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Seed Treatment for Control of Wheat Insects and Its Effect on Yield[1]

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ABSTRACT The efficacy of three insecticides as seed treatments for control of insect pests on winter wheat were evaluated over a 4-year period at two locations in Kansas. Their effects on yield also were measured. Imidacloprid (Gaucho®) and thiamethoxam (Adage®) were effective in controlling early season (fall) infestations of the greenbug, *Schizaphis graminum* (Rondani), and the Russian wheat aphid, *Diuraphis noxia* (Mordvilko) at all rates tested. Control of spring infestations with those two compounds was less consistent. Fipronil (Regent®) was not effective against these two aphid species. All three compounds effectively controlled fall infestations of Hessian fly, *Mayetiola destructor* (Say), but none were effective against the sole spring infestation that occurred. Imidacloprid had no insecticidal effect on fall armyworm, *Spodoptera frugiperda* (Smith), in a single fall test. Under low or nonexistent natural pest infestations, no yield benefits were observed from any of the seed treatments. No phytotoxicity or negative effects on yield were detected.

KEY WORDS Russian wheat aphid, greenbugs, Hessian fly, fall armyworm, seed treatment, winter wheat

Introduction

Insect pests are a major factor limiting wheat production in the United States and elsewhere. The greenbug, *Schizaphis graminum* (Rondani), the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), the Hessian fly, *Mayetiola destructor* (Say), and the fall armyworm, *Spodoptera frugiperda* (Smith), often are present in numbers that limit yield. Several different methods, including resistant varieties, natural enemies, and foliar-applied insecticides, have been used to control these pest species. Recently, the use of seed treatments has been suggested as an alternative control method. The use of a systematic seed treatment, such as imidacloprid (Gaucho®) has been demonstrated to protect wheat and barley from Russian wheat aphids for 27–85 days (Pike et al. 1993, Archer 1994, Kroening et al. 1998). However, few data are available on the effects of other seed treatments, other insects controlled, or effects on yield. Seed treatments would be particularly useful in situations where one or more of these pests cause chronic problems. The objectives of this study were to evaluate several insecticides currently registered

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or being tested as seed treatments for control of these major wheat pests and to determine their effects on yield of two common varieties of winter wheat grown throughout the Great Plains.

Materials and Methods

Field plots were established at Manhattan and Hesston, KS, over a 4-year period (1995–1999). Planting was accomplished using a small-plot planter that drilled 'Karl 92' (1995–1997) or 'Tomahawk' (1997–1999) wheat at a seedling rate of ca. 67 kg/ha. Plots were 1.5 by 12.2 m and replicated four times in a randomized complete block design. Detailed information for each location and year is listed in Table 1. Insecticides tested and the number of treatments and rates varied from year to year. A completely untreated check (no fungicide and no insecticide) was included in 1997-1998, but not in any other year. Treated seeds were provided by the companies that produce the different compounds tested. The respective companies established insecticide rates. Carboxin (Vitavax®) and difenoconazole (Dividend®) were the fungicidal treatments used as checks.

All plots were examined twice in the fall and twice in the spring for the presence of the target insect pests. Because natural field infestations of these insects did not occur, treatments were evaluated in the fall and spring by digging up two wheat plants from each plot, transplanting each plant into 1.5 cm-diameter pots, and transferring them to a greenhouse where they were watered and maintained at 25 ± 2°C. Two days after transplanting, all plants were artificially infested with approximately 20 greenbugs or Russian wheat aphids from colonies reared in the greenhouse using the Davis inoculator (Harvey et al. 1985). Hessian fly infestations were obtained by aspirating a sufficient number of adults to release 10 per pot and left undisturbed for 3 days to oviposit. Hessian flies and caging materials were obtained from a United States Department of Agriculture laboratory at Manhattan, KS. Fall armyworms also were collected from laboratory colonies and transferred to the test plants using a Davis inoculator according to the procedure developed by Davis & Oswalt (1979). Insect counts and/or damage ratings were made 20–30 days after infestation. Damage ratings for greenbugs, Russian wheat aphids, and fall armyworms were made using a subjective 0–5, 0–9, or 0–10 scale where 0 indicated no damage was evident and 5,9, or 10 indicated plant death depending on the respective scale used. The same person (G. Wilde) evaluated throughout the 4-year test period. Plants were carefully examined and arbitrarily divided into equal portions depending on the scale used. For the 0–5 scale, the plants were divided into 5 approximately equal parts and visually rated for the damage characteristics of the target pest, either greenbugs, Russian wheat aphids, or fall armyworms. Hessian fly counts and other counts where the actual insects were counted were conducted by placing the test plants under magnification and counting the number of insects. No damage rating scheme was used for the Hessian fly. A previous study by Archer (1994) indicated these artificial infestation techniques correlated well with field studies and are an effective method for evaluating insect control. Analysis of variance was performed on the data collected. Mean separations were based on an LSD test at a probability level of 0.05 (SAS Institute 1987).

Table 1. Relevant planting, agronomic, and insect evaluation practices for evaluation of seed treatments to control insects in Kansas wheat, 1995–1999.

Year	Location	Planting date	Pest	Evaluation		Evaluation date
				Fall	Spring	
1995–96	Manhattan	10/9	Greenbug	X		10/30
			Russian wheat aphid	X	X	10/30, 5/31
			Hessian fly	X		12/8
	Hesston	10/15	Greenbug	X		12/7
			Russian wheat aphid	X		12/7
			Hessian fly	X		12/8
1996–97	Manhattan	10/21	Greenbug	X	X	11/26, 5/13
			Russian wheat aphid	X	X	11/26, 5/13
			Fall armyworm	X		11/26
	Hesston	10/18	Greenbug	X	X	11/26, 5/13
			Russian wheat aphid	X	X	11/26, 5/13
1997–98	Manhattan	10/7	Greenbug	X	X	12/5, 5/5
			Russian wheat aphid	X	X	12/19, 5/5
			Hessian fly	X	X	12/5, 5/5
	Hesston	10/7	Greenbug	X	X	12/5, 5/12
			Russian wheat aphid	X	X	12/19, 5/12
1998–99	Manhattan	9/29	Greenbug	X	X	12/10, 3/31
			Russian wheat aphid	X	X	12/10, 3/31
			Hessian fly	X		12/10
	Hesston	10/15	Greenbug	X	X	12/10, 3/31
			Russian wheat aphid	X	X	12/10, 3/31

Results

Tests Conducted: 1995–1996. Evaluation during the fall of 1995 showed that all four rates of imidacloprid reduced numbers of greenbug (Table 2), Russian wheat aphid (Table 4), and Hessian fly (Table 6) and reduced or eliminated damage (Tables 3 and 5). Evaluation of Russian wheat aphid damage in spring 1996 (Table 4) showed a dose response to the treatments. Wheat treated with the three highest rates had less damage from all the insects than wheat treated with carboxin only. However, wheat treated with the lowest concentrations showed damage from Russian wheat aphid feeding was not different from that of the

Table 2. Effect of various seed treatments on greenbugs (GB) in winter wheat at Manhattan, Kansas, 1995–1999.

Treatment	Rate g AI/100 kg seed	Fall 95–96 GB[a]/ plant[b]	Fall 96–97 GB DR[cd]	Spring 96–97 GB DR[cd]	Fall 97–98 GB DR[fg]	Spring 97–98 GB DR[eg]	Fall 98–99 GB DR[eh]	Spring 98–99 GB DR[eh]
Vitavax	88.0	31.3b	5.0b	4.2c	9.0b	9.2d	9.5c	10.0c
Gaucho 480FS	31.2				0.0a	4.3a		
Gaucho 480FS	32.5	1.8a	0.0a	3.2b	0.1a	6.5bc	0.3a	0.5a
Gaucho 480FS	48.7	0.0a	0.0a	2.5ab	0.3a	5.6ab	0.0a	0.0a
Gaucho 480FS	62.4				0.0a	4.1a		
Gaucho 480FS	65.2	0.0a	0.0a	2.0a	0.3a	4.6a	0.3a	0.5a
Gaucho 480FS	97.5	0.0a						
Regent 4FC	5.0				8.7b			
Regent 4FC	10.0						9.0c	
Regent 4FC	15.0				8.7b		9.7c	
Regent 4FC	20.0						10.0c	
Regent 4FC	35.0				9.0b			
Adage 5FS	29.5				0.4a	7.5c	2.7b	5.2b
Adage 5FS	34.0						0.3a	0.5a
Adage 5FS	39.0				0.4a	7.8cd	0.3a	0.5a
Adage 5FS	58.5				0.3a	6.7bc		
Dividend lot 1	12.0				8.7b		9.5c	10.0c
Dividend lot 2	12.0				9.0b		9.7c	
Untreated					9.0b			
F		12.3	∞	8.9	702.2	8.1	176.2	21.7
df		4,3	3,3	3,3	14,3	8,3	11,3	7,3
P		0.0001	<0.0001	0.0018	0.0001	0.0001	0.0001	0.0001

[a]GB—Greenbug.
[b]Based on potted plants brought back to greenhouse on 10/23/95. GB counts on 10/30/95.
[c]DR—Damage rating scale ranges from 0–5, where 0 = no damage to 5 = plant death.
[d]Based on potted plants brought back to greenhouse on 11/9/96 and 4/19/97, evaluated on 11/26/96 and 5/13/97.
[e]DR—Damage rating scale ranges from 0–10, where 0 = no damage to 10 = plant death.
[f]DR—Damage rating scale ranges from 0–9, where 0 = no damage to 9 = plant death.
[g]Based on potted plants brought back to greenhouse on 11/19/97, evaluated on 12/5/97 and 5/5/98.
[h]Based on potted plants brought back to greenhouse on 11/18/98 and 3/4/99, evaluated on 3/31/99.
Means in a column followed by the same letter are not significantly different at $P = 0.05$ (LSD).

Table 3. Effect of various seed treatments on greenbugs (GB) in winter wheat at Hesston, Kansas, 1995–1999.

Treatment	Rate g AI/100 kg seed	Fall 95–96 GB[a]/ DR[bc]	Fall 96–97 GB DR[de]	Spring 96–97 GB DR[de]	Fall 97–98 GB DR[fg]	Spring 97–98 GB DR[fg]	Fall 98–99 GB DR[ch]	Spring 98–99 GB DR[ch]
Vitavax	88.0	8.0b	4.5b	5.0b	7.8c	7.6b	9.6bc	10.0e
Gaucho 480FS	31.2				0.0a	5.6ab		
Gaucho 480FS	32.5	0.0a	0.0a	4.0ab	0.1a	6.1ab	0.4ab	3.1ab
Gaucho 480FS	48.7	0.0a	0.0a	3.2a	0.0a	6.7ab	0.9a	4.0abc
Gaucho 480FS	62.4				0.0a	5.7ab		
Gaucho 480FS	65.2	0.0a	0.3a	3.2a	0.3a	5.1a	0.3ab	4.7bcd
Gaucho 480FS	97.5	0.0a						
Regent 4FC	5.0				7.8c			
Regent 4FC	10.0						9.2bc	
Regent 4FC	15.0				6.2b		9.9c	
Regent 4FC	20.0						9.0bc	
Regent 4FC	35.0				7.6c			
Adage 5FS	29.5				0.0a	6.6ab	0.4ab	5.0cd
Adage 5FS	34.0						0.6ab	6.0d
Adage 5FS	39.0				0.0a	6.1ab	0.1a	2.5a
Adage 5FS	58.5				0.0a	5.1a		
Dividend lot 1	12.0				7.8c		9.8c	9.9e
Dividend lot 2	12.0				8.0c		9.6bc	
Untreated					8.1c			
F		15.7	83.8	4.8	484.6	1.5	417.5	21.8
df		4,3	3,3	3,3	14,3	8,3	12,3	7,3
P		<0.0001	<0.0001	0.0183	0.0001	0.176	0.0001	0.0001

[a]GB—Greenbug.
[b]Based on potted plants brought back to greenhouse on 11/17/95, evaluated on 12/7/95.
[c]DR—Damage rating scale ranges from 0–10, where 0 = no damage to 10 = plant death.
[d]DR—Damage rating scale ranges from 0–5, where 0 = no damage to 5 = plant death.
[e]Based on potted plants brough back to greenhouse on 11/8/96 and 4/7/97, evaluated on 11/26/96 and 5/13/97.
[f]DR—Damage rating scale ranges from 0–9, where 0 = no damage to 9 = plant death.
[g]Based on potted plants brought back to greenhouse on 11/19/97 and 4/22/98, evaluated on 12/5/97 and 5/5/98.
[h]Based on potted plants brought back to greenhouse on 11/17/98 and 3/4/99, evaluated on 12/10/98 and 3/31/99.
Means in a column followed by the same letter are not significantly different at $P = 0.05$ (LSD).

Table 4. Effect of various seed treatments on Russian wheat aphids (RWA) in winter wheat at Manhattan, Kansas, 1995–1999.

Treatment	Rate g AI/100 kg seed	Fall 95–96 RWA[a]/ plant[b]	Spring 95–96 RWA DR[bc]	Fall 96–97 RWA DR[cd]	Spring 96–97 RWA DR[cd]	Fall 97–98 RWA DR[fg]	Spring 97–98 RWA DR[fg]	Fall 98–99 RWA DR[eh]	Spring 98–99 RWA DR[eh]
Vitavax	88.0	22.8b	3.9b	4.5b	4.7b	7.5b	6.8c	7.2de	9.5d
Gaucho 480FS	31.2					0.6a	5.1ab		
Gaucho 480FS	32.5	2.0a	2.9b	0.3a	2.7a	0.1a	6.3bc	1.5ab	0.5ab
Gaucho 480FS	48.7	0.0a	1.8a	0.0a	2.5a	0.0a	5.0ab	0.0a	0.3a
Gaucho 480FS	62.4					0.0a	4.7a		
Gaucho 480FS	65.2	0.0a	1.9a	0.0a	2.0a	0.0a	5.0ab	0.5a	0.8ab
Gaucho 480FS	97.5	0.0a	1.1a						
Regent 4FC	5.0					8.8c			
Regent 4FC	10.0							9.0ef	
Regent 4FC	15.0					8.2bc		7.3de	
Regent 4FC	20.0							9.8f	
Regent 4FC	35.0					7.8bc			
Adage 5FS	29.5					0.0a	6.7c	5.0cd	1.7c
Adage 5FS	34.0							3.5bc	1.3bc
Adage 5FS	39.0					0.0a	7.0c	0.5a	0.8ab
Adage 5FS	58.5					0.0a	7.3c		
Dividend lot 1	12.0					8.9c		10.0f	10.0d
Dividend lot 2	12.0					7.1b		9.0ef	
Untreated						7.9bc			
F		13.2	21.2	24.8	7.5	98.5	4.1	23.3	161.1
df		4,3	4,3	3,3	3,3	14,3	8,3	11,3	7,3
P		<0.0001	0.0076	<0.0001	0.0081	0.0001	0.0006	0.0001	0.0001

[a]RWA—Russian wheat aphid.
[b]Based on potted plants brought back to greenhouse on 10/23/95. RWA counts on 10/30/95.
[c]DR—Damage rating scale ranges from 0–5, where 0 = no damage to 5 = plant death.
[d]Based on potted plants brought back to greenhouse on 11/9/96 and 4/19/97, evaluated on 11/26/96 and 5/13/97.
[e]DR—Damage rating scale ranges from 0–10, where 0 = no damage to 10 = plant death.
[f]DR—Damage rating scale ranges from 0–9, where 0 = no damage to 9 = plant death.
[g]Based on potted plants brought back to greenhouse on 11/19/97, evaluated on 12/5/97 and 5/5/98.
[h]Based on potted plants brought back to greenhouse on 11/18/98 and 3/4/99, evaluated on 3/31/99.
Means in a column followed by the same letter are not significantly different at $P = 0.05$ (LSD).

Table 5. Effect of various seed treatments on Russian wheat aphids (RWA) in winter wheat at Hesston, Kansas, 1995–1999.

Treatment	Rate g AI/100 kg seed	Fall 95–96 RWA[a] DR[bc]	Fall 96–97 RWA DR[de]	Spring 96–97 RWA DR[de]	Fall 97–98 RWA DR[fg]	Spring 97–98 RWA DR[fg]	Fall 98–99 RWA DR[ch]	Spring 98–99 RWA DR[ch]
Vitavax	88.0	8.7b	3.8b	5.0b	9.0d	7.6b	9.6c	8.2bc
Gaucho 480FS	31.2				0.0a	5.6ab		
Gaucho 480FS	32.5	0.3a	0.0a	4.0a	0.7b	6.1ab	0.1a	3.7c
Gaucho 480FS	48.7	0.8a	0.5a	4.0a	0.3ab	6.7ab	0.1a	5.9c
Gaucho 480FS	62.4				0.0a	5.7ab		
Gaucho 480FS	65.2	0.0a	0.0a	3.5a	0.3ab	5.1a	0.1a	2.7a
Gaucho 480FS	97.5	0.3a						
Regent 4FC	5.0				9.0d			
Regent 4FC	10.0						9.5c	
Regent 4FC	15.0				9.0d		9.2bc	
Regent 4FC	20.0						8.7bc	
Regent 4FC	35.0				7.8c			
Adage 5FS	29.5				0.0a	6.6ab	0.4a	5.3c
Adage 5FS	34.0						0.5a	5.3c
Adage 5FS	39.0				0.1ab	6.1ab	0.1a	2.5a
Adage 5FS	58.5				0.1ab	5.1a		
Dividend lot 1	12.0				9.0c		9.7c	9.6c
Dividend lot 2	12.0				9.0c		9.4c	
Untreated					8.8c			
F		10.3	37.8	3.7	361.8	1.8	551.9	11.0
df		4,3	3,3	3,3	14,3	8,3	11,3	7,3
P		<0.0001	<0.0001	0.0134	0.0001	0.099	0.0001	0.0001

[a]RWA—Russian wheat aphid.
[b]Based on potted plants brought back to greenhouse on 11/17/95, evaluated on 12/7/95.
[c]DR—Damage rating scale ranges from 0–10, where 0 = no damage to 10 = plant death.
[d]DR—Damage rating scale ranges from 0–5, where 0 = no damage to 5 = plant death.
[e]Based on potted plants brough back to greenhouse on 11/8/96 and 4/7/97, evaluated on 11/26/96 and 5/13/97.
[f]DR—Damage rating scale ranges from 0–9, where 0 = no damage to 9 = plant death.
[g]Based on potted plants brought back to greenhouse on 11/19/97 and 4/22/98, evaluated on 12/5/97 and 5/5/98.
[h]Based on potted plants brought back to greenhouse on 11/17/98 and 3/4/99, evaluated on 12/10/98 and 3/31/99.
Means in a column followed by the same letter are not significantly different at $P = 0.05$ (LSD).

Table 6. Effect of various seed treatments on Hessian fly (HF) in winter wheat at Manhattan, Kansas, 1995–1999 and Hesston, Kansas, Fall, 1995.

		Manhattan, Kansas				Hesston, Kansas
Treatment	Rate g AI/100 kg seed	Fall 95–96 HF[a]/ plant[b]	Fall 97–98 HF/ plant[c]	Spring 97–98 HF/ plant[c]	Fall 98–99 HF/ plant[d]	Fall 1995 HF/ plant[e]
Vitavax	88.0	15.2b	3.2fg	29.8a	10.8c	7.3c
Gaucho 480FS	31.2		0.8abc	26.3a		
Gaucho 480FS	32.5	0.2a	2.1cdef	37.5a	0.1a	2.5b
Gaucho 480FS	48.7	0.0a	0.6ab	25.7a	0.0a	0.4a
Gaucho 480FS	62.4		0.3a	27.5a		
Gaucho 480FS	65.2	0.0a	0.1a	29.8a	0.0a	0.0a
Gaucho 480FS	97.5	0.0a				0.0a
Regent 4FC	5.0		1.6abcd	26.3a		
Regent 4FC	10.0				2.5b	
Regent 4FC	15.0		0.5ab	22.3a	1.6ab	
Regent 4FC	20.0				1.7ab	
Regent 4FC	35.0		0.5ab	26.2a		
Adage 5FS	29.5		1.9cdef	26.4a	1.2ab	
Adage 5FS	34.0				0.0a	
Adage 5FS	39.0		1.1abcd	23.6a	0.0a	
Adage 5FS	58.5		1.1abcd	24.9a		
Dividend lot 1	12.0		2.9efg		9.7c	
Dividend lot 2	12.0		2.3defg		14.3c	
Untreated			3.5g			
F		15.3	6.07	0.47	40.5	1.6
df		4,3	14,3	11,3	11,3	4,3
P		<0.0001	0.0001	0.90	0.0001	0.0198

[a]HF—Hessian fly.
[b]Based on potted plants brought back to greenhouse on 10/23/95. HF counts on 12/8/95.
[c]Based on potted plants brough back to greenhouse on 11/19/97, evaluated on 12/5/97 and 5/5/98.
[d]Based on potted plants brought back to greenhouse on 11/18/98 and 3/4/99, evaluated on 3/31/99.
[e]Based on potted plants brought back to greenhouse on 11/17/95, evaluated on 12/7/95.
Means in a column followed by the same letter are not significantly different at $P = 0.05$ (LSD).

carboxin check at Manhattan. Yield data from both locations indicated no differences between various treatments and the check treated only with carboxin.

Tests Conducted: 1996–1997. Results of the 1996–1997 planting were very similar to those of the previous year. Both greenbug (Tables 2, 3) and Russian wheat aphids (Tables 4, 5) were controlled extremely well by imidacloprid in the fall, but plants showed some damage in the spring evaluation. However, damage was reduced in all but one treatment. No differences in yield were detected among the various treatments and the carboxin only check. Artificially infesting fall

armyworms during the fall showed that imidacloprid had no insecticidal activity against this lepidopteran pest because all treatments were severely defoliated (Table 7).

Tests Conducted: 1997–1998. Fifteen treatments involving various rates of three compounds, imidacloprid, thiamethoxam, and fipronil, as well as carboxin and difenoconazole, were evaluated for their effects in the fall and spring on various pests. Imidacloprid and thiamethoxam were very effective in the field against greenbug (Tables 2, 3) and Russian wheat aphids (Tables 4, 5) at all rates. Fipronil showed no aphicidal activity at the rates tested. In the spring, imidacloprid reduced damage by greenbug and Russian wheat aphids at the higher rates, but some feeding damage occurred for all treatments. Fipronil was not evaluated for aphid damage in the spring, because it exhibited no insecticidal activity in the fall. All three compounds were effective against Hessian fly in the fall (Table 5), especially at the higher rates tested. Fipronil and imidacloprid were more effective than thiamethoxam. However, none of the treatments showed insecticidal activity against Hessian fly in the spring evaluation at Manhattan. No differences in yield were detected between the untreated fungicide check and fipronil or imidacloprid at Hesston. A small but significant difference in yield was detected between the untreated fungicide checks and insecticides (fipronil, imidacloprid or thiamethoxam) imidacloprid at 31.2 and 62.4 g AI/100 kg of seed or all rates of thiamethoxam. Because each of these three groups (fipronil, imidacloprid or thiamethoxam) represented three different seed lots, the results point out the importance of seed source when conducting yield trials.

1998–1999. Thirteen treatments involving various rates of the same three insecticides evaluated in the previous growing season, as well as two fungicides, were evaluated in the fall and spring. As in 1997–98, various rates of imidacloprid and thiamethoxam gave excellent control of Russian wheat aphids (Tables 4 and 5) and greenbugs (Tables 2 and 3) in the fall, but fipronil again was not toxic to these two pests. In the spring, some damage occurred to the plants treated with thiamethoxam and imidacloprid, but plant injury was reduced from that of the fungicide treated checks, especially at the higher rates. As in 1997–98, all insecticide treatments were effective in reducing Hessian fly numbers in the fall evaluation at Manhattan (Table 6). No differences in yield for various treatments occurred at either Hesston or Manhattan.

Discussion

Results of these tests over a 4-year period indicated that seed treatments with imidacloprid and thiamethoxam can provide outstanding early season (fall) control of greenbugs and Russian wheat aphids at all rates tested. Control of spring aphid infestations with these two compounds was less effective and inconsistent, but the highest rates tested reduced damage. Fipronil did not control either aphid species. All three compounds tested provided good early season (fall) control of Hessian fly but none showed insecticidal activity against spring infestation in one test. Imidacloprid had no effect on fall armyworms in one test. These results agree with those of Archer (1994), Pike et al. (1993), and Kroening et al. (1998) who showed various degrees of initial and residual control of Russian wheat aphids in their tests. Tharp et al. (2000) found that imidacloprid controlled the cereal leaf beetle in barley at the four-leaf stage, but was ineffective at the early tillering or

Table 7. Effect of imidacloprid (Gaucho®) seed treatment on fall armyworms (FAW) in winter wheat at Manhattan, Kansas, Fall, 1996.

Treatment	Rate g AI/100 kg seed	FAW[a] Damage rating[bc]
Vitavax	88.0	4.0a
Gaucho 480FS	32.5	4.3a
Gaucho 480FS	48.7	4.3a
Gaucho 480FS	65.2	4.0a
F		0.23
df		3,3
P		0.87

[a]FAW—Fall armyworm.
[b]Damage rating scale 0–5 where 0 = no damage and 5 = plant death.
[c]Based on potted plants brought back to greenhouse on 11/9/96, evaluated on 11/26/96.

flag-leaf stage. Further tests are needed to determine how different amounts of rainfall or irrigation effect initial and residual control with these and other compounds. This is especially true since the tests were conducted under artificial infestation in the greenhouse and the results may vary with field-related environmental conditions and natural insect infestations. Yields from treated seed were similar to those from fungicide treated or untreated seed in all 4 years under the low or nonexistent natural infestation of these experiments. No phytotoxicity or negative effects on yield were observed with the seed treatments. Similar results were obtained by Kroenig et al. (1998) with seed treatments for wheat and Wilde et al. (1999) for sorghum. The current cost to treat wheat seed based on common seeding rates and recent price quotations from dealers is $11.70 per hectare. Therefore, the rise of infestation by any of these insects will be an important consideration in the decision to use seed treatment as a tool for control of aphids and other pests in winter wheat. The use of seed treatments is economically risky where insect populations are variable. Foliar treatments based on established action thresholds would be stronger alternatives from both economic and IPM perspectives. However, seed treatments could be most useful in several situations: if a significant fall infestation is likely, if aphid or Hessian fly densities are locally significant on winter grain and spring wheat or barley is about to be planted, or if an insect pest is a chronic problem in a given area (Legg et al. 1993).

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Monitoring the Effects of Granular Insecticides for Argentine Ant Control in Nursery Settings[1]

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ABSTRACT The effects of broadcast granular insecticide formulations on Argentine ant, *Linepithema humile* (Mayr), foraging rates were measured in two types of nursery settings. In an artificial nursery setting, colonies of Argentine ants were collected and introduced into the soil of potted oleander plants (*Nerium oleander* L.). Plants were relocated into 12 field plots that were subsequently treated with granular formulations of fipronil or bifenthrin, or left untreated. Two or three days after treatment of artificial nurseries, the number of foraging ants was reduced 80% or more in all treated plots compared with controls. Ant foraging rates in fipronil treated plots were reduced for about 3 wk after treatments and began to recover by week 4. Bifenthrin treatments almost eliminated foraging ants for the duration of the trial. The number of ants in control plots increased in the first three days after treatment, suggesting ants may have initially moved from treated plots to untreated areas. In commercial nursery settings, similar sized plots contained plants naturally infested with *L. humile* of varying size colonies. Plots were treated with a granular formulation of fipronil, or left untreated. Foraging rates were measured in each plot. Trials in commercial nurseries showed a similar response in to fipronil treatments compared to the artificial nursery. Fipronil granules were effective in significantly reducing ant foraging rates, but did not completely eliminate them from treated plots. In both types of studies, it was difficult to space plots far enough apart so that treatments did not interfere with each other. Fluctuations in control foraging rates indicated that ants were probably moving from plot to plot during the course of the study.

KEY WORDS bifenthrin, Formicidae, fipronil, Hymenoptera, insecticide, nursery, *Linepithema humile*

California is the leading producer of nursery and greenhouse products in the Untied States producing 20% of total U. S. nursery crops (USDA 1999). In 1999, nursery products ranked 3rd in the state among agricultural commodities with a value of $2 billion (CDFA 2000). The recent introduction of the red imported fire ant, *Solenopsis invicta* Buren, into southern California has increased the need for testing materials for ant control in production nurseries (Newell & Barber 1913, Lewis et al. 1992). Infestations of container plants with any ant species can result in delay or rejection of inter-regional shipments due to quarantines presently in

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place for fire ants (*Solenopsis* spp.) and other pests. Container stock in quarantine areas must be treated before shipping. The potential loss of insecticides such as chlorpyrifos and diazinon presently used for ant control in nurseries, and the enforcement of regulations associated with water quality protection, necessitate testing new materials for ant control in nursery situations.

Outside of areas quarantined for the red imported fire ant, the Argentine ant, *Linepithema humile* (Mayr), is the major ant pest reported in nursery crops in southern California. Argentine ants are difficult to control for several reasons. Little is known about their foraging habits. They are polydomous, meaning that individual colonies of ants over a large area are interrelated, and individuals can move easily from one nest site to another if a colony is disturbed (Markin 1968). In addition, *L. humile* is polygynous and colonies reproduce by budding or sociotomy. This makes it difficult to experimentally evaluate the effectiveness of materials used to control them.

Liquid sprays for ant control are commonly used in urban and nursery settings because of their ease of application. Barrier applications around homes effectively excluded ants for up to 30 d (Rust et al. 1996) and in some instances 60 d (Suoja et al. 2000). However, in nursery environments, frequent (sometimes daily) overhead irrigation can dilute or remove insecticides from ground surfaces and exposure to sunlight can result in relatively short residual efficacy (Rust et al. 1996). Similarly, insecticides mixed in bait formulations such as MaxForce (hydramethylnon), become moldy and unattractive to Argentine ants if broadcast in areas with overhead irrigation (Krushelnycky & Reimer 1998). For this reason, we are testing granular formulations of two products found to be effective for red imported fire ant control (Greenberg et al. unpublished data).

A variety of complications arise when conducting field experiments with ants in nursery environments. Observations of reduced foraging rates of ants in treated sites may not give a true indication that ants have been controlled. Ant foragers may avoid the treated area and shift their foraging range into new, or other existing nest sites (Costa & Rust 1999). It is also difficult to find a situation where sufficient numbers of ant colonies are present in similar environments (container size, crop species and spacing), and colonies are rarely found evenly distributed throughout potential test plots. In addition to inherent difficulties associated with ant behavior and biology, problems conducting field trials in commercial nurseries can result from a variety of business and cultural management practices. Frequent movement of blocks of plants from one location to another, and the sale of plants during the course of studies creates problems by artificially moving ants. Day to day operating procedures such as movement of equipment, employee and vehicular traffic, and overhead irrigation can destroy or interfere with monitoring stations designed to measure foraging rates. Overhead irrigation practices and the use of weed mats or gravel can affect the distribution and efficacy of materials, particularly granular materials, on the variable surfaces. Treatments for other insect pests in the nursery during the study can also directly affect ant foraging rates.

In this study, we measured the effects of broadcast granular insecticide formulations on Argentine ant foraging rates, and tested methods of monitoring ant foraging rates in two types of nursery settings: commercial nurseries, and a smaller artificial nursery setting constructed on a university research farm.

Material and Methods

Artificial Nursery Treatment Area. A total of 12 plots, each 15.2 × 15.2 m (232 m^2) were measured on a flat, well drained area on the research farm at University of California, Riverside to create a nursery-like environment (Fig. 1). A 6 × 6 m section of black woven plastic weed barrier (Lumite Weed Mat, McCalif Grower Supply, Vista, California) was secured with metal staples in the center of each plot (Fig. 1). Plots had at least 9 m of buffer space on all sides. Each plot was irrigated with two overhead sprinklers that applied a total of approximately 7 cm of water per week.

Introduction of Ants in Artificial Nursery. Colonies of Argentine ants were collected during June and July from the field in Riverside, California and maintained in plastic containers (25 × 30 × 10 cm) with the inner walls coated with a DuPont Teflon® to prevent the ants from escaping. Each colony was provided with an artificial nest, food and water (Costa & Rust 1999). Thirty-six colonies, each containing approximately 3,000 worker ants and several queens, were assembled. After the ants had acclimated for at least one week, one oleander (*Nerium oleander* L.) plant (ca. 2-years-old) in a 11-L plastic pot was introduced into each ant colony. Ants immediately moved their nest including brood and queens into the potted plant.

Once the ants had established their colonies in the pots for at least two weeks, the pots were removed from the laboratory and placed into the experimental plots (Fig. 1). Three plants, each infested with an ant colony, were placed into the center of each plot by the end of July. Three additional plants without ant colonies were interspersed with infested plants for a total of 6 plants per plot. All plants were placed on top of the black weed block located in the center of the plot. Plants were maintained with overhead irrigation which covered the entire area to be treated.

Monitoring Ant Foraging Rates in Artificial Nursery. To monitor foraging workers of *L. humile*, monitoring stations were set up next to the infested pots in each plot (three per plot, total of 36 stations). An additional 24 stations were set up in a perimeter around experimental plots to monitor ant activity outside the treated plots (Fig. 1). Each monitoring station consisted of a 50-mL screw cap centrifuge tube filled about half way with a 50% sucrose solution. A 50% sucrose solution was used to reduce the amount of water evaporation because the monitoring tubes were left uncapped. Each tube was placed uncapped on its side and supported by a plaster mold designed to hold the tube at an angle to allow ants to enter and feed. Each tube was covered with a 15-cm diam. clay pot weighted with a brick to keep larger animals out of the stations. For each evaluation date, the clay pot cover was removed and the number of ants feeding in the sucrose water tube, and the number of ants on the ground in the area under the clay pot was counted. Sucrose solutions were refilled or replaced as needed. A pre-treatment count was made on the date of application. Post-treatment evaluation of colonies was done at the end of the study, one month after treatment.

Treatment Application in Artificial Nursery. Four treatments were arranged in a completely randomized design, with three replicates for each treatment. The entire 15.2 × 15.2 m area of each plot (232 sq. m) was treated with material or left as an untreated control. The treatments used in this study were: an untreated control; a granular formulation of bifenthrin (sand carrier), Tal-

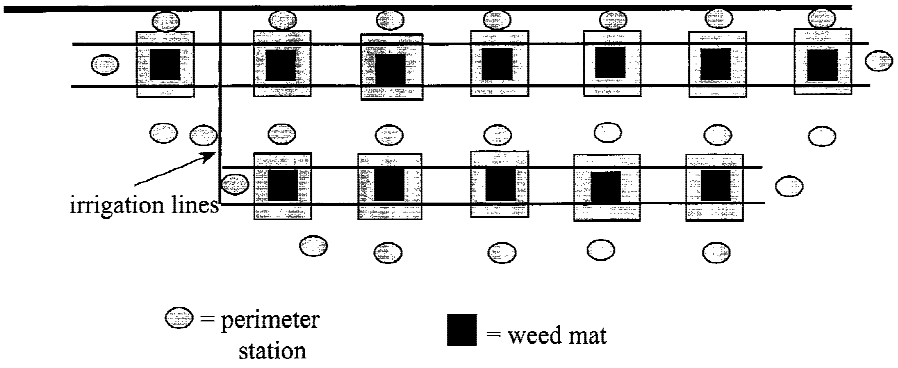

Fig. 1. Plot design for artificial nurseries. Twelve plots each 15.2 × 15.2 m were measured on a flat, well-drained area. A section of black weed mat (6 × 6 m) was placed in the center of each plot. Six, 11-L containers with oleander plants were placed in each plot, half with ant colonies and half without. Each plant with an ant colony had a sucrose water monitoring station associated with it. An additional 24 monitoring stations were placed around the perimeter of the plots.

star® 0.2 G (FMC Corp., Princeton, New Jersey), 225 g AI/ha (2.59 kg formulation per plot); and two rates of a granular formulation of fipronil, EXP61748A 0.0143 (clay carrier) (Aventis Environmental Science, Montvale, New Jersey), 14 or 28 g AI/ha (2.27 or 4.54 kg formulation per plot, respectively). None of these materials contain food attractants. The total amount of material required for each plot was weighed in advance then applied evenly by walking through the plot using a hand-held applicator (Scotts HandyGreen II, Scotts Co., Marysville, Ohio) making two to three passes over the plot until all the material was distributed. Granules fell on top of plants and into containers as the applicator walked between plants during application.

Artificial Nursery Soil Residual Bioassay. Soil samples were collected from each plot 12 wk after treatment and stored at – 4°C. Samples were collected from the soil surface (<3 cm) underneath the black weed mat or from bare soil in the treated areas of each plot. Ants were exposed for 24 h to each soil sample. Vials (60 mL) were coated with DuPont Teflon® to prevent ants from escaping, and a 2-cm-diameter section of moist filter paper was placed on the bottom of each vial. Soil (1.2 cm^3) was added to cover the filter paper, and 10 ants were placed into each vial. The vials were covered with parafilm and held at ambient temperature. After 24 h, the vial contents were emptied into cool water to count the number of live ants. The mean percent mortality of ants at 24 h was compared among treatments.

