-Bt isoline, 'Bonus' were arranged in a randomized complete block design and replicated 4 times. Each plot was 23 m square (30 rows wide and 23 m long) with 0.77 m row spacing; plots were separated by 1.5–3.0 m alleys. The seeding rate was 64,220 seeds/ha for both varieties at all locations.

Each sweet corn hybrid was evaluated for insect damage by examining 200 to >8000 primary ears per sample date (50 to >2000/replicate). Ears were harvested at maturity (≈90 d after planting, Sept. 1999) for both Bt and non-Bt varieties at all locations. Ears were hand-harvested, placed into burlap sacks, and taken to a field edge for immediate evaluation. Ear evaluation included removing the husks and examining for the presence of lepidopteran larvae; the presence of larvae and feeding damage were noted (e.g., Bartels and Hutchison 1995). Confirmation that plant tissue was expressing Bt was made using the Gene Check strip test (Monsanto 1995) on selected ear samples. Larval specimens were identified as *R. albicosta* by three characteristic short, dark stripes on the first segment behind the headcapsule (e.g., Keaster 1999). *Richia albicosta* larvae were transported to the University of Minnesota and maintained on artificial diet until all locations had been sampled. A total of 5 larvae were shipped to the University of Nebraska, Lincoln, for confirmation of identification as *R. albicosta*.

Larvae of *R. albicosta* were collected from four of the five University of Minnesota Agricultural Research and Outreach Centers surveyed (Table 1). The greatest number of larvae were observed in southwestern Minnesota at the Lamberton site, Sept. 8–9 Sept. Larvae were also collected at Rosemount (Sept. 16–17), Waseca (Sept. 14 and 30) and Morris (Sept. 21–22). To our knowledge, this is the first documented occurrence of *R. albicosta* in Minnesota. Neither adult nor larval specimens of *R. albicosta* exist in the Insect Museum, Department of Entomology, University of Minnesota. In addition to *O. nubilalis* and *H. zea,* the primary species of interest for resistance monitoring in Bt corn, the only other lepidopteran we recovered was variegated cutworm, *Peridroma saucia* (Hübner), a common species in Minnesota (Noetzel & Ostlie 1986).

Larvae of *R. albicosta* were observed feeding on kernel tissue of both Bt and non-Bt sweet corn hybrids, 'GH-0937' and 'Bonus,' respectively (Table 1). More larvae (227 of 234) were found on Bt sweet corn because more Bt ears were sampled (36,120 versus 1,405 non-Bt sweet corn ears). Across all locations, mean cutworm densities (± SD) of 0.0080 (± 0.018) larvae/ear in the Bt sweet corn and 0.0050 (± 0.013) larvae/ear in the non-Bt sweet corn were not significantly different ($t = 0.35$, $df = 11$, $P = 0.73$, Table 1).

The widespread distribution of *R. albicosta* from west-central to southern Minnesota during 1999 may reflect an expansion of the cutworm's range, or may simply be due to unusual, temporary weather patterns that facilitated migration into Minnesota. When incorporating field observations for 2000, potential expansion of the cutworm's range remains unclear. Although one northwestern Iowa farm reported >95% of ears affected with *R. albicosta* larvae or damage (Rice 2000), sampling in southwestern Minnesota (near Lamberton) revealed fewer larvae in 2000 than in 1999. Continued monitoring will be necessary to determine the extent to which this cutworm may have expanded its range in the Midwest.

Our detection of a low-density infestation of *R. albicosta* also suggests that the BT11 event (Cry1Ab toxin) does not appear to provide significant control of this

Table 1. Number of western bean cutworm, *R. albicosta*, larvae in Minnesota sweet corn, Sept. 1999.

		Bt corn					Non-Bt corn		
Location	Harvest Date	No. Ears	Small	Medium	Large	Total No./ear	No. Ears	Large	Total No./ear
Becker	23 Sep	6821	0	0	0	0	200	0	0
Lamberton	08 Sep	3875	8	60	120	0.0485	200	7	0.035
Morris	21 Sep	8184	0	3	2	0.0006	200	0	0
Rosemount	03 Sep	4488	0	0	0	0	205	0	0
Rosemount	16 Sep	6102	0	12	0	0.0020	200	0	0
Waseca	14 Sep	4978	1	5	15	0.0042	200	0	0
Waseca	30 Sep	1672	1	0	0	0.0006	200	0	0
Total	—	36,120	10	80	137	0.0080	1405	7	0.005
Mean/ear (±SD)						0.008 (± 0.018)[y]			0.005 (±0.013)[y]
Mean/100 ears	—	—	—	—	—	0.80	—	—	0.50

[y]Means were not significantly different, Student's t test ($t = 0.35$, df = 11, $P = 0.73$).

cutworm. Failure to control *R. albicosta* is not surprising given the known differential susceptibility of Lepidoptera to *B. thuringiensis* toxins and that Bt corn was developed primarily for *O. nubilalis* (e.g., Ostlie et al. 1997). These data also suggest that *R. albicosta* would not be an economic concern in dent corn at current infestation levels in Minnesota (Appel et al. 1993). In addition, densities of <1.0 larva/100 ears (Table 1) would not likely be a concern to most sweet corn producers. However, late-instar infestations approaching 5% of ears infested, such as those for Lamberton, would be an economic concern for both fresh-market sweet corn growers and processors (Bartels & Hutchison 1995, Bartels 1998). As with the black cutworm, *Agrotis ipsilon* (Hufnagel), or the common stalk borer, *Papaipema nebris* (Guenée), *R. albicosta* is another lepidopteran pest that growers should be aware of in both Bt and non-Bt corn, particularly in states where the cutworm is a more prevalent pest (Keaster 1999).