Commercial Nurseries Experimental Area. Experiments were conducted at two commercial nurseries, one in San Diego County, the other in Orange County, California. At each site, six plots, each 15.2 × 15.2 m were set up so that at least three trees per plot were associated with ant activity. Trees were planted in containers approximately 1.2 m diameter. Plots were placed at least 15.2 m

apart, but within the same section of the nursery. Trees were watered with drip irrigation.

Monitoring Ant Foraging Rates in Commercial Nurseries. Ant activity was monitored at each tree by measuring the consumption of a 25% sucrose water solution from a 15-mL disposable centrifuge tube with the open end covered by a semi-porous material "WeedBlock®" (Easy Gardener, Waco, Texas) secured with a rubber band (Klotz et al. 2000). The size of the holes in this material allowed ants to feed through it on the sucrose solution when the tube was inverted and reduced the amount of water evaporation. Consequently, 25% sucrose solutions were used. Inverted tubes were taped to trees and the initial volume of liquid was recorded. The amount of sucrose solution consumed after 24 h was recorded and converted to mL/hr.

Treatment Application in Commercial Nurseries. Treatments were arranged in a complete randomized design, with three replicates per treatment. Plots were treated with a granular formulation of fipronil with a clay carrier (Fipronil 0.05, Aventis Environmental Science, Montvale, New Jersey) at the rate of 28 g AI/ha (1.3 kg/plot) or left as untreated controls. This material does not contain any food attractants. Materials were applied evenly by walking through plots using a hand-held applicator (Scotts HandyGreen II) making two to three passes over the plot until all the material was distributed. Granules fell on top of plants and into containers as the applicator walked between plants during application.

Statistical Analysis for Artificial and Commercial Nurseries. Data were analyzed using a repeated measures analysis-of-variance (Systat 1999). This technique is sometimes called "profile analysis" and gives an interaction term that compares the slopes of the control and treatment curves. Preliminary data analysis showed that the data were positively skewed; a logarithmic transformation on the original data improved their fit to a normal curve, making the stated probabilities valid. In the artificial nursery data we compared the pretreatment data with that of each subsequent date and determined whether the slopes for each treatment differed from the control. Within each date we used the Bonferroni correction to correct for multiple slope comparisons and to maintain the stated probability level. For the commercial nursery data the trends were more consistent and we did one overall repeated measures analysis-of-variance for the entire data set. Soil bioassay data were analyzed using a general linear model followed by Fisher least significant difference test (Minitab 1996). Data were arcsine square-root transformed before analysis.

Results

Artificial Nursery. Initial ant counts per plot ranged between 30–450 ants feeding at sucrose solution stations, and between 90–400 ants on the ground under monitoring station covers. Ants appeared to prefer some sections of the experimental area relative to others. To compensate for differences in starting foraging rates in each plot, the changes in the number of ants feeding at monitoring stations (Fig. 2A), and the number of ants present on the ground under station covers (Fig. 2B) are reported as the percent change relative to the pretreatment count. Overall, the fluctuations in the number of ants feeding in su-

crose stations and the number of ants foraging on the ground under the monitoring stations were similar.

All treatments resulted in significant reductions in number of ants foraging compared to controls. Two days after treatment, ant counts at sucrose water stations in all treated plots had declined significantly with respect to the control (bifenthrin: $F = 31.6$, df = 1,2, $P < 0.001$; low fipronil: $F = 19.1$, df = 1,2, $P = 0.003$; high fipronil: $F = 7.96$, df = 1,2, $P = 0.045$; P values reported with Bonferroni correction). On subsequent days only the bifenthrin provided significant control of ants (Fig. 2A). Similar results were observed using counts of ants on the ground under monitoring stations (Fig. 2B). Three days after treating the plots, ant counts in all treated plots had declined significantly with respect to the control (bifenthrin: $F = 107.6$, df = 1,4, $P < 0.001$; low fipronil: $F = 31.1$, df = 1,4, $P < 0.001$; high fipronil: $F = 14.3$, df = 1,4, $P < 0.006$). Again, only the bifenthrin provided significant reduction of ants on the ground on subsequent days. Bifenthrin treatments quickly reduced the numbers of foraging ants almost completely for the duration of the trial (8 wk). The number of ants in control plots initially increased in the first days after treatment (more ants foraging in plots after treatment than in pre-treatment counts). Four days after application, ant foraging rates in control plots showed more than a 50% reduction in foraging, however, 7 d after treatment, the number of ants foraging in control plots returned to pretreatment levels.

At the end of the study all potted plants were evaluated for the presence of ants in the soil, and underneath the pot. Of the nine pots originally infested with ants for each treatment (three per plot), only four still had ants living in the soil, three in control plots and one in a high rate fipronil plot. When ants were present, most were found on top of the weed mat, underneath pots that were originally infested rather than in the container soil. This is particularly noticeable in the plots treated with the high rates of fipronil when in 8/9 cases, the ants had moved from the soil to the area underneath the container. We found, however, that six of the nine plants originally infested with ants in control plots also had ants underneath the potted plant.

Artificial Nursery Soil Residual Bioassay. Bioassays of soil treated with bifenthrin collected 12 wks after treatment showed significant residual activity, providing 17 ± 7% kill of workers compared with 1 ± 1% in controls ($F = 3.29$; df = 3,40; $P = 0.03$). The high and low rates of fipronil did not provide significant mortality. Samples of soil treated with bifenthrin collected from underneath the plastic weed mat provided greater kill (20 ± 13%) than did samples collected from uncovered soil (5 ± 5%). A similar trend was observed with the high rate of fipronil (12 ± 5% under mat, 5 ± 5% on uncovered soil).

Commercial Nurseries. Estimates of the numbers of foraging ants in commercial nursery settings showed a similar response to fipronil treatment as was found in the artificial nursery. At both commercial sites, fipronil granules were effective in significantly reducing ant foraging rates, but did not completely eliminate them from treated plots (Fig. 3 A and B). The repeated measures analysis-of-variance interaction term between the controls and treatments was highly significant at both sites over the entire test period (San Diego County: $F = 3.1$, df = 4,36, $P = 0.027$; Orange County: $F = 8.4$, df = 3,30, $P < 0.001$). Previous studies (Hooper 1998, Reierson et al. 1998) have demonstrated that each worker ant consumes approximately 0.3 μl of liquid per visit. The amount of sucrose

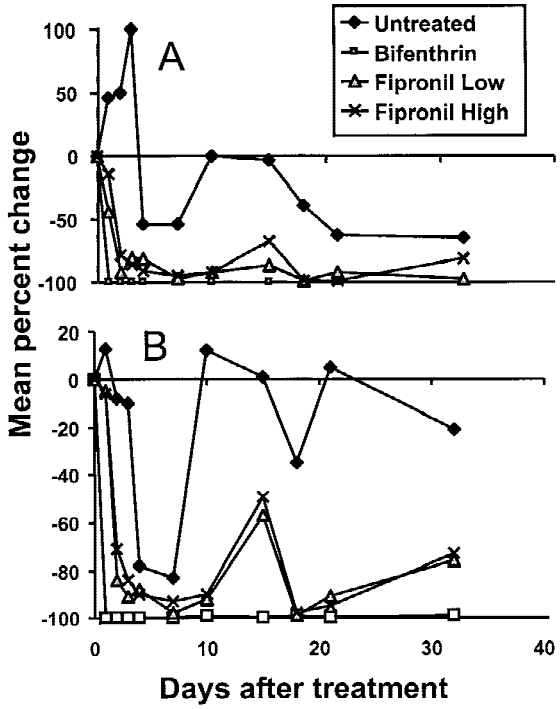

Fig. 2. Ant foraging rates in the artificial nursery. Mean percent change in number of Argentine ants foraging at various times after treatment relative to the number of ants foraging in the same plot in pretreatment counts. **A.** Ants feeding in sucrose water tubes. **B.** Ants present on the ground under monitoring station covers.

water consumed at stations during pretreatment counts indicates that about 1,300 ant visits occurred per hour at each station before treatment.

Discussion

The experiments conducted in the artificial nursery were successful in evaluating granular insecticides for ant control. The experimental design allowed for more consistent conditions among replicated plots, and more control over the nurseries environmental and cultural practices than in commercial settings. Although plots were spaced at least 9 m apart, fluctuations in control foraging rates indicated that ants may have been affected by surrounding treatments. Initial increases in numbers of ants in control plots suggests that ants moved into untreated areas to avoid treated plots. Subsequent reductions in the numbers of ants foraging at 4 d after treatment could be the result of ants that eventually foraged from untreated into treated areas. In replicated studies of Argentine ants in the field, it is difficult to space plots far enough apart so they do not interfere with each other, while trying to maintain similar cultural and environmental

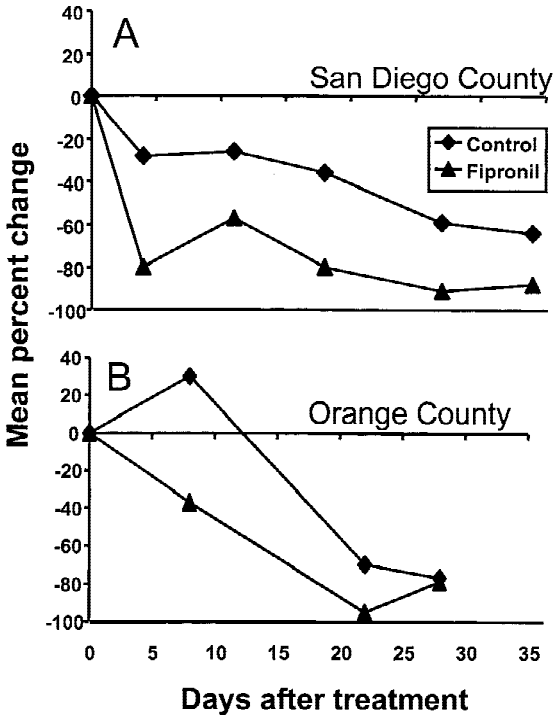

Fig. 3. Ant foraging rates in commercial nurseries. Mean percent change in the amount of sucrose water consumed by Argentine ants at various times after treatment relative to the amount consumed in pretreatment measurements. **A.** Nursery field site San Diego County, California, **B**. Nursery field site Orange County, California.

conditions. Argentine ants are capable of foraging more than 61 m around a nest site (Ripa et al. 1999, Vega & Rust 2001). The use of repellent sprays between plots to keep ants from moving from one plot to another may allow better evaluation of treatments for research purposes, however, such limitations on the natural foraging behavior of ants must be considered when evaluating the efficacy of materials.

Materials were initially very evenly distributed in treated plots, however, the use of overhead irrigation resulted in uneven distribution of granules on the ground after one or two irrigation cycles. Granules were washed off certain areas of the weed mat and accumulated in others, mostly in depressions or seams. Clumping of material was more noticeable on the weed mat surface than on the bare soil. The type of irrigation being used and type of substrate under containers, such as gravel or cement, should be considered as factors when evaluating the efficacy of insecticides or selecting a formulation for use in a nursery environment.

Materials were applied by broadcasting over the tops of plants. Thus, the soil surface in the pot was also sprinkled with the material. The only surface areas in

the treated plots that were not initially sprinkled with material were the areas under the potted plants and the areas under the monitoring stations. One explanation of why ants accumulated underneath pots is that they were avoiding contact with the granular material. However, because we found that six of the nine plants originally infested with ants in control plots also had ants underneath the potted plant, there are probably additional reasons why ants tended to accumulate there. Environmental conditions, such as temperature fluctuations in the pot, may also have contributed to ants leaving the soil and moving under the pots. It was evident that in some cases ants had built colonies directly in the ground within and around treated plots. Counts at perimeter stations around the experimental area indicated a resurgence of foraging rates just outside the experimental area near the untreated plots that had the highest numbers of ants at the start of the experiment (data not shown).

Bifenthrin treated plots had little or no foraging activity during the course of our experiments. Results of the soil bioassay suggest that some residual activity remained after 12 wk in plots treated with bifenthrin. This may explain why Argentine ants had not moved back into bifenthrin treated plots at the end of the experiment in the artificial nursery. Broadcast applications of a granular formulation of fipronil were effective in reducing overall foraging rates of Argentine ants in treated areas. The foraging rates in fipronil treated plots began to recover during the test period.

In field trials, the effects of repellency and contact mortality cannot be entirely separated. We can only report the relative change in number of ants foraging in treated plots. It is not possible to determine if the reduction is a result of ants avoiding treated plots and feeding in other areas, or a result of ant mortality. The initial increase followed by a decrease in foraging activity in untreated plots indicates that both are occurring.

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Occurrence of Greenbug Biotype K in the Field[1]

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ABSTRACT The success of managing greenbugs, *Schizaphis graminum* (Rondani), with plant resistance has been challenged by the repeated occurrence of resistance-breaking biotypes. Biotypes recognized as seriously damaging to sorghum, *Sorghum bicolor* (L.) Moench, hybrids are C, E, I, and K. In 1995, biotype K, which damages sorghum resistant to biotype I, was first identified in a biotype I greenbug colony being reared in the greenhouse. This colony originated from greenbugs collected in a field of wheat in Haskell County, Kansas, in April 1992, and was maintained on susceptible sorghum. Documenting the presence of biotype K in the field was imperative. Field samples of greenbugs collected from wheat and sorghum in Kansas and Oklahoma from 1996 to 1998 were identified to biotypes. These samples indicated that biotype I was the dominant biotype on both crops. Biotypes E and K were present in about 21 and 12% of the samples collected from sorghum in 1998, respectively. Biotype K was identified in samples collected from 11 counties in Kansas and 10 counties in Oklahoma during the sampling period. It is a potential threat to future sorghum hybrids that may rely on PI 550610 for protection against greenbug damage. Studies conducted at constant temperatures of 22, 27, and 32°C indicated that biotypes C, E, I, and K had similiar reproductive capacity and survival at 22 and 27°C.

KEY WORDS Host plant resistance, intrinsic rate of increase, *Schizaphis graminum*, *Sorghum bicolor*, temperature

Since 1975, resistant hybrids of sorghum, *Sorghum bicolor* (L.) Moench, have been used to reduce damage by greenbugs, *Schizaphis graminum* (Rondani). However, the success of managing greenbugs with plant resistance has been challenged by the occurrence of resistance-breaking biotypes. Greenbug biotypes were described by Puterka & Peters (1990), Harvey et al. (1991), and Beregovoy & Peters (1994). Known biotypes occurring in the field and recognized as seriously damaging to sorghum are C, E, and I. Biotype C was the first one considered a major pest of sorghum (Harvey & Hackerott 1969). Biotype E (Porter et al. 1982)

This article reports the results of research only. Mention of trade names of commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U. S. Department of Agriculture.

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replaced biotype C in the 1980s, when it was found to be virulent to sorghum hybrids resistant to biotype C. Biotype I (Harvey et al. 1991), which is virulent to biotype E-resistant sorghum hybrids, is gradually replacing biotype E in the field (Bowling et al. 1994, Peters et al. 1997).

Greenbugs collected from a field of wheat in Haskell County, Kansas, in April 1992 and identified as biotype I were used to start a colony that was maintained on susceptible sorghums in a greenhouse. In 1993, an isolate from the Haskell County collection was sent to NC+ Hybrids in Hastings, Nebraska, where it was used to evaluate sorghum germplasm produced from the biotype I-resistant parent PI 550610 (Andrews et al. 1993). During the spring of 1995, personnel at NC+ observed that lines previously resistant to the isolate were susceptible. Isolates from the NC+ Hybrids collection were designated as biotype K, and sorghums resistant to biotype I (Kofoid et al. 1991, Andrews et al. 1993, Harvey et al. 1994) and being utilized in public and private breeding programs were reported to be susceptible to the new biotype (Harvey et al. 1997). For this reason, documenting the distribution of known biotypes in the field each year is important. Various individuals over the years have made efforts to keep track of the biotypes, and several publications exist that document those efforts (Puterka et al. 1982, Kindler et al. 1984, Bush et al. 1987, Kerns et al. 1987, Bowling et al. 1994, Peters et al. 1997).

Once biotype K was discovered, documenting its presence in the field was imperative. From 1996 to 1998, we collected greenbugs from wheat and sorghum in Kansas and Oklahoma and identified the biotypes to determine their relative abundance and distribution. Also, we determined the survival and reproductive capacity of greenbug biotypes, C, E, I, and K at constant temperature to see if high temperatures would favor one biotype over another.

Materials and Methods

Greenbugs were collected during 1996, 1997, and 1998 from fields of wheat, *Triticum aestivum* L., and sorghum in Kansas and Oklahoma. The field collections were brought to a laboratory and placed on barley, *Hordeum vulgare* L., grown in poly-cast tubes (16 by 3.8 cm, Cone-Tainer Company, Cambry, Oregon) containing a greenhouse soil mixture. The collections were maintained on the barley until numbers were sufficient to test. To identify the presence of biotype K, we compared biotype I-resistant PI 550610 with Deltapine 'G550E' (resistant to biotype E but susceptible to biotype I) in a free choice test. The free-choice test involved four replicates of two plants of each genotype at the two-leaf stage grown in poly-cast tubes (16 by 3.8 cm), which were infested with 10 apterous aphids of a single field collection either from wheat or sorghum, and then covered with clear plastic cylinder cages. Individual plants were scored for greenbug damage at 7–14 d after infestation. In addition, we compared the aphid isolate from the same field collection on Deltapine 'G550E' (resistant to biotype E but susceptible to biotype I) and Deltapine 'G550' (susceptible to both biotypes E and I) in order to verify the identifications of both biotypes. The methods for identifying the field collections to biotype were identical to the ones described by Harvey et al. (1997), including the 1–9 damage rating system.

Temperature. We conducted a life history analysis to measure effects of constant high temperatures on different stages of the aphid life cycle on susceptible grain sorghum plants. Cultures of biotypes C, E, I, and K were maintained

in the greenhouse on greenbug-susceptible sorghum and barley grown in plastic pots, 15.2 cm diam., covered with cylindrical cellulose nitrate cages.

The test plants, 'SG-858' greenbug-susceptible grain sorghum, were seeded in 7.6-cm (= 3½-inch) diam. pots and caged to prevent natural infestation. Soon after seedling emergence, the plants were thinned to one/pot. Each entry was replicated five times. When the seedlings were in the two-leaf stage, three apterous adult aphids of each biotype were caged on five separate plants, which were placed in a growth chamber preset at constant temperatures of 22, 27, and 32°C. When progeny occurred, the adults were removed, and five nymphs less than 24 h old were left on each plant. When the nymphs matured, all but one specimen were removed. Thereafter, the progeny of the test specimen were removed every 48 h, and the numbers were recorded until reproduction ceased and adult aphid died.

Life history parameters calculated directly from the data included (1) time to start reproduction, (2) total reproduction, (3) life span of each adult aphid, and (4) intrinsic rate of increase (r_m) or the female progeny per female per day. The latter was estimated separately for each plant entry, using the method of Wyatt & White (1977): $r_m = 0.74 (\log_e M_d / d)$, where d is the preproductive time, M_d is the number of progeny produced in a time equal to the preproductive time, and 0.74 is the constant.

The data were analyzed using PROC MEANS (SAS Institute 1990). Mean comparisons were made using the Ryan-Einot-Gabriel-Welsh multiple range test (SAS Institute 1990).

Results and Discussion

From 1996 to 1998, biotype I was the predominant greenbug biotype collected from wheat and sorghum in Kansas and Oklahoma (Figs. 1 and 2; Table 1). Biotype K was identified in samples collected from 11 counties in Kansas and 10 counties in Oklahoma during the 1996–1998 sampling period (Figs. 3 and 4). Biotype K occurred in the field at about the same frequency as biotype E, but both biotypes were found less frequently than biotype I.

In Oklahoma, biotype K was identified in 12 of 51 collections taken from wheat in 1996. In 1997, biotype K was identified in 2 of 14 collections from sorghum, but no biotype K was identified in the 26 collections from wheat. In 1998, biotype K was not identified in the 11 collections taken from wheat or the 13 collections taken from sorghum.

In Kansas, biotype K was not identified positively in the 48 collections from sorghum in 1996 nor in the 7 collections from wheat in 1996. In 1997, biotype K was present in 4 of the 22 collections from wheat and sorghum. In 1998, biotype K was identified in 7 of 47 collections from sorghum. It was not identified in the two collections from wheat.

A summary of the data for greenbug biotypes collected on sorghum from 1968 to 1998 showed that biotype C was the dominant biotype in the field from 1968 to 1980 (13 years). Biotype E replaced C as the dominant biotype in 1981 and continued to be dominant until 1994, a total of 14 years. Biotype I replaced biotype E in 1995 and continued to be the dominant biotype in the field through 1998.

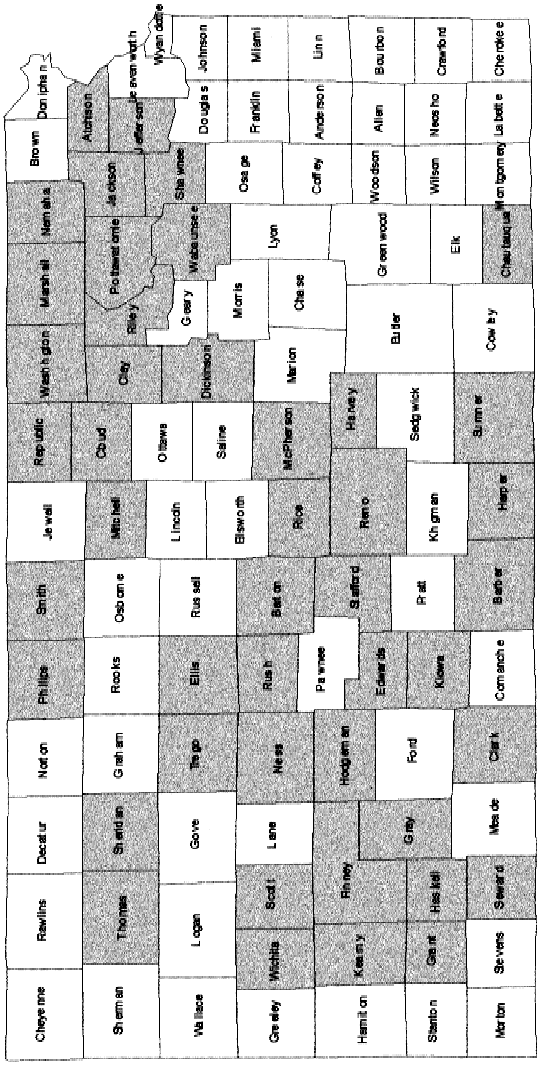

Fig. 1. Distribution by county of greenbug biotype I in Kansas for 1996–1998.

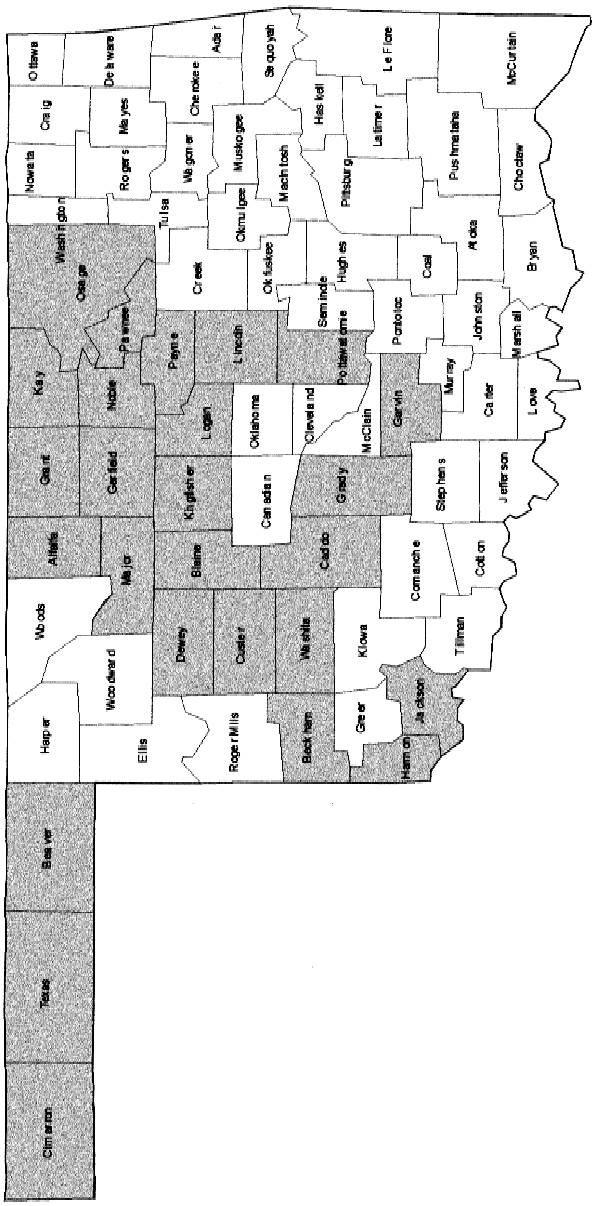

Fig. 2. Distribution by county of greenbug biotype I in Oklahoma for 1996–1998.

TABLE 1. Collections of Greenbug biotypes E, I, and K, 1991–1998.

	Number of collections[a]				% of collections[d]		
Year	Total	E	I[b]	K[c]	E	I	K
1991	130	97	33	—	75	25	
1992	63	34	29	—	54	46	
1993	74	54	20	—	73	37	
1994	61	31	30	—	51	49	
1995	145	15	130	—	10	90	
1996	76	8	68	13	9	89	15
1997	62	2	60	6	3	97	9
1998	73	13	70	7	18	96	10

[a]Data from 1991–1995 were from greenbug collections made from wheat and sorghum, primarily from Kansas, Oklahoma, & Texas; data from 1996–1998 were from collections in wheat and sorghum fields in Kansas and Oklahoma.
[b]Greenbug biotype I discovered in 1990.
[c]Greenbig biotype K discovered in 1995.
[d]Some individual collections for the years 1996, 1997, and 1998 had mixtures of biotypes E, I, and K; therefore, the percentages do not total 100%.

The percentage of sorghum hybrids resistant to greenbug biotypes is presented in Table 2. The first resistant hybrids became available to sorghum growers in 1975, 7 years after biotype C greenbug was found damaging sorghum. In 1980, 83% of the sorghum hybrids available to growers were resistant to biotype C greenbug. In 1982, the year after biotype E became dominant, the first sorghum hybrids resistant to this biotype were available on a limited basis. During the years 1993 to 1998, 65 to 76% of the available sorghum hybrids were resistant to biotype E and these hybrids also had resistance to biotype C. Soon after biotype I became dominant in 1995, about 6% of the sorghum hybrids available were resistant to that biotype, and by 1998 this had increased to about 10%.

By 1980, an estimated 90% of the grain sorghum acreage planted in the Southern Plains was resistant to biotype C (Starks et al. 1983, Dixon et al. 1991). Estimates of seed sales in Oklahoma in 1986 indicate that approximately 91% of the total acreage of sorghum fields was planted to biotype C-resistant hybrids that year (Kerns et al. 1987). They estimated that 53% of seed was resistant to biotype C, and 38% of the seed had combined resistant to both biotypes C and E. Because antibiosis (Painter 1951) is one of the resistance mechanisms characterized in greenbug-resistant sorghum (Kindler & Spomer 1986), the total acreage of resistant hybrids grown probably has the most effect on biotype frequency.

By 1998, 10% of the sorghum entries in Kansas State University's performance tests evaluated for resistance to greenbug biotypes were resistant to biotype I, whereas 76% were resistant to biotype E (Table 2). The difference in availability to sorghum growers of sorghum hybrids resistant to biotypes E and I should account for the higher frequency of biotype I in the field because there should be less suppression pressure provided by resistant sorghum to biotype I than to biotype E.

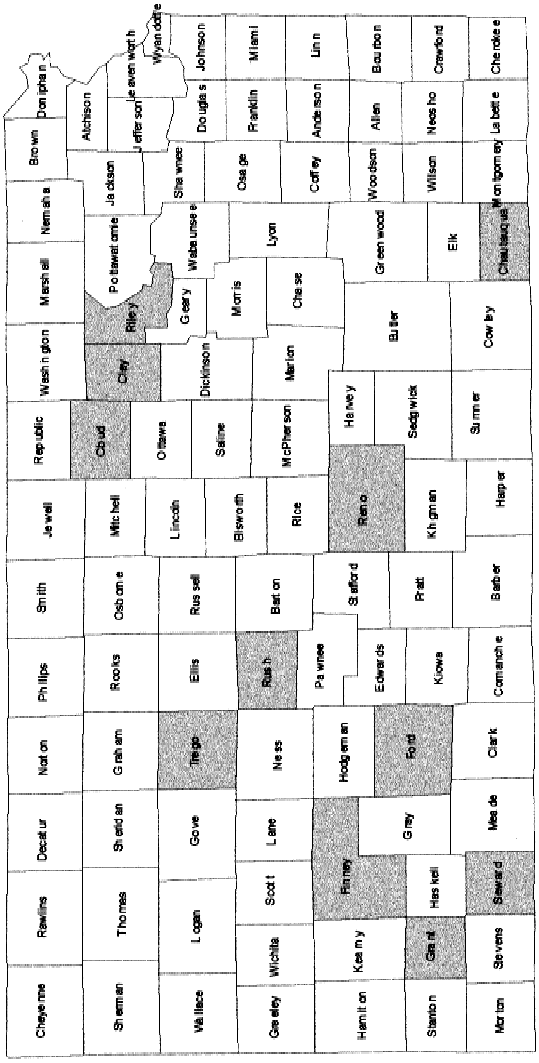

Fig. 3. Distribution by county of greenbug biotype K in Kansas for 1996–1998.

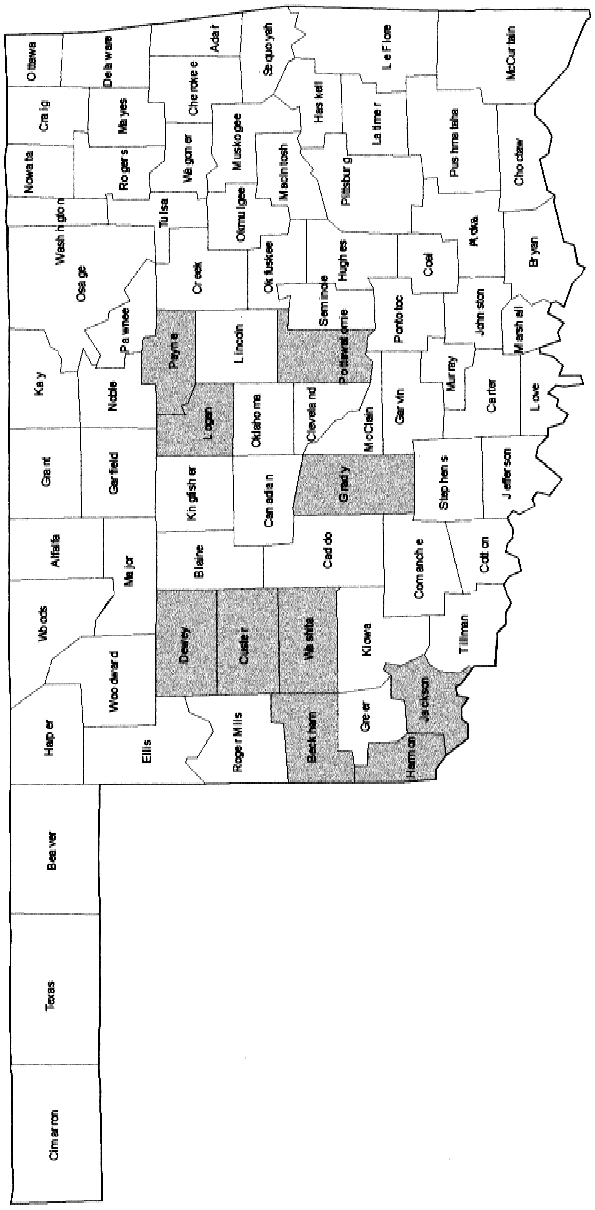

Fig. 4. Distribution by county of greenbug biotype K in Oklahoma for 1996–1998.

TABLE 2. Percent of sorghum hybrids resistant to greenbugs.[a]

Biotype C[b]		Biotype E[b]		Biotype I[b]	
Year	%	Year	%	Year	%
1975	2	1982	3	1991	1
1977	61	1984	4	1992	2
1978	73	1986	8	1993	2
1979	82	1988	16	1994	3
1980	83	1990	33	1996	6
		1992	61	1997	7
		1993–98	65–76	1998	10

[a]Based on hybrids entered in Kansas State University annual sorghum performance tests and data reported by entrants. Resistance to greenbugs was determined by procedures outlined by Starks and Burton (1977).
[b]Year first reported: C = 1968, E = 1980, I = 1990.

It is apparent that the sorghum industry is not releasing sorghum hybrids at the same rate for biotype I as for biotypes C and E (Table 2). From 1975 to 1980, the percentage of sorghum hybrids resistant to biotype C increased from 2 to 83%, and from 1982 to 1998, the percentage of sorghum hybrids resistant to biotype E increased from 3 to 76%. However, the percentage of hybrids resistant to biotype I increased from 1% in 1991 to only 10% by 1998.

Temperature. The parameters calculated from the constant temperature/greenbug interaction test are presented in Table 3. Based on the intrinsic rate of increase (r_m), the most favorable temperature was 27°C, the next was 22°C, and the least desirable was 32°C (all biotypes ceased to reproduce and perished rapidly). Biotype I at both 22 and 27°C had a lower intrinsic rate of increase (r_m) and total fecundity but the means were not significantly different from the means of biotypes C, E, and K. At 22°C, adult longevity of biotype I was significantly lower than the adult longevities of biotypes C, E, and K. However, the 22°C temperature did not significantly affect the number of days to reproductive maturity and number of nymphs produced per adult per day for the four greenbug biotypes. Before the temperature study, the absence of biotype K in the collections made in Oklahoma during 1998 was thought to be due to greater sensitivity to extreme climatic conditions compared with biotype I. Record high temperatures and extreme drought occurred throughout most of the state in that year.

Two general theories have been proposed for the development of greenbug biotypes (Porter et al. 1997). The prevailing theory proposed that the development of resistant cereals drives the development of virulent biotypes. The second proposed that biotype development is driven by the selective pressure from noncultivated host plants such as perennial grasses.

What then drives the development of new resistance-breaking biotypes on sorghum, such as biotype K? The development and release of greenbug-resistant wheat do not appear to be responsible. In every case, no resistant cultivars were in use against the prevailing biotype prior to the report of a new biotype (Porter et al. 1997). Although sorghum hybrids resistant to the prevailing biotypes were

TABLE 3. Life history parameters of greenbug biotypes on grain sorghum at 3 temperatures (Means ± SEM[a]).

Temperature Greenbug biotype		No. days to reproductive maturity	No. nymphs/ reproductive day	Adult longevity (days)	Total adult fecundity	Intrinsic rate of increase r_m
22°C	*N*					
Biotype C	5	7.2 ± 0.2a	3.6 ± 0.1a	39.0 ± 0.4a	74.2 ± 1.6a	0.308 ± 0.010a
Biotype E	5	7.2 ± 0.2a	3.0 ± 0.1a	3.78 ± 0.8a	62.6 ± 1.6ab	0.304 ± 0.007a
Biotype I	5	7.0 ± 0.0a	2.3 ± 0.6a	30.4 ± 1.8a	41.6 ± 11.3b	0.267 ± 0.050a
Biotype K	5	7.2 ± 0.2a	3.0 ± 0.2a	37.4 ± 1.1a	62.2 ± 5.4ab	0.306 ± 0.009a
F (3,12)		0.38	2.22	9.63	3.67	0.52
27°C	*N*					
Biotype C	5	5.0 ± 0a	4.5 ± 0.2a	24.8 ± 0.5a	69.6 ± 2.4a	0.422 ± 0.024a
Biotype E	5	5.0 ± 0a	3.7 ± 0.6a	24.8 ± 2.9a	60.2 ± 13.3a	0.408 ± 0.015a
Biotype I	5	5.0 ± 0a	2.8 ± 0.8a	22.4 ± 1.9a	41.8 ± 12.7a	0.357 ± 0.034a
Biotype K	5	5.0 ± 0a	4.1 ± 0.3a	25.6 ± 0.7a	64.0 ± 3.6a	0.410 ± 0.009a
F (3,12)		[b]	2.04	0.86	1.78	1.83
32°C	*N*					
Biotype C	4	6.5 ± 0.5a	0.3 ± 0.1a	14.5 ± 0.5a	3.5 ± 0.9a	0.113 ± 0.038a
Biotype E	5	6.4 ± 0.4a	0.4 ± 0.1a	14.2 ± 0.5a	3.8 ± 1.3a	0.122 ± 0.051a
Biotype I	5	5.6 ± 0.2a	0.3 ± 0.1a	15.0 ± 0.5a	3.6 ± 1.0a	0.077 ± 0.036a
Biotype K	5	5.6 ± 0.2a	0.5 ± 0.1a	15.4 ± 0.2a	5.2 ± 0.9a	0.206 ± 0.206a
F (3,12)		2.36	0.79	1.34	0.56	2.14

[a]Means within column followed by same letter are not significantly different; Ryan-Einot-Gabriel-Welsch multiple range test at $\alpha = 0.05$ (SAS Institute 1990).
[b]All aphids reached reproductive maturity at the same number of days. Therefore, no variance existed.

in production prior to the report of a new biotype, the level of resistance in sorghum is low in comparison to that found in wheat. This intermediate level of resistance is thought to cause little selective pressure for the development of biotypes in greenbug (Porter et al. 1997). Those authors suggested that the development of new biotypes is driven by the greenbugs' long-standing interaction with native grasses.

Biotype occurrence in the field in Kansas and Oklahoma during 1998 indicates that planting biotype C-resistant sorghums would have no benefit in managing greenbug populations in either state. Planting sorghum with biotype E resistance also would contribute very little for management of greenbugs. If growers are concerned about greenbugs, planting sorghum hybrids that have resistance to biotype I would be beneficial.

Continued evaluation of sorghum germplasm for resistance to new biotypes as they occur is important if we want to use greenbug-resistant sorghum as a tool to manage greenbugs in an integrated pest management system. Programs to collect greenbugs in the field and determine the biotypes and their frequency in the total population also should be maintained. Finally, we must continue to design diagnostic tests for identifying and determining biotypes as the occur in the field.

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Host Range Differences Between Two Strains of Wheat Curl Mites (Acari: Eriophyidae)[1]

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ABSTRACT Previous greenhouse studies to determine the host range of the wheat curl mite, A*ceria tosichella* Keifer, sometimes have produced conflicting results. One explanation for the reported differences is that collections of wheat curl mites from different locations may vary in their ability to colonize various grasses. Our objective was to test this possibility by comparing two collections of wheat curl mites, one each from Kansas and Nebraska, known to differ in virulence to resistant wheat, for their ability to colonize 28 species of cultivated and wild grasses. Twenty of the 28 grasses were nonhosts based on the criteria of no increase in numbers of wheat curl mites (less than the initial infestation of 10 per plant) on these species. Small, but statistically significant, differences in survival between the two collections of wheat curl mites occurred on three of these 20 grasses considered as nonhosts. With the exception of rye, the Kansas and Nebraska wheat curl mites increased equally on the eight grasses that were considered as hosts. The Kansas wheat curl mites increased significantly more on rye than the Nebraska wheat curl mites. This difference may be explained by the development of a Kansas wheat curl mite strain virulent to 'TAM 107' wheat, which has a rye source of resistance. These results provide little support for the theory that the major differences between previously reported results are due to the use of different collections or strains of wheat curl mite. Further studies are needed to better define the host range of the wheat curl mite.

KEY WORDS Wheat curl mite, Acaria, Eriophyidae, *Aceria tosichella,* Gramineae, wheat, *Triticum aestivum,* host range

The wheat curl mite (WCM), *Aceria tosichella* Keifer, is an important pest of wheat and corn in the western plains of the United States and Canada. It is the only vector of wheat streak mosaic virus (Slykhuis 1955) and High Plains virus (Seifers et al. 1997 1998), which are important diseases of wheat and corn. The WCM also may cause losses in wheat when large WCM populations infest the spikes (Harvey et al. 2000).

Alternate hosts for the WCM may be important in the epidemiology of the vectored viruses and to maintain WCM populations when wheat is not readily available. Previous greenhouse studies have evaluated the potential for wild and cultivated grasses to serve as alternate hosts for the WCM (Slykhuis 1955, Connin 1956, Staples & Allington 1956, Somsen & Sill 1970). These studies have

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produced conflicting results. One explanation for differences is that different collections or strains of WCM may vary in their ability to survive or increase on potential hosts. Slykhuis (1955) used WCM from Canada, Staples and Allington (1956) from Nebraska, Connin (1956) from Kansas, and Somsen and Sill (1970) also from Kansas but 14 y later than Connin. WCM collected at different times and locations varied in their responses to resistant wheat (Harvey et al. 1999) and likewise may differ in their ability to survive on other grasses. Our objective was to determine from greenhouse tests whether two collections or strains of WCM, known to vary in virulence to resistant wheat, also vary in their ability to colonize different grass species.

Materials and Methods

The two collections of WCM compared in this study were designated KS and NE. The KS collection was obtained from wheat at Ellis County, Kansas in 1996. The NE collection, supplied by Dr. Talat Mahmood in 1996, originated from wheat in western Nebraska. Both colonies were identified as *Aceria tosichella* Keifer by J. W. Amrine (West Virginia University, Morgantown, West Virginia) The colonies were maintained as previously described (Seifers et al. 1997) on 'Tomahawk' wheat grown in poly-cast tubes 2.5 cm in diameter and 14 cm long (Container Company, Canby, Oregon 97333) and covered with WCM-proof cylinder cages as described by Seifers et al. (1997). Both colonies were maintained in the same greenhouse under natural light at 17 to 32°C.

The cultivated and wild grass species tested are listed in Tables 1 and 2. Seeds were supplied by the following: Kansas State University Agricultural Experiment Station–Hays, Kansas 67601; Valley Seed Service, Fresno, California 93791; Sharp Bros. Seeds, Healy, Kansas 67850; National Small Grains Collection, USDA-ARS, Aberdeen, Idaho 83210; and Townsend Seeds, Inc., Townsend, Montana 59644. Plant identifications were verified with the Flora of the Great Plains (Great Plains Flora Association, 1991). Seedlings were established in poly-cast tubes and covered with WCM-proof cages as described above (Seifers et al. 1997). Ten seedlings per accession, five each infested with 10 KS or NE WCM per plant, constituted a test, and each test was repeated four times for each grass species with variable time periods between tests. The WCM were transferred from the rearing host, Tomahawk wheat, to the test plants in the 2- to 3-leaf stage by collecting them on the tip of a hair glued to a dowel and releasing them into the whorl of the second leaf of each plant. Details of the WCM transfer technique were reported previously (Harvey et al. 1999). Only adult or second instars displaying normal movement were used. The WCM were transferred and counted by using a dissecting microscope with 20× magnification and a fiber optic system to provide cold light. After infestation, the caged plants were randomized into supportive racks, which were set in watering trays and held on a greenhouse bench with natural light and variable temperatures (17 to 32°C). Total numbers of WCM adults and nymphs per plant were recorded 7 to 8 d after infestation. Longer periods of infestation on susceptible hosts allow WCM numbers to become too large to be counted accurately. Grasses were considered as hosts or nonhosts depending of the WCM's ability to increase to more than the initial infestation of 10 per plant during the 7–8 d infestation period.

TABLE 1. Host ranges (greenhouse experiments) for wheat curl mites (WCM) based on results of Slykhuis (1955), Connin (1956), and Somsen and Sill (1970).

Species	Common name or variety	WCM Survival Ratings[a]		
		Slykhuis	Connin	Somsen
Aegilops cylindrica Host	Jointed goatgrass	NT	Fair–Good	S
Agropyron elongatum (Host) Beauv.	Tall wheatgrass	0	None	S
A. smithii Rybd.	Western wheatgrass	0	Poor–Fair	S
Alopecurus pratensis L.	Meadow foxtail	NT	None	NT
Avena sativa L.	Oat	0	Very Poor	NT
Bouteloua gracilis (H.B.K.) Lag. ex Steud.	Blue grama	NT	None	S
Bromus tectorum L.	Downy brome	0	None	S
Cenchrus pauciflorus Benth	Sandbur	NT	Good	S
Dactylis glomerata L.	Orchard grass	NT	None	R
Digitaria ischaemum (Schreb.) Schreb. Ex Muhl.	Smooth crabgrass	NT	Fair–Good	NT
D. sanguinalis (L.) Scop.	Crabgrass	0	Very Poor	S
Eleusine indica (L.) Gaertn.	Goosegrass	NT	None	S
Eragrostis cilianensis (All.) E. Mosher.	Stinkgrass	NT	Poor	S
Hordeum vulgare L.	Barley	+	Very Good	NT
Poa pratensis L.	Kentucky bluegrass	0	NT	S
Panicum capillare L.	Witchgrass	0	None	S
P. virgatum L.	Switchgrass	NT	None	S
Pennisetum glaucum (L.) R. Br.	Pearl millet	NT	NT	S
Phalaris arundinacea L.	Reed canary grass	NT	None	NT
Secale cereale L.	'Dakold' rye	+	NT	NT
Setaria glauca (L.) Beauv.	Yellow foxtail	0	NT	S
S. viridis (L.) Beauv.[b]	Green foxtail	+	None	S
Sorghastrum nutans (L.) Nash	Indiangrass	NT	None	R
Sorghum bicolor (L.) Moench	'Atlas' sorghum	NT	Poor–Good	NT
S. halapense (L.) Pers.	Johnsongrass	NT	Good	R
Triticum aestivum L.	Wheat	+++	Very Good	S
Zea mays L.	Corn	0	Poor–Fair	NT

[a]Slykhuis–0 = no WCMs, + = few WCMs, +++ = abundance of WCMs; Connin–None, Poor, Fair, and Good refer to WCM increases; Somsen–R = resistant, S = susceptible refers to the reaction of host where WCMs were able (S) or not able (R) to complete a reproductive cycle. NT = not tested.
[b]Reported as a good host (Del Rosario & Still 1965).

Values presented are mean numbers ± SEs of WCM per plant for each entry. Data were subjected to analysis of variance with treatment means separated by the Student-Newman-Keuls (Steele & Torrie 1960) multiple range test at $P < 0.05$.

Results and Discussion

A comparison of the results of Slykhuis (1955), Connin (1956), and Somsen and Sill (1970) for WCM survival on some of the same grass species tested in this

TABLE 2. Host ranges for wheat curl mites (WCM) collected from wheat in Kansas (KS) and Nebraska (NE) based on survival and reproduction in the greenhouse.

Species	Common name or variety	Avg. No. WCM ± SE[a]	
		KS	NE
Aegilops cyclindrica Host.	Jointed goatgrass	48.8 ± 13.5a[b]	43.8 ± 6.6a
Agropyron elongatum (Host) Beauv.	Tall wheatgrass	0.1 ± 0.2a	8.9 ± 5.2b
A. smithii Rybd.	Western wheatgrass	0.0 ± 0.0a	1.4 ± 1.3a
Alopecurus pratensis L.	Meadow foxtail	0.2 ± 0.1a	0.0 ± 0.0a
Avena sativa L.	'Clinton' Oat	0.0 ± 0.1a	0.0 ± 0.0a
Bouteloua gracilis (H.B.K.) Lag. ex Steud.	Blue grama	0.1 ± 0.1a	0.7 ± 0.7a
Bromus tectorum L.	Downy brome	0.0 ± 0.0a	7.0 ± 6.6a
Cenchrus pauciflorus Benth	Sandbur	10.6 ± 14.5a	9.8 ± 13.8a
Dactylis glomerata L.	Orchard grass	0.0 ± 0.0a	0.7 ± 0.8a
Digitaria ischaemum (Schreb.) Schreb. Ex Muhl.	Smooth crabgrass	0.0 ± 0.0a	0.0 ± 0.0a
D. sanguinalis (L.) Scop.	Crabgrass	0.0 ± 0.0a	0.0 ± 0.0a
Eleusine indica (L.) Gaertn.	Goosegrass	2.5 ± 1.5a	2.3 ± 3.3a
Eragrostis cilianesis (All.) E. Mosher	Stinkgrass	0.0 ± 0.0a	0.3 ± 0.3a
Hordeum vulgare L.	'Westford' barley	21.8 ± 17.1a	31.3 ± 25.2a
Oryza sativa L.	'Drew' rice	0.0 ± 0.0a	0.4 ± 0.7a
Panicum capillare L.	Witchgrass	0.0 ± 0.0a	0.0 ± 0.0a
P. virgatum L.	Switchgrass	0.0 ± 0.0a	0.0 ± 0.0a
Pennisetum glaucum (L.) R. Br.	Pearl millet	16.1 ± 20.4a	5.7 ± 6.5a
Phalaris arundinacea L.	Reed canary grass	4.7 ± 3.8a	0.2 ± 0.3a
Poa pratensis L.	Kentucky bluegrass	0.0 ± 0.0a	0.0 ± 0.0a
Secale cereale L.	'Bonel' rye	32.8 ± 17.0a	8.6 ± 7.2b
Setaria glauca (L.) Beauv.	Yellow foxtail	6.8 ± 6.1a	0.0 ± 0.0b
S. viridis (L.) Beauv.	Green foxtail	9.4 ± 4.6a	0.4 ± 0.5b
Sorghastrum nutans (L.) Nash	Indiangrass	0.0 ± 0.0a	0.0 ± 0.0a
Sorghum bicolor (L.) Moench	'Atlas' Sorghum	13.7 ± 9.4a	9.0 ± 16.5a
S. halapense (L.) Pers.	Johnsongrass	0.0 ± 0.0a	0.0 ± 0.0a
Triticum aestivum L.	'Tomahawk' wheat	44.9 ± 17.9a	37.6 ± 19.1a
Zea mays L.	'Spirit' corn	8.5 ± 2.1a	10.8 ± 3.4a

[a]Number per plant one wk after an initial infestation of 10 WCM/plant.
[b]Means within a row (same grass species) followed by the same letter are not significantly different (Student-Newman-Keuls, $P < 0.05$).

study are shown in Table 1. Results of Staples & Allington (1956) were omitted because they tested only four species, green foxtail, yellow foxtail, stink grass, and witchgrass and reported that "these grasses are not hosts on which WCM can survive for any length of time." Although these four studies used different methods to estimate WCM populations, some valid comparisons should be possible. The results of Slykhuis (1955) and Connin (1956) were in good agreement for the 10 grass species that they both tested, except that Slykhuis reported a few WCM on green foxtail, whereas Connin (likewise, Staples & Allington 1956) found none.

Also, Connin (1956) reported barley as a very good host, but Slykhuis (1955) found only a few WCM. Their use of different cultivars could account for their lack of agreement for WCM survival on barley. Somsen and Sill (1970) classified 17 of the same grass species tested by Connin (1956) as either resistant (R) or susceptible (S) based on the ability of WCM to complete a full reproductive cycle. Somsen and Sill's results were markedly different from Connin's in that seven grasses he listed as nonhosts were considered susceptible by Somsen and Sill; conversely, they listed Johnsongrass as resistant, but Connin reported it as a good host.

Survival of two WCM strains (KS and NE) on 28 wild and cultivated grasses is shown in Table 2. Based on the average number of WCM present 1 wk after infestation with 10 WCM per plant, grasses were assigned to three categories in which no increase (less than 10 per plant) of either strain of WCM occurred: (1) no survival—oat, smooth crabgrass, crabgrass, witchgrass, switchgrass, Kentucky bluegrass, Indiangrass, and Johnsongrass; (2) less than one WCM per plant—meadow foxtail, blue grama, orchard grass, stinkgrass, and rice; and (3) less than 10 per plant—tall wheatgrass, western wheatgrass, downy brome, goosegrass, reed canary grass, yellow foxtail, and green foxtail. The 20 grass species listed above were considered as nonhosts for the KS and NE strains because the numbers of WCM decreased during 1 wk of infestation. Only three significant differences between WCM strains occurred on these 20 grasses; the NE strain survived significantly better on tall wheatgrass than the KS strain, and the KS strain survived better than the NE strain on yellow and green foxtails.

Based on WCM increases (more than 10 mites per plant for at least one strain), the following eight grasses were considered as hosts: jointed goatgrass, sandbur, barley, pearl millet, sorghum, corn, wheat, and rye. With the exception of rye, no significant differences in survival occurred between WCM strains. A possible explanation for the difference for rye is that the KS WCM strain has become virulent to and able to colonize 'TAM 107' wheat, which has a rye source of WCM resistance (Harvey et al. 1995).

All domesticated grasses except oat and rice were WCM hosts whereas only two of 20 wild grasses (sandbur and jointed goatgrass) were hosts. Jointed goatgrass is a close relative of wheat, and sandbur met only minimal standards to qualify as a host. However, sandbur also was reported as a favorable host by both Connin (1956) and Somsen & Sill (1970).

Host ranges for the KS and NE strains were in good agreement with those published by Slykhuis (1955) and Connin (1956), but not with those reported by Somsen and Sill (1970) (Table 1). One exception was that Johnsongrass did not support either the KS or NE WCM but was rated as a good host by Connin (1956) and resistant by Somsen and Sill (1970). Kentucky bluegrass is a host that was reported to be infested with WCM in the field (Slykhuis 1963, Somsen and Sill 1970, Brey et al. 1998), but supported inconsistent survival in the greenhouse. The KS and NE WCM did not survive on bluegrass (Table 2), and Slykhuis (1955) also reported that WCM did not become established on bluegrass in the laboratory. On the other hand, Slykhuis (1965) and Somsen and Sill (1970), reported bluegrass as susceptible to WCM in the greenhouse.

Besides possible variation among WCM strains, other conditions that may affect differences in WCM survival are temperature, humidity, and maturity of the grasses. Slykhuis (1955) and Connin (1956) reported that the degree of maturity of grass species may influence their suitability as WCM hosts. Connin

(1956) infested 2-week-old seedlings, but Somsen and Sill (1970) did not report the growth stage of the grasses used in their tests. However, because grasses tend to become more resistant as they mature (Slykhuis 1955, Staples & Allington 1956) and because Connin (1956) used young seedlings, this would not explain the greater number of susceptible hosts reported by Somsen and Sill (1970). The effects of temperature and humidity on WCM survival on potential grass hosts are unknown but should be investigated to determine if they could account for the host range differences reported.

Minor differences like those reported for green foxtail by Slykhuis (1955) and Connin (1956) may be explained by strain differences because the survival of the KS and NE mites were significantly different on this species. However, the minor differences in survival between the KS and NE strains provide little support for the theory that the major differences between the results of Connin (1956) and Somsen and Sill (1970) are due to their use of different WCM strains. On the other hand, the KS and NE strains, even though different with respect to resistance in wheat, may not represent the range of WCM variability used in previous studies. Better methods are needed to determine the survival of WCM on grasses so that the variation among larger samples of WCM collections may be determined. The cause of differences among results in WCM survival on various grasses is still unresolved and needs further study.

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Sticky Traps for Large Scale House Fly (Diptera: Muscidae) Trapping in New York Poultry Facilities[1]

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ABSTRACT Large sticky traps were evaluated under field conditions in two commercial high-rise, caged-layer poultry facilities in New York. To determine the longevity of trap efficacy in capturing house flies, *Musca domestica* L., we exposed varying lengths of adhesive coated ribbon to the flies, dust, and debris common in caged-layer poultry facilities. One side of each poultry facility received traps with 1.2 m of exposed adhesive, whereas traps with 2.4 m of exposed ribbon were placed on the opposite side. Significantly more house flies were captured using 2.4-m traps on 4 of the first 5 weeks when fly densities were highest. During all sampling weeks, traps exposed for 3- and 4-day intervals captured significantly more flies per day per meter of trap than the 7-day trap intervals. Furthermore, on 7 of the 10 weeks, 3-day trapping captured significantly more flies per meter per day than 4-day trapping, indicating a rapid deterioration in trap efficacy. Spot card data documented the reduction in fly densities at the bird level (upstairs) as the study progressed. The estimated number of house flies captured during this 10-wk study was greater than 9 million.

KEY WORDS *Musca domestica*, poultry, sticky traps, physical control

The house fly, *Musca domestica* L., is the primary pest in New York poultry facilities. The potential for fly outbreaks on farms combined with a highly mobile adult insect and the possibility of nuisance fly litigation intensify poultry producers' anxiety. Insecticide resistance (Scott et al. 2000) and loss of insecticides due to regulatory actions have reduced pesticide options available for fly control. Proven biological and cultural fly control options offer cost-effective alternatives to the use of insecticides (Legner 1975, Axtell & Rutz 1986, Meyer 1990). However, these methods often do not provide a reduction in adult house fly outbreaks that commonly occur 4–8 wk following repopulation or in fresh manure following a mid-year cleanout. An effective trapping device may allow for the capture of migrating flies before these flies leave the buildings.

Light-trapping of flies has been examined by several researchers (Tarry 1968, Skovmand & Mouvier 1986, Rutz et al. 1988, Pickens et al. 1994). However, little research has been conducted with alternative trapping devices in poultry facili-

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[2]Atlantic Paste & Glue Co., Inc., 170 - 53rd St. Brooklyn, New York 11232

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ties. Pickens et al. (1994) reported that light traps placed in darkened poultry manure pits captured 1% of the fly population per day and required cleaning every 30 days. Baited traps and pyramidal traps have been shown to capture large numbers of house flies on dairy farms (Pickens & Miller 1987).

A new sticky trap, the Spider Web™ (Atlantic Paste & Glue Co., Inc, Brooklyn, New York), captures large numbers of house flies when fly densities are high. However, like other sticky devices, effectiveness can be reduced under dusty conditions that are commonly found in poultry facilities. To determine the longevity of trap efficacy, we evaluated the Spider Web™ in two commercial high-rise, caged-layer poultry facilities in New York.

Materials and Methods

This study was conducted in two commercial, conventionally ventilated, high-rise, caged-layer poultry facilities located in Sullivan County, New York. Adult house fly densities were monitored using two sets of 10 spot cards (76 × 127 mm white file cards) that were positioned equidistant on each side of the facility on the bird level, 1.7 m above the floor (Axtell 1970). Each Friday, two cards were placed at each location. On the following Monday, one of the two cards was removed and a fresh card placed. This provided fly density data for the 3-, 4-, and 7-day time periods.

The Spider Web™ trap is a spooled ribbon, 30 cm wide by 7.3 m long, that when pulled exposes increasing lengths of adhesive coated ribbon. The attractiveness of the adhesive is enhanced by the addition of several house fly attractants. Spider Web™ traps were positioned horizontally, between the fluorescent lamps, 8 to 15 cm below the ceiling above the birds in the walkway (Fig. 1). Label directions for these traps suggest that 6.1 m (20 ft) of trap be placed for every 56 m^2 (600 ft^2) of building. Each facility was divided lengthwise providing three aisles per side. The outer aisle on each side was not included in the trial because of dust accumulation caused by close proximity to ventilation baffles. On one side, 1.2 m of each trap was exposed, whereas 2.4 m of each trap was exposed on the opposite side of the facility (2 aisles each). Facility 1 held 30,000 birds and contained 12, 2.4-m traps and 12, 1.2-m traps (6 traps per aisle). Twenty-four 2.4-m traps and 24, 1.2-m traps were placed in facility 2, which contained 60,000 birds (12 traps per aisle). This arrangement resulted in one-half the linear distance (i.e., total area) of trap being present on the 1.2-m sides of the facility on a given day. However, because the 1.2-m traps were stretched twice each week, an equal amount of "unexposed" trap was presented each week.

All traps were changed weekly (new trap placed or an additional 1.2 or 2.4 m exposed). In addition, 1.2 m traps were refreshed (by pulling an additional 1.2 m of ribbon) following a 3-day exposure, providing an alternating 3- and 4-day exposure, equaling the single "fresh" 2.4 m exposure per week. Traps were initially placed on 2 July 1999 and the study concluded on 10 September 1999 (10 wk).

Following exposure, all traps were removed from poultry facilities and the numbers of house flies estimated. To determine the numbers of flies captured, each trap was unrolled and hung vertically such that both the top and bottom of the trap could be observed from the side. Trap exposures were measured to de-

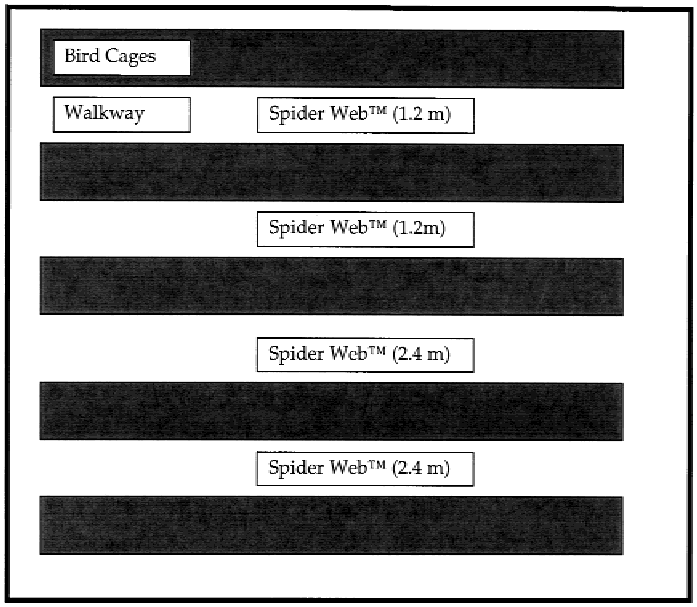

Fig. 1. Layout of poultry facility and positions of Spider Web™ trap placement.

termine the exact length presented at each time period. To estimate the numbers of flies on a trap, transparent acetates (7.6 × 28 cm) were positioned on opposite sides of the trap and flies observed through the acetate were counted. Acetates were positioned randomly on each trap with three and six areas counted on 1.2-m and 2.4-m traps, respectively. The numbers of flies per side of each trap were determined by multiplying the length of each trap (cm) by the average number of flies per cm per respective side.

To make comparisons as to the relative loss in effectiveness of the trap over time, we converted the trap catch data to a constant, based on the estimated number of flies captured per meter of trap per day. Analyses were conducted on the fly catch data to determine the impacts of linear distance exposed and to uncover a time-dependent reduction in effectiveness (accumulation of dust, feathers, etc.). To determine the impact of linear distance, the total numbers of flies captured on 3- and 4-day traps were summed and compared to the total fly catch on 7-day traps. This allowed for a comparison between an equal time exposure, with only 50% of the linear distance presented with the 3- and 4-day traps. A two-way analysis of variance was performed on these data with poultry facility and exposure length in the linear model (SAS 1996).

To determine the influence of time of exposure (duration) on trap efficiency, weekly fly catch data from the 3-, 4-, and 7-day traps were converted to a number of flies per meter of trap per day constant. Data were examined using a two-way analysis of variance with poultry facility and days of exposure in the linear model (SAS 1996). A Tukey's comparison was used to separate significant differences among treatment means. Spot card data from 7-day cards were log transformed and analyzed by week using a two-way analysis of variance with poultry facility

and side of facility in the linear model (SAS 1996). Spot card data were reverse transformed, and the mean numbers of spots per card are presented. For each analysis presented, the lowest F-value that provided a significant difference over the 10-week study along with the associated degrees of freedom and *P*-value for that study week are provided in the text.

Results and Discussion

House fly populations in these poultry facilities were very high when traps were initially placed (Fig. 2). Insecticides were not applied to either facility and manure conditions were very favorable for house fly production throughout the study.

Significantly more flies were captured using 2.4-m traps on 4 of the first 5 weeks (Fig. 3) when fly densities were highest ($F = 5.86$, df = 139, $P < 0.0168$). During weeks 9 and 10, significantly more flies were captured on the 1.2-m traps ($F = 8.89$, df = 141, $P < 0.0034$). These study weeks also corresponded to weeks with lower fly catch rates and indicated that dust accumulation on traps may have reduced trap efficiency in as little as 7 days.

During all sampling weeks, both the 3- and 4-day traps captured significantly more flies per day than the 7-day traps ($F = 28.55$, df = 210, $P < 0.0001$) (Fig. 4). Furthermore, on 7 of the 10 weeks, 3-day traps captured significantly more flies per meter per day than 4-day traps, indicating a rapid deterioration in trap efficacy due to dust accumulation.

Although the trap was not as efficient after a few days, it was certainly not ineffective. The display of 2 or 3 m of trap has surface area advantages over shorter presentations, especially when fly populations are high. Placing and stretching traps was labor intensive. Although shorter traps captured significantly more flies per meter per day, the additional labor required to maximize catch rates would render frequent trap maintenance impractical. The incorporation of an automatic stretching device would provide poultry producers with a labor saving investment as well as improve trap effectiveness. However, following an initial 2- or 3-m exposure, the "refresh" or roll-up rate should be designed to offer a slow delivery. This would offer an excellent balance between trap effectiveness and economics.

The estimated total number of house flies captured on 3-day–exposed, 1.2-m traps was 2,167,046 flies, on 439 linear m of trap (equivalent to 60 traps). The estimated total number of house flies captured on 4-day–exposed, 1.2-m traps was 2,353,086 flies, on 439 linear m of trap (equivalent to 60 traps). The estimated total number of house flies captured on 7-day-exposed, 2.4-m. traps was 4,649,162 flies, on 878 linear m of trap (equivalent to 120 traps). The estimated total number of house flies captured during this 10 wk study was 9,169,294.

The use of electrocuting black light devices in poultry facilities was reported by Rutz et al. (1988) who noted that traps were more effective when placed in the manure pit. During an eight wk period when house fly densities were high, manure pit placed devices killed over 29,000 flies per device per week. When the electrocution device was combined with muscalure (Z-9-tricosene), a sex attractant, the efficacy of the device was increased up to 76%. Pickens et al. (1994) reported that a Hodge window trap fitted with a black light and placed in a

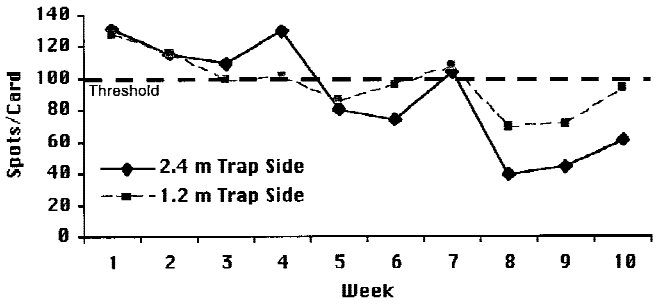

Fig. 2. Mean number of fly spots per card placed on opposite sides of two high-rise, caged-layer poultry facilities while Spider Web™ traps were present.

poultry manure pit captured 10,500 house flies per day, or 1% of the population. The Spider Web™ traps captured ca. 25,000 house flies per trap per week at the manufacture recommended rate (6.1 m of trap for every 56 m^2 area). These capture levels are comparable to those reported by Rutz et al. (1988) for electrocuting black light devices placed in manure pits; however, the Spider Web™ traps were positioned at the bird level.

Spot card data documented the reduction in fly densities at the bird level (upstairs) (Fig. 2). There were no significant differences between fly populations on either side of the facility. This is not surprising given the high mobility of adult house flies. As discussed earlier, trap catches also decreased as the study progressed, further demonstrating the effectiveness of the Spider Web™ in reducing fly populations (Figs. 2 and 3).

Cornell University Poultry Pest Management Recommendations suggest a treatment threshold of 100 spots per card (Kaufman et al. 2000). The numbers of spots per card in this study were above this threshold for the first 4 wks, and for 5 of the 10 wks of the study (Fig. 2). This suggests that house fly populations can be reduced; however, they cannot be satisfactorily managed using only trapping.

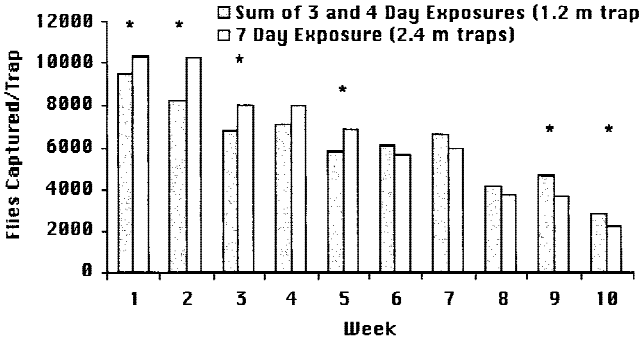

Fig. 3. Estimated number of house flies captured using the Spider Web™ in two high-rise, caged-layer poultry facilities in New York. (*Indicates significant differences between treatments, $\alpha = 0.05$)

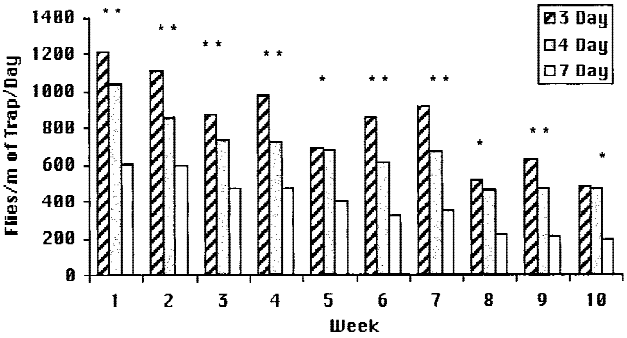

Fig. 4. Estimated number of house flies captured/meter of Spider Web™/day in two high-rise, caged-layer poultry facilities in New York. (**Indicates significant differences between all treatment means, *indicates significant differences between one treatment mean and remaining 2 treatment means, $\alpha = 0.05$)

Furthermore, use of the Spider Web™ would be most advantageous during house fly outbreaks when producers are most concerned about off-farm fly movement. The Spider Web™ provides an additional effective management tool to poultry producers facing severe fly outbreaks.

Acknowledgment

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Timing Insecticide Sprays for Control of Pod-sucking Bugs (Pentatomidae, Coreidae, and Alydidae) in Cowpea (*Vigna unguiculata* [L.] Walpers)[1]

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ABSTRACT Field experiments were conducted at Charleston, South Carolina, during 1997 and 1998 using foliar applications of endosulfan to evaluate damage by pod-sucking bugs (Pentatomidae, Coreidae and Alydidae) to cowpea at different pod-fill stages. Also, seasonal dynamics of bugs were assessed in untreated cowpea. Cowpea pod and seed damage was significantly lower in most plots treated with endosulfan than in untreated plots. Damage by sucking bug pests in 1997 was generally lower in treatment combinations that included spraying at early pod-fill. In 1998, pod damage was lower when treatments were made at early pod-fill than at late pod-fill. Also, seed damage in plots treated at late pod-fill was the same as that from the untreated check. However, treatment at early pod-fill gave a significantly lower seed damage than the control. Total seed yield was higher from plots with treatment combinations that included early pod-fill compared to untreated plots in 1998. Buildup of pod-sucking bugs, composed of *Nezara viridula* (L.), *Leptoglossus phyllopus* (L.), and the congeneric species *Alydus eurinus* (Say) and *A. pilosulus* Herrich-Schaeffer, started during the late vegetative stage. Bug populations peaked around late pod-fill and declined near dry-pod harvest. *Nezara viridula* was the first and most abundant species of pod-sucking bug to infest the crop. Populations of the other bugs, particularly *A. eurinus* and *A. pilosulus*, occurred toward late pod-fill. Our results suggest that early pod-fill is the most susceptible stage of cowpea to damage by pod-sucking bugs and that *N. viridula* is the major pest.

KEY WORDS Endosulfan, Nezara viridula, Leptoglossus phyllopus, Alydus eurinus, Alydus pilosulus, Pod-sucking bugs, cowpea.

Cowpea (*Vigna unguiculata* [L.] Walpers) is an important food crop worldwide and one of the main sources of plant protein for many people in developing countries. The tender leaves and green pods are eaten as vegetables and the dry seeds are a staple food (Okigbo 1978, Fery 1981, Parh 1993). Several arthropod pests seriously reduce cowpea yields, particularly postflowering insect pests (Raheja 1976, Singh & Jackai 1985, Jackai et al. 1992). Pod-sucking bugs (PSBs) (Pentatomidae, Coreidae, and Alydidae) are the most serious postflowering pests of

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cowpea in many areas of cowpea production (Singh 1980, Dreyer et al. 1994). Both adults and nymphs feed on pods and developing seeds, resulting in premature drying and shriveling of pods and seeds (Taylor 1968, Singh 1980, Fery & Schalk 1981, Nilakhe et al. 1981a, b, Schalk & Fery 1982, Jackai et al. 1989, Dreyer & Baumgartner 1995). In addition to direct injury, sucking bug feeding also results in the transmission of the fungus *Nemastospora coryli* Peglion that further contributes to damage (Clarke & Wilde 1970). Cowpea losses between 60–90% have been attributed to these insects in West Africa (Jackai et al. 1989).

In Africa, as many as six insecticide applications are used after the onset of flowering to control pod-sucking insect pests in cowpea (Dina 1976, Raheja & Apeji 1980). Three insecticide sprays are used for control of stink bugs in cowpea in the southeastern United States (Griffin 1999). Apart from the high costs of these chemicals, there are health and environmental hazards associated with their use (Luckmann & Metcalf 1994, Amatobi 1995). Abudulai (1998) found that endosulfan use in cowpea negatively impacts natural enemies. Although insecticides are recommended for the control of PSBs in cowpea (Stacey 1979, Parh 1993), information is limited about the extent of damage in relation to different pod-fill stages. Moreover, there is inadequate information on the seasonal dynamics of these insect pests in cowpea. Such information would be useful for appropriate timing of insecticide sprays to maximize their effectiveness with a minimum amount of material (Luckmann & Metcalf 1994).

The reproductive period of cowpea comprises overlapping developmental periods of individual pods, each of short duration (Wien & Ackah 1978). Schalk & Fery (1982) reported that cowpea infestations by *Leptoglossus phyllopus* (L.) and *Nezara viridula* (L.) at early bloom caused more damage to cowpea pods and seeds than infestations at late bloom. Dreyer & Baumgartner (1995) reported that *Clavigralla tomentosicollis* Stål caused heavy seed losses in cowpea when feeding occurred in early stages of pod formation. Duncan & Walker (1968) investigated the effect of *N. viridula* damage on the quality and yield of soybean. They used field cages to show that injury during the early stages of seed development caused the greatest weight loss.