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The Termite (Isoptera) Fauna of South Carolina[1]

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ABSTRACT Eight species of termites are now recorded from South Carolina. *Reticulitermes flavipes* (Kollar) and *R. virginicus* (Banks) were the two most abundant subterranean species (Rhinotermitidae), each distributed throughout the state. *Reticulitermes hageni* (Banks) was distributed sporadically over the state, with records from six counties. *Coptotermes formosanus* Shiraki was found primarily in Charleston County, but new records extended the range to Beaufort, Dorchester, Berkeley, and Orangeburg Counties. Drywood termites (Kalotermitidae) were not encountered as frequently as subterranean species. *Cryptotermes brevis* (Walker) was recorded in seven counties throughout the state. *Incisitermes snyderi* (Light) was found in seven coastal counties and once in Pickens County, which is inland. *Kalotermes approximatus* (Snyder) was recorded primarily from natural habitats in 18 counties, and a western drywood species, *Incisitermes minor* (Hagen), was collected from Greenville and Beaufort Counties, representing a state record.

KEY WORDS Rhinotermitidae, Kalotermitidae, distribution, new records

Five species of termites were recorded previously from South Carolina, including four subterranean (Rhinotermitidae) and one drywood (Kalotermitidae) species (Light 1934). Native subterranean termites included *Reticulitermes flavipes* (Kollar), *R. hageni* (Banks), and *R. virginicus* (Banks). *Reticulitermes flavipes, R. hageni,* and *R. virginicus* are found throughout the eastern United States (Weesner 1965). The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is an introduced species found along the Gulf Coast and sporadically in the southern U.S. (Potter 1997). The drywood termite, *Incisitermes snyderi* (Light), is widely distributed throughout the southeastern U.S. (Snyder 1954).

The powderpost termite, *Cryptotermes brevis* (Walker), is the most widely distributed termite species in the world (Gay 1967). *Kalotermes approximatus* (Snyder) is found mainly along coastal areas in the southeastern states of the U.S. (Potter 1997). Although these two species are not specifically stated to occur in South Carolina, *C. brevis* and *K. approximatus* are assumed to be found in the state according to the statements in the previous references. *Incisitermes schwarzi* (Banks) is a drywood termite that was thought to occur in South Caro-

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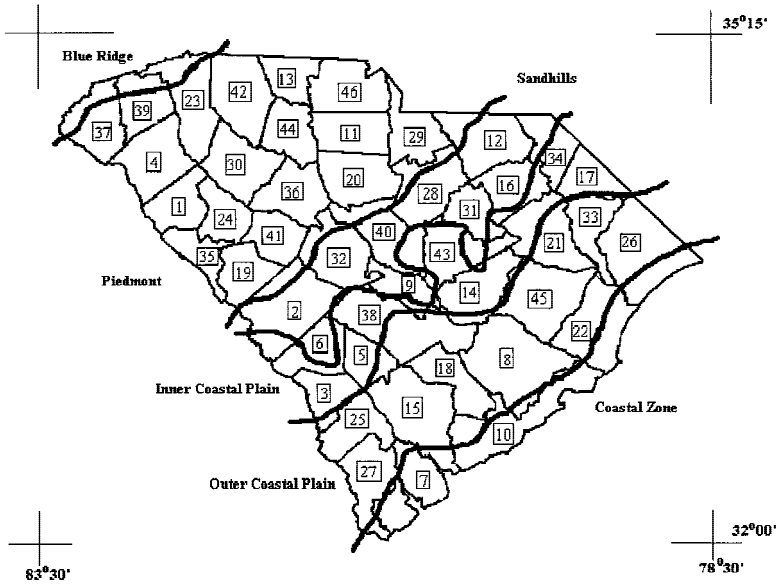

Counties

1	Abbeville	9	Calhoun	17	Dillon	25	Hampton	33	Marion	41	Saluda
2	Aiken	10	Charleston	18	Dorchester	26	Horry	34	Marlboro	42	Spartanburg
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7	Beaufort	15	Colleton	23	Greenville	31	Lee	39	Pickens		
8	Berkeley	16	Darlington	24	Greenwood	32	Lexington	40	Richland		