A pod-sucking bug complex of *N. viridula*, *L. phyllopus*, and the congeneric species *Alydus eurinus* (Say) and *A. pilosulus* Herrich-Schaeffer is considered the most important group of pod-sucking insect pests of cowpea in the United States (Underhill 1943, Yonke & Medler 1968, Fery & Schalk 1981, Nilakhe et al. 1981a, b, Schalk & Fery 1982). The objectives of this study were to determine the time during the pod-filling stage when pods were most susceptible to damage by pod-sucking bug feeding and to determine the seasonal population trends of pod-sucking bugs on cowpea.

Materials and Methods

Field experiments were conducted during 1997 and 1998 at Clemson University's Coastal Research and Education Center, Charleston, South Carolina.

Response of Sucking Bugs to Insecticide Sprays. Cowpea cultivar 'Pink-eye Purple Hull', which is susceptible to pod-sucking bugs, was planted in the field on 26 May in 1997 and 1998. A completely randomized block design was used with four blocks. Blocks were separated by four rows (4 m wide). Plots within blocks consisted of four 9-m-long rows with 1 m spacing between rows and 0.05 m

between plants in a row. Plots were 2 m apart. Treatments consisted of endosulfan foliar applications at: (1) early pod-fill; (2) mid- pod-fill; (3) late pod-fill; (4) early and mid- pod-fill; (5) early and late pod-fill; (6) mid- and late pod-fill; and (7) early, mid- and late pod-fill. Untreated plots served as controls. Each treatment was replicated four times.

Insecticide treatments were applied to two rows at a time using a CO_2-pressurized backpack sprayer with a 4 nozzle Tee Jet tips (Spraying Systems Co. Wheaton, Illinois 60189–7900) boom at 4.21 kg/cm^2 and at the recommended rate of 0.56 kg a.i./ha of endosulfan (in 278 L water/ha). Application of insecticide began at early pod-fill which occurred 46 days after planting (DAP). Applications also were made on 53 DAP (mid pod-fill), and 59 DAP (late pod-fill). Endosulfan was applied between 0800 and 0930 h Eastern Daylight Savings time.

Pods were harvested 5–7 August by hand-picking mature pods from the two middle rows of each plot. Pods were dried in a Fisher oven (The Grieve Corporation, Found Lake, Illinois 60073-9989) at 100°C for 5 days. A subsample of 40 pods from each plot was examined for visible stink damage and percent pod damage was calculated. A pod was considered damaged when it had one or more stink bug feeding scars. Pods from this subsample were shelled to determine the weight of 100 seeds and number of seeds per pod. Aborted, wrinkled seeds and those having one or more visible stink bug feeding scars were considered damaged seeds (Dreyer et al. 1994). Percent damaged seeds per plot was calculated as weight of damaged seeds divided by the total weight of seeds from the plot, multiplied by 100. Seed yield in each plot was calculated from the weight of total seeds shelled from the plot, expressed in kg/ha. All weights were taken on a sensitive scale (± 0.1 g) (Sartorius Ag, Göttingen, Germany).

Seasonal Abundance of Sucking Bugs. Bug density was determined by weekly sampling field populations of *N. viridula*, *L. phyllopus*, *A. eurinus,* and *A. pilosulus* in a separate but adjacent unsprayed cowpea plot (0.10 ha). Sampling commenced 28 DAP and continued until harvest. Samples were taken in the morning (from 0700–0900) by counting nymphs and adults in 1 m rows at 10 randomly selected sites (Ogunwolu 1992).

Statistical Analyses. The cowpea data were analyzed using analysis of variance and means were separated using Fisher's Least Significant Difference (LSD) test at $P = 0.05$ (SAS Institute 1990). Weekly counts of individual bug species were averaged and the means were used to show the seasonal population trends.

Results

Response of Sucking Bugs to Insecticide Sprays. In 1997, pod-sucking bugs caused significantly greater ($P < 0.05$) damage to pods in untreated control than in most treated plots (Table 1). There were no significant differences in percent pod damage among plots treated at mid- pod-fill, mid- and late pod-fill and the untreated check. Pod damage was generally lower compared to the untreated check in plots that were treated with combinations that included early pod-fill treatment.

Mean percent seed damage was significantly higher ($P < 0.05$) in untreated plots than those treated at early pod-fill, early and mid- pod-fill, early and late pod-fill, and early, mid- and late pod-fill. There were no significant differences ($P > 0.05$) in number of seeds per pod among the treatments. Seed weight was

TABLE 1. Effects of different endosulfan spray schedules to control pod-sucking bugs on 'Pinkeye Purple Hull' cowpea, Charleston, South Carolina, 1997.

Spray schedule	% Pod damage	% Seed damage	No. of seeds/pod	Wt/100 seeds (g)	Total seed yield (kg/ha)
Control	30 ± 7.8a	41.8 ± 5.2a	7.8 ± 0.6a	12.3 ± 0.4b	487 ± 153a
Early pod-fill	11.3 ± 0.7b	18.1 ± 1.6c	8.7 ± 0.3a	13.8 ± 0.2a	1031 ± 175a
Mid- pod-fill	18.0 ± 4.2ab	34.9 ± 6.6ab	8.8 ± 0.5a	12.5 ± 0.9b	692 ± 253a
Late pod-fill	16.3 ± 3.3b	28.3 ± 4.3abc	8.7 ± 0.4a	13.0 ± 0.6ab	646 ± 107a
Early & mid-pod-fill	10.0 ± 1.8b	18.8 ± 4.6c	9.4 ± 0.6a	13.6 ± 0.2a	895 ± 189a
Early & late pod-fill	17.5 ± 2.7b	26.4 ± 1.7bc	8.9 ± 0.2a	13.3 ± 0.5a	937 ± 146a
Mid- & late pod-fill	19.4 ± 3.3ab	28.3 ± 8.3abc	7.9 ± 0.7a	13.2 ± 0.3ab	701 ± 98a
Early, mid- & late pod-fill	8.1 ± 1.2b	19.2 ± 2.6c	9.3 ± 0.3a	13.7 ± 0.2a	1042 ± 98a
F	3.19	3.04	1.56	2.52	2.06
df	7	7	7	7	7
P	0.0183	0.0227	0.2014	0.0437	0.0942

Treatment means ± SEM (n = 4) within a column followed by the same letter are not significantly different by Fisher's LSD ($P = 0.05$) test.
Plot size was 4 × 9 m with four replications per treatment. Early pod-fill spray applications were made 46 days after planting (DAP), mid- pod-fill at 53 DAP, and late pod-fill at 59 DAP. Plants were harvested on 71 DAP.

significantly greater ($P < 0.05$) in all plots that included a treatment at early pod-fill than the control. Seed weights from plots treated at mid- pod-fill, late pod-fill, and mid- and late pod-fill were not significantly different ($P > 0.05$) from the control. Total seed yield did not differ significantly ($P > 0.05$) among treatments (Table 1).

Significantly greater ($P < 0.05$) pod damage occurred in untreated control plots in 1998 (Table 2). Pod damage was not significantly lower ($P > 0.05$) in plots that received three insecticide applications at early, mid- and late pod-fill than those that were treated at early and late pod-fill. Additionally, there were no significant differences in pod damage between plots treated at early and late pod-fill and those treated at early pod-fill, early and mid- pod-fill, and mid- and late pod-fill.

Mean percent seed damage was significantly greater ($P < 0.05$) in untreated plots than in treated ones except where plots were treated once at late pod-fill. Percent seed damage was not significantly lower ($P > 0.05$) in plots that received insecticide at early, mid- and late pod-fill than those treated at early and mid-pod-fill, early and late pod-fill, and mid- and late pod-fill. Significantly fewer ($P < 0.05$) seeds per pod were recorded in untreated plots than those treated at early and mid- pod-fill, early and late pod-fill, and early, mid- and late pod-fill. Seed weight was significantly lower ($P < 0.05$) in untreated plots than in treated ones. Seed weight from plots that received three insecticide applications at early, mid- and late pod-fill was not significantly greater ($P > 0.05$) than those treated at early and mid- pod-fill, early and late pod-fill, and mid- and late pod-fill.

In 1998, seed yield in untreated plots was not significantly lower ($P > 0.05$) than those that were treated at mid- pod-fill and late pod-fill. Seed yield in plots treated at early, mid- and late pod-fill was not greater than those treated at early and mid- pod-fill. Yield in the latter plots also was not significantly greater ($P > 0.05$) than those treated at early pod-fill, early and late pod-fill, and mid- and late pod-fill.

Seasonal Abundance of Pod-sucking Bugs. Abundance of bug species varied during the crop season in 1997 (Fig. 1). The first evidence of bug occurrence was observed 49 DAP, which coincided with early pod-fill. Only *N. viridula* was recorded at that time with densities of 4.1 nymphs/m row and 0.1 adults/m row. All four species of bugs together first were detected on 63 DAP, which was during late pod-fill. Adult populations generally peaked on 63 DAP (Fig. 1) and declined as the crop matured.

In 1998, adults of *N. viridula* (0.1 adults/m row) were first recorded in the field on 28 DAP (Fig. 1). Populations of *L. phyllopus* (0.1 adults/m row) were recorded from 42 DAP. *Alydus eurinus* and *A. pilosulus* were not recorded until 56 DAP at 0.1 adults/m row and 0.2 adults/m row, respectively. Adult *N. viridula* populations peaked on 56 DAP (1.6 adults/m row). Adults of *L. phyllopus* had a low population peak on 49 DAP (0.5 adults/m row)). Nymphs of *N. viridula* had two population peaks, one on 42 DAP (4.5 nymphs/m row) and the other on 56 DAP (2.2 nymphs/m row) (Fig. 1). As in the previous year, populations of *A. eurinus* and *A. pilosulus* were low throughout the season.

Discussion

Results of the 2-year study demonstrate that endosulfan applications reduced cowpea damage by pod-sucking bugs. The effectiveness of this protection de-

TABLE 2. Effects of different endosulfan spray schedules to control pod-sucking bugs on 'Pinkeye Purple Hull' cowpea, Charleston, South Carolina, 1998.

Spray schedule	% Pod damage	% Seed damage	No. of seeds/pod	Wt/100 seeds (g)	Total seed yield (kg/ha)
Control	28.0 ± 2.9a	29.8 ± 2.3a	7.3 ± 0.4c	12.9 ± 0.3a	543 ± 154e
Early pod-fill	13.0 ± 2.5cd	17.5 ± 5.6b	8.1 ± 0.4bc	13.7 ± 0.1bcd	968 ± 81bcd
Mid- pod-fill	17.5 ± 1.9bc	17.8 ± 2.8b	7.8 ± 0.4bc	13.4 ± 0.3d	883 ± 126cde
Late pod-fill	18.3 ± 3.2b	20.5 ± 5.7ab	8.0 ± 0.2bc	13.5 ± 0.0cd	786 ± 155de
Early & mid- pod-fill	12.5 ± 1.8d	10.4 ± 1.8bc	8.7 ± 0.2ab	14.2 ± 0.1ab	1302 ± 160ab
Early & late pod-fill	11.8 ± 0.9de	12.4 ± 2.6bc	8.5 ± 0.4b	14.3 ± 0.1ab	1093 ± 145bcd
Mid- & late pod-fill	14.0 ± 1.4bcd	12.8 ± 2.8bc	7.8 ± 0.4bc	14.0 ± 0.2abc	1145 ± 108bc
Early, mid- & late pod-fill	7.8 ± 1.7e	6.6 ± 1.6c	9.7 ± 0.5a	14.3 ± 0.1a	1585 ± 103a
F	14.89	4.27	3.33	7.77	7.71
df	7	7	7	7	7
P	0.0001	0.0045	0.0151	0.0001	0.0001

Treatment means ± SEM (n = 4) within a column followed by the same letter are not significantly different by Fisher's LSD ($P = 0.05$) test.
Plot size was 4 × 9 m with four replications per treatment. Early pod-fill spray applications were made 46 days after planting (DAP), mid- pod-fill at 53 DAP, and late pod-fill at 59 DAP. Plants were harvested on 71 DAP.

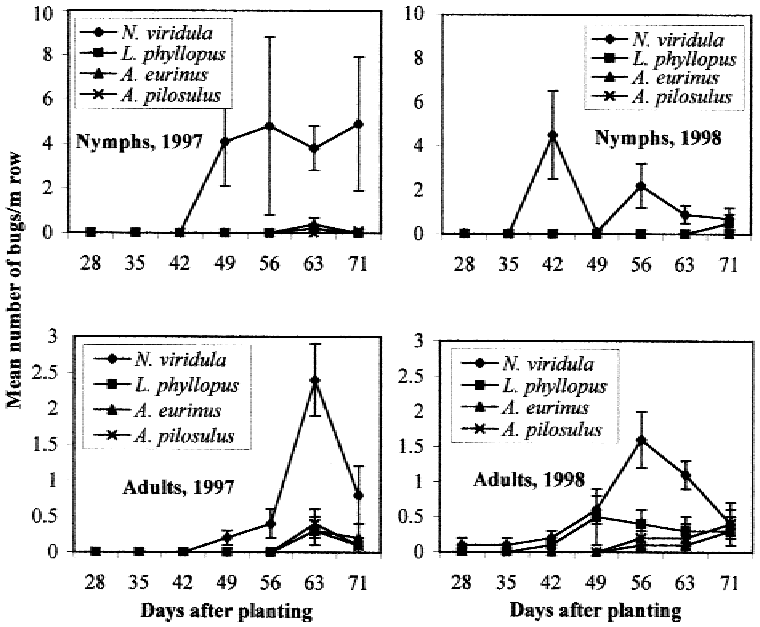

Fig. 1. Seasonal abundance (mean ± SEM) of *Nezara viridula*, *Leptoglossus phyllopus*, *Alydus eurinus,* and *A. pilosulus* in relation to phenology of 'Pink-eye Purple Hull' cowpea. Charleston, South Carolina. June-August 1997 and 1998. Vegetative stage = less than 42 days after planting (DAP), flowering stage = 42 DAP, early pod-fill = 49 DAP, mid- pod-fill = 56 DAP, late pod-fill = 63 DAP, and harvest = 71 DAP.

pended greatly upon the spray schedule or pod-fill stage at which spray was applied. In 1997, pod damage was not different from the untreated check when insecticide was applied at mid- pod-fill or at mid- and late pod-fill. Seed damage also was not reduced compared to untreated plots when insecticide treatments were made at mid- pod-fill, late pod-fill or at mid- and late pod-fill. Seed damage was lower in treatments that included spraying at early pod-fill. In 1998, pod damage was lower when treatments were made at early pod-fill than at late pod-fill. Seed damage in plots treated at late pod-fill also did not differ from the untreated check. Similarly, yields were not greater when plots were treated at mid- pod-fill or late pod-fill compared to the control. Yields were greater in the early pod-fill treatments. This finding agrees with what is reported in the literature. Many workers have demonstrated in cowpea (Schalk & Fery 1982, Jackai et al. 1989, Dreyer et al. 1994, Dreyer & Baumgartner 1995), soybean (Duncan & Walker 1968) and sorghum (Sharma & Lopez 1989, Hall & Teetes 1982a, b) that pod-sucking bug infestations at early seed development caused greater damage to pods and seeds, and loss in total seed yield than infestations made during later development. Pods punctured at the early endospermic stage are often drained of their contents which result in shriveled and underdeveloped seeds (Todd & Turnipseed 1974).

Damage and yields clearly are related to pest occurrence in this study. Early pod-fill generally coincided with the build up of *N. viridula* populations on the crop. In soybean, *N. viridula* first was recorded from the late vegetative to early seed development stage (Schumann & Todd 1982). Adult bug populations peaked on 63 DAP (late pod-fill) in 1997 (Fig. 1). There was an earlier peak, 56 DAP (mid-pod-fill), in 1998 apparently because bugs appeared in the field much earlier than in the previous year (Fig. 1). These phenologies are similar to that reported for *N. viridula* in rice (Kiritani et al. 1965) and in soybean (Schumann & Todd 1982). Those authors found that populations of *N. viridula* increased as pods began to fill, then moved to more succulent hosts as seeds matured (Todd & Herzog 1980).

Based on field population estimates, it is likely that the major contribution to damage was by *N. viridula*. It was the first bug to be recorded and appeared to be the most abundant. Fery & Schalk (1981) observed that *N. viridula* was a more serious pest of cowpea than *L. phyllopus*. In their study, at the same level of infestation of three adults per plant, *N. viridula* caused 94.7–96.2% loss in cowpea seed yield whereas *L. phyllopus* reduced seed yield by 37.2–71.4%. Similarly, Hall & Teetes (1982b) noted that *N. viridula* caused more reductions in grain yield in sorghum than did *L. phyllopus*. They observed that the damage threshold for these pests was four *N. viridula* and six *L. phyllopus*, per panicle of sorghum. Populations of *A. eurinus* and *A. pilosulus* were low in this study. They probably caused less damage to cowpea than did *N. viridula* and *L. phyllopus* that occurred in higher populations. Fracker (1918) reported that *A. eurinus* and *A. pilosulus* are numerous late in the summer. It is likely they may not cause much damage in cowpea, particularly those planted in early summer. However, they could be serious pests of late-planted cowpea. Underhill (1943) reported they cause significant damage to maturing seedpods.

Nymphal populations generally were higher than adults in this study. Nilakhe et al. (1981b) in cowpea, and Simmons & Yeargan (1988) in soybean, reported that 4^{th} and 5^{th} instars and adult stink bugs cause more qualitative and quantitative loss than younger instars. In our studies, nymphs were not categorized into various instar groupings. Thus, given their high populations, it is likely that nymphs inflicted more damage to cowpea pods than did adults.

In conclusion, proper monitoring of populations of pod-sucking bugs, especially *N. viridula*, and timing insecticide applications to coincide with early pod-fill can reduce damage and increase yield in cowpea. A single insecticide application at the early pod-fill stage can be an important saving and an economically feasible alternative.

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One or two authors are listed alphabetically; three or more authors should be listed chronologically:

Elsey, K. C. & J. A. Klun, 1989. Pickleworm sex pheromone: potential for use in cucumber pest management. J. Agric. Entomol. 6: 275–282.

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

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1980. Title. Abbr. J. 00: 00–00. (for another Jones, Burns and Curtin citation).

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Lower Developmental Threshold and Degree-Day Prediction of Larval Emergence of Citrus Cutworm, *Egira curialis* (Grote) (Lepidoptera: Noctuidae)[1]

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ABSTRACT The lower developmental threshold and the developmental rate of eggs and 5 larval instars of citrus cutworm *Egira curialis* (Grote) were calculated using five temperature regimes: 10°, 15°, 20°, 25° and 30°C. At 10°C, eggs did not hatch after 20 days and neonate larvae introduced to this temperature experienced high mortality and were unable to attain the fifth larval instar 76 days after eclosion. The developmental rate of citrus cutworm from oviposition until initiation of pupation was linearly correlated with temperature over the range of 15° to 25°C and larval survivorship was greater than 80% for the duration of the experiment. The rate of development of citrus cutworm larvae exposed to the 30°C treatment was similar to those exposed to 25°C; however, larval survivorship at 30°C was significantly reduced. The linear regression equation for the period of oviposition through 50% second instar larvae gave a theoretical lower developmental threshold for citrus cutworm of 7.6°C. Based on this value, *E. curialis* requires approximately 258 (C) degree-days (DD) to advance from oviposition to 50% second-instar larvae in the laboratory. A dosage of 100 micrograms synthetic pheromone/lure was found to effectively monitor weekly flights of male citrus cutworm. Field studies indicate that first capture of mixed populations of first and second instar larvae begins approximately 203 DD (C) after a biofix of the second consecutive week of moth capture using a 7.6°C lower developmental threshold. Third instar larvae began appearing an average of 315 DD (C) after the biofix. Using degree-days to predict first appearance of larvae and first appearance of 3rd instar larvae should improve efficacy of selective pesticides because the younger larval instars will be detected and treated.

KEY WORDS cutworm, phenology, degree-day, developmental threshold, pheromone

The univoltine citrus cutworm, *Egira* (=*Xylomyges*) *curialis* Grote, has been an important economic pest of citrus in the San Joaquin Valley of California since the 1930s (Woglum & Lewis 1935, Keifer 1935). Moths emerge during the period of January through April and deposit eggs on the leaves and bark of citrus (Atkins 1958). Eggs begin to hatch in March and the larvae feed on citrus leaves, flowers, and fruit. Larvae complete a series of instars from April through June, when they

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drop to the soil surface and form a puparium. Pupae aestivate from June through December enclosed within a cell in the top five to ten centimeters of soil.

The first larval instar of citrus cutworm feeds on the surface of tender young citrus leaves. Later citrus cutworm instars prefer to feed on the ovary of flowers and the rind of the newly developing green fruit (Quezada et al. 1976). Citrus cutworm larvae are difficult to see and can cause significant damage in a very short period of time. Larvae chew on the surface of the immature fruit and, if the fruit falls off the tree, a yield loss results. When scarred fruit remains on the tree until harvest, it is downgraded in the packinghouse. Citrus cutworm larvae will also bore into mature fruit. Thus, damage by this pest is both direct and cosmetic.

Sampling of citrus cutworm larval populations can be accomplished using an insect net, shaking the foliage over a cloth, or using a time search method (Pehrson et al. 1991, Haney et al. 1992, Grafton-Cardwell et al. 1997). The economic threshold varies depending on whether or not fruit are present. Citrus trees tolerate high levels of leaf damage and so prebloom treatment thresholds are relatively high; 15 larvae per hour search, per 20 trees shaken over a 61 by 92 cm beating sheet, or per 25 net shakes is the threshold. Post bloom, the larvae preferentially feed on fruit and so the action threshold decreases to 3–5 larvae per sampling method.

The current broad-spectrum pesticide program for citrus cutworm recommends a single foliar application of an organophosphate or carbamate insecticide (Grafton-Cardwell et al. 1997). These insecticides are highly effective against citrus cutworm larvae yet they disrupt parasitoids and predators that are needed for management of other citrus pests such as California red scale, *Aonidiella aurantii* (Maskell) (Bellows & Morse 1993, Bellows et al. 1993). In orchards where integrated pest management (IPM) is practiced, growers prefer to use selective insecticides such as *Bacillus thuringiensis* (Bt) or cryolite to control citrus cutworm (Grafton-Cardwell et al. 1997). Selective insecticides allow most natural enemies to survive (Bellows & Morse 1993, Bellows et al. 1993), however, they are sometimes less effective and slower acting than the broad spectrum insecticides, especially against the larger citrus cutworm larval instars (Grafton-Cardwell & Reagan 1994, 1995, 1996). Low temperatures can also reduce the efficacy of both Bt and cryolite because these pesticides are stomach poisons and larvae must be actively feeding to be poisoned. Therefore, the timing of application for these selective insecticides is a critical factor in maximizing their performance.

Early in the season, there is uncertainty as to when the citrus cutworm larvae will appear and so sampling often begins before citrus cutworm larvae emerge. When the larvae do emerge, first and second instar citrus cutworm are very difficult to see because they are small, light green, and position themselves on leaf edges and veins. An estimate of the date of larval emergence based on a degree-day model would improve the efficiency of sampling efforts and enhance the effectiveness of selective insecticides by targeting the smaller larval instars.

The purpose of our study was to develop and validate a degree-day model to predict larval emergence of citrus cutworm in San Joaquin Valley, California citrus. First we determined the developmental rate of citrus cutworm at various temperatures in the laboratory to calculate the lower developmental threshold. From this information, a degree-day model was developed and used to predict first appearance of various instars of larvae. A synthetic pheromone for attracting male citrus cutworm had already been identified (McDonough et al. 1982). Phero-

mone traps and temperature recorders were placed in commercial citrus orchards and moths and larvae were sampled to determine the degree-day accumulation that best predicted larval emergence and development in the field.

Materials and Methods

Laboratory developmental threshold. The initial laboratory stock of *E. curialis* was obtained by blacklight trapping of male and female adult moths at the peak of their flight activity during 1–14 March 1995 in a commercial citrus orchard near Ivanhoe, California. The moths were placed in oviposition chambers consisting of wire mesh cylinders lined with paper toweling (Ignoffo 1963) and were supplied with a 10% honey-water solution. The oviposition chambers were kept at temperatures of 20 ± 2°C with fluorescent light on a 12:12 [L:D]h photoperiod and a relative humidity of 75–85%. Eggs deposited on the paper lining were collected daily. These eggs were disinfected in a 0.15% sodium hypochlorite solution for five minutes and rinsed in deionized water (Mangat 1971). The eggs were separated into plastic Petri dishes lined with filter paper to which 1 mL of distilled water was added once to prevent dehydration.

To determine the lower developmental threshold of citrus cutworm, six replicates of 24 eggs per replicate were placed in growth chambers held at 10°, 15°, 20°, 25°, and 30°C. The eggs and larvae were maintained without light and a relative humidity of 75 ± 5% using open pans with water placed inside each chamber. After hatching, pairs of neonate larvae were transferred to individual 30-mL containers (Solo, Urbana, Illinois) with artificial diet. The diet consisted of a commercial *Trichoplusia ni* formula (Bioserv, Frenchtown, New Jersey) augmented with ≈10 grams of navel orange (*Citrus sinensis* Osbeck) leaves and 1 mL of formalin per liter of diet. Each rearing container was provisioned with 6–10 mL of diet. Larvae were transferred to fresh rearing containers every six to ten days as their food supply was depleted or to prevent spoilage. As there was no hatch of eggs held at 10°C, a second group of six replicates were incubated at 20°C until eclosion and then transferred to the 10°C chamber as neonate larvae to determine the rate of development of larval stages at this temperature.

As the larvae developed at the various temperature regimes, head capsule widths were measured every 1–2 days using an ocular micrometer fitted in a dissecting microscope to determine instar progression. The results of the 15–25°C regimes were used to calculate mean head capsule widths for each instar. The larvae were allowed to remain in their rearing containers during measurement to minimize mortality due to handling.

Forty-eight to 96 hours after the larvae had reached the 5th instar, they were transferred to pupation chambers consisting of a 180-mL waxed-cardboard cup with a paper lid. Each pupation chamber was partially filled with autoclaved fine vermiculite moistened once with 100 mL of distilled water per liter vermiculite and provisioned with one gram of artificial diet. This diet was replaced every two days to prevent mold development. The larvae would then complete their development in the chambers set for their original temperature regime and construct a pupal cell using the vermiculite as a matrix. After remaining in their cells for six weeks, pupae were extracted and viable pupae were identified as to sex (McDonough et al. 1982).

Head capsule width measurements for the various larval instars were analyzed using analysis of variance (SAS Institute 1996). Data for larvae reared at 15, 20, and 25°C were used for the head capsule width analysis since significant mortality occurred to larvae reared at the other temperatures. Larvae may have had more than one head capsule width measurement for each instar, if the head capsule width increased measurably during that instar. Mean head capsule widths were separated using the Ryan-Einot-Gabriel Welsch multiple range test. Mean days from oviposition to each larval instar and the sex ratio of viable pupae in each temperature regime were calculated. Developmental rate was calculated using the reciprocal of the number of days to complete a stage multiplied by 100 to give an integer value. The developmental rate for each individual in each temperature regime was analyzed and linear regression equations calculated using a general linear models procedure (SAS Institute 1996).

Field validation of the pheromone concentration. Preliminary tests in 1991 suggested that 100 micrograms of 95% *cis*-11-Hexadecen-1-ol (Aldrich Chem. Co., Milwaukee, Wisconsin) was an appropriate concentration to monitor male citrus cutworm moth activity. In 1999, the effectiveness of 0, 10, 100, and 1000 microgram dosages of synthetic pheromone mixed in acetone were compared in a replicated trial. For each dosage, 50 μL of solution was applied to each 8 mm natural rubber septa (Aldrich, Milwaukee, Wisconsin) using a micropipette. Each septa was mounted in a plastic bucket trap (Gempler's, Belleville, Wisconsin) and septa were replaced every 4 weeks. A 2 by 2 cm piece of Prozap Pest Strip (18.6% dichlorvos, Loveland, Greeley, Colorado) was tacked to the inside of the trap to kill the male moths. Traps were emptied and moths were counted once per week. Four traps each of 0, 10, 100, and 1000 micrograms pheromone were placed in a commercial citrus orchard in Exeter, CA in a randomized block design. Each pheromone trap was separated by 7 trees and each treatment replication was separated by 10 rows. Moths were monitored for a 12-week period from 28 January until 12 May 1999.

Field validation of the degree-day model. Four to nine citrus orchards each year were monitored during 1993–1999 in the Exeter and Ivanhoe areas of the San Joaquin Valley of California. These orchards were were separated from each other by 1–10 km. Moths were sampled on a weekly basis from January through May. Moth populations were monitored using traps as described above and septa baited with 100 microgram pheromone. Traps were emptied and moths were counted once per week. A single trap was placed in each orchard.

Ambient air temperature was monitored using DataPod (Omnidata, Ogden, Utah) or DataScribe (Avatel, Fort Bragg, California) electronic temperature recorders. Degree-day accumulations were calculated using an averaging degree-day model (University of California Publication 21373, Wilson & Barnett 1983, Zalom et al. 1983). Occasionally, individual moths were caught during December and early January, weeks before the full moth flight that signaled the initiation of the biofix. Therefore the model biofix was initiated using the second consecutive week of more than one moth captured and 7.6°C was used as the lower developmental threshold.

Larvae were sampled weekly from March through June. Sampling for citrus cutworm larvae was accomplished by vigorously shaking a citrus branch 3 times over a 61 by 92 cm black canvas sheet. During 1993 each of 30 trees were sampled, during 1994 each of 25 trees were sampled, and during 1995–1999 each of 20 trees

were sampled per orchard. During 1993, the total number of larvae was recorded and during 1994–1999 the instar of larvae collected was noted as well. The linear regression relationship between the total number of moths collected (x) and the total number of larvae sampled (y) was calculated for 37 orchards (SAS Institute 1996). The mean number of degree-day units for 1st larval capture and first 3rd instars captured were calculated using a total of 29 orchards. Orchards with low numbers of larvae (<25 total larvae collected during the season) were excluded from the larval analysis.

Results and Discussion

Laboratory developmental threshold. Five larval instars of citrus cutworm were identified using head capsule width measurements (Table 1, Figure 1) combined with presence of cast head capsules. Mean head capsule widths were 0.34, 0.61, 1.12, 2.10, and 3.07 mm and were significantly different ($F = 85{,}300$; df = 4, 1980; $P < 0.0001$) for the five larval instars. The total number of larval instars had not been previously described. The sex ratio of the combined population from all temperature regimes was 53% female based on identification of 380 viable pupae examined.

Citrus cutworm eggs held at 10°C remained viable for a period of time but none were hatched at 20 days (Table 2). For all other temperatures, egg hatch proceeded with no mortality and required from 2.0 to 10.7 days for completion by 50% of the population. Using citrus cutworm eggs hatched in the 20°C regime and then placed as neonate larvae in a 10°C environment, it was found that larval development did occur at this temperature but at a very slow pace. Advancement from the first to the second instar at 10°C did not begin until 35 days after oviposition, and no larvae reared at this temperature completed pupation at 87 days. Larval mortality at 10°C rapidly increased from 20% at 38 days from oviposition to 88% at the end of the experiment (Fig. 2). Absence of egg hatch after 20 days, inability to mature past the fifth larval instar, and poor survivorship of the larvae held at 10°C suggest that the lower developmental threshold for citrus cutworm was near this temperature.

Larvae exhibited increasing rates of development from 15° to 25°C for each of the instars evaluated (Table 2) and mortality of ≤20% (Fig. 2) during the 80 days of the experiment. Fifty percent of the larvae initiated pupation after 62 days at 15°C, 48 days at 20°C, 26 days at 25°C, and 28 days at 30°C (Table 2). While the rate of larval development at 30°C was similar to larvae reared at 25°C, mortality of larvae held at 30°C was much higher (Fig. 2) reaching 100% mortality 36 days after oviposition. Although some larvae did build puparia when reared at 30°C, none developed beyond the prepupal stage. High mortality of larvae reared at 30°C suggests that the upper developmental threshold for citrus cutworm is near this temperature, however, this temperature is never reached during the spring in the San Joaquin Valley when larvae are developing.

The developmental rate from egg deposition to each instar in each temperature regime was plotted and regression equations calculated (Table 3). The various developmental periods and equations yielded theoretical lower developmental thresholds (DT_L) of 7.6° to 11.4°C. Data for larvae reared at 10°C and 30°C were not included in the regression analyses due to poor survivorship and inability to complete egg hatch or pupation.

Table 1. Head capsule measurements of *E. curialis* reared at constant temperatures of 15, 20, and 25°C in the laboratory.

Instar	Number of larvae examined per stage	Head capsule widths (mm)	
		Mean[a] ± SE	Range
Instar 1	431	0.34 ± 0.002e	0.2–0.4
Instar 2	405	0.61 ± 0.002d	0.5–0.9
Instar 3	394	1.12 ± 0.004c	1.0–1.6
Instar 4	379	2.10 ± 0.005b	1.8–2.6
Instar 5	375	3.07 ± 0.005a	2.9–3.5

[a]Mean head capsule widths followed by different letters are significantly different ($P < 0.0001$) according to the Ryan-Einot-Gabriel-Welsch multiple range test.

Degree-day calculations were performed using the basic model of average daily temperature minus the lower developmental threshold (Wilson & Barnett 1983). This was chosen as the appropriate equation because the calculated lower developmental threshold for most instars (Table 3) was below the lowest experimental temperature regime (10°C), and the approximate upper developmental threshold of 30°C was found to be above that normally attained in the field. However, this method may not be as accurate as other methods when field temperatures fall very much below the developmental threshold. Temperature data for the period of January through May for the years 1977 to 1997 were downloaded from the California Irrigation Management Information System (CIMIS) weather station in Lindsay, California. These data showed that the average daily temperature never exceeded 30°C and only exceeded 25°C 61 times in that 20 year span. In this

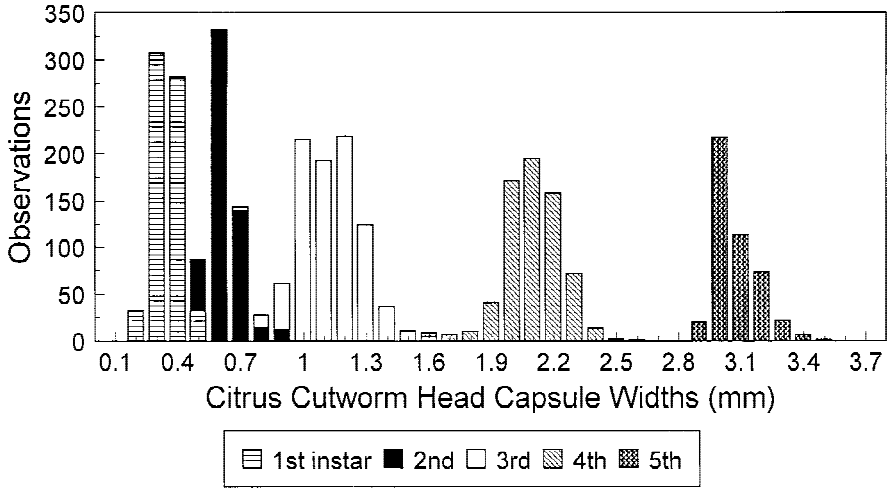

Fig. 1. Head capsule widths for 5 instars of citrus cutworm reared at temperatures of 15, 20 and 25°C.

Table 2. Days required for 50th percentile of citrus cutworm larvae to complete each growth stage for five different temperatures.

Temp. °C	Oviposition to Eclosion	Oviposition to 2nd instar	Oviposition to 3rd instar	Oviposition to 4th instar	Oviposition to 5th instar	Oviposition to pupation
10	—[a]	34.7[b]	49.5[b]	68.3[b]	81.4[b]	—[c]
15	10.7	22.2	29.2	40.2	51.7	62.3
20	6.7	17.5	25.8	36.2	41.0	48.0
25	2.0	8.3	13.0	18.0	21.2	26.0
30	2.0	7.2	10.7	15.5	22.4	28.3

[a]Individuals reared at this temperature did not complete this stage at 20 days after eggs were deposited.
[b]Individuals reared to eclosion in 6.7 days at 20°C.
[c]Individuals reared at this temperature did not complete this stage at 87 days after eggs were deposited.

situation, use of the basic equation results in degree-day values that are not statistically different from values derived from either the triangulation or sine methods of degree-day calculation, which are more accurate when environmental temperatures fall above or below the respective developmental thresholds (Wilson & Barnett 1983).