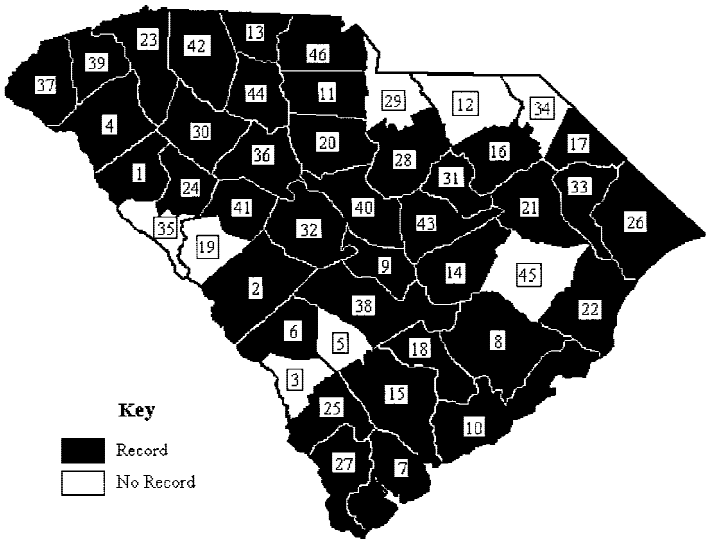
Fig. 1. South Carolina counties and landform regions.

lina. However, when *C. formosanus* was first collected in 1957, it was misidentified as *I. schwarzi* (Chambers 1988), and no other records of *I. schwarzi* exist for the state. Snyder (1924) reported collections of the drywood species, *Kalotermes* (*Incisitermes*) *marginipennis* Latreille from cedar telegraph poles in Charleston, but the species was confused with *I. snyderi* (Scheffrahn et al. 1988).

The only survey of a termite species in South Carolina was limited to *C. formosanus*, first introduced into the continental United States through the port of Charleston (Chambers et al. 1988). All other references list species occurrence by state, and not county by county. The objective of our study was to document the current termite fauna and their distributions, by county, in South Carolina.

Materials and Methods

Our research was conducted using five survey methods: (1) a review of data from the Clemson University Arthropod Collection (CUAC); (2) a mail survey of pest control firms throughout the state; (3) New Jersey light trap (NJLT) collections; (4) field collections; and (5) a review of records from the Clemson University Plant Problem Clinic (CUPPC).



Counties

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7	Beaufort	15	Colleton	23	Greenville	31	Lee	39	Pickens		
8	Berkeley	16	Darlington	24	Greenwood	32	Lexington	40	Richland		

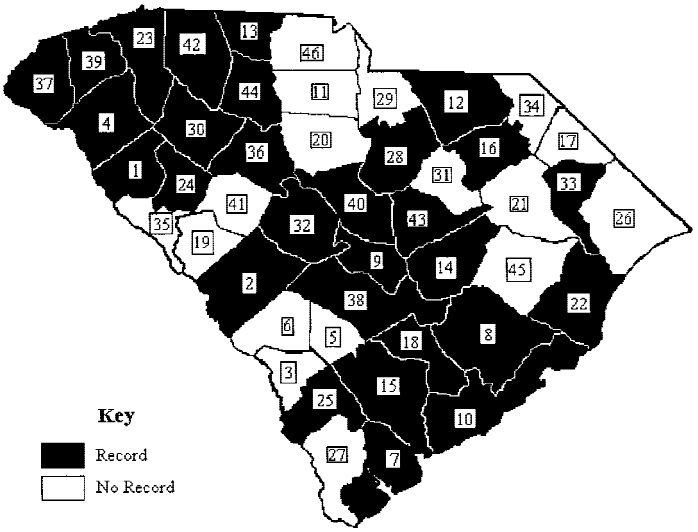
Fig. 2. Distribution of *Reticulitermes flavipes* in South Carolina by county.

Clemson University Arthropod Collection Records. The CUAC contains voucher and study specimens used in University research and extension programs. The identified specimens of the collection serve as reference material for identification of samples of insects and other arthropods submitted from throughout South Carolina. CUAC specimens were reviewed for new county records of termites to November 2000.

Mail Survey. A survey, two vials with 95% ethyl alcohol, and instructions to collect termite soldiers and alates were mailed on February 22, 1999 to 153 pest control firms in all regions of South Carolina. These firms were all members of the South Carolina Pest Control Association in 1998. Questions on the survey were general by design and the answers were not used as data for this project. The questions asked if the pest control company had encountered drywood termites, aerial nests, or Formosan subterranean termites within the past five years. Instructions asked to include collection date and locality with the samples collected. A follow-up postcard was mailed on May 27, 1999. The survey concluded on September 1, 1999. Voucher specimens were deposited in the CUAC.

New Jersey light trap collections.

In 1998, from June to October, a total of 111 NJLTs were monitored for termite alates. These traps were monitored by mosquito control programs from six South



Counties

1 Abbeville	9 Calhoun	17 Dillon	25 Hampton	33 Marion	41 Saluda
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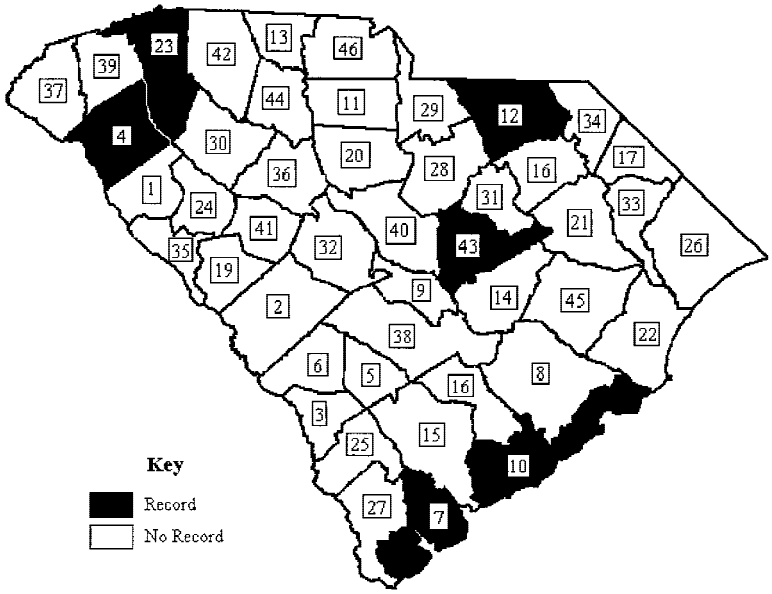
7 Beaufort	15 Colleton	23 Greenville	31 Lee	39 Pickens	
8 Berkeley	16 Darlington	24 Greenwood	32 Lexington	40 Richland	

Fig. 3. Distribution of *Reticulitermes virginicus* in South Carolina by county.

Carolina counties: Charleston, Beaufort, Georgetown, Williamsburg, Berkeley, and Bamberg. In 1999, trapping in Bamberg was discontinued and four other counties were added: Lexington, Florence, Orangeburg, and Richland. All of these counties are located in the Sandhills, Coastal Plain, and Coastal Zone regions of South Carolina (Kovacik & Winberry 1987). Three NJLTs also were placed in the Piedmont region, and two in the Blue Ridge region to give a representation of all regions in the state (Fig. 1). In 1999, 155 NJLTs were monitored from April to October. Termite alates from light traps monitored by the mosquito control programs were collected twice weekly and held in freezers until they were returned to Clemson University and identified using the key by Scheffrahn & Su (1994). Voucher specimens were deposited in the CUAC.

Field Collections. A systematic grid-sampling scheme was originally attempted for field collecting but proved to be ineffective. Many of the randomly selected grids were located on inaccessible or non-wooded properties. Ultimately, field collecting consisted of traveling to counties not previously recorded for a given termite species, and searching for the species in natural wooded habitats. Voucher specimens were deposited in the CUAC.

Extension Records. The Clemson University Plant Problem Clinic (CUPPC) is an extension office that identifies samples submitted by county extension agents and the public. Extension specialists verify the identifications and



Counties

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7	Beaufort	15	Colleton	23	Greenville	31	Lee	39	Pickens		
8	Berkeley	16	Darlington	24	Greenwood	32	Lexington	40	Richland		

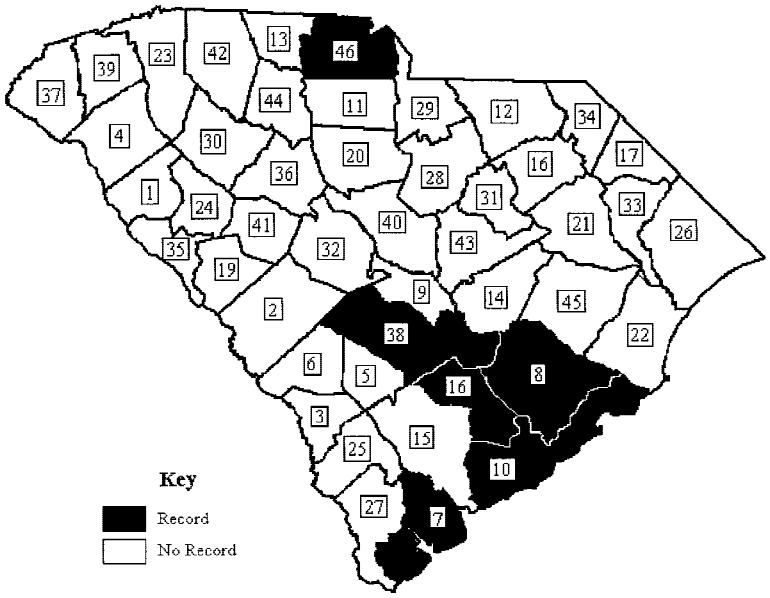
Fig. 4. Distribution of *Reticulitermes hageni* in South Carolina by county.

provide control or management recommendations. CUPPC records from 1986 to 1999 were reviewed for new county records of termites. Unfortunately, voucher specimens for these records are not available to be viewed for corroboration. The authors are aware of the implications of this and exercise extreme caution when reporting new records.