Because we are interested in predicting the presence of 2nd instar larvae to time *B. thuringiensis* sprays more effectively, we chose to use 7.6°C (includes development from oviposition to 50% of second instars) as the lower developmental threshold for field validation of degree-day units (Table 3). Using the calculated lower developmental threshold of 7.6°C, we found that, in the laboratory, the citrus cutworm required approximately 57°D from oviposition to 50% hatch

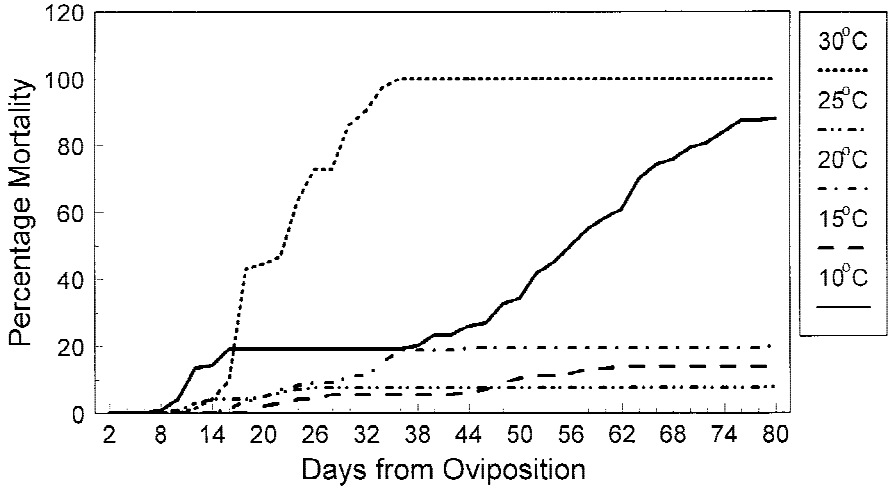

Fig. 2. Percentage mortality of citrus cutworm larvae reared at five different temperatures.

Table 3. Lower developmental thresholds for citrus cutworm by growth stage, using data from the 15°, 20°, and 25°C treatments.

Growth stages	No. tested	Regression equation	r^2	DT_L	Degree-day (C) accumulation (1/slope) for 50% of each stage
Oviposition to Eclosion	432	y = −0.1944 + 0.0171x	0.97	11.4	56.6
Oviposition to Instar 1	427	y = −0.0657 + 0.0068x	0.77	9.7	147.7
Oviposition to Instar 2	407	y = −0.0296 + 0.0039x	0.84	7.6	257.9
Oviposition to Instar 3	393	y = −0.0238 + 0.0030x	0.91	7.9	335.6
Oviposition to Instar 4	384	y = −0.0232 + 0.0027x	0.94	8.7	375.7
Oviposition to Instar 5	368	y = −0.0176 + 0.0021x	0.90	8.3	471.5

and 148 DD, 258 DD, 336 DD, 376 DD, and 472 DD to 50% completion of the first, second, third, fourth and fifth larval instars, respectively.

Field validation of the pheromone concentration. Figure 3 shows the attraction of male citrus cutworm moths to concentrations of 10, 100, and 1,000 micrograms synthetic female pheromone. No moths were caught in the pheromone traps in which the septa were treated with acetone alone. The traps with septa treated with 10 micrograms pheromone per lure attracted fewer than 12 moths in any trap and moths were found on only 4 dates during the 12-week sampling period. Thus, this pheromone concentration was too low for acceptable attraction of male citrus cutworm. The concentrations of 100 and 1,000 micrograms per septa showed similar peaks and valleys of moth activity. The 1,000 microgram rate attracted significantly more moths than the 100 microgram rate on 7 dates of the 12 week collection period. For reasons of economy of pheromone cap production and ease of counting moths in the traps, the 100 microgram rate was chosen as the best rate for detecting citrus cutworm moths and monitoring their flight activity.

Field validation of the degree-day model. Degree-day accumulations using the 7.6°C developmental threshold were applied to adult male pheromone trap and larval sampling data taken from groves in the Exeter and Ivanhoe citrus growing regions over a seven year period (Table 4). A total of 37 orchards were monitored from 1993 through 1999. Data from 8 orchards were eliminated from the degree-day analysis because larval populations of <25 total larvae were found during the 3 month collection period. In these orchards, densities of larvae were too low to detect first larval emergence.

The biofix dates (2^{nd} consecutive week of moth capture) varied from year to year with the earliest biofix observed on 14 Jan. 1994 and the latest observed on 18 Feb. 1993 (Table 4). Within a given year, the biofix date varied ≤2 weeks between orchards in the region. Moth densities in the early part of the season (December – early January) are often very low and erratic. Therefore, pest control advisors are advised to place pheromone traps in orchards on January 1, empty the traps weekly, and start their temperature accumulations when they have two consecutive trap catches of more than one moth in the same orchard.

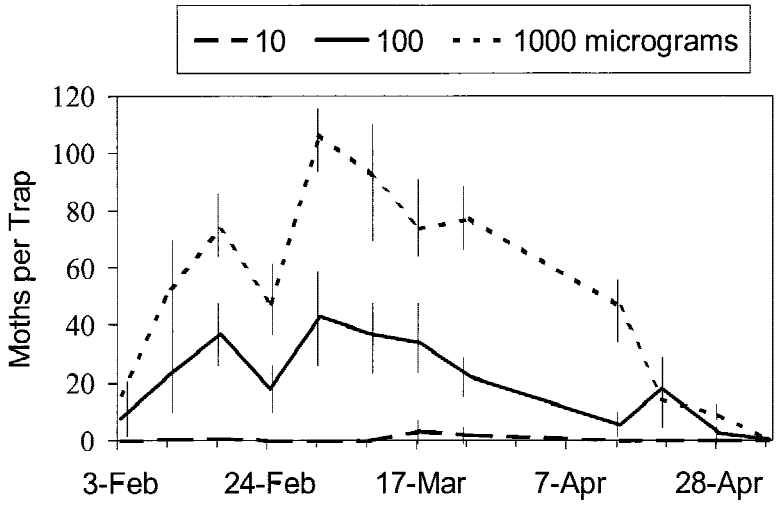

Fig. 3. The number of citrus cutworm male moths collected in traps in a commercial citrus orchard and baited with 10, 100, or 1000 micrograms synthetic female pheromone. Bars indicate the standard error of the mean.

Figures 4a and 4b show the typical pattern of the appearance of moths and larvae in a citrus orchard in Exeter, California during 1996. The moths began emerging during the week of 20 Jan. and the biofix was initiated on 27 Jan. during the second week of male moth capture. Varying numbers of male moths were captured from late January until the end of April. Rainstorms and cold weather are likely to have had an influence on male moth flight patterns. Egg masses of citrus cutworm are extremely difficult to find and first instar larvae are not easily observed in the field. The first larvae found in an orchard are usually a mixture of first and second instars. Also, fifth instar larvae are rarely observed in the field as they drop to the soil surface to begin construction of their puparia shortly thereafter. In the Exeter orchard (Fig 4b) we were able to detect a mixture of first and second instar cutworm larvae 155 DD (C) after the biofix of the second week of consecutive moth capture. Third instar larvae began appearing 388 DD (C) after the biofix.

Using data from 29 orchards sampled for cutworm moths and larvae over a seven year period (Table 4), we were able to detect citrus cutworm larvae in citrus orchards using a beating sheet an average of 203 DD (C) after the biofix using 7.6°C as the lower developmental threshold. The field average of 203 DD (C) for first emergence of first and second instar larvae lies between the estimated degree-day accumulations of first and second instars (Table 3) observed in the laboratory. Thus, the field data approximates the laboratory data.

The earliest date that the first cutworm larvae were found was 4 March 1997 and the latest was 5 April 1998. The minimum number of degree-days from moth biofix to first larval capture observed was 138 DD and the maximum was 301 DD (C). This is a 5–10 day range in results. However, the larvae were only sampled once each week and so we expect fairly high variation in the observed accumulated degree-days.

Table 4. Comparison of biofix and degree-day accumulations for citrus orchards in eastern Tulare County, California during 1993–1999.

Year	Number of orchards	Biofix dates	First larval capture dates	Mean (±se) DD (C) to first larval capture (DT_L = 7.6°C)	First capture of 3rd instars	Mean (±se). DD (C) to 3rd instar larvae (DT_L = 7.6°C)
1993	4	2 Feb–18 Feb	23 Mar–5 Apr	246.2 ± 21.7	—[a]	—[a]
1994	9	14 Jan–28 Jan	15 Mar–2 Apr	188.6 ± 12.8	22 Mar–15 Apr	266.1 ± 17.9
1995	4	5 Feb–12 Feb	5 Mar–12 Mar	187.5 ± 19.7	12 Mar–9 Apr	291.8 ± 22.4
1996	2	1 Feb–8 Feb	8 Mar–15 Mar	191.0 ± 36.0	5 Apr	376.5 ± 11.5
1997	4	21 Jan	4 Mar–25 Mar	162.5 ± 11.6	18 Mar–1 Apr	315.5 ± 29.1
1998	4	21 Jan	18 Mar–25 Mar	240.5 ± 12.6	1 Apr–8 Apr	300.8 ± 2.8
1999	2	28 Jan	1 Apr	205.0 ± 1.0	22 Apr	340.0 ± 2.0
Grand mean				203.0 ± 16.5		315.1 ± 14.3

[a]Larval instars were not recorded during this year.

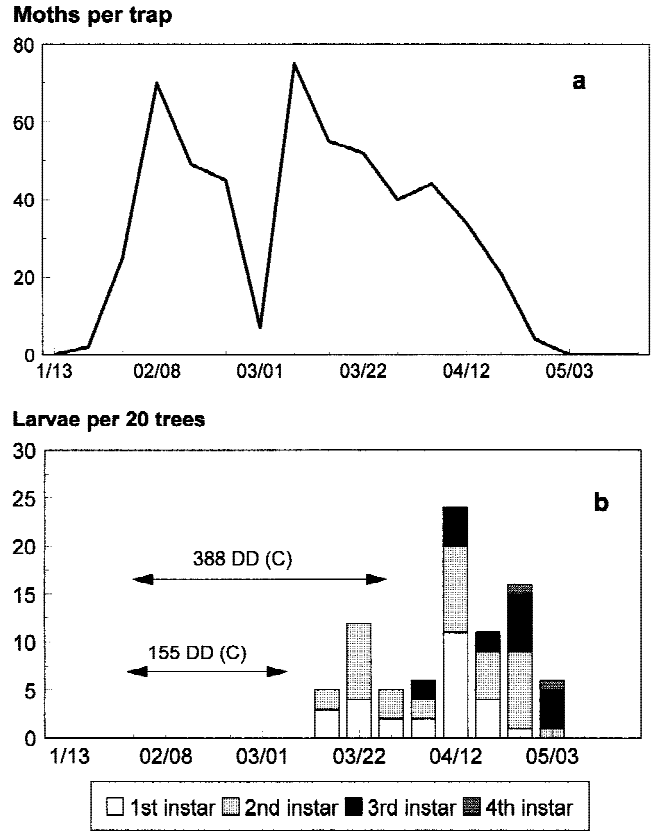

Fig. 4. Citrus cutworm emergence and degree-day unit accumulation in an orchard in Exeter, CA during 1996 (a) collection of moths in pheromone traps and (b) number of larvae in various instars sampled with a beating sheet. Larvae were first captured at 155 DD and 3rd instar larvae were first captured at 388 DD.

The first objective of the proposed model, is to use degree-days to predict first citrus cutworm larval capture to maximize pest control advisor monitoring efficiency. We suggest that pest control advisors begin sampling for citrus cutworm larvae at 138 DD (C) after the biofix, which is the earliest recorded capture of larvae sampled during 1993–1999. Degree-days accumulate fairly slowly (15–30 DD [C] per week) during January and February as the moths are flying and the larvae are just beginning to appear. If pest control advisors begin sampling at 138 DD, then they should, on the average, see larvae appear 2–3 weeks after they begin sampling.

The appearance of 3rd instar larvae (Fig. 4b) is also a significant event for management of citrus cutworm. Citrus cutworm eggs continuously hatch throughout the early spring and 3rd instar larvae begin to appear in late March and early April. Selective insecticides such as the microbial *B. thuringiensis* and

the stomach poison cryolite should be timed for when the majority of the larval population consists of 1st and 2nd instars. Table 4 shows the degree-day accumulations for the 25 orchards in which larval instars were recorded. The average degree-day accumulation for first collection of 3rd instar larvae was 315 DD (C). The 315 DD average emergence of 3rd instar larvae lies between the estimated degree-day accumulations of second and third instars (Table 3) observed in the laboratory. Thus, the field data approximates the laboratory data for this instar as well.

The earliest first capture of 3rd instar cutworm larvae was 12 Mar 1995 and the latest was 22 Apr 1999. For all orchards, the proportion of 3rd instars in the population was observed to be <50% in the first 3 weeks after larvae were first detected. Thus, pest control advisors have a short period of time to assess the situation and schedule an insecticide treatment. The degree-day estimation of the arrival of 3rd instar citrus cutworm larvae, coupled with sampling for economically important densities, should assist in scheduling application equipment. Improved timing of applications will improve efficacy of selective insecticides.

Moth densities were not a good predictor of larval densities ($n = 37$, $r^2 = 0.22$, $y = -39 + 0.19x$). This may be because the larvae experience greater mortality due to weather, parasites, predators, and pathogens compared to the moths. Thus, the utility of the pheromone trap collection coupled with a temperature recorder is not to predict which sites will have economically important densities of citrus cutworm larvae, but rather to improve the timing of the initiation of sampling by the pest control advisor and the timing of insecticide use by the grower.

Citrus growers use the relatively high threshold of 15 larvae per 20 net shakes before petal fall and a more conservative, lower threshold after petal fall because the cutworm exact greater damage when small fruit are on the tree (Grafton-Cardwell et al. 1997). Citrus growers would like to avoid broad spectrum pesticides early in the season so that they can preserve natural enemies such as *Aphytis melinus* DeBach and *Comperiella bifasciata* Howard for California red scale and the predacious mite *Euseius tularensis* (Congdon) for citrus thrips *Scirtothrips citri* (Moulton) and citrus red mite *Panonychus citri* (McGregor) control. If the Bt or cryolite treatment for citrus cutworm is successful, then they can avoid a broad spectrum insecticide treatment for citrus cutworm and can also choose a more selective insecticide for citrus thrips control at petal fall. If the Bt or cryolite treatment is not successful in reducing citrus cutworm larvae by petal fall, the grower will choose a broad spectrum insecticide to control both citrus cutworm and citrus thrips. The model described here is designed to improve the reliability of sampling and the efficacy of selective insecticides during the period before petal fall. Improved control of citrus cutworm with selective insecticides before petal fall, should eliminate citrus cutworm as a consideration after petal fall. This would allow growers to use insecticides that selectively control citrus thrips in the post petal fall period and reduce the use of broad spectrum pesticides for both pests.

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Field Tests of the Varroa Treatment Device Using Formic Acid to Control *Varroa destructor* and *Acarapis woodi*[1]

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ABSTRACT The Varroa Treatment Device (VTD) filled with 85% formic acid (FA) was field tested for honey bee parasitic mite control in the Piedmont region of South Carolina from February–October, 1996. Three apiaries with 28 honey bee colonies were used in this test. Each colony was housed and managed in one, 10-frame Langstroth hive body and one 10-frame Illinois super. Two VTD/FA treatments, one Apistan® treatment and one control were replicated seven times for comparison of varroa and tracheal mite levels. Treatments were: (1) two 60-day treatments with the VTD/FA; (2) continuous VTD/FA treatment except during the 2-month nectar flow period; (3) two 42-day treatments with Apistan®; and (4) VTD with sawdust but no FA as a control. Initial treatments were placed in colonies on 19 February, and the second treatments of VTD/FA and Apistan® were administered on 6 August. The VTD/FA treated colonies were serviced at approximately two week intervals during the treatment periods. Samples of approximately 300 adult bees were collected for mite diagnosis (alcohol wash method) on 23 January, 11 April, 10 June, 6 August, and 15 October. One hundred pupae from each colony were extracted and checked for varroa on the same dates beginning 11 April. Thirty-three adult bees from each sample were also diagnosed for tracheal mites by the thoracic disc method. Varroa mite counts on adult bees collected from all treatments were significantly less than the control for the August and October samples. Although varroa mite counts on extracted bee pupae from most treatments were significantly less than the control for the August sample, the Apistan® treatment was the only treatment that maintained significant varroa control in the brood for the October sample. Although the results of this test indicate that the VTD/FA is less effective than Apistan® in controlling varroa mites, the VTD/FA provides a viable alternative varroa mite control in combination with other mite control measures, especially as an early season treatment.

KEY WORDS honey bee, *Apis mellifera, Varroa destructor, Acarapis woodi*, formic acid, varroa, treatment device

Parasitic mites have become a primary concern for beekeepers over the past several years in the United States. Although EPA-registered products are available for honey bee (*Apis mellifera* L.) mite control in the U.S. (Apistan®, Check

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Prod. #18202

Mite +® [only in some states], and Apicure® for varroa mites, *Varroa destructor* [Anderson], and Mite-A-Thol® and Apicure® for tracheal mites, *Acarapis woodi* [Rennie]), other means of mite chemotherapy are being investigated. At present, Apicure® is the only registered product in the U.S. for both tracheal and varroa mite control.

Formic acid is a natural product found in honey (Liu 1991). Several field and laboratory tests (Hoppe et al. 1989, Bracey & Fischer 1989, Fries 1991, Liu & Nasr 1992, Ritter & Ruttner 1980, Wilson & Collins 1993a, Feldlaufer et al. 1997) have shown formic acid to have acaricidal properties in controlling parasitic honey bee mites including *Acarapis woodi* (Rennie), *Tropilaelaps clarae* (Delfinado-Baker) and *Varroa destructor* (Anderson). Wilson and Collins (1993b) reported only minor varroa control when colonies were fumigated twice in the fall with 65% formic acid. Formic acid is a very volatile organic compound and residues found in honey decrease rapidly following treatment (Liu 1991.)

Various means of formic acid application to control parasitic bee mites have been investigated. Fries (1991) used a thin (1.5 mm) plate of soft treefiber material (286 × 200 mm) soaked with 50 and 100 ml of 85% formic acid to treat varroa mites in frames of bee brood stored in plastic foam boxes in Sweden. Effective varroa control (mortality of 100% of mother mites) was accomplished using the 50 ml rate for two hours with more than 90% of the brood surviving. A higher bee brood rate loss occurred with the 100 ml rate treatment.

Hoppe et al. (1989) used absorbent cardboard plates (Illertissen mite plates®) soaked with 20 ml of 65% formic acid to control bee mites on *Apis mellifera carnica* colonies in the Schwarzwald/Fed. Rep. of Germany. Optimum varroa control (94% varroa mortality) was reported when four formic acid treatment plates (30 × 20 × 0.15 cm) were placed on the bottom board at four day intervals. With a single plate treatment for *Tropilaelaps clareae* infested colonies, an average mite mortality rate of 100%, 99%, 88%, and 95% occurred in larvae, protodeutonymph and adults stages, respectively. Two single plate treatments at 7 d intervals resulted in a 91% adult *Acarapis woodi* mortality rate.

Bracey & Fischer (1989) used a 200 ml plastic container having an air tight lid with a slit sufficient to hold an absorbent pad. The container was filled with 70% formic acid and placed on the top brood frame bars inside an empty shallow super (16.8 cm). The formic acid level was checked and refilled at 8–10 d intervals during the 1-month treatment period. The field tests were conducted in Dubai, part of the United Arab Emirates, in June with average temperatures ranging 28°–40°C. Highly effective varroa mite control was achieved without causing any adverse effects on treated colonies.

Wilson & Collins (1993a), using three weekly applications, treated colonies for tracheal mites in Arkansas and Texas in September and February, respectively, with 65% formic acid soaked filter pads placed over the top bars. Following treatments, live and dead tracheal mites were counted 21 days post-treatment with an average mite mortality of 94%.

Nelson et al. (1994) field tested five application methods of formic acid and one treatment of menthol paste for tracheal mite control in Alberta, Canada. The methods of formic acid application were: (1) 30 ml of 65% liquid formic acid in three weekly applications; (2) a polymer gel-strip with 30 g of formic acid; (3) gel-strip with 60 g formic acid; (4) Illertissen mite plates (IMP) and; (5) 30 ml of liquid formic acid (applied five times and during a nectar flow). Treatment ap-

plications were conducted from May–June, and mite levels were measured in the summer. All five tested colony applications reduced tracheal mite levels compared to non-treated colonies, but liquid formulations, IMP pads, and menthol gave superior control. Significantly increased bee kill was reported on days following the liquid formic acid treatment.

Imdorf et al. (1996) reported varroa mite treatment efficiency of 95%–97% in Switzerland using soft fiber plates (Pavatex) impregnated with formic acid and sealed plastic bags of 0.15 mm thickness. Treatment efficiency was dependent on the formic acid concentration in the hive and the duration of the treatment.

The Beltsville formic acid gel pack, commercially known as Apicure®, is now registered in the U.S. for control of parasitic honey bee mites. Although a spring application of Apicure® gave near 100% tracheal mite control, only 70% varroa mite control was reported (Feldlaufer et al. 1997). Guzman et al. (1999) tested the Beltsville formic acid gel pack and reported several negative effects on drone production and a decrease in number of drones found in treated colonies.

Although various methods of formic acid application for parasitic mite control have been investigated and one formic acid product (Apicure®) is registered in the U.S., a convenient, highly effective, and safe method of application is desirable. The purpose of this study was to evaluate the acaricidal effect of the Varroa Treatment Device (VTD) using an experimental 85% formic acid solution. The VTD treated colonies were compared with colonies receiving Apistan® and colonies receiving no miticide. Tracheal and varroa mite levels were monitored bimonthly during the 10-month test period.

Materials and Methods

Three apiaries with 28 honey bee colonies (12 in yard one and 8 in yards two and three) in Anderson and Pickens counties, South Carolina, were used for this study. Apiaries were separated by a minimum distance of 1 km. Each colony was housed and managed in one 10-frame deep Langstroth hive body with queen excluder and one 10-frame Illinois super. The colonies were managed for honey production with extra supers added during he April and May nectar flow. Surplus honey supers were removed, extracted and stored following the 2-month nectar flow.

Colonies in each apiary were randomly selected to receive one of four treatments: (1) two 42 day treatments with Apistan® beginning on 19 February and 6 August; (2) Varroa Treatment Device (Popodi Development, 2498 Gammon Rd, Site 11 Comp 55 RR1, Naramata, B.C. V0H 1N0) administering 85% formic acid for two sixty day treatments beginning on 19 February and 6 August; (3) Varroa Treatment Device (VTD) administering 85% formic acid beginning on 19 February and ending on 15 October, except removal of the device during the primary nectar flow (11 April–10 June) and; (4) control VTD with sawdust without formic acid. Each treatment was replicated seven times. Colonies for each treatment group were identified with identical color-coded 8 cm geometric wooden blocks centered 2.5 cm above the hive entrance to reduce bee drift. Colors used were blue, brown, green and yellow.

The plastic VTD (152 × 80 × 25mm (Fig. 1)) was tested according to the manufacturer's specifications. The base of the device was attached to a solid bottom bar

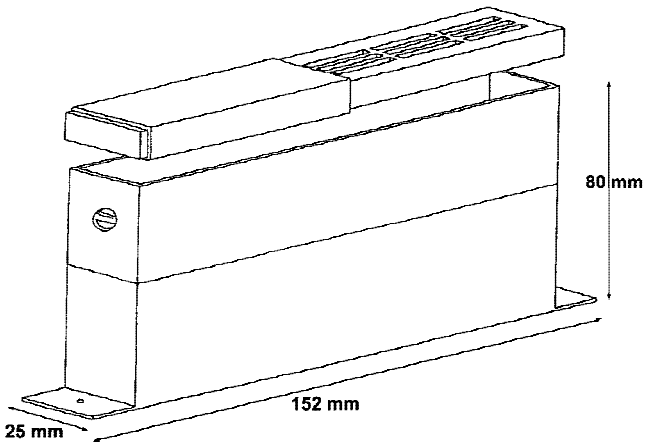

Fig. 1. The Varroa Treatment Device.

of a brood frame using two (1.27 cm) Phillips head sheet metal screws. The VTD was filled with dry softwood sawdust to the indicator fill line to allow absorption and controlled evaporation of the acid through the removable vented lid. A sliding vented cover allowed the user to regulate the amount of acid evaporation. For the duration of this study, vents were left opened at the two-thirds mark. One hundred and forty ml of 85% formic acid were poured into the sawdust to the fill line, and the device was placed next to the first broodless frame in the hive body. Treated colonies were serviced biweekly to refill the device with formic acid to the fill line and to adjust the location of the device in relation to the brood. The amount of formic acid necessary to refill the device was measured and recorded at each service. The amount of comb formation in the void area surrounding the VTD at the end of each treatment period was measured by use of a plexiglass cm^2 grid.

An Apistan® hive strip per five frames of bees was placed in each colony of the Apistan® treated colonies. The control colonies received one VTD (dry softwood sawdust only) placed in the hive body as an exterior frame for the duration of the test. All colonies were treated three times at five day intervals beginning 12 March with Terramycin/powdered sugar mixture for foulbrood prevention.

Samples of approximately 300 adult bees were collected from each test colony and placed in 0.47 liter glass jars half filled with 70% alcohol on 23 January, 11 April, 10 June, 6 August, and 15 October. Varroa mite diagnosis was conducted on all samples by the alcohol shaking method described by Shimanuki & Knox (1991). Adult mite infection was expressed as the number of mites/300 adult bees. For bee brood varroa analysis, one frame of brood was removed temporarily from each colony from the brood nest center on all of the above listed sample dates except 23 January. Fifty worker pupae on each side of the frames were uncapped and removed with forceps for varroa examination.

Tracheal mite diagnosis was conducted on adult bees by a modified thoracic disk method (10% KOH instead of 5%) as described by Shimanuki & Knox (1991) method no. 4. The large prothoracic tracheal trunks from 33 bees/colony were

examined microscopically (105X) for mite presence. Mite prevalence was calculated as a percentage of bees infested by tracheal mites in each sample.

For both pupal and adult varroa mite infection, a two-way analysis of variance with repeated measures, was performed. Main effects were treatment and sample date; hive was the repeated measure. The intent was to detect any significant differences in mite control among treatment groups for each sample date (January, April, June, August, October). Therefore, following analyses of variance, LSD mean separations were conducted for differences among treatment groups for each sample date. The dependent variables (number of mites/300 adult bees or /100 bee pupae) were log transformed (natural log) prior to analysis (Zar 1984). All significance tests were set at $P < 0.01$.

Results

Varroa mite counts on both adults and pupae were significantly different among treatments and sample date (Table 1). Mean separations among treatment groups for each sample date are shown in Table 2. Changes in mite infection over time for adults and pupae are shown in Figs. 2 and 3, respectively. With the exception of the extended VTD/FA treatment, varroa mite counts on adult and pupal samples taken from all treatments for the April and June samples were not significantly different ($P > .01$) compared to the control. Varroa mite counts on adult bees collected from all treatments were significantly less ($P < .01$) than the control for the August and October samples. Although varroa mite counts on extracted bee pupae from the Apistan® and FA min treatments were significantly less ($P < .01$) than the control for the August sample, only the Apistan® treatment maintained significant ($P < .01$) varroa control in the brood to October. The extended VTD/FA treatment did not result in increased varroa control when compared to the other VTD/FA treatment.

Tracheal mite levels remained extremely low throughout this test with 20 colonies (71%) having mite levels of 10% or less during the test period. Three of the control colonies (43%) were found to have no tracheal mites throughout the test. Therefore, treatment effects on tracheal mites were inconclusive.

The average amount of total FA used during this test was 460 ml for the extended VTD/FA treated colonies and 425 ml for the two 60-day treated colonies. The higher FA evaporation rates from the VTD occurred during July and August (Table 3). The highest daily FA evaporation rate of 2.5 ml occurred during the 12-day period between 10 July and 22 July, when daytime high temperatures averaged 32.2°C (90.2°F).

Bees began to propolize the vents of the VTD/FA treated colonies in June with all devices receiving some degree of propolization. An average of 84.3% of the vent area was covered at the end of the 24 June sample period. The highest degree of propolization was recorded during the 22 July and the 6 August sample dates when 100% of the vent area was propolized. A drastic decrease in vent propolization (35.4%) was recorded at the end of the 3 October treatment period.

During each service visit at biweekly intervals, propolis was removed from the VTD vents using a hive tool to allow free FA evaporation. No effort was made to quantify propolization over time between service visits. Although propolization of the VTD vents played an unknown roll in FA evaporation during these tests, the highest FA evaporation rate was recorded in July when 100% propolization was recorded during the service visit (Table 3).

Table 1. Two-way analysis of variance with repeated measures for varroa mite control in bee adults and pupae.

Source	d.f.	SS	MS	F	P
Adults					
Treatment group	3	13.583	4.538	5.01	0.0078
Hive (treatment)	24	21.715	0.905	2.56	0.0007
Sample date	4	159.368	39.842	112.87	0.0001
Treatment × time	12	25.425	2.119	6.00	0.0001
Error	94	33.184	0.353		
Pupae					
Treatment group	3	23.027	7.676	7.41	0.0011
Hive (treatment)	24	24.873	1.036	2.06	0.0109
Sample date	2	192.432	64.144	27.48	0.0001
Treatment × time	9	7.104	0.790	1.57	0.1428
Error	67	33.713	0.503		

Discussion

The VTD/FA treated colonies resulted in similar varroa mite control as compared to the Apistan® treated colonies through the August sampling period. But, a significant increase ($P < .01$) in the level of varroa mites in brood and adult bees occurred in the VTD/FA treated colonies compared to the Apistan® treated colo-

Table 2. Mean separation (LSD) for varroa mite control on bee adults and pupae. For each sample date, means with the same different letters are not significantly different at $P > 0.01$.

	Sample date[b]				
Treatment	January	April	June	August	October
Adults					
Apistan	1.432a	0.012a	0.105a	1.2044a	2.026a
Control	0.929ab	0.456a	0.556a	2.8201b	4.366b
FA_max	0.601ab	0.277a	0.855a	1.2045a	3.385c
FA_min	0.443b	0.394a	0.747a	1.3908a	3.328c
Pupae					
Apistan	—	0.141a	0.157a	1.915a	2.626a
Control	—	0.773a	1.443b	3.290b	4.433b
FA_max	—	0.355a	1.011ab	2.231ab	3.935b
FA_min	—	0.093a	0.301a	1.630a	3.899b

[a]FA = 85% Formic acid in the Varroa Treatment Device (see Fig. 1).
[b]For each treatment adult mite infection was expressed as the log number (natural log) of mites per 300 bees examined and pupal mite infection rate as the log number (natural log) of mites per 100 pupae examined. Means per treatment are based on a sample size (n) = 7.

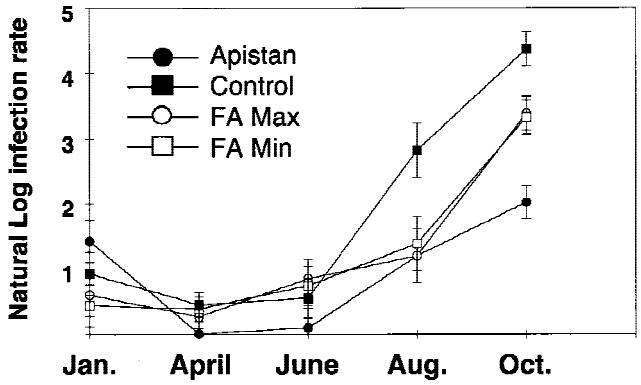

Fig. 2. Log transformed (natural log) adult bee varroa infestation rate (i.e., number of mites/300 bees) under 4 different treatment regimes. FA = 85% formic acid in the Varroa Treatment Device. Vertical bars represent 95% confidence intervals.

nies during the next 60 days. Although propolis was removed from the vents at each biweekly service, the propolization which began in June may have contributed to the reduced mite control. However, the reduction in propolis accumulation on vents in late season from early August to mid October did not result in increased varroa control when compared to the Apistan® treatment.

Although the results of this test indicate that the VTD filled with experimental 85% formic acid is less effective than Apistan® in controlling varroa mites, the VTD/FA provides a viable alternative for varroa mite control especially as an early season treatment when no propolization of vents occurred. The highest FA

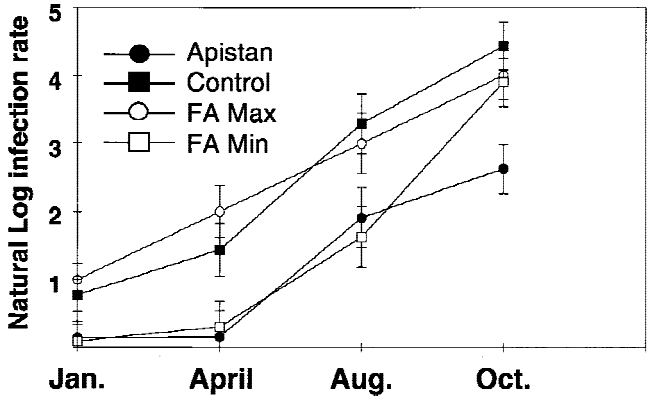

Fig. 3. Log transformed (natural log) bee pupae infestation rate (i.e., number of mites/100 pupae) with varroa mite under 4 different treatment regimes. FA = 85% formic acid in the Varroa Treatment Device. Vertical bars represent 95% confidence intervals.

Table 3. Mean (±SD) daily evaporation rate of 85% formic acid and maximum temperature (±SD) before the approximately biweekly VTD service period, and VTD vent average % propolization at service.

	Date of service								
	12 Mar	25 Mar	8 Apr	22 July	6 Aug	22 Aug	5 Sept	19 Sept	3 Oct
Daily average FA evap. (ml)	1.78 ± .51	1.43 ± .39	1.16 ± .52	2.50 ± .95	2.12 ± .89	2.27 ± 1.02	2.05 ± .74	1.91 ± .68	1.17 ± .45
Daily average high temp. (°C)	14.8 ± 5.76	16.6 ± 5.36	17.7 ± 5.75	32.3 ± 5.75	31.1 ± 2.37	30.8 ± 2.16	30.6 ± 2.87	28.9 ± 3.16	24.4 ± 3.87
Average % vent propolization	0.0	0.0	0.0	100	100	87.1	90.0	81.4	35.4

evaporation rate occurred during periods of higher temperatures which likely resulted in the bees covering the vents with propolis. Other mite control measures may be preferred for late season treatment or areas of hot climates, particularly in areas where VTD vent propolis accumulation is expected. Or, a less concentrated FA solution used in hot weather or warmer climates may reduce the VTD vent propolization. The extended FA exposure treatment (FA max) did not result in superior varroa control over the other FA treatment (FA min) and may contribute to a general decline in colony health.

The VTD system may serve as a varroa mite trap as colonies built drone comb in the area of the frame not occupied by the device. On average, the VTD/FA treated colonies built 26.6 cm^2 of drone comb on frames during the initial 60-day period. The seven extended period treated colonies, where devices were returned to the colonies on 10 June, had an average end of experiment comb production of 399 cm^2. The other seven 60-day fall treatment colonies had an average end of experiment drone comb production of 73 cm^2. This comb production was often followed by queen oviposition in the cells. The removal of this comb when drone pupae are present may offer the beekeeper an additional alternative form of varroa control (Dung et al. 1995).

We report here test results which were conducted in the Piedmont region of South Carolina only; therefore, the effectiveness of the VTD/FA treatment for varroa mites may vary in other regions of the U.S. Future tests of the VTD/FA should be conducted to further evaluate mite control efficiency and treatment effects on brood in other areas of the U.S.

Acknowledgment

This research was supported in part by a grant received from the National Research Council in Canada. We thank Doug Bidlack, Kevin Hathorne and Denny Smith for their assistance in the field and the laboratory. We thank Bill Bridges for his assistance with the statistical analysis of the data. The editorial comments of Don Manley and Albert Johnson were greatly appreciated. This is Technical Contribution no. 4292 of the South Carolina Agricultural Experiment Station.

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Relationship Between Temperature and Rate of Parasitism by *Eretmocerus* sp. (Hymenoptera: Aphelinidae), a Parasitoid of *Bemisia tabaci* (Homoptera: Aleyrodidae)[1]

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ABSTRACT Knowledge of the ecology of beneficial organisms can help in determining their role in managing insect pests. The effect of temperature on rate of parasitism by *Eretmocerus* sp. (Hymenoptera: Aphelinidae), an indigenous nymphal parasitoid of the B-biotype sweetpotato whitefly, *Bemisia tabaci* (Gennadius), was studied in the laboratory. The parasitoids were confined on collard leaves in plastic Petri dish cages and examined over a range of constant temperatures (15 to 45°C). Host density was 25 to 50 whitefly nymphs per test arena. Temperature influenced percentage parasitism. The rate of parasitism by the parasitoid at 30 and 35°C was higher than at 15, 20, 40, and 45°C. There was a quadratic relationship between temperature and percentage parasitism. No parasitism was observed at 15°C. At 45°C, the adult parasitoids did not survive during the 12 h oviposition exposure period, and no parasitism was observed at this temperature. Among the temperatures tested, rate of parasitism ranged from 0 to 29%. These results define the temperature range which is optimum (25 to 35°C) for parasitism by this species of *Eretmocerus*.

KEY WORDS whitefly, *Bemisia tabaci,* parasitoid, *Eretmocerus,* vegetable, greenhouse, Homoptera, Aleyrodidae, Hymenoptera, Aphelinidae

The B-biotype sweetpotato whitefly, *Bemisia tabaci* (Gennadius) [=*B. argentifolii* Bellows & Perring (Stoetzel 1989, Perring et al. 1993, Bellows et al. 1994)] has a wide host range which includes field and greenhouse crops (Greathead 1986, Byrne and Bellows 1991, Cock 1993, Riley et al. 1996, Simmons et al. 2000). It is a polyphagous pest species in the tropics and subtropics that occurs on all continents (Brown et al. 1995), and survives the mild winters of coastal South Carolina (Simmons & Elsey 1995). Economic losses occur when the whitefly debilitates plants, contaminates plants with honeydew and associated molds, transmits plant pathogens, or causes other plant disorders (Hill 1994). It is important to manage this pest in its early life stages because of the need to suppress early feeding and to reduce the likelihood of plant disorders and transmission of pathogens by the adults.