Results

Termite species distribution maps show their current status in South Carolina (Figs. 2–8). Previous and new records are noted.

Clemson University Arthropod Collection. There are 41 combined county records of *R. flavipes* and *R. virginicus* for South Carolina in the CUAC. The only record of *R. hageni* for South Carolina in the CUAC was for Greenville County. There are samples of *C. formosanus* from Charleston, Beaufort, and Orangeburg Counties. There are records of *I. snyderi* from the counties of Georgetown, Horry, Charleston, Berkeley, Dorchester, and Pickens. With the exception of Pickens County, this species was recorded only in the coastal region (Fig. 1). Samples of *C. brevis* are recorded from Spartanburg, Beaufort, and Charleston



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7	Beaufort	15	Colleton	23	Greenville	31	Lee	39	Pickens		
8	Berkeley	16	Darlington	24	Greenwood	32	Lexington	40	Richland		

Fig. 5. Distribution of *Coptotermes formosanus* in South Carolina by county.

Counties, and there are records of *K. approximatus* from Charleston, Pickens, and Beaufort Counties in the CUAC.

Mail Survey. At the end of the survey period, 23% of the pest control firms had sent a total of 71 samples. Five samples contained only worker termites which cannot be identified to species using available taxonomic keys. Fifty samples were identified as *R. flavipes*, with new county records from Union, Dorchester, Lexington, and Marion Counties. Nine samples were identified as *R. virginicus*, with new county records from Aiken and Marion Counties. Six samples of *C. formosanus* were received from Charleston County and one from Burton, located near the city of Beaufort in Beaufort County. Two samples were identified as *Incisitermes minor* (Hagen), which are new state records for South Carolina. One sample was collected from a couch moved from California to the city of Greenville, Greenville County, and the other was collected from a house in the city of Beaufort, Beaufort County.

New Jersey light trap collections. The 1998 and 1999 light traps captured termites in Berkeley, Charleston, Georgetown, and Beaufort Counties, all located in the Outer Coastal Plain and Coastal Zone regions (Table 1). Most collections were in late May to late June. Exceptions to this were single collections of *R.*

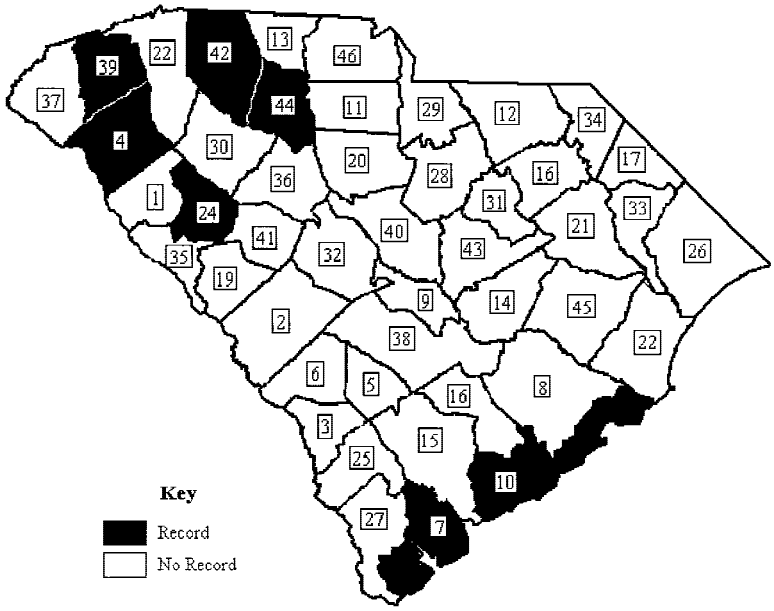

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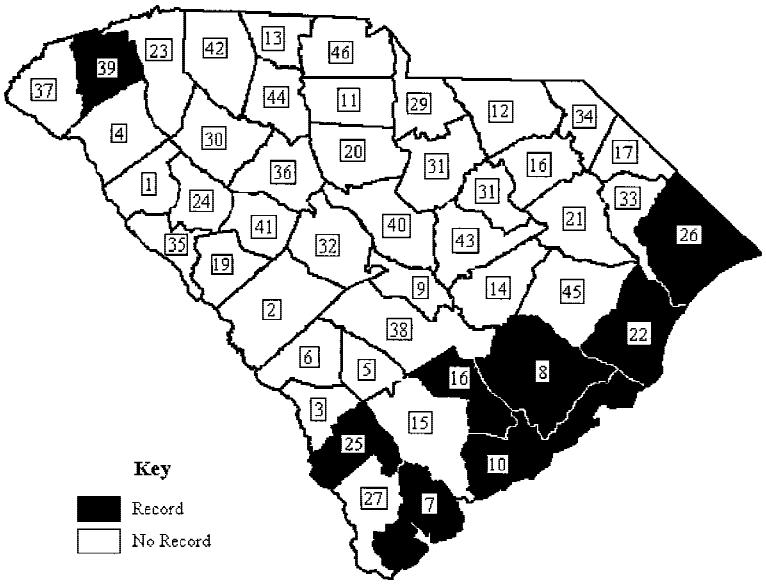
Fig. 6. Distribution of *Cryptotermes brevis* in South Carolina by county.

flavipes and *I. snyderi* in late September, and *I. snyderi* and *C. formosanus* in early October (Table 1).The majority of samples were either *C. formosanus* or *I. snyderi*. New county records included *C. formosanus* from Berkeley County, and *I. snyderi* from Beaufort County.

Field Collections. Of the 46 counties in South Carolina, 27 were field sampled for termites. A total of 87 samples of termites were field collected from 22 of the counties visited. New records of *R. flavipes* were found in collections from Anderson, Calhoun, Jasper, and Lee Counties. New records of *R. virginicus* were found in collections from Calhoun, Darlington, Dorchester, Hampton, and Orangeburg Counties. One new record of *R. hageni* was found in collections from Anderson County. *Kalotermes approximatus* was found in natural habitats and 15 new records were added from Berkeley, Calhoun, Colleton, Darlington, Dorchester, Florence, Georgtown, Horry, Jasper, Lee, Lexington, Marion, Newberry, Orangeburg, and Union Counties.

Extension Records. After reviewing CUPPC records from 1986 to 1999, 16 new county records were noted (Robinson, personal communication). Because there are no voucher specimens to verify these records, the authors have only reported those records that can be justified. *Reticulitermes flavipes* was docu-



Counties

1 Abbeville	9 Calhoun	17 Dillon	25 Hampton	33 Marion	41 Saluda
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6 Barnwell	14 Clarendon	22 Georgetown	30 Laurens	38 Orangeburg	46 York
7 Beaufort	15 Colleton	23 Greenville	31 Lee	39 Pickens	
8 Berkeley	16 Darlington	24 Greenwood	32 Lexington	40 Richland	

Fig. 7. Distribution of *Incisitermes snyderi* in South Carolina by county.

mented from six counties previously unrecorded for the species: Aiken, Chester, Fairfield, Florence, Laurens, and Orangeburg. Likewise, *R. virginicus* was documented from six counties previously unrecorded for the species: Anderson, Cherokee, Clarendon, Greenwood, Lexington, and Sumter. *Reticulitermes flavipes* and *R. virginicus* are found throughout the state and those counties listed above as new records all have surrounding counties with verified presence. Two new county records for *R. hageni* were found from Beaufort and Charleston Counties. Samples of *R. hageni* have been sent in to Clemson University from pest control companies in these areas. Three new records for *C. formosanus* were documented from Monck's Corner in Berkeley County, and St. George and Ridgeville in Dorchester County. These records were verified by the Department of Pesticide Regulation.

Discussion

New county records were added for each species of termite known to occur in South Carolina. The most commonly recorded termite in the state is *R. flavipes*, now recorded in 38 of 46 counties (Fig. 2). Very likely, if field collecting was conducted in the remaining eight counties, these too would yield *R. flavipes*.

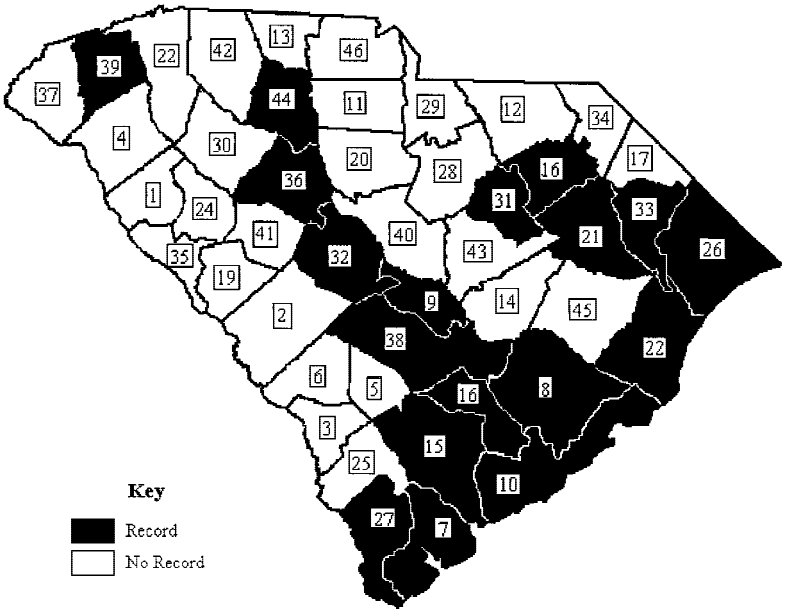

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Fig. 8. Distribution of *Kalotermes approximatus* in South Carolina by county.

Reticulitermes virginicus is now recorded in 29 of 46 counties (Fig. 3). It is probable that this species also could be collected in those counties which currently do not have records. These two species are cosmopolitan in distribution throughout all regions of the state. *Reticulitermes hageni* is recorded in six counties which represent all landform regions of South Carolina (Fig. 4). This species is rarely recognized. The alates are sometimes misidentified as a drywood species because of their lighter color (Potter 1997). In a study by Scheffrahn et al. (1988) on the termites of peninsular Florida, *R. hageni* was reported as rare. This species is probably more widespread in South Carolina than indicated on the map (Fig. 4).