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Parasitoids of *Bemisia* are active in numerous ecosystems. Parasitism rates have exceeded 70% by aphelinid parasitoids in cotton, *Gossypium hirsutum* (L.) in the Imperial Valley, California (Gerling 1967, Natwick & Zalom 1984). In other research in the Imperial Valley, Coudriet et al. (1986) monitored seasonal abundance of aphelinid parasitoids, *Eretmocerus* spp. and *Encarsia* spp. In their study, the most predominant parasitoids were *Eretmocerus* spp. on field bindweed and wild sunflowers. Parasitoids of *Bemisia* have been reported from both cultivated and wild host plants in Florida and the Neotropics (Bennett et al. 1990). McAuslane et al. (1993) reported high rates of parasitism in peanut plots in Florida when no insecticide was used. From early August through early November, *Encarsia nigricephala* Dozier was the most abundant parasitoid in their study. Later in the season, *Eretmocerus* nr. *californicus* Howard became more abundant. Simmons (1998) identified five spp. of *Bemisia*-associated parasitoids in a survey in coastal South Carolina on sweetpotato, *Ipomea batatas* (L.). The abundance of those parasitoids varied during the production season and among widely separated locations. The parasitoids in this study were *E. pergandiella* Howard, *E. nigricephala* Dozier, *E. strenua* (Silvestri), *E. quaintancei* Howard, and *Eretmocerus* sp. In addition, a hyperparasitoid, *Signophora* sp. was collected. In the same survey, *Eretmocerus* sp. represented 5 and 18% of the parasitoids in 1993 and 1994, respectively, from sites in coastal South Carolina. In a study on several vegetable crops in South Carolina, approximately 45% of whitefly parasitoids were *Eretmocerus* sp. during season-long surveys (Simmons & Jackson 2000). However, Jackson et al. (2000) observed up to 99% of the parasitoids as *Eretmocerus* spp. in a collard field during June in 1999 in South Carolina. The taxonomic status of the *Eretmocerus* sp. detected from sweetpotato in South Carolina is under review by Mike Rose (Department of Entomology, Montana State University). Headrick et al. (1995) reported that females of *Eretmocerus* sp. nr. *californicus* Howard (= *E. eremicus* Rose & Zolnerowich) (Rose & Zolnerowich 1997) preferred probing the second instar among all instars. They reported that among the hosts probed, females chose all stages for oviposition with the same relative frequency. McAuslane & Nguyen (1996) reported that after confining parasitoids for 24 h with whitefly nymphs, significantly more eggs were found under second and third instars than under first and fourth instars.

While several studies have documented the seasonal occurrence, ovipositional preference, and fecundity of *Eretmocerus* spp., few studies have been conducted to determine the effect of environmental factors on the parasitoids. Many of the concerns about economic losses due to *B. tabaci* infestations are in the area of greenhouse management. It has been suggested that if greenhouse temperatures were lowered to reduce energy costs, the biological control of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), with *Encarsia formosa* Gahan may be impossible (van Lenteren & Woets 1988). Research was conducted to evaluate the performance of several species of parasitoids at low temperatures. As a result, *E. formosa* proved to be the best parasitoid at low temperatures. Powell & Bellows (1992) conducted studies under controlled environmental conditions with two populations of *Eretmocerus* sp. which developed on *B. tabaci*. Parasitoid fertilities were higher on cucumber than on cotton at 29°C. They concluded that biological control of *B. tabaci* by *Eretmocerus* sp. would probably be more effective at higher temperatures because the *Eretmocerus* species was native to Hawaii.

Environmental conditions may have a great impact on the behavior and population dynamics of *Eretmocerus* sp., one of the most abundant parasitoids of whiteflies on vegetables. Thus, we conducted a study to determine the effect of temperature on rate of parasitism on *B. tabaci* by an indigenous *Eretmocerus* sp. in South Carolina.

Materials and Methods

Insect rearing. Greenhouse colonies of B-biotype *B. tabaci* and *Eretmocerus* sp. were maintained on plants in plastic pots containing Jiffy Mix (Hummert's, St. Louis, Missouri) at the USDA, ARS, U.S. Vegetable Laboratory, Charleston, South Carolina. Both colonies originated from indigenous populations. Host plants included collard, *Brassica oleracea* ssp. *acephala* de Condolle; cotton, *Gossypium hirsutum* L.; cowpea, *Vigna unguiculata* (L.) Walpers ssp. *unguiculata*; bell pepper, *Capsicum annuum* L. ssp. *annuum*; soybean, *Glycine max* Merrill (L.); melon, *Cucumis melo* L.; and tomato, *Lycopersicon esculentum* Miller. The plants were watered daily and fertilized weekly with liquid fertilizer (Peter's Excel, Hummert's, St. Louis, Missouri). Collards were seeded weekly from July 1998 through June 1999 into 65-ml black plastic cell trays. Collard plants (second and third leaf stage) were infested with eggs of *B. tabaci* by exposing the plants to the adults in a greenhouse colony. The underside of the collard leaves was checked at 30-minute intervals for 2–3 h to determine when an appropriate infestation level was reached (Table 1). After the desired egg population was obtained, the adults were removed from the plants by placing them in a stream of air from an electric fan, tapping the plants gently by hand, and by using an aspirator. The egg-infested plants were then placed in covered plexi-glass containers (38.0 × 38.0 × 48.2 cm) in a growth chamber free of adult whiteflies. The photoperiod was 16:8 (L:D) h; temperature was maintained at 25°C; and relative humidity was ca. 55%. Butler et al. (1983) reported that the egg stage of *B. tabaci* varied from 22.5 to 5.0 d at 16.7 to 32.5°C, respectively, with the egg stage lasting 7.6 d at 25°C. Therefore, after 7–10 days, the whitefly nymphs (first to second instars) were counted on the undersurface of collard leaves. Using a No. 10 blade scalpel, excessive nymphs were removed. Similarly, all nymphs from the second or third leaf were removed from the top surface of the leaf. Other nymphs were removed from surfaces of all other leaves. Infested leaves were labeled with a tie-on paper tag, and each plant was assigned an identification number. The actual number of nymphs left on the undersurface of the leaf was recorded. The number of second instar nymphs per plant ranged from 25 to 50 (Table 1). While the target number of nymphs per plant was 40, it was not always possible to maintain consistency from trial to trial because of differences in rate of oviposition and eclosion. The number of nymphs was constant across temperature treatments within each trial with the exception of trial 3 (Table 1). In trial 3, 25 nymphs were set up for exposure to parasitoids on half the plants, and 50 nymphs were set up for exposure on the other half.

Parasitism of whiteflies. Females of *Eretmocerus* sp. were collected with aspirators and held individually in medicine dropper glass tubes. The ends of the tubes were sealed with parafilm, and the sex of each parasitoid was confirmed using a microscope. The test leaf, which was intact with a potted plant turned on its side, was confined in a cage made from a 15.0 × 2.5 cm plastic Petri dish. A

Table 1. Numbers of collard plants exposed to *Bemisia tabaci* adults for oviposition and numbers of *B. tabaci* nymphs and *Eretmocerus* sp. adults used in eight trials in the laboratory.

Trial	No. of plants tested	No. of 2nd instar nymphs per plant	No. of parasitoids per plant
1	20	33	3
2	20	30	1
3	16	25, 50	1
4	20	50	3
5	40	40	1
6	40	40	1
7	30	40	1
8	30	40	1

U-shaped opening (for the stem) was made along one side of the lid of the Petri dish. A 0.5 cm hole was drilled in the lid about 2 cm from the edge and opposite the side with the U-shaped opening. Each parasitoid was gently tapped by hand from the glass tube and into each cage which was placed on top of a black 54.0 × 40.0 × 7.6 cm plastic tray turned upside down. Depending on the trial, one or three parasitoids were caged (Table 1). Six treatments (15, 20, 25, 30, 35, and 40°C) were replicated 5 to 8 times in each of 8 trials. Two of those trials were conducted using an additional treatment of 45°C. A tray, containing parasitoids and caged whitefly nymphs on collard, was placed in an environmental unit with continuous flourescent lighting along the side of the chamber and 60–80% RH at each temperature treatment level. After 12 h, the parasitoids were removed from the cages with an aspirator, and the numbers of dead, live, and missing parasitoids were recorded. The potted collard plants were subsequently held in covered plexi-glass containers as described above in a growth chamber at 25°C, 14:10 (L:D) h, and ca. 55% RH. The plants were maintained by watering from the bottom within each plastic canister. They were held 3 wk for whitefly and parasitoid emergence. The numbers of whiteflies and parasitoids which emerged were determined by the type of exit hole in the exuviae. Because parasitism was determined by circular-shaped exit holes in the exuvia, only the parasitoids that developed to the adult stage were included in the analysis of data.

Statistical analysis. Percentage parasitism was transformed using Parasitism = Arcsin (SQRT (Number Parasitized/100) before analysis. Mean parasitism among temperature treatments were separated according to the Least Significant Difference Method (Analytical Software 1998). The relationship between temperature and percentage parasitism was described by regression analysis (SPSS Inc., 1998).

Results and Discussion

Survival of parasitoids. The whiteflies oviposited readily on the collard plants, and the desired infestation was reached within 2–3 h. The percentage of

adult female *Eretmocerus* sp. that was recovered alive following the 12-h exposure period for parasitism ranged from 50.4 to 60.8%. However, none of the parasitoids survived 12 h at 45°C, which was included to make a better assessment of behavior of the parasitoid at high temperature readings. This is important because of the potential use of the parasitoid in greenhouses. While there were no significant differences in percentage survival of the adult parasitoids among the other various temperatures (15, 20, 25, 30, 35, and 40°C), the lowest numerical percentage survival was at 40°C, and the highest numerical percentage survival was at 15°C, where all parasitoids were recovered alive. Because of the size (ca. 0.3 mm) of the adult wasp, we do not know if human handling contributed to any mortality. Nevertheless, such a factor would have been common across temperature treatments.

Incidence of parasitism. There were significantly higher rates of parasitism of *B. tabaci* by the *Eretmocerus* sp. at temperatures between 30 and 35°C than at 15, 20, 40, and 45°C ($F = 7.02$, df = 6,49; $P < 0.05$) (Table 2). Rates of parasitism at 25, 30, and 35°C were not significantly different. No parasitism occurred at 15 and 45°C. There was a quadratic relationship between temperature (15–45°C) and percentage parasitism (Fig. 1). In the regression analysis, the equation for transformed data is Arcsin($\sqrt{}$% Parasitized) = −1.600 + (0.139Temperature) − (0.00229Temperature^2); $F = 68.98$; $P < 0.001$; $r^2 = 0.563$. The data indicate that the rate of parasitism declines above 35°C. Another report indicated that at temperatures above 36°C, the eggs of *B. tabaci* (A-strain) failed to hatch (Butler et al. 1983). Therefore, economic loss caused by whiteflies may be lessened in greenhouses at these high temperature regimes, although the temperature would be expected to be favorable to the whiteflies during the night. Adult longevity reported for *E. californicus* was highest at 16°C and lowest at 38°C (Gerling 1966). Therefore, coupling the survival performance and ovipositional behavior across temperatures for the parasitoid in this study, it appears that a temperature of 30°C would be suitable for high parasitoid efficacy.

Table 2. Rates of parasitism of *Bemisia tabaci* by indigenous *Eretmocerus* sp. during 12 h on collard at constant temperatures in the laboratory.

Temperature (°C)	No. of nymphs	% Parasitism ($\bar{x}$ ± SD)	% Parasitism Minimum	% Parasitism Maximum
15	640	0.0a	0.0	0.0
20	1,773	6.78 ± 6.1a	0.0	20.3
25	1,801	20.03 ± 16.5bc	0.0	43.3
30	1,696	25.39 ± 17.9c	0.0	59.3
35	1,780	28.98 ± 20.8c	2.2	62.0
40	954	9.87 ± 8.5ab	2.4	19.4
45	640	0.0a	0.0	0.0

Means followed by the same letter are not significantly different; $F = 7.02$, df = 6,49, $P < 0.05$; Least Significant Difference Method (Analytical Software 1998). No survival of adult parasitoids at 45°C.

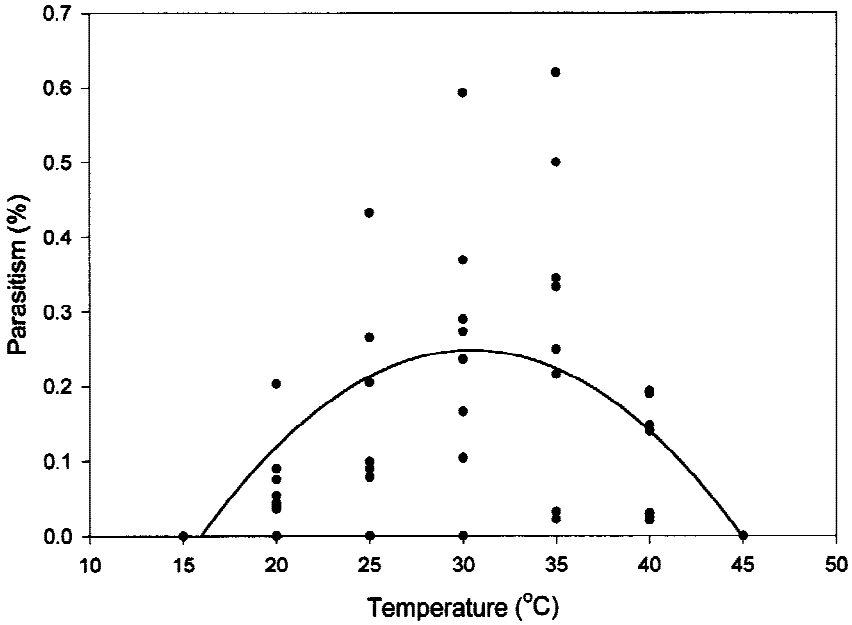

Fig. 1. Quadratic relationship of temperature and percentage parasitism of *Bemisia tabaci* by *Eretmocerus* sp. The equation for transformed data is Arcsin($\sqrt{}$% Parasitized) = −1.600 + (0.139Temperature) − (0.00229Temperature^2); $F = 68.98$; df = 6,49; $P < 0.001$; $r^2 = 0.56$. The numbers of *B. tabaci* nymphs tested at 15, 20, 25, 30, 35, 40, and 45°C were 640, 1773, 1801, 1696, 1780, 954, and 640, respectively.

The observed differences in parasitism apparently resulted from the effect of temperature on parasitoid searching behavior. The available leaf area was similar among treatments, and nymphs were only on the lower leaf surface. Although no data were recorded on the location of the adult parasitoids at the end of the exposure period, they were generally found on the nymph-infested lower leaf surface. However, some were found on the upper leaf surface or on the Petri dish. Eggs of *B. tabaci* are generally deposited on the lower leaf surface of collard, and the subsequent whitefly crawlers tend to stay on the side of the leaf where they are deposited (Simmons 1999).

Whereas the *Eretmocerus* sp. appears to have great potential as a biological control agent of *B. tabaci*, both in the greenhouse and in the open field, much information is still needed to determine its behavioral aspects. Van Lenteren & Woets (1988) reported on potential biological and integrated pest control in greenhouses where greenhouse whiteflies are a problem, and the use of parasitoids was quite successful. In a laboratory study (Headrick et al. 1995), an *Eretmocerus* sp. spent most of its time (59%) feeding, grooming, and resting. The remaining 41% of the total time was spent searching for hosts, assessing the host, probing, and ovipositing. Host searching occurred directly before host feeding, and oviposition occurred directly after host feeding, but not in the same host on which the par-

asitoid fed. In fields of coastal South Carolina, B-biotype *B. tabaci* survives the mild winters in low abundance (Simmons & Elsey 1995). Parasitoids of the pest increase during the warmer times of the year and decrease during the cool times (Simmons 1998, Simmons et al. 2000). Additional parasitoid studies are needed on host selection, courtship and mating, and larval development under various environmental conditions, particularly the climatic conditions under which the parasitoids will be used as biological control agents. As producers begin to depend more on biological agents to help manage whiteflies, it is important that these issues be addressed so that the highest quality control agent can be made available for use in a whitefly management program.

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Parasitism and Predation on Eggs of *Leptoglossus phyllopus* (L.) (Hemiptera: Coreidae) in Cowpea: Impact of Endosulfan Sprays[1]

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ABSTRACT The impact of endosulfan sprays in cowpea on parasitism and predation of *Leptoglossus phyllopus* (L.) eggs was determined by placing egg masses in the field after insecticide treatment at early, mid- and late pod-fill in 1997 and 1998. The parasitoids, *Gryon carinatifrons* (Ashmead), *G. pennsylvanicum* (Ashmead), and *Ooencyrtus ?leptoglossi* Yoshimoto were recovered from the egg masses. *G. carinatifrons* was the most abundant species, composing 83.9% of the parasitoids recorded. Predators included fire ants, *Solenopsis invicta* Buren, that tended to remove the eggs entirely and the snowy tree cricket, *Oecanthus fultoni* Walker, which chewed eggs and left chorion fragments on the substrate. Parasitism of *L. phyllopus* eggs was significantly reduced by endosulfan treatments, but predation was not affected. Parasitism generally was low early in the season and increased as the season progressed. Predators caused more egg mortality than did parasitoids and *S. invicta* was the major predator. These results support the hypothesis that parasitism and predation are important mortality factors of *L. phyllopus* in cowpea.

KEY WORDS *Leptoglossus phyllopus, Gryon carinatifrons, G. pennsylvanicum, Ooencyrtus ?leptoglossi, Solenopsis invicta, Oecanthus fultoni*, cowpea

Indigenous insect parasitoids and predators are important sources of mortality for herbivorous insect pests (Smith & Mittler 1967, Batra 1982, Jones 1982). Often, however, the effectiveness of these entomophagous species is limited in the field by insecticides (DeBach 1974). Most insecticides are toxic to non-target beneficial arthropods as well as to the target pests (Barlett 1963, Stern 1963, Croft & Brown 1975, Wilkinson et al. 1975, Orr 1988, Smart et al. 1989, Terry et al. 1993). Van den Bosch (1966) reported that applications of insecticides destroy more beneficial arthropods than any other agricultural practice and most beneficials are more susceptible to insecticides than are the target pests (Croft 1990). Smilanick et al. (1996) reported that methamidophos residue significantly affected pentatomid egg parasitoids, *Trissolcus basalis* (Wollaston) and *T. utahensis* (Ash-

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mead) in tomato (*Lycopersicon esculentum* Mill.). Also, chlorfenapyr and methyl parathion applied to soybean (*Glycine max* [L.] Merrill) was highly toxic to the heteropteran predators, *Geocoris punctipes* (Say), *Nabis roseipennis* Reuter and *Podisus maculiventris* (Say) (Boyd & Boethel 1998). Similarly, Barlett (1963) reported that endosulfan was highly toxic to parasitic Hymenoptera and coccinellids. Thus, pest resurgence and outbreaks of secondary pests are often major consequences of destruction of natural enemies by insecticides (Metcalf 1994). Despite these negative effects, insecticides are still the most commonly-used control measure against insect pests of cowpea (*Vigna unguiculata* [L.] Walpers) (Chalfant 1976, Stacey 1979).

The leaffooted bug, *Leptoglossus phyllopus* (L.), is a pest of cowpea in the southeastern United States (Bissell 1929, Fery & Schalk 1981). Feeding by both nymphs and adults greatly reduces seed quality and yield (Schalk & Fery 1982). Control of this bug and other sucking bug pests is necessary to prevent substantial reductions in the marketable yield of cowpea in the southern United States (Stacey 1979). One of the most frequently used insecticides is endosulfan, which is used mainly for control of the cowpea curculio (*Chalcodermus aeneus* Boh.). However, the impact of endosulfan on natural enemies of sucking bugs in cowpea has not been investigated.

The objectives of this study were to determine parasitism and predation on eggs of *L. phyllopus* in cowpea and to evaluate the impact of endosulfan sprays on parasitism and predation on *L. phyllopus* eggs.

Materials and Methods

Field tests were conducted in 1997 and 1998 at Clemson University's Coastal Research and Education Center, Charleston, South Carolina. The two treatments were endosulfan-treated and untreated control. Endosulfan was applied using a backpack sprayer at 4.21 kg/cm^2 at the recommended rate of 0.56 kg a.i./ha. Three applications were made in a season, at early pod-fill (46 days after planting [DAP]), mid- pod-fill (53 DAP), and at late pod-fill (59 DAP). The plot size used in 1997 was 4 by 9 m with 1 m between plots and 4 m between blocks. A second test was conducted on larger (20 by 35 m) plots in 1998, in addition to carrying out tests on the smaller plots as in 1997. Plots and blocks in the larger plots were separated by 4 m alleys. The larger plots were included in 1998 to try to reduce the possible effects of arthropod movements from sprayed into adjacent control plots. Plots were laid out in randomized complete block design with four replications. All plots were planted on 26 May in 1997 and 1998.

Adults and nymphs of *L. phyllopus* were collected from the field on several host plants such as thistle (*Cirsium* spp.), *Pyrrhopappus carolinianus* (Walter) DC., and tomato. Bugs were reared in the laboratory in plexiglass cages (51 by 53 by 36 cm) at 28.7 ± 0.5°C and 55.8 ± 2.0% RH with 14: 10 [L:D]h photoperiod. Fresh green beans (*Vigna* spp.) and sunflower seeds (*Helianthus* spp.), supplemented with corn (*Zea mays* L.) and okra (*Abelmoschus esculenta* [L.]), were given as food. Water was provided by a moistened wick in a plastic cup of water. Wooden applicator sticks (15 cm long by 0.2 cm diameter), inserted in lumps of modeling clay, were placed in the cages as oviposition sites (Mitchell & Mitchell 1986). Applicator sticks bearing egg masses were removed from the cages daily and kept in a freezer at –20°C until ready to be placed in the field. Powell & Shepard (1982)

found that freezing eggs of *Nezara viridula* (L.) did not affect their acceptability as hosts for the parasitoid, *T. basalis*.

The number and location of eggs on the stick were noted. Often, there was more than one egg mass per stick but these were treated as one mass. Mean number of eggs per stick ranged from 10 to 101 eggs in 1997 and 13 to 135 in 1998. Five egg masses were placed in each plot. Sticks bearing eggs were attached to stems or petioles with paper clips and marked with surveyor's tape for ease of locating eggs later. Eggs were placed in the field during early pod-fill, mid- pod-fill and late pod-fill 24 h after chemical applications were made, and collected five days later. Eggs remaining on sticks were counted and all missing eggs and those that were partially removed were presumed attacked by predators. Recovered eggs were held in test tubes covered with nylon mesh and maintained in the rearing room at 28.7 ± 0.5°C and 55.8 ± 2.0% RH with 14: 10 [L: D] h photoperiod until parasitoids emerged. Parasitism was determined by counting parasitoids that emerged or their exit holes and by dissecting whole eggs to detect parasitoids that had not emerged. Eggs were classified as: 1) eaten, 2) chewed, with part of the chorion remaining, 3) parasitized, with the presence of an exit hole or parasitoid, 4) intact, hemicylindrical in shape with no exit hole or parasitoid found within and, 5) collapsed, triangular in shape with collapsed sides and no exit hole or parasitoid (Mitchell et al. 1999). Percent parasitism per egg mass was calculated as parasitized eggs divided by parasitized plus intact eggs × 100. Chewed, eaten and collapsed eggs were excluded from calculations of parasitism. Scelionid parasitoids were identified using keys provided by Masner (1983) and were confirmed by Dr. Walker A. Jones of the USDA, Weslaco, Texas. Dr. J. B. Woolley of Texas A&M University, College Station, identified the encyrtid parasitoids. Percent predation per egg mass was calculated as number of eggs that were chewed plus number of completely eaten eggs, divided by total number of eggs in the mass placed in the field × 100.

Estimates of predator density were determined by visual observations made during the time eggs were in the field at 0730 h, 1230 h, and at 1730 h, and any predator near the egg masses or feeding on them was recorded. Observation data were augmented by pitfall traps in 1998. Traps were made from plastic cups (11.5 × 10 × 4 cm), which were half-filled with ethylene glycol (Super Tech Antifreeze/ Coolant, Alsip, Illinois). Cups were buried into the soil so that the lip of the cup was at ground level. Two traps were placed in each plot, two times during the season. Trap contents were collected after 5 d and arthropods were identified.

Statistical analyses. The data were averaged over number of egg masses to obtain means for analysis. Percentages were transformed to arcsine square root values to ensure homogeneity of variances before analyses (Gomez and Gomez 1984). Data for parasitism and predation were analyzed using the multivariate analysis of variance (MANOVA)/ANOVA for repeated measures, with pod-fill stages as the repeated measures (SAS Institute 1996). Means for main factors were separated using Fisher protected least significant difference (LSD) test ($P < 0.05$).

Results

The scelionid parasitoids, *Gryon carinatifrons* (Ashmead) and *G. pennsylvanicum* (Ashmead) were reared from the field-exposed egg masses in 1997. In

addition, an encyrtid, *Ooencyrtus* ?*leptoglossi* Yoshimoto was recovered from the eggs in 1998. *Gryon carinatifrons* was the most abundant parasitoid species, making up 83.9% of all parasitoids that emerged. *Gryon pennsylvanicum* and *O.* ?*leptoglossi* comprised 13.9% and 2.2% respectively. Only one parasitoid of all species emerged from a single egg. Voucher specimens of parasitoids were deposited in the Entomology Department Arthropod Museum at Clemson University.

The red imported fire ant, *Solenopsis invicta* Buren, was the most abundant predator based on pitfall trap collections (Table 1) and visual observations in the field. The ants were regularly seen removing eggs from the substrate. The snowy tree cricket, *Oecanthus fultoni* Walker chewed *L. phyllopus* eggs leaving chorion fragments on the substrate. The results from pitfall traps and visual observations also revealed that *Hippodamia convergens* Guérin-Méneville (Coccinellidae), *Coleomegilla maculata* (De Geer) (Coccinellidae), *Labidura riparia* (Pallas) (Labiduridae), *Gryllus* spp., *Geocoris* spp., *Nabis* spp., *P. maculiventris,* and *Pardosa milvina* (Hentz) (Lycosid spiders) were common in the field (Table 1), but they were not observed feeding on *L. phyllopus* eggs. Predators (probably *S. invicta*) that removed the entire eggs were responsible for 85.5% of total predation while chewing predators accounted for 15.5%.

In 1997, there was no significant interaction between the main factors of spray treatment and pod-fill stage (MANOVA, Wilks Lamda value = 0.5964; F = 1.6217, df = 4, 22; P = 0.2043). The main effects or season-long effects of treatment on percent parasitism and predation for the 2-yr study are summarized in Table 2. Although no significant effects of endosulfan treatment were detected when parasitism and predation were considered simultaneously (MANOVA, Wilks Lambda value = 0.1329; F = 6.5215, df = 2, 2; P = 0.1330), separate ANOVAs revealed that the insecticide significantly reduced percent parasitism (F = 14.10, df = 1, 12; P = 0.0330), but not percent predation (F = 0.03, df = 1, 12; P = 0.8713). Partial correlation matrices generated with the PRINTE option in the MANOVA for treatment showed that parasitism and predation were not significantly correlated with each other for either year. There was a significant effect of pod-fill stage (MANOVA, Wilks Lambda value = 0.1468; F = 8.8543, df = 4, 22; P = 0.0002). Parasitism was significantly lower at early pod-fill than at mid- or late pod-fill (F = 31.19, df = 2, 12; P = 0.0001). However, predation was not different at any of the pod-fill stages ((F = 0.74, df = 2, 12; P = 0.4964) (Fig. 1).

In 1998, in the 4 × 9 m plots, there was no significant treatment by pod-fill stage interaction (MANOVA, Wilks Lambda value = 0.5166; F = 2.1523, df = 4, 22; P = 0.1082). Neither insecticide treatment (MANOVA, Wilks Lambda value = 0.2888; F = 2.4617, df = 2, 2; P = 0.2889) nor pod-fill stage (MANOVA, Wilks Lambda value = 0.7796; F = 0.7293, df = 4, 22; P = 0.5816; Fig. 2) significantly influenced rates of parasitism and predation. The apparent lack of significant effects of endosulfan treatment in these plots was probably due to the large variation in percent parasitism, which ranged from 0-100% in control plots (Table 2).

In the larger plots (20 × 35 m) in 1998, no significant overall treatment effect on parasitism and predation were detected (MANOVA, Wilks Lambda value = 0.0716; F = 12.9720, df = 2, 2; P = 0.0716). However, examination of the ANOVAs again showed a significant reduction in percent parasitism (F = 16.08, df = 1, 12; P = 0.0278) but not percent predation (F = 1.10, df = 1, 12; P =

Table 1. Predator densities from pitfall traps in cowpea in 1998.

Predator species	Total density[a]
Solenopsis invicta	87
Labidura riparia	56
Geocoris punctipes	1
Coccinellid larvae	2
Pardosa milvina	26
Gryllus spp.	35

[a]The sum of predators from sixteen traps total in the field. Two traps were placed in each of four plots two times in the season and were collected after every 5 d.

0.3712) with endosulfan treatment (Table 2). Pod-fill stage (MANOVA, Wilks Lambda value = 0.3149; $F = 4.3004$, df = 4, 22; $P = 0.0101$) and treatment by pod-fill stage interaction (MANOVA, Wilks Lambda value = 0.2677; $F = 5.1308$, df = 4, 22; $P = 0.0457$) both showed significant effects on rates of parasitism and predation. Parasitism was significantly lower at early pod-fill than at mid- or late pod-fill ($F = 5.26$, df = 2, 12; $P = 0.0229$). Percent predation was higher at late pod-fill than at mid pod-fill ($F = 4.14$, df = 2, 12; $P = 0.0430$) (Fig. 3).

Neither percent chewed eggs nor those removed differed significantly between endosulfan-treated and untreated plots in 1997 (chewed: $F = 0.20$, df = 1, 12; $P = 0.6849$; removed: $F = 0.01$, df = 1, 12; $P = 0.9240$), 1998 small plots (chewed: $F = 0.01$, df = 1, 12; $P = 0.9567$; removed: $F = 2.47$, df = 1, 12; $P = 0.2140$) or 1998 large plots (chewed: $F = 7.36$, df = 1, 12; $P = 0.0730$; removed: $F = 0.49$, df = 1, 12; $P = 0.5355$) (Table 3).

Table 2. Season-long percent parasitism and predation (mean ± SEM) on eggs of *L. phyllopus* in cowpea plots receiving endosulfan sprays and in control plots.

Treatment	N	Percent parasitism (Mean ± SEM)	Percent predation (Mean ± SEM)
		1997 (4 by 9 m plots)	
Sprayed	12	7.1 ± 2.2a	18.5 ± 4.3a
Control	12	14.5 ± 3.2b	22.0 ± 6.0a
		1998 (4 by 9 m plots)	
Sprayed	12	2.6 ± 1.0a	32.1 ± 5.0a
Control	12	21.8 ± 8.7a	48.3 ± 7.9a
		1998 (20 by 35 m plots)	
Sprayed	12	4.2 ± 1.9a	24.6 ± 4.8a
Control	12	14.4 ± 4.2b	34.3 ± 4.8a

Within column means ± SE followed by the same letter are not significantly different ($P > 0.05$).

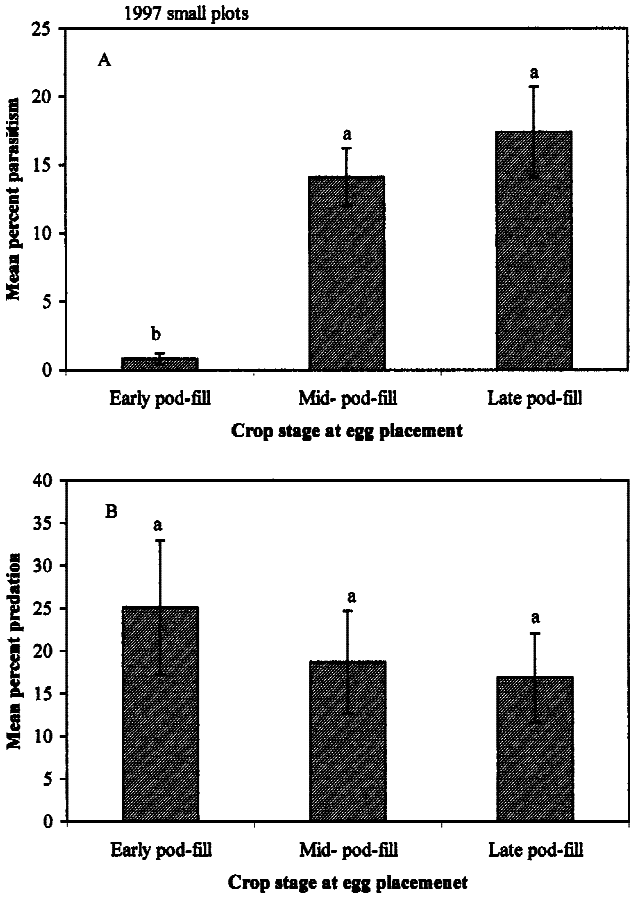
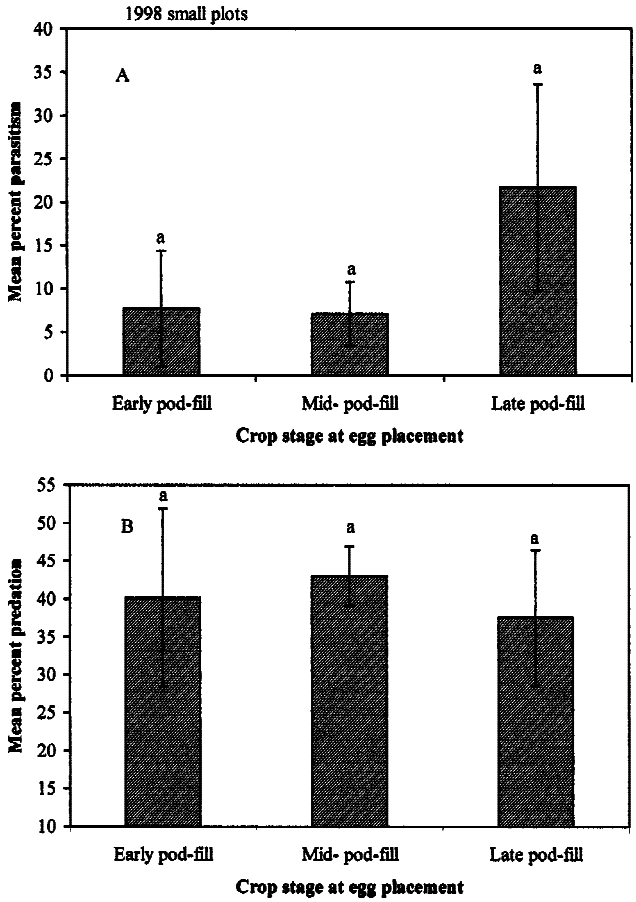


Fig. 1. Mean (± SEM) percent parasitism and predation on eggs of *L. phyllopus* at different stages of pod-filling in cowpea in 1997. Parasitism and predation were measured by placing five egg masses at each pod-fill stage in treated and untreated plots for 5 d. Means are for eight plots. (A) Percent parasitism, (B) Percent predation. Bars with different letters are significantly different ($P < 0.05$; LSD).

Discussion

These studies revealed that three endosulfan treatments generally impacted negatively on parasitoids but not predators of *L. phyllopus* eggs (Table 2). In two out of the three tests, parasitism was lower in treated plots compared with controls. These results agree with those of Justo (1994), who reported that parasitism of *N. viridula* eggs by *T. basalis* in tomato was significantly reduced by esfenvalerate treatments compared with controls. Likewise, he found that predation was not significantly different between these treatments. Croft & Brown (1975) reported that parasitoids are more susceptible to insecticides than predators. Per-



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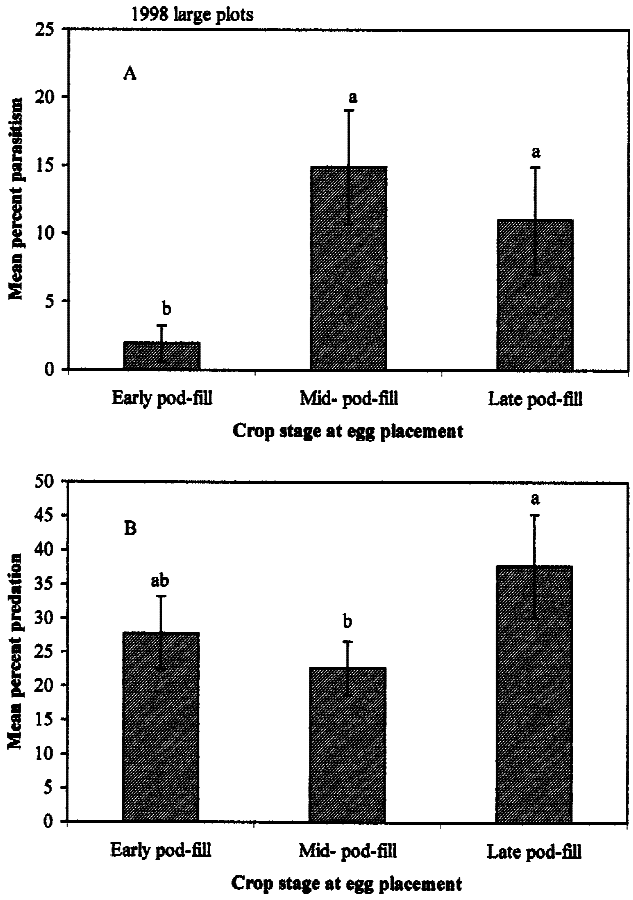
haps this is because parasitoids, unlike generalist predators, search extensively for their prey, which increases their contact with insecticide residues. Barlett (1963) tested the toxicity of several insecticides and reported that endosulfan was highly toxic to hymenopteran parasitoids.

Parasitism was generally low at early pod-fill and increased as the season progressed. Similar phenologies were reported for *Gryon flavipes* (Ashmead) on eggs of the rice ear bug, *Leptocorisa oratorius* (Fabricius) (Rothschild 1970), *G. carinatifrons* and *G. pennsylvanicum* on *L. phyllopus* eggs in cowpea (Mitchell et al. 1999), and for egg parasitoids of the harlequin bug, *Murgantia histrionica* Hahn (Ludwig & Kok 1998). Taylor (1975) and Matteson (1981) also reported that



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parasitism on eggs of *Clavigralla tomentosicollis* Stål by *Gryon gnidus* Nixon was low when bug eggs first appeared in the field but peaked late in the season. They observed that this mortality did not prevent the bug population from reaching damaging levels. A previous study showed that early pod-fill is the most susceptible stage of cowpea to pod-sucking bug damage (Abudulai & Shepard 2001). Thus, the low rates of parasitism at early pod-fill in the present study may not have reduced *L. phyllopus* populations enough to prevent economic damage in cowpea.

More eggs were removed than were chewed, which indicated that predators such as *S. invicta* that removed eggs contributed more to *L. phyllopus* egg mor-

Table 3. Season-long percent predation (mean ± SEM) on eggs of *L. phyllopus* by predators that chewed eggs and left part of chorion on substrate and by predators that removed eggs, in cowpea plots receiving endosulfan sprays and in control plots.

Treatment	N	Predation (%) (eggs chewed) (Mean ± SEM)	Predation (%) (eggs removed) (Mean ± SEM)
		1997 (4 by 9 m plots)	
Sprayed	12	2.1 ± 1.0a	16.3 ± 4.5a
Control	12	4.1 ± 1.7a	18.0 ± 6.0a
		1998 (4 by 9 m plots)	
Sprayed	12	3.0 ± 1.3a	29.4 ± 5.0a
Control	12	2.8 ± 1.2a	45.5 ± 8.1a
		1998 (20 by 35 m plots)	
Sprayed	12	5.9 ± 2.1a	18.6 ± 3.6a
Control	12	10.0 ± 2.2a	24.2 ± 4.4a

Within column means ± SE followed by the same letter are not significantly different ($P > 0.05$).

tality than did chewing predators such as *O. fultoni*. Other workers also have reported that *S. invicta* are major predators of insect eggs in the field (Regsdale et al. 1981, Stam et al. 1987, Justo 1994, Zenger & Gibb 2001). Although we did not observe many of the predator species that were collected in pitfall traps feeding on egg masses, it is possible they contributed to total egg predation in the field. Using an enzyme linked immunosorbent assay (ELISA), Regsdale et al. (1981) reported that *G. punctipes*, *C. maculata* and *P. maculiventris* were important predators of *N. viridula* eggs in soybean.

Overall percent predation on *L. phyllopus* eggs was higher than was percent parasitism (Table 2). However, predators probably removed both parasitized and unparasitized eggs, which complicates calculation of parasitism.

In conclusion, endosulfan treatment affected *L. phyllopus* egg mortality by parasitoids, but not by predators. However, both predators and parasitoids are important sources of *L. phyllopus* egg mortality. Because parasitoid populations build up late in the season after most bug damage to cowpea has occurred, their potential as a source of mortality may not be fully realized. Therefore, augmentative or inundative releases of parasitoids early in the season, coupled with cultural techniques that enhance the build-up of natural enemies in alternate hosts and judicious use of less toxic insecticides may be part of an integrated pest management program for *L. phyllopus* in cowpea.

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Effects of Temperature on Development and Reproduction of a Predatory Beetle, *Nephus includens* Kirsch (Coleoptera: Coccinellidae)[1]

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ABSTRACT The effect of different temperatures on some biological properties of *Nephus includens* Kirsch (Coleoptera: Coccinellidae) was investigated. This species is one of the most important predators of *Planococcus citri* Risso (Homoptera: Pseudococcidae). The development time, mortality and fecundity were determined at constant temperatures of 15, 20, 25, 30, and 35°C and at the variable temperatures, 25–35°C (12 hours 25°C, 12 hours 35°C). Life tables were also constructed for 25, 30, 35, and 25–35°C. The mortality was lower and the mean generation time was shorter at 30°C than at all other temperatures except 35°C. The intrinsic rate of increase was the highest at 30°C (0.081), followed by 0.076 at 25–35°C. The net reproductive rate was higher at 25–35°C than at 30°C. From biological data and population growth parameters calculated from the life tables, 30°C and 25–35°C were determined to be the most suitable temperatures for mass rearing of *Nephus includens*. However, mass rearing at a temperature as high as 35°C could cause deterioration of sprouted potatoes on which the citrus mealybug is reared. Therefore, 30°C would be better than 25–35°C.

KEY WORDS *Nephus includens*, citrus mealybug, mass-rearing, biological control

The citrus mealybug, *Planococcus citri* Risso (Homoptera: Pseudococcidae) is one of the most important citrus pests in Turkey. It causes significant damage by feeding on all parts of citrus except the roots. It sometimes causes 30–60% fruit drop, which ranks it as a key pest in orchards where the natural balance of the pest predator complex has been damaged by overspraying of pesticides (Ozkan et al. 1991).

Biological control against the citrus mealybug has been carried out since 1972, with releases of the ladybeetle, *Cryptolaemus montrouzieri* Mulsant (Coleoptera; Coccinellidae) and the parasitoid, *Leptomastix dactilopii* Howard (Hymenoptera: Encyrtidae). These beneficial insects have been mass reared and sold to farmers. However, these nonindigenous species are not capable of overwintering in Tur-

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key, probably due to inability to survive low temperature and/or lack of alternative prey (Bodenheimer 1951, Ebeling 1959, Kececioglu 1975, Uygun 1981, Soylu et al.1983). Therefore, they must be mass-reared and released every year. On the other hand, about 27 natural enemies of citrus mealybug have been reported in the Mediterranean region and they suppress this pest in orchards where broad-spectrum pesticides are not used (Soylu & Urel 1977, Kansu & Uygun 1980, Uygun et al. 1991, Uygun et al. 1992). One of the most promising of these is the ladybeetle, *Nephus includens* Kirsch (Soylu & Urel 1977, Kansu & Uygun 1980, Uygun 1981, and Soylu et al.1983).

The objective of this study was to assess some biological properties of *N. includens* at different temperatures to serve as a basis for the use of this predatory coccinelid in a biological control program. Development time and mortality of different immature stages, longevity and fecundity were determined. Life tables were constructed using these data.

Materials and Methods

Maintenance of citrus mealybug colony. Immature stages were collected from Washington navel citrus trees in the orchard of Adana Plant Protection Research Institute, Adana, Turkey in 1993 and were used to infest sprouted potatoes in crispers (26 by 35 by 6 cm). This colony was maintained throughout the study.

Maintenance of *Nephus includens*. Sprouted potatoes infested with citrus mealybugs in two crispers were put into rearing cages (45 by 60 by 60 cm) and predators were released into cages at the rate of 30 individuals/crisper, 60 individuals/cage. When needed, additional food was added and new cages were established by the same method when potatoes became too old to support the mealybugs.

Development times and mortality rates of *Nephus includens*. This test was conducted at constant temperatures of 15, 20, 25, 30, 35°C and at fluctuating temperatures of 25–35°C (12 hours 25°C, 12 hours 35°C). 20–25 adults of *N. includens* were placed for 24 hours in plexiglas containers (25 cm diameter × 30 cm height) containing sprouted potatoes infested with citrus mealybug. Eggs were collected from the containers and put in petri dishes. The larvae that hatched were placed singly on navel citrus fruits infested with citrus mealybugs, using a thin brush. A plastic ring (3-cm diam. by 2-cm height) which had mesh cloth on the top was placed over the larvae on each fruit. Eggs and larvae were checked twice daily and development time and mortality of eggs, larvae, and pupae were determined. Trials were conducted using a completely randomized design, with 50 replicates of the egg stage and 30 replicates of the larval and pupal stages.

Preoviposition, oviposition, postoviposition, and progeny of *Nephus includens*. Adults of *N. includens* that had newly emerged were put onto navel citrus fruit infested with citrus mealybugs. The fruit was placed in plexiglas cells 4 cm in diameter, and 3 cm in height. The predators were supplied with more citrus mealybugs daily than they could consume and they were kept in these cells until they died. They were checked daily for preoviposition, oviposition, postoviposition and progeny. There were 5 replicates of the treatments in a completely randomized design. Data were analyzed using the MSTAT-C computing package.

Table 1. Development times for immature stages of *Nephus includens* reared at different temperatures in the laboratory.

Temp. (°C)	n	Mean development time (days) and range for each stage[a]						Total
		Egg	1st ins.	2nd ins.	3rd ins.	4th ins.	Pupa	
15	30	26.1a (23–29.3)	No larval development					
20	30	9.7b (8.9–11.3)	6.2a (4.2–7.8)	3.9a (2.8–4.8)	4.5a (3.5–5)	6.9a (4.2–8.7)	17.6a (16.8–19)	48.8a (44.1–52.2)
25	30	7.3c (5.6–8.6)	3.3b (3.0–4.1)	2.3b (1.9–3.0)	2.6b (1.9–3.4)	3.6b (3.0–4.9)	12.3b (9.9–14.1)	31.4b (29.3–33.8)
30	30	5.4e (4.4–6.1)	3.0cd (2.4–3.3)	2.0cd (1.4–2.6)	2.2c (1.9–2.6)	3.1c (2.1–4.0)	8.9c (7.0–10.2)	24.6d (21.7–26.3)
35	30	4.6f (3.4–5.3)	2.8d (2.0–3.3)	1.9d (1.1–2.4)	2.1cd (1.3–3)	2.5d (2.0–3.4)	8.1d (6.2–9.1)	22.0e (20.2–24.6)
25–35	30	6.1d (5.3–6.4)	3.2bc (3.0–3.9)	2.2c (1.8–3.0)	2.0d (1.8–3.1)	3.1c (2.3–3.9)	8.8c (7.7–9.8)	25.4c (23.7–26.8)

[a]Means followed by same letter within a column are not significantly different, at $\alpha = 0.05$.

Table 2. Mortality rates of *Nephus includens* for immature stages reared at different temperatures in the laboratory.

Temp. (°C)	%Mortality for egg stage		%Mortality for larval and pupal stages						Total mortality (%)
	n	%	n	1^{st}	2^{nd}	3^{rd}	4^{th}	Pupa	
15	50	80	30	—	—	—	—	—	—
20	50	26	30	23.3	3.3	6.7	0.0	13.3	55.6
25	50	24	30	16.7	0.0	0.0	0.0	0.0	36.7
30	50	18	30	20.0	0.0	0.0	0.0	0.0	34.4
35	50	34	30	23.3	10.0	13.3	10.0	20.0	71.6
25–35	50	28	30	26.0	0.0	0.0	0.0	0.0	46.7

Life tables of *Nephus includens* reared at different temperatures. The method of Southwood (1978) was used to construct the life tables. The formula is $\Sigma\ l_x.m_x.e^{-\ r\ m\ \cdot\ x} = 1$ where: l_x = percentage survival at age x, m_x = expected number of daughters per female at age × (female/female/day), e = natural log base, r_m = intrinsic rate of increase, × = age of females in days.

R_o is a parameter that defines the number of daughters that replace an average female during her life. The method of estimating R_o from information in the life table is the sum of the reproduction expectation ($\Sigma l_x.m_x$) for each age group. The mean period elapsing from birth of parents to birth of offspring (T) was estimated by the formula of Laing (1968): $T = \log_e R_o/r_m$

Result and Discussion

Development times and mortality rates of *N. includens*. The incubation period of eggs decreased significantly as temperature increased. The period was 26.1 days at 15°C and 4.6 days at 35°C (Table 1). Although 5–6 eggs hatched at 15°C, larval development was not observed. Larval and pupal development times at other temperatures decreased as temperature increased. Development time for immature stages was the longest at 20°C (48.8 days) and shortest at 35°C (22 days) (Table 1), and differences between temperatures were statistically significant.

Tranfaglia and Viggianni (1973) reported that the development time from hatching to adult of *N. includens* was 25.9 days at 25–27°C. This period is between the 31.4 days at 25°C and 24.6 days at 30°C reported in this test. Soylu et al. (1983) found development times to be 32.6 and 23.3 days at 25 and 28°C, respectively which are similar to the results that we obtained.

The mortality rate of eggs was higher than that of any other stage except at 30°C. Total mortality decreased as temperature increased up to 30°C. Mortality was highest at 35°C with 71.6% (Table 2). Temperatures higher than 30°C are not suitable for development of *N. includens.* Tranfaglia and Viggiani (1973) found that the mortality rate of eggs was 29% at 25–27°C, which is similar to the 24% at 25°C found in this test. Yigit (1989) stated that the mortality rate of eggs and 1^{st} instar larvae of the coccinellid *Stethorus punctillum* Weise decreased as temperature increased to 30°C; these results are similar to ours.

Table 3. Preoviposition, oviposition, and postoviposition periods and longevity of *Nephus includens* adults reared at different temperatures in the laboratory.

Temp (°C)	Mean longevity of females and range for each period (days)[a]				Mean longevity and range (days)[b]			
	n	Pre.	Ovi.	Post	n	Female	n	Male
15	—	—	—	—	11	105aA (24–201)	13	85aA (28–184)
20	—	—	—	—	10	91abA (52–157)	10	80aA (51–147)
25	9	5.7a (4–7)	45.8a (19–66)	21.7a (6–56)	14	70bcA (19–129)	9	78abA (17–134)
30	11	4.6a (3–9)	41.2a (22–62)	21.1a (1–55)	15	69cA (26–117)	12	77abA (35–105)
35	15	3.2b (2–7)	33.8a (12–55)	18.0a (2–37)	18	57aC (12–89)	21	55bA (11–84)
25–35	10	5.4a (4–11)	48.2a (12–72)	20.5a (1–36)	14	61cA (23–117)	12	67abA (17–120)

[a]Means within a column followed by the same letter (lower case) are not significantly different at $\alpha = 0.05$.
[b]Means within a line followed by the same letter (upper case) are not significantly different at $\alpha = 0.05$ (t test).

Table 4. Total and daily eggs produced by *Nephus includens* at different temperatures in the laboratory.

Temperatures (°C)	n	Mean no. total eggs/female[a]	Mean no. eggs/ female/day
25	14	133.5a (52–264)	2.4 (0.0–4.0)
30	15	123.0a (56–257)	2.1 (0.8–3.5)
35	18	50.8b (9–103)	1.7 (0.0–5.9)
25–35	14	123.4a (23–244)	2.3 (0.7–4.8)

[a]Means within a column followed by the same letter are not significantly different at $\alpha = 0.05$.

Preoviposition, oviposition, postoviposition, and progeny of *Nephus includens*. Increasing temperature decreased longevity of adults (Table 3). The highest female longevity was 105 days at 15°C and the lowest was 57 days at 35°C. Longevity for females at 15°C was significantly different from longevity of females reared at other temperatures except 20°C. Male longevity was longest at 15°C (85 days) and decreased as temperature increased. Differences in male longevity between temperatures were not significant except between 15 and 35°C.

Tranfaglia and Viggiani (1973) reported that the female longevity of *N. includens* was 74 days and the male longevity 62 days at 25–27°C and 50–70% relative humidity. While the 74 days for female longevity is similar to our result (70 days) at 25 C°, male longevity was quite different in the two studies (62 versus 78).

The preoviposition, oviposition and postovoposition periods decreased as temperature increased (Table 3). The 10.8 days of preoviposition at 25-27°C determined by Tranfaglia and Viggiani (1973) is different from the 5.7 days at 25°C in this study.

The total number of eggs laid by *N.includens* at 25, 30, and 35 also decreased with increasing temperature (Table 4). Egg numbers were highest (133.5) at 25°C and lowest (50.8) at 35°C. However, temperature did not influence total egg numbers except at 35°C, which resulted in significantly lower numbers (50.8). Tranfaglia and Viggiani (1973) stated that *N. includens* laid 150.9 eggs at 25-27°C. This amount differs from 133.5 at 25°C and 123 at 30°C in this study.

Life tables of *Nephus includens* reared at different temperatures. Life graphs of *N. includens* for different temperatures were constructed using life tables (Fig. 1). One of the important factors that affects population growth is starting time for egg deposition. Females at 35, 30, 25–35 and 25°C started to lay eggs on the 26^{th}, $29^{th,}$ 31st and 38^{th} day, respectively (Fig. 1). Early egg deposition is another important parameter for population growth. Females at 25, 30, 35, and 25–35°C laid about 50 % of their total eggs by the 58^{th}, 52^{nd}, 32^{nd} and 55^{th} day, respectively.

Total mortality rates were higher at 35 and 25–35 °C than at 25 and 30°C, and mortality was seen earlier. Mortality rates were 36.7, 34.4, 71.6 and 46.7 at 25, 30, 35 and 25–35°C, respectively (Table 2).

The net reproductive rate (R_o), the mean length of a generation (T) and the intrinsic rate of increase (r_m) were calculated from life tables (Table 5). R_o, the

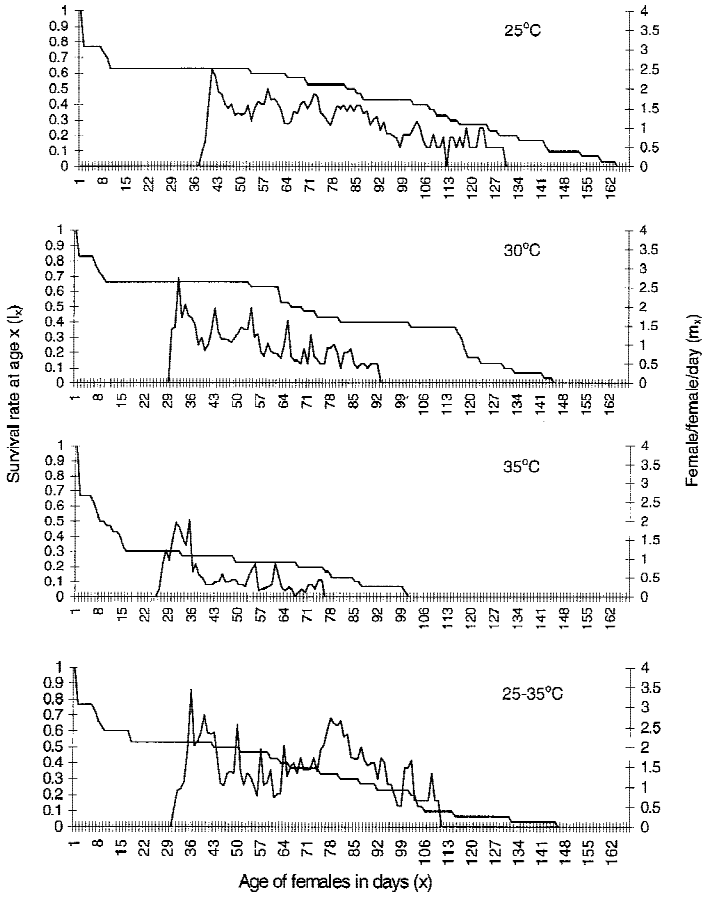

Fig. 1. Life graphs of *Nephus includens* reared at different temperatures in the laboratory.

number of daughters that replace an average female during her life, was highest at 25°C, with 54. 4. R_0 was 38, 7.7 and 46.1 at 30, 35 and 25–35°C, respectively. Mean generation time (T) was longest (59.6 days) at 25°C, and was 44.9, 38.4 and 50.4 days at 30, 35 and 25–35°C, respectively. The intrinsic rate of increase (r_m) was highest at 30°C (0.081), followed by 0.076 at 25–35°C, 0.067 at 25°C and 0.053 at 35°C.

Conclusions

As one of the most effective indigenous predators of the citrus mealybug, *N. includens* could be a valuable component of a biological control program if suitable mass-rearing procedures can be developed for augmentative releases. In this study we demonstrated that the most suitable rearing temperatures for *N. includens* were 30 and 25–35°C because the intrinsic rate of increase (r_m) was

Table 5. Net reproductive rate (R_o), intrinsic rate of increase (r_m) and mean generation time (T) of *Nephus includens* reared at different temperatures in the laboratory.

Temp. (°C)	R_o	r_m	T (days)
25	54.4	0.067	59.6
30	38.0	0.081	44.9
35	7.7	0.053	38.4
25–35	46.1	0.076	50.4

highest at 30°C and very close to the highest at 25–35°C. In addition, mortality rates for immature stages were less and mean generation time (T) was shorter at 30°C than for other temperatures. However, mass rearing at temperatures as high as 35°C could cause deterioration of sprouted potatoes on which the citrus mealybug grows. Therefore 30°C would be better than 25–35°C for mass rearing of *N. includens*

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Efficacy of Daily Oral Treatments of Ivermectin Administered to Cattle Infested with *Boophilus microplus* (Acari: Ixodidae)[1,2]

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ABSTRACT The efficacy of ivermectin administered orally to cattle infested with all parasitic stages of *Boophilus microplus* (Canestrini) ticks was evaluated. Ivermectin capsules were administered to two separate groups of cattle at a dose rate of either 25 or 50 μg/kg for a period of 21 consecutive days. A third group of calves received a placebo capsule each day and served as a control. Although the overall control achieved at both doses of ivermectin was >99% against all parasitic stages, the 50 μg/kg/d dose was significantly more effective than the 25 μg/kg/d dose against each developmental stage of the tick. Each ivermectin treatment dose produced a significantly higher percentage reduction in female tick numbers against ticks that were adults at the time of treatment onset than was observed against immature ticks (nymphs and larvae), however the 50 μg/kg/d treatment was significantly more effective in reducing tick numbers, regardless of the developmental stage of the ticks. Both engorgement weight and egg mass weight of females were significantly lower in the ivermectin treated groups than were observed in the untreated group. The potential applicability for treating tick-infested cattle with different delivery systems, such as daily oral treatments, boluses, and medicated feed that contain ivermectin or other macrocyclic lactone compounds is also discussed.

KEY WORDS ivermectin, *Boophilus microplus,* oral treatment, macrocyclic lactone, cattle tick, acaricidal activity

The discovery and development of avermectin endectocides, of which ivermectin is perhaps the foremost example (Hotson 1981), has provided the opportunity for evaluation of these compounds for the control and management of livestock ectoparasites. The avermectins offer the dual advantage of having broad spectrum activity and efficacy at extremely low concentrations (Putter et al. 1981). Numerous investigations with various tick species have been conducted which demonstrate that avermectins would be excellent candidates for use in programs

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where control of the pest species is the ultimate objective (Nolan et al. 1981, Drummond et al. 1981, Lancaster et al. 1982, Horak et al. 1983, Pegram & Lemche 1985, Cramer et al. 1988, Miller et al. 1989; 1997, Taylor & Kenny 1990, and Soll et al. 1989; 1990). However, with perhaps one notable exception (Miller et al. 1999), there is a paucity of information regarding the potential applicability of avermectins in a program where eradication of the pest is the goal.

The United States *Boophilus* Eradication Program has been in continuous operation in the continental U.S. since 1906. Presently *Boophilus* spp. ticks have been eradicated throughout the country, except for 8 counties that lie along the Texas-Mexico border, where a permanent quarantine is maintained by the U.S. Department of Agriculture, Animal Plant Health Inspection Service, Veterinary Services. Program operational procedures, as they are currently applied, rely solely on the use of an organophosphate (OP) acaricide, coumaphos, as the principal means of preventing the re-introduction of *Boophilus* ticks. Due to several factors associated with the reliance on this single acaricide, such as re-registration of the material and the widespread occurrence of OP resistance in tick populations in Mexico, there is a critical need to identify and develop alternative acaricides and treatment methods which may have potential for use in the Cattle Fever Tick Eradication Program in the United States.

In the current study, the primary objective was to determine whether the sustained application of ivermectin administered to tick-infested cattle provides a standard of control that would be acceptable in an eradication program. The secondary objective was to obtain data that can be used in the future development of sustained release technologies, such as boluses or medicated feed systems for use in a systematic treatment eradication program.

Materials and Methods

This study was conducted at the USDA, Agricultural Research Service, Cattle Fever Tick Research Laboratory in Mission, Texas, a certified quarantine facility where research on *Boophilus* spp. ticks is conducted in support of the eradication program.

Experimental design. Nine Hereford heifer calves weighing approximately 190 kg each were randomly assigned to three groups of three each. Throughout the study each calf was stanchioned individually in a 3.3 × 3.3 m stall in an open-sided barn under ambient conditions, except that no direct rainfall or sunlight reached the cattle. Fourteen days before the initiation of oral ivermectin treatments each calf was infested with ca. 5,000 *B. microplus* larvae that were 2–4 wk of age. Additional larval infestations of ca. 5,000 each were made at 7 d and before the beginning of oral treatments. This infestation regime provided the means for evaluating the effects of oral treatments against ticks that were in the early stages of adult development (14-d pretreatment infestation), nymphal development (7-d pretreatment infestation) and newly infesting larvae (0-d pretreatment infestation) at the time oral treatments were initiated.

Engorged female ticks were collected and counted daily beginning on the first d when they began to detach from the calves (21 d after the first pretreatment infestation and 7 d after the oral treatments were initiated). The daily tick collections were continued for 28 d after the last pretreatment infestation (7 d after the oral treatments were terminated). Random samples of up to 10 females

per day per calf (whenever possible) were saved to obtain data on the oviposition capability of the surviving ticks. Females within each sample (≤10) were weighed collectively, placed in a coded 25 × 95 mm (8-dram) shell vial with a cotton stopper and stored in an incubator at 27 ± 2°C, 92% RH, under a 12:12 [L:D]h photoperiod and allowed to oviposit for 20 d. After oviposition was complete females were discarded and the eggs produced by females in each sample were weighed and returned to the incubator. After 4 wk the percentage egg hatch of each sample group was visually estimated by observing the contents of the vial under a stereo microscope with a grid background and comparing the proportion of larvae to the proportion of unhatched eggs within the vial.

When all data on daily tick counts and engorgement weight, egg mass weight, and percentage egg hatch of saved females were complete, the daily index of fecundity (IF) of the ticks recovered from each calf in each of the three groups of animals was calculated. The IF is an estimate of the reproductive potential of the ticks that survived to repletion following the onset of oral ivermectin treatments, and is derived from the index of reproduction (IR) formula described by Drummond et al. (1967):

$$\text{No. of } ♀♀ \text{ collected} \times \frac{\text{Weight of eggs (g)}}{\text{No. of } ♀♀ \text{ saved}} \times \text{Egg hatch (\%)} = \text{IF}$$

Treatment procedures. The efficacy of two different oral doses of ivermectin administered daily was evaluated in the study. Before initiation of the daily oral treatments each calf was weighed individually, so that the appropriate treatment dose could be calculated. Formulation of each treatment dose for each calf was conducted by weighing the appropriate amount of drug (ivermectin), based on the individual weight of each calf, then loading the ivermectin into a gelatin capsule with enough whole wheat flour to fill the remainder of the capsule. The formulated capsules were placed in prescription bottles marked for use on each individual calf. A sufficient number of capsules were made so that each calf could be treated for a period of 21 consecutive days. A standard balling gun was used to administer a single individual capsule to each animal for a period of 21 consecutive days following initiation of the oral treatments.

One group of cattle (n = 3) received a daily oral dose of 25 μg of ivermectin per kg of body weight. A second group of cattle (n = 3) received a dose of 50 μg/kg/d of ivermectin, and the third group of calves (n = 3) received a placebo containing only the whole wheat flour, thus serving as an untreated control group.

Blood sample procedures. Immediately before initiation of the daily oral treatments (day 0) and at 3, 7, 10, 14, 17, 21, 24, and 28 d after oral treatments began, blood samples were collected from the jugular vein of each calf. Whole blood samples were collected in 13-mL SST vacutainer tubes (Becton Dickinson, Franklin Lakes, New Jersey). Blood samples were allowed to clot for 1 h at room temperature, then centrifuged at 2,500 rpm for 30 min to separate the serum. The serum (5 mL) was poured into a coded plastic holding vial, sealed and frozen at –20°C for later analysis. Samples were analyzed for ivermectin concentration by placing 5 μL of serum in a liquid chromatography column and determining the absorption rate for ivermectin in an HPLC analyzer according to the technique described by Oehler & Miller (1989) that enables quantification of as little as 2 ppb of ivermectin in 5 μL of serum.

Data classification. The timing of pretreatment infestations (14, 7, and 0 d before onset of treatment), the interval between infestations (7 d), and the known parasitic development and detachment patterns of *B. microplus* (95% of all ticks infested at a given time detaching at 21–27 d following infestation (Hitchcock 1955)), provided a means for classifying and estimating the effect of the ivermectin treatments on tick numbers and the IF (index of fecundity) values by individual parasitic development stage. Using this information, a classification system was devised such that females collected 7–13 d after treatments began were considered to be adults at the time treatments were initiated. Females collected 14–20 d after treatments began were classified as nymphs at the time of treatment initiation; and females recovered 21–27 d after treatments began were classified as larvae at the time of treatment initiation.

Once the daily tick numbers and IF values for each calf within each treatment or control group over the entire study period were obtained, the numbers and values were summed across each of the classification categories described above to provide a mean total tick number and IF value for each treatment or control group. The percentage control afforded by each treatment against the various parasitic life stages of the tick were calculated by comparing the total IF value of each treated group with the total IF value of the untreated group within the same classification category using the following modified Abbott's formula (Abbott 1925):

$$\frac{\text{Total IF of untreated} - \text{Total IF of treated}}{\text{Total IF of untreated}} \times 100 = \% \text{ Control}$$

Data analysis. Data obtained on concentration of ivermectin in blood serum in the two treated groups were subjected to *t*-test analysis on each day samples were taken (SPSS, Inc. 1997). Remaining data were subjected to a two-way analysis of variance with life stage and treatment dose as the main factors to determine differences. Differences among means was determined by Tukey's test (SPSS, Inc. 1997). Arcsine transformation was applied to the data expressed as percentages (percentage reduction of the number of ticks per animal and percentage control of the IF) prior to analysis.

Results

The ivermectin concentration in the blood serum of cattle treated at 50 μg/kg/d was significantly higher ($t = 4.3$; df = 4; $P = 0.02$) than the 25 μg/kg/d treatment group at all sampling periods between 3-28 d after oral treatments were initiated, with the exception of the 21 d sample, which occurred on the day oral treatments were terminated (Fig. 1). However, even on this sampling day (21 d), although the difference was not significant between the two treatment groups ($t = 2.5$; df = 4; $P = 0.07$), it was substantial. At the 25 μg/kg/d treatment dose, the ivermectin concentration did not reach 6 ppb until 10 d following the initiation of oral treatments, which is the approximate concentration at which it remained through day 21. By day 28 (7 d after oral treatments were terminated), the ivermectin concentration decreased to undetectable levels (<2 ppb). At the 50 μg/kg/d treatment dose the ivermectin concentration in the blood reached 6 ppb at 3 d following the initiation of oral treatment and continued to increase to a

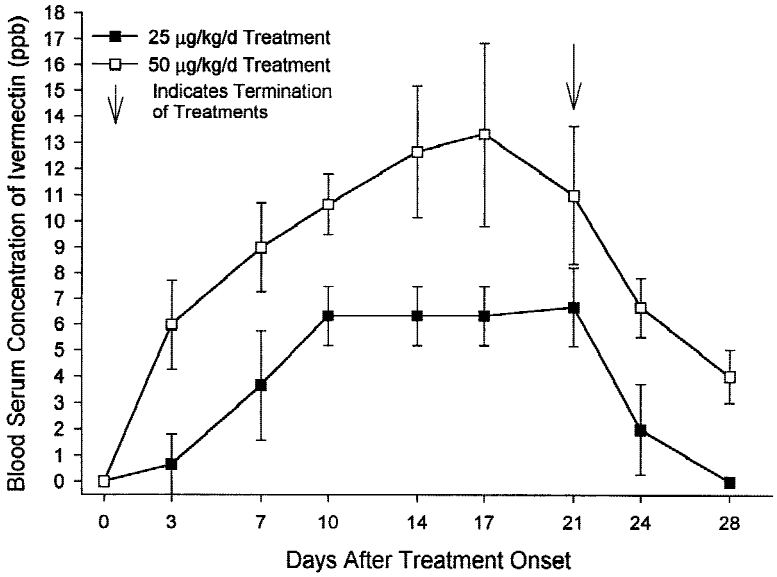

Fig. 1. Mean and standard deviation of the ivermectin concentration in the blood serum of two groups of Hereford heifer calves administered daily oral treatments at dosages of 25 and 50 µg (active ingredient) per kg of body weight for 21 consecutive days.

peak concentration of 13.3 ppb at 17 d after treatments began. The ivermectin concentration did not drop below 6 ppb until 24 d following the onset of treatment, and it was still at 4 ppb on day 28 (7 d after oral treatments were terminated) when the study ended.

Both the life stage of the ticks and the treatment dose responded independently of each other (no interaction effect; $F = 2.9$; df = 2,12; $P = 0.1$) in their effect on the percentage reduction of the number of ticks per animal that reached repletion following the onset of daily oral ivermectin treatment (Table 1). Comparison of three life stages within each treatment dose showed there was a significantly greater percentage reduction in tick numbers ($F = 20.6$; df = 2,12; $P < 0.01$) for ticks that were adults at the time treatments were initiated than was observed for ticks that were either nymphs or larvae at treatment initiation. Comparison of the two treatment doses within each life stage showed that 50 µg/kg/d of ivermectin provided significantly greater percentage reduction ($F = 118.2$; df = 1, 12; $P < 0.0\ 1$) in the number of ticks per animal than the 25 µg/kg/d treatment, regardless of the life stage of the ticks at treatment onset.

The percentage control of the IF did not differ ($F = 0.5$; df = 2,12; $P > 0.6$) between the three life stages within either treatment dose (Table 1). However, the 50 µg/kg/d treatment provided a significantly higher level of control ($F = 51.5$; df = 1,12; $P < 0.01$) than was achieved against ticks treated at 25 µg/kg/d, regardless of the stage the ticks were in when treatment began. Analysis showed that control was consistently high (no interaction effect; $F = 0.2$; df = 2, 12; $P = 0.8$) against adults, nymphs, and larvae at both dosages.

Table 1. Mean ± standard deviation of the percentage reduction in ticks per animal, index of fecundity (IF), and percentage control of the IF obtained against *Boophilus microplus* females that were in different parasitic life stages at the time daily oral ivermectin treatments were initially administered to tick-infested cattle.

Parasitic life stage	Treatment dose			Dose within life stage
	Untreated	25 μg/kg/d	50 μg/kg/d	
	Percentage reduction of number of ticks per animal recovered[a]			
Adult	– (731)	92.4 ± 5.1 (56)	99.7 ± 0.3 (2)	
Nymph	– (1327)	82.9 ± 3.7 (227)	97.6 ± 1.3 (32)	$F = 118.2$; df = 1, 12; $P < 0.001$
Larva	– (1151)	71.9 ± 6.2 (323)	96.9 ± 0.8 (36)	
Life stage within dose		$F = 20.6$; df = 2, 12; $P < 0.001$		Stage × dose interaction: $F = 2.9$; df = 2, 12; $P > 0.05$
		Percentage control of the IF		
Adult	–	99.7 ± 0.2	>99.9 ± 0.006	
Nymph	–	99.7 ± 0.2	>99.9 ± 0.001	$F = 51.2$; df = 1, 12; $P < 0.001$
Larva	–	99.6 ± 0.2	>99.9 ± 0.001	
Life stage within dose		$F = 0.5$; df = 2, 12; $P > 0.06$		Stage × dose interaction: $F = 0.2$; df = 2, 12; $P > 0.8$

[a]Numbers in parentheses are the actual mean number of ticks per animal recovered.

The engorgement weight of females recovered from untreated and ivermectin treated cattle was significantly dependent (interaction effect; $F = 9.9$; df = 4, 17; $P < 0.01$) on both life stage and treatment dose (Table 2). Analysis of the life stage within each treatment dose showed that the engorgement weights of females recovered from untreated cattle were similar, regardless of the life stage they were in when ivermectin treatments were begun ($F = 12.7$; df = 2, 17; $P < 0.001$). However, in the 25 μg/kg/d treatment ticks that were adults at treatment onset weighed more than larval ticks, whereas ticks in the nymphal stage at treatment onset produced engorgement weights that were intermediate between that of adults and larvae. At the 50 μg/kg/d treatment, ticks treated as adults weighed more than immature ticks (nymphs or larvae), which had similar engorgement weights. Comparison of the effects of the three treatment doses within the various life stages showed that against ticks that were adults at treatment onset, engorgement weight of untreated females was higher than either of the ivermectin treated groups of females, which were similar ($F = 1156.0$; df = 2, 17; $P < 0.001$). Treatments initiated against both nymphs and larvae showed a dose response with untreated females weighing more than females treated at 25 μg/kg/d, which in turn weighed more than ticks treated at 50 μg/kg/d.