Documenting the spread of *C. formosanus* was an important aspect of this research because of its destructive nature. Since the study by Chambers et al. (1988), *C. formosanus* has become established on Hilton Head Island in Beaufort County, in the city of Orangeburg in Orangeburg County, and as far north as Rutherford County, North Carolina. These infestations might have been established through the transport of infested lumber brought in from *C. formosanus* established areas. A review of CUPPC records shows accounts of *C. formosanus* in two areas in Dorchester County and one in Berkeley County. These infestations

Table 1. Termite species captured in New Jersey light traps used by mosquito control programs in South Carolina in 1998 and 1999.

Species	Date	County
C. formosanus	05 JUN 1998	Charleston
	11 JUN 1999	Berkeley
	25 MAY 1999	Charleston
	15 JUN 1999	Charleston
	19 JUN 1999	Charleston
	15 OCT 1999	Charleston
I. snyderi	22 MAY 1998	Berkeley
	24 MAY 1999	Georgetown
	03 JUN 1999	Beaufort, Georgetown
	04 JUN 1999	Berkeley
	08 JUN 1999	Georgetown
	15 JUN 1999	Charleston
	18 JUN 1999	Georgetown
	19 JUN 1999	Charleston
	22 JUN 1999	Berkeley
	13 JUL 1999	Charleston
	27 SEP 1999	Georgetown
	02 OCT 1999	Georgetown
K. approximatus	15 JUN 1999	Charleston
R. flavipes	21 SEP 1999	Georgetown
R. hageni	25 MAY 1999	Charleston

also are attributed to infested lumber brought into these areas. *Coptotermes formosanus* were collected from the city of Burton, which indicates they have moved inland into Beaufort County. An alate collected from a NJLT in Wando, Berkeley County, is believed to be from the nearby city of Mt. Pleasant in Charleston County. The Formosan subterranean termite is spreading, but mainly through the transport of infested materials.

Cryptotermes brevis is now recorded in seven counties in South Carolina. These counties cover all regions of the state except the Sandhills and Inner Coastal Plain. This species has never been recorded in natural habitats in South Carolina, or anywhere else (Gay 1967), and only occasionally in structures. It might be more widespread than indicated as a result of infestations not being reported or samples not identified to species.

Incisitermes snyderi was found in seven counties in the coastal region of South Carolina. There is one record of this species in a structure from Pickens County, indicating it is not restricted to the coast. All samples from South Carolina were taken from structures or light traps.

Kalotermes approximatus was collected from natural habitats in 18 counties in South Carolina. This species is not a major pest, but occasionally infests structures. All but three specimens were collected from live and dead hardwood trees.

The samples of *I. minor* received from Greenville and Beaufort Counties are a

new state record. This species, a major pest in the western U.S., appears to be spreading to the eastern states. It is established in Florida and Alabama (Scheffrahn, personal communication) and could establish in South Carolina.

The terrain of the five landform regions in South Carolina does not appear to be a contributing factor in the distribution of termite species. The coastal region of the state has more termite pressure than other regions. This is attributed to the warm, humid, sub-tropical climate. Other termite species eventually may be introduced into this region of South Carolina.

Whenever a species of termite is encountered that is not previously known to occur in an area, it should be reported to the appropriate extension entomologist. It is important to be familiar with the species that occur in an area to develop better management programs for pest species, to recognize and potentially stop a new pest species that might establish in an area, and to keep a current catalogue for future research.

Acknowledgments

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E R R A T U M

The article identification information for the following two articles was inadvertently omitted from the previous issue of the journal:

Averting-Cost Measures of the Benefits to South Carolina Households of Red Imported Fire Ant Control

Stephen E. Miller, Mark S. Henry, Brenda J. Vander Mey, and Paul M. Horton

Department of Agricultural and Applied Economics, Clemson University,
Clemson, South Carolina 29634-0355 USA

J. Agric. Urban Entomol. 17(3): 113–123 (July 2000)

Integration of Chlorfenapyr into a Management Program for the German Cockroach (Dictyoptera: Blattellidae)

Abdullahi Ameen, Walid Kaakeh and Gary Bennett

Center for Urban and Industrial Pest Management, Department of Entomology, 1158 Smith Hall,
Purdue University, West Lafayette, Indiana 47907

J. Agric. Urban Entomol. 17(3): 135–142 (July 2000)

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NOTICE TO CONTRIBUTORS

The Editorial Committee of *Journal of Agricultural Entomology* considers the timely publication and documentation of research endeavors critical to the advancement of agricultural and urban entomology. As such, JAUE places a high priority on prompt processing of submitted works, as well as prompt publication of accepted works. However, the overall turnaround time is to a large extent in the hands of authors making revisions.