As with the engorgement weight of females, the egg mass weights produced by females recovered from untreated and ivermectin treated cattle was significantly dependent on both life stage and treatment dose (interaction effect; F = 4.1; df = 4, 17; $P < 0.02$) (Table 2). The life stage of the ticks within each of the three treatment groups had only a minimal effect on subsequent egg mass weights ($F = 1.7$; df = 2, 17; $P > 0.2$). The egg mass weights obtained from both untreated females and females treated at 50 μg/kg/d were similar, regardless of the life stage of the females at treatment onset. In the 25 μg/kg/d treatment group, females that were adults at treatment onset produced egg masses that weighed more than egg masses produced by females that were larvae at treatment initiation, with egg masses of nymphal ticks being intermediate between that of adults and larvae. Comparison of the effects of each treatment dose within each of the three life stages showed that against ticks in the adult stage at treatment initiation, there was a dose response, in which the weights of eggs produced by untreated females were higher than the 25 μg/kg/d group, which in turn were higher than the 50 μg/kg/d group ($F = 712.1$; df = 2, 17; $P < 0.001$). Against nymphal and larval ticks, the subsequent egg mass weights of untreated females were higher than either ivermectin treated group, which had similar egg mass weights.

Discussion

Results of the study demonstrated that daily oral treatments of ivermectin were highly efficacious against *B. microplus,* regardless of the life stage the ticks were in at the onset of treatment. Both treatment dosages (25 and 50 μg/kg/d) provided >99% control of ticks. However, the 50 μg/kg/d dose reached the maximum blood serum level achieved by the 25 μg/kg/d about three times faster, and was significantly more effective. The greater effectiveness of the 50 μg/kg/d dosage was probably because the ivermectin concentration in the blood of calves reached a lethal level more rapidly, was maintained at a higher level during treatment, and remained at a lethal level for a longer interval after treatment

Table 2. Mean ± standard deviation of female weight and egg mass weight of *Boophilus microplus* that were in different parasitic life stages at the time daily oral ivermectin treatments were initially administered to tick-infected cattle.

Parasitic life stage	Treatment dose			
	Untreated	25 μg/kg/d	50 μg/kg/d	Dose within life stage
		Engorged female weight (mg)		
Adult	354 ± 9.5	130 ± 9.7	123 ± 20.5	
Nymph	368 ± 16.1	102 ± 21.3	54 ± 14.6	$F = 1156$; df = 2, 17; $P < 0.001$
Larva	376 ± 14.0	83 ± 10.5	51 ± 1.5	
Life Stage within Dose	$F = 12.7$; df = 2, 17; $P < 0.001$			Stage × Dose Interaction: $F = 9.9$; df = 4, 17; $P < 0.001$
		Egg mass weight (mg)		
Adult	163 ± 15.3	45 ± 13.5	21 ± 2.8	
Nymph	176 ± 10.3	25 ± 11.5	5 ± 1.0	$F = 712.1$; df = 2,17; $P < 0.001$
Larva	181 ± 10.8	22 ± 6.0	4 ± 3.0	
Life Stage within Dose	$F = 1.7$; df = 2, 17; $P > 0.2$			Stage × Dose Interaction: $F = 4.1$; df = 4, 17; $P < 0.02$

was terminated, thus providing more time for the chemical to produce adverse effects on the reproductive processes in the ticks. These results were consistent with other studies conducted with ivermectin against various tick species. In a similar study, it was reported that a daily oral dose of Merck MK-933 (ivermectin) administered to cattle at 50 μg/kg/d was 95% effective against the 1-host tick, *Dermacentor albipictus* (Packard), as well as numerous 3-host ticks (Drummond et al. 1981). In another study, dosages of 20–80 μg/kg/d of ivermectin administered to cattle intraruminally (similar to daily oral doses) provided >99% control against *Rhipicephalus appendiculatus* Newmann, *R. evertsi* Newmann, and *Hyalomma truncatum* Koch (Soll et al. 1989). When Spanish goats, *Capra hircus* (L.) were administered daily oral doses of ivermectin at 20–50 μg/kg/d the blood serum level remained at ≥2 ppb, resulting in >95% reduction of larvae in the 3-host tick *Amblyomma americanum* (L.) (Miller et al. 1989).

In the majority of studies conducted with ivermectin against various tick species it has been reported that perhaps the most important measure of control was associated with the reduction of the reproductive potential of the female ticks (Drummond et al. 1981, Lancaster et al. 1982, Cramer et al. 1988, Soil et al. 1989; 1990). While the results of our study were consistent with the observations of these investigators, it was also evident that mortality of ticks was also an important factor in the overall control achieved. Within each treatment dose, a higher percentage reduction in tick numbers was observed in ticks that were in the adult stage when treatments were initiated than was observed for ticks in either the nymphal or larval stage at treatment initiation (Table 1). However, even though a higher percentage of female ticks were subsequently able to reach repletion when treatments were initiated against ticks in the nymphal or larval develop-

ment stage, these females had lower engorgement and egg mass weights as compared to ticks that were adults at treatment initiation (Table 2). Thus, although the overall control achieved was essentially the same during each parasitic stage, the level of control at different stages was apparently obtained by a different effect. When daily ivermectin treatments were initiated against ticks that were in the early stage of adult development, high mortality of the females occurred. This high mortality of early stage adult ticks may have been because these ticks were imbibing relatively large volumes of ivermectin in their blood meal as they underwent rapid engorgement at approximately the same time that ivermectin blood serum levels in the animals were at peak levels. On the other hand, it is possible that since immature ticks underwent one or two molts after ivermectin treatments were initiated (a time during which little or no blood is taken (Tatchell & Moorhouse 1968, Seifert et al. 1968)), a higher percentage of the ticks were able to survive because they did not imbibe enough ivermectin contaminated blood to cause death. By the time these ticks reached the adult stage, during which relatively large volumes of blood would have been taken in, it is possible that the ivermectin blood serum level in the animals was either constant or declining (Fig. 1), which allowed a greater percentage of ticks to reach repletion. However, the reduction in reproductive potential of these ticks suggests that although they may not have imbibed enough ivermectin contaminated blood to kill them, they were subjected to a sublethal level of the drug over a long enough period of time to cause a dramatic adverse effect on engorgement, fecundity, and fertility of the females. Our results seem to contrast with a study in which it was reported that there was no difference in death of adult female *Ixodes ricinus* (L.) on ivermectin treated cattle, as compared to untreated cattle (Taylor & Kenny 1990). Other research with *B. microplus* reported that either the adult ticks were less susceptible to ivermectin or there was a lag phase after treatment, during which the drug did not reach the engorging adult ticks in lethal amounts (Nolan et al. 1981). Our results stand in stark contrast to the first part of this statement, as adult ticks in our study were highly susceptible to ivermectin. Our findings neither confirm or refute that there is a lag phase that allows engorging adults to survive, but our results clearly show that high mortality will occur when early stage adult ticks are exposed to ivermectin. Our findings of lower reproductive capacity associated with ticks that were in the nymphal and larval stage of development at treatment initiation appeared to be in agreement with other studies regarding immature stages of ticks. Soll et al. (1990) reported that a reduction in the percentage of nymphs of the 2-host tick, *R. evertsi* was probably a result of having the two immature stages exposed to ivermectin over a prolonged period. Similarly, it was reported that efficacy of ivermectin against the 1-host tick, *B. decoloratus* (Koch) was at least partially due to the effect against the immature stages of the tick, which were exposed to the drug for an extended period of time (Horak et al. 1983).

As stated previously, the highly positive aspect of this study is that it clearly demonstrated that sustained daily oral treatments of ivermectin produced a very high degree of control against *Boophilus* ticks. On the other hand, the fact that even small numbers of viable ticks in all stages of development at the initiation of treatments were able to survive indicated that there could be a potentially serious negative impact with this treatment regime. There is little doubt that ivermectin levels in the blood serum of the animals acted to produce a very strong

selective pressure on the ticks that survived. Consequently, the use of this type of sustained release treatment method has the distinct possibility of initiating the emergence and future development of acaricide resistance in the surviving tick population. If a large enough segment of the tick population in a given area or region were subjected to this type of selection pressure for an extended period of time, it could result in the elimination of ivermectin or other avermectins from consideration as candidates for use in tick control programs in treatment regimes that might otherwise be successful. Thus, serious forethought should be given before using these potent endectocides in a manner that could induce the development of resistance in a short time.

Aside from the concern over development of acaricide resistance, the results of the study otherwise provide encouraging possibilities for the potential use of ivermectin and other macrocyclic lactone compounds in the Cattle Fever Tick Eradication Program as a means of eliminating *Boophilus* ticks on infested cattle. However, the potential use of this specific sustained release treatment (daily oral treatments) is unlikely to ever achieve wide acceptability in the program because of the necessity for treating the animals on a daily basis for such an extended period of time. It is possible that future circumstances could occur that would necessitate the consideration of this type of treatment regime (daily oral treatment) as a potential candidate for use on cattle within an infested premises to achieve eradication of the ticks.

Perhaps the most important aspect of this study is that it provides a model for the development of other sustained release delivery systems using macrocyclic lactone compounds. Treatment methods, such as pour-on, bolus, and injectable formulations, some of which provide sustained release of ivermectin, have produced good results in the control of *Boophilus* ticks (Pegram & Lemche 1985, Cramer et al. 1988, Taylor & Kenny 1990, Soll et al. 1990, Miller et al. 1997, 1999). However, only one of these studies (Miller et al. 1999) has demonstrated the total elimination of ticks in the field. The success of these studies has not resulted in a commercial registration of the drug or the various treatment methods for use in the U.S. eradication program. But if sustained release systems, such as long-term boluses, medicated feed systems, or medicated baits can be designed to provide a dosage level of at least 50 μg/kg/d for an extended period of time, then perhaps macrocyclic lactones or specifically ivermectin can be used to achieve eradication of a *Boophilus* population, as evidenced by this study. Thus, these acaricidal compounds show considerable potential for future applicability in an eradication program, and additional studies are warranted to provide the data necessary for obtaining a commercial registration for their use.

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Parasitoids of the Hop Aphid (Homoptera: Aphididae) on *Prunus* during the Spring in Washington State[1]

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J. Agric. Urban Entomol. 18(3): 141–147 (July 2001)

ABSTRACT In 1999, 802 primary parasitoids and 1,448 hyperparasitoids were reared from 83 samples of hop aphids, *Phorodon humuli* (Schrank), collected from *Prunus* sp. at 47 sites in the hop-growing area of south central Washington. In 2000, we collected 94 primary parasitoids and 180 hyperparasitoids in 59 samples from 28 sites. Parasitoids (primary plus hyperparasitoids) were reared from over 86% of the samples in 1999 and 61% of the samples in 2000. *Lysiphlebus testaceipes* (Cresson) was the most abundant primary parasitoid, accounting for 81.6% of the primary parasitoids in 1999 and for 52.1% in 2000. *Praon unicum* Smith was second in abundance with 14.3% in 1999 and 37.2% in 2000. Other primary parasitoids were Aphelinidae (0.9% in 1999 and 4.3% in 2000), *Aphidius ervi* Haliday (1.0% in 1999 and 1.1% in 2000), *Diaeretiella rapae* (M'Intosh) (0.3% in 1999 and 0% in 2000), and *P. occidentale* Baker (0.4% in 1999 and 0% in 2000). Aphelinidae have not been reported previously from hop aphids. *D. rapae* and *P. occidentale* Baker are new records for the hop aphid on *Prunus*. Hyperparasitoids were in the genera *Alloxysta* (Charipidae), *Asaphes* and *Pachyneuron* (Pteromalidae), and *Dendrocerus* (Megaspilidae). This initial study indicates that the primary parasitoids have potential as biological control agents.

KEY WORDS Homoptera, Aphididae, *Phorodon humuli*, *Humulus lupulus*, hops, *Prunus*, parasitoids, *Lysiphlebus testaceipes*, *Brachycaudus helichrysi*, *Praon unicum*

The hop aphid, *Phorodon humuli* (Schrank), alternates between hop, *Humulus lupulus* L., in the summer and certain *Prunus* spp. in the winter (Wright et al. 1995), and is a major pest of hops in most of the hop-growing areas of the Northern Hemisphere (Neve 1991). Heavy infestations reduce hop yield and lower quality by producing honeydew, which allows sooty mold to grow in the hop cones (Neve 1991). In Washington, hops are grown in the south central part of the state, where growers usually apply insecticides at least once during the growing season to control hop aphids. On fruit trees, growers typically apply a delayed-dormant spray to control aphids. Large numbers of hop aphids on ornamental trees are a

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nuisance because of the honeydew they produce and their tendency to reduce tree growth.

Hop aphid parasitoids are rare on hops in Washington (Campbell & Cone 1994, Pike & Starý 1995) and England (Copland 1979). The use of pesticides has been given as a reason for the low numbers on hops, but parasitization is also rare on unsprayed hops (Pike & Starý 1995). The hop aphid is an introduced insect, therefore, the indigenous parasitoids may not have adapted to the aphid on hops. However, hop aphids are commonly parasitized during the spring on *Prunus* trees. The purple-leaf ornamental tree, *Prunus cerasifera* Ehrhart, also known as cherry plum or Myrobalan plum ('Thundercloud' is probably the most popular variety), appears to be the major source of hop aphids in the spring in south central Washington (L. C. W., unpublished data). At least part of the reason for this is because ornamental trees are more abundant than fruit trees. The ornamental trees are not usually treated with insecticides, so they may be good sites for parasitoids to increase and reduce the number of hop aphids.

Pike & Starý (1995) listed the known hop aphid parasitoids from around the world. Pike et al. (2000) list eight primary parasitoids (Braconidae: Aphidiinae) of hop aphids from the northwest United States, four of which were found only on hops. No work has been reported on the relative numbers of the primary parasitoids or on the hyperparasitoids. Our objective was to identify and determine the relative abundance of primary parasitoids and hyperparasitoids attacking hop aphids on *Prunus* during the spring in the hop growing areas of Washington.

Materials and Methods

Leaf samples were collected from the hop growing areas of the Yakima and Moxee Valleys in south central Washington. We sampled from 22 April to 16 June 1999 and from 31 March to 14 June 2000. Most of the hop aphids had emigrated from *Prunus* by the middle of June in both years. *Prunus* trees were located by driving the roads of the area with observers visually searching for trees. The sampling method was similar to that used by Pike & Starý (1995). Trees were examined for hop aphids, and infested leaves were clipped and placed in 10 cm diameter × 4 cm deep (300 ml) round plastic containers covered with fine nylon screen. One sample was taken from each infested site. No site was more than about 0.5 ha. The number of leaves per sample was not constant because the number of infested leaves varied among trees and the number of trees varied among sites. The aphids were identified and the relative number of each species was estimated. Parasitoids were allowed to emerge in the containers, which were stored in the laboratory at 20–26°C. Parasitoids were removed from the containers every 2 to 3 days and were placed in vials of 70% ethanol. About 2 months after the last collection, we removed the dead parasitoids that remained in the containers and placed them in the ethanol vials. In 1999, 83 samples were collected from 47 sites. Seventy-eight samples were from purple-leaf ornamental varieties and five were from green-leaf varieties. In 2000, 59 samples were taken from 28 sites, all from purple-leaf plum trees. We identified the parasitoids using the key in Pike et al. (1997). The key in Pike & Starý (1995) was also used to confirm the identification of some of the primary parasitoids. We used the keys in Goulet & Huber (1993) to confirm the identifications of the Aphelinidae to family. The keys allowed identification of *Praon* and *Aphidius* males only to genus. If a

sample had only one species of *Praon* or *Aphidius* female, we assumed the males were the same species. In mixed-species samples or samples that had only males, the males were identified to genus only. Hyperparasitoids (secondary parasitoids), which parasitize primary parasitoids, were identified to genus. The keys did not separate *Asaphes* from *Pachyneuron*, therefore, they are combined in this paper. Relative abundance is defined as (P / T) × 100, where P = the number of individuals of a species or genus and T = total number of parasitoids. Primary parasitoids and hyperparasitoids were calculated separately.

Results and Discussion

In 1999, the hop aphid was found alone in 36 samples, the leaf-curling plum aphid, *Brachycaudus helichrysi* (Kaltenbach), was found alone in one sample, and 46 samples had both species. The mealy plum aphid, *Hyalopterus pruni* (Geoffroy), was found in two samples with the other two aphids. Hop aphids were more abundant than the leaf-curling plum aphid in all but five of the mixed colonies. In 2000, hop aphid was the only species in 52 samples, *P. humuli* and *B. helichrysi* were found together in the remaining seven samples, and of those, *P. humuli* numbers were the largest in five. No *H. pruni* were found in 2000.

Parasitoids were very common in both years, but the percentage of samples with parasitoids declined dramatically from 1999 to 2000 (Table 1). We collected about 30% fewer samples in 2000 compared with 1999, but the number of parasitoids declined over 87%. Parasitism apparently varies considerably from year to year. Hyperparasitoids outnumbered primary parasitoids both years at a ratio of approximately 65% to 35% (Table 1).

Lysiphlebus testaceipes (Cresson) was the most common primary parasitoid both years (Table 2). It accounted for over 81% of the primary parasitoids and was found in over 62% of the samples in 1999. In 2000, just over one-half of the primary parasitoids were *L. testaceipes* and it was found in almost 24% of the samples (Table 2). This parasitoid previously has been found parasitizing hop aphids on *Prunus* and hops (Pike & Starý 1995, Pike et al. 2000). It has a wide host range, is probably native to North America, and is common in Washington (Mackauer & Starý 1967, Pike et al. 1997, 2000).

Hop aphid eggs hatch in February and March (L. C. W., unpublished data), therefore, parasitoids must be able to reproduce and develop during the late winter and early spring when temperatures are still low. *L. testaceipes* completed development at 12.8°C (Tyler & Jones 1974a) and emerged from aphid mummies at temperatures as low as 3.3°C (Tyler & Jones 1974b). *L. testaceipes* is active from April through November in Washington (Pike et al. 1997). Our earliest collections were on 26 April 1999 and 11 April 2000. Carroll & Hoyt (1986) found the parasitoid was most active during midsummer in north central Washington apple orchards and it overwintered on the shrub *Viburnum opulus* L., a host of *Aphis fabae* Scopoli. *L. testaceipes* appears to be well adapted to parasitizing hop aphids on *Prunus*.

Praon unicum Smith was second in abundance, accounting for over 14% of the primary parasitoids in 1999 and 37% in 2000 (Table 2). It was found in over 33% of the samples in 1999 and in over 11% in 2000 (Table 2). Because most of the *Praon* females were *P. unicum*, most of the *Praon* males are also likely to have been this species. Carroll & Hoyt (1986) studied the biology of *P. unicum* in north

Table 1. Number of samples with hop aphid parasitoids and total number of parasitoids collected from *Prunus* in 2 years of sampling.

	Year	
	1999	2000
Total no. of samples	83	59
Samples with parasitoids	72 (86.8%)	36 (61.0%)
Samples with no parasitoids	11 (13.3%)	23 (39.0%)
Samples with primary parasitoids	58 (69.9%)	26 (44.17%)
Samples with hyperparasitoids	61 (73.5%)	23 (39.0%)
No. of primary parasitoids	802 (35.6%)	94 (34.3%)
No. of hyperparasitoids	1,448 (64.5%)	180 (65.7%)

central Washington and concluded that it was the most important parasitoid of the apple aphid, *Aphis pomi* De Geer, mainly because it was active early in the season. *P. unicum* has been reported on hop aphids on hops and on *Prunus salicina* (Pike et al. 2000).

Aphidius ervi Haliday, *Diaeretiella rapae* (M'Intosh), *P. occidentale* Baker, and the Aphelinidae together comprised less than 3% of the primary parasites in 1999 (Table 2). Only one *Aphidius* sp., a male, was collected in 2000. Aphelinidae were present in low numbers both years (Table 2). Neither *D. rapae* nor *P. occidentale* were recovered in 2000. All of the primary parasitoids except *D. rapae* and *A. ervi* were found in samples containing only hop aphids; this is verification that they parasitize hop aphids. The one sample with *B. helichrysi* alone (1999) contained *L. testaceipes* and a *Praon* male.

We found some parasitoids not previously recorded for the hop aphid or they have not been found on *Prunus*. Aphelinidae have not been reported from the hop aphid. *Aphidius ervi* was listed as an uncertain parasitoid of *P. humuli* on *Prunus* in western Washington (Pike et al. 2000). *D. rapae* has been reported attacking *P. humuli* and *B. helichrysi* on hops, but not on *Prunus* (Mackauer & Starý 1967, Pike & Starý 2000). *Praon occidentale* was found parasitizing hop aphids on hop, but not on *Prunus* (Pike & Starý 2000).

Several parasitoids have been reported parasitizing the hop aphid, but were not found in this survey. *Aphidius matricariae* Haliday has been found parasitizing hop aphids on hops in Washington, Europe, and Iran (Pike & Starý 1995, Pike et al. 2000). *Binodoxys conei* Pike & Starý has been recovered from Washington hops (Pike & Starý 1995). *Monoctonus campbellianus* Pike & Starý was reared from a mixed collection of aphids on *Prunus*, which included *P. humuli*. *Ephedrus persicae* Froggatt, *E. plagiator* (Nees), *P. volucre* (Haliday), and *Trioxys humuli* Mackauer have been reported as parasitoids of hop aphids in Europe (Pike & Starý 1995).

Wasps in the genus *Alloxysta* were the most numerous hyperparasitoids in 1999, followed by the pteromalids, *Asaphes* and *Pachyneuron*, and finally by *Dendrocerus* (Table 2). In 2000, *Asaphes* and *Pachyneuron* were the most abundant, closely followed by *Alloxysta* and only one *Dendrocerus*. Hyperparasitoids

Table 2. Primary parasitoids and hyperparasitoids reared from aphid samples collected from *Prunus* sp. in the hop growing areas of Washington during the spring of 1999 and 2000.

	Primary parasitoids																			
			Aphidiidae												Hyperparasitoids					
	Aphelinidae		*Aphidius ervi*		*Diaeretiella rapae*		*Lysiphlebus testaceipes*		*Praon occidentale*		*P. unicum*		*Praon* males		Charipidae *Alloxysta* sp.		Pteromalidae *Asaphes* and *Pachyneuron* sp.		Megaspilidae *Dendrocerus* sp.	
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
No. of parasitoids	7	4	8	1[a]	2	0	654	49	3	0	115	35	13	5	845	80	514	99	89	1
Percent of parasitoids	0.87	4.26	1.00	1.06	0.25	0	81.55	52.13	0.37	0	14.34	37.23	1.62	5.32	58.36	44.44	35.50	55.00	6.15	0.56
No. of samples with parasitoids	4	4	3	1	2	0	52	14	2	0	28	7	6	5	51	16	42	13	12	1
Percentage of samples with parasitoids	4.82	6.78	3.61	1.69	2.41	0	62.65	23.73	2.41	0	33.74	11.86	7.23	8.47	61.45	27.12	50.60	22.03	14.46	1.70

[a]This specimen was a male, which could be identified only to genus.

exceeded primary parasitoids in both years. Over 73% of the samples in 1999 and 39% in 2000 contained hyperparasitoids (Table 1), indicating that they were well distributed among locations. *Alloxysta* wasps are endohyperparasitoids, which tend to be host specific and are generally the most abundant hyperparasitoids in native systems. The other hyperparasitoids we collected are ectohyperparasitoids, which are generalists and usually most numerous in exotic primary parasitoids (Sullivan & Völkl 1999).

This initial survey indicates that *L. testaceipes* and *P. unicum* may be promising biological control agents for the hop aphid on *Prunus*. The abundance and efficacy of overwintering parasitoids is likely to be a critical factor in regulating hop aphid populations on *Prunus* during the spring. The large number of hyperparasitoids may be a cause for concern, but the negative impact of hyperparasitoids on biological control is not necessarily of primary importance (Mackauer & Völkl 1993, Sullivan & Völkl 1999).

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Repellency and Toxicity of Mint Oil to American and German Cockroaches (Dictyoptera: Blattidae and Blattellidae)[1]

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ABSTRACT The repellency and toxicity of mint oil to American, *Periplaneta americana* (L.), and German, *Blattella germanica* (L.), cockroaches were evaluated in a series of laboratory experiments. In topical application experiments, mint oil was toxic to both species with toxicities (LD_{50}s) of 2.57 (1.98–4.20) in 10 μl and 3.83 (2.35–7.34)% in 2 μl for American and German cockroaches, respectively. In continuous exposure experiments, Mortality (LT_{50}) values for American cockroaches ranged from 246.8 min with 3% mint oil to 64.2 min with 100% mint oil. LT_{50} values for German cockroaches ranged from 318 to 5.6 min for 3% and 30% mint oil, respectively. American and German cockroaches had knockdown (KT_{50}) values of ≈7.4 and 9.2 h, respectively, when fumigated with 50 μl of 100% mint oil; 100% of both species were killed after 24 h. Mint oil deposits were ≈100% repellent in Ebeling choice boxes to both species during each day of the 14-d experiment. Mint oil-based formulations could provide another integrated pest management tool for cockroach management, especially in situations in which conventional insecticides would be inappropriate.

KEY WORDS *Blattella germanica*, *Periplaneta americana*, mint oil, essential oil, Ebeling choice box, toxicity

American, *Periplaneta americana* (L.), and German, *Blattella germanica* (L.), cockroaches remain two of the most important insect species to homeowners and in food-handling facilities (Bennett et al. 1997). Even though a wide variety of insecticidal products are available for cockroach control, most contain synthetic organic insecticides. With homeowner's increased awareness and concern about traditional insecticides, there is a greater potential for use of less toxic materials for cockroach control.

Naturally occurring insecticides have been used in pest control for centuries (Ebeling 1971, Coats 1994). Many of these compounds, including alkaloids, quinones, essential oils (including terpenoids), glycosides, and flavonoids, are secondary plant substances (Raven et al. 1992). Monoterpenoids are present in cedar, citrus, eucalyptus, mints, and a variety of spices. Many monoterpenoids are

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used as cosmetic, food, and pharmacological additives where they provide flavors and fragrances. Not unexpectedly, these compounds also induce a variety of responses in insects. For example, several monoterpenoids (Inazuka 1982) and cedar oils (Appel & Mack 1989) are repellent to German cockroaches, affect insect growth and development (Hink & Fee 1986, Karr & Coats 1992), or are acutely toxic to insects (Smith 1965, Coyne & Lott 1976, Coats et al. 1991, Rice & Coats 1994). Monoterpenoids are considered neurotoxic because of their speed of action and their effects on neurotransmission (Coats et al. 1991).

The purpose of this study was to determine the repellency and toxicity of mint oil against American and German cockroaches. Toxicity was determined using topical application and continuous exposure methods. Repellency was determined using Ebeling choice boxes and was compared with conventional insecticides.

Materials and Methods

Insects. Insecticide-susceptible American and German cockroaches (American Cyanamid Co., Clifton, New Jersey) were used in the laboratory experiments. Cockroaches were reared in plastic trash cans with cardboard harborage at 25–28°C and 40%–55% RH, were exposed to a photoperiod of 12:12 (L:D) h, and were supplied water and dry dog chow (Purina Dog Chow, Ralston Purina, St. Louis, Missouri) *ad libitum*. Adult males were used in both toxicity and choice box tests. Cockroaches were anesthetized briefly (<5 min) with CO_2 to facilitate handling.

Toxicity tests. Corn mint, *Mentha arvensis* L., oil with a density of 882.54 μg/μl was obtained from Woodstream Corporation, Lititz, Pennsylvania. The oil contained 24.79% *l*-menthol, 24.83% menthone, 8.55% isomenthone, 3.56% neomenthone, and a variety of other terpenoids each comprising <1% of the mixture. Serial dilutions of a stock (100%) mint oil extract were prepared in Fisher Scientific Certified ACS acetone (99.7% purity; Fisher Scientific, Fair Lawn, New Jersey) and were used in both topical application and continuous exposure bioassays. For the topical application experiment, at least four concentrations of mint oil that produced between 1% and 100% mortality were used. Either 2 or 10 μl (for German or American cockroaches, respectively) of a concentration were applied to the abdominal sternites with a Burkard Manufacturing Co. hand microapplicator (Hertfordshire, United Kingdom). A minimum of 60 cockroaches were used for each concentration. After treatment, groups of 10 cockroaches were placed in 0.95-liter glass jars with a small (about 1 cm) piece of moistened cotton wick. The upper inside surface of the jar was lightly greased with petroleum jelly to prevent escape. Mortality was assessed 24 h after treatment and was scored as the inability of a cockroach to move when prodded. There were two different control groups: one treated with either 2 or 10 μl of acetone and the other with either 2 or 10 μl of mineral oil.

Continuous-exposure bioassays were conducted by pipetting 1 ml of a mint oil concentration (prepared as above) into the bottom of a 9-cm diameter glass petri dish and by allowing the acetone to completely evaporate in a laboratory hood (≈1 h). Six petri dishes with either 3 (American) or 10 (German) cockroaches were used for each concentration. Mortality (see above) was assessed after 15, 30, 60, 120, and 240 min, and 24 h of continuous exposure to the residue.

Fumigant activity of mint oil was assessed by sealing either 6 (American) or 10 (German) adult male cockroaches in 0.95-liter glass jars with a 1-cm diameter

cotton ball treated with 50 μl of 100% mint oil. Mint oil was injected into the center of each cotton ball to allow volatilization while preventing the cockroaches from contacting the residue. Knockdown (inability to move in a coordinated manner) was recorded hourly, and mortality was recorded at 24 h. Control jars had cockroaches and an untreated cotton ball. There were 6 mint oil and 6 control replicates for each species.

Choice box tests. Cockroach repellency and mortality were determined in Ebeling choice boxes (Ebeling et al. 1966) as described by Appel (1990, 1992). Food and water were placed in the lighted compartment of the choice box. Mint oil (2 ml of stock solution) was pipetted onto an aluminum foil-covered insert (15 × 30.5 × 0.5 cm) that fit snugly into the floor of the dark compartment. Control boxes were also fitted with aluminum foil-covered inserts, but were treated with 2 ml of acetone. Both mint oil- and acetone-treated inserts were dried for 1 h under a laboratory fume hood before being placed into choice boxes. Either 10 American or 20 German cockroaches were released into the untreated compartment of the choice box and were allowed to enter the treated compartment after 4 h. Cockroaches were able to move freely between the dark (treated) and the lighted (untreated) compartments.

Choice boxes were exposed to a photoperiod of 12:12 (L:D) h at 25–28°C. Banks of white florescent lights were 1.6 m above the choice boxes and produced a light intensity in the untreated compartment of 300–350 lux (INS Digital Lux Meter, Markson Scientific, Phoenix, Arizona). The number of live and dead cockroaches in each compartment at 3 to 4 h into the photophase was recorded daily for 14 d. Repellency was defined as the mean percentage of live cockroaches present in the light compartment during the photophase. Six replicates were used for each treatment in a completely randomized design.

Data analysis. Mortality (LT_{50}) in the continuous exposure, and knockdown (KT_{50}) in the fumigation tests were analyzed by probit analysis for correlated data (Throne et al. 1995) because multiple observations were taken on the same individuals. Probit analysis for independent data (SAS Institute 1985) was used to estimate the toxicity (LD_{50}) in the topical application tests. Significant differences in LD_{50}, LT_{50}, and KT_{50} were based on nonoverlap of the 95% confidence intervals (CI). A three parameter exponential decay model of the form: $LT_{50} = a\ [exp(-bx)] + c$, was used to quantify the relationship between LT_{50} and mint oil concentration in the continuous exposure tests (SigmaPlot 5.0; SPSS 1998). In this equation, LT_{50} is the estimated time (min) to reach 50% mortality, a is the estimated augmentation LT_{50} (min) of an untreated control population (i.e., a population treated with a concentration of 0%), b is the rate constant, and c is the asymptotic limit of the minimum LT_{50} for a population exposed to a concentration of 100%. The numerical value of the LT_{50} at 0% concentration is $a + c$ and defines the y-intercept of the model. Biologically, a represents the natural mortality, in LT_{50} terms, of a control population, and c represents the minimum time, again in LT_{50} terms, necessary to kill at the maximum concentration. Speed of penetration, time required to reach the active site(s), and the mechanism of toxicity all contribute to the value of c. This regression model was selected because a linear model was not significant ($P > 0.1$), an exponential model closely resembled a plot of the data, one and two parameter exponential models were not significant ($P > 0.05$), but had increasing R^2 values, and additional variables did not add significantly to the P or R^2 values. Repellency (percentage of live cockroaches in the

light side of the choice box) was analyzed using a Mann-Whitney rank sum test (SigmaStat 2.03; Jandel Scientific 1997).

Results

Toxicity. When applied topically, mint oil was toxic to both American and German cockroaches. However, mint oil concentrations ≤1% did not cause any mortality at 24 h for either species. The LD_{50} value was 10 μl of 2.57% mint oil for American cockroaches and 2 μl of 3.83% mint oil for German cockroaches (Table 1). Homogeneity of response (slope of the log-dose probit relationship) was 9.36 for American cockroaches and 5.95 for German cockroaches. There was no control mortality for both species.

In the continuous exposure tests, LT_{50} values for American cockroaches ranged from 469.9 min for 3% mint oil to 10.4 min for 30% mint oil (Table 2). LT_{50} values for German cockroaches ranged from 3,318 to 1.0 min for 3% and 100% mint oil, respectively (Table 2). As with the topical application tests, mint oil concentrations ≤1% did not cause any mortality and there was no control mortality. LT_{50} values for both species declined exponentially with increasing concentration of mint oil. For American cockroaches, LT_{50} = 1,598 (±15) {*exp*[-0.42 (±0.003) *x*]} + 10.73 (±0.32); F = 368,679; df = 2, 3; P = 0.0012; R^2 = 0.999; residual mean square error = 0.21. For German cockroaches, LT_{50} = 31,700 (±3,600) {*exp*[-0.75 (±0.038) *x*]} + 3.6 (±2.6); F = 4,296,766; df = 2, 3; P = 0.001; R^2 = 0.999; residual mean square error = 13.83. Both models indicate that mint oil has relatively low toxicity at 3%, but toxicity increases at a threshold value of about 10% and increases slightly with increasing concentration.

American and German cockroaches had KT_{50} values of 7.4 and 9.2 h, respectively, when held in sealed jars with 50 μl of 100% mint oil (Table 3). Mortality of both species was 100% after 24 h of exposure to the mint oil; no control knockdown or mortality was observed.

Repellency. Mean repellency of male German cockroaches exposed to mint oil ranged from 92.3% to 100%, and was 100% for male American cockroaches (Fig. 1). Control repellency over the entire 14-d experiment was 2.02% ± 0.19% for American and 13.3% ± 2.5% for German cockroaches. Using daily means as replicates, mint oil was significantly repellent to both American [Mann-Whitney rank sum test; T = 301.00; n (smaller) = 14, n (larger) = 14; $P < 0.001$] and German cockroaches [Mann-Whitney rank sum test; T = 105.00; n (smaller) = 14, n (larger) = 14; $P < 0.001$].

Discussion

In concentrations of ≥3%, mint oil is toxic and repellent to both American and German cockroaches. Mint oil also volatilizes rapidly in an open environment unlike most conventional insecticides and leaves little or no residue (Unpublished data). These characteristics, together with its relatively low mammalian toxicity (Reynolds 1982, Dreisbach 1983) and wide spread use in perfumery, liqueurs, flavorings, cough drops, and as a topical anti-puritic, make mint oil an ideal insecticide for the rapid control of insect pests in areas where conventional insecticides are not appropriate.

TABLE 1. Toxicity of mint oil applied topically to adult male American and German cockroaches.

Species	*n*	Slope ± SE	LD_{50}, % (95%CI)	χ^2	*P*
American	60	9.36 ± 1.34	2.57 (1.98–4.20)	89.31	0.0001
German	60	5.95 ± 0.72	3.83 (2.35–7.34)	67.78	0.0001

Mint oil was applied in 10 μl for American cockroaches and 2 μl for German cockroaches.

Unlike conventional insecticides such as carbamates, organophosphates, and pyrethroids, mint oil is much less toxic and therefore requires greater concentrations. For example, bendiocarb, chlorpyrifos, and cyfluthrin have LD_{50} values of 10.89, 3.70, and 0.53 μg/g, respectively, for insecticide-susceptible male German cockroaches (Abd-Elghafar et al. 1990) compared with 1,126.71 μg/g for mint oil. Even though mint oil was more toxic to American (LD_{50} of 250.50 μg/g) than German cockroaches, conventional insecticides are also generally more toxic to American cockroaches (Cornwell 1976). These relatively large LD_{50} values are similar to those found by Lee et al. (1997) for a variety of monoterpenoids tested against adult house flies, *Musca domestica* L. Assuming a mean body mass of 0.0175 g per fly (Liu & Scott 1995), the LD_{50} value