In light of this, the Committee requests that authors return revised manuscripts to their subject editor within three months of receipt. Manuscripts held for revisions for six months or more will, at the discretion of the editor, be subject to a 10% surcharge over the normal cost of publication.

The Committee recognizes that extenuating circumstances arise, and will consider special arrangements for authors in such situations.

J. H. Cochran Excellence in Entomology Award

Peter H. Adler

Dr. Peter H. Adler was the 1999 recipient of the South Carolina Entomological Society's J. H. Cochran, Excellence in Entomology Award. In 1976, he received his Bachelor's Degree in Biology from Washington & Lee University. He earned his Master's Degree in Zoology in 1979 and in 1983, his Ph.D. in Entomology both from Pennsylvania State University. Dr. Adler was awarded a Postdoctoral Research Fellowship at the University of Alberta, Canada in 1984, and later that year he accepted an associate professor position at Clemson University.

Dr. Adler is a tenured professor in his 16th year at Clemson. His research focuses on arthropods of medical and agricultural significance, with emphasis on black flies, the second most important group of blood-sucking insects in the world. Dr. Adler has pioneered the tandem cytogenetic-morphological-ecological approach to the study of black flies while expanding his research geographically. As a result of Dr. Adler's thorough investigations, black flies have become one of the best known and biologically understood groups of insects in North America, the Amazon Basin, Russia, China, the Caucasus and Europe. His research has been funded in part through grants from the NSF, NIH, USDA, NATO, Civilian Research Foundation, and the U.S. Fish & Wildlife Service. An accomplished writer and presenter, Dr. Adler has published 90 research papers and authored or coauthored 153 professional presentations. He has collaborated on research projects with colleagues in 11 countries, hosted 8 international postdoctoral researchers, trained 24 graduate students, and served on 66 additional graduate student committees.

Dr. Adler continues to teach 3 core subjects in addition to special topics courses, special problems, and directed research. Almost 400 students, mostly graduate students, have taken his courses; approximately 90% of them have ranked him in the highest category ("one of the best") of instructors at Clemson University. To honor their mentor, two of his doctoral students dedicated their dissertations to Dr. Adler, an authoritative researcher, caring mentor, and outstanding teacher.

Prod. #

J. H. Cochran Memorial Graduate Student Award and Scholarship

Will Karlisle Reeves

Will Karlisle Reeves was the South Carolina Entomological Society's 1999 winner of the J. H. Cochran Memorial Scholarship for the Outstanding Graduate Student in Entomology. Will received a BS in applied biology at Georgia Tech in 1997 and completed his Master's degree at Clemson University in August 1999. Under the direction of Dr. Peter H. Adler, Will's thesis was entitled "Ecology of Invertebrate Necrophages in Caves of Northwestern Georgia." Will has given 10 presentations at professional meetings, published six papers in refereed journals, and submitted five additional papers to peer-reviewed journals. He also has published two nonrefereed papers, presented three invited talks, given an invited interview on National Public Radio about his research in caves of the Great Smoky Mountains National Park, and received five competitive mini-grants to support his research. Will is a member of nine professional societies and is currently a teaching assistant in Clemson University's Biology Program.

Will has received an award for the best student presentation at the 1999 meeting of the South Carolina Entomological Society. In March 2000, he received the Kirby L. Hays Memorial Award for the outstanding entomology student in the Southeastern Branch of the Entomological Society of America. Will is an expert on caves and has led expeditions in a number of cave systems, most recently to the Maje Mountains of Panama. He has discovered several new species of invertebrates in various caves, especially in the southeastern United States, and recently had a new species of fly named in his honor. Will is beginning his doctoral program with Dr. Adler in the Department of Entomology at Clemson University.

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South Carolina
Entomological Society, Inc.

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Clemson, South Carolina 29633

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I hereby make application for membership in the South Carolina Entomological Society, Inc. with the status of active ($30), student ($15), emeritus ($10) membership.

Signature: _____ Date: _____
Return form with membership dues to address above.

ATTENTION

The South Carolina Entomological Society (http://entweb.clemson.edu/scesweb) proudly introduces its new Journal title:

Journal of Agricultural and Urban Entomology

Recognizing the growing importance of urban entomology in today's global society, we have unanimously approved a proposal to incorporate a broader range of interests in our scientific publication and close the link between urban and agricultural entomology.

The Journal of Agricultural and Urban Entomology (formerly: *Journal of Agricultural Entomology* * Jan 1984 through Oct 1998) is published quarterly under the auspices of the South Carolina Entomological Society, Inc. The Journal publishes contributions of original research concerning insects and other arthropods of agricultural and urban importance to include those affecting humans, livestock, poultry, and wildlife. The Journal is particularly dedicated to the timely publication of articles and notes pertaining to applied entomology, although it will accept suitable contributions of a fundamental nature related to agricultural and urban entomology.

Single issues and complete sets of the Journal of Agricultural Entomology, volume 1 through 15 are available. Single issues cost $20 each. Complete sets (1984–1998) are offered at $1000 plus shipping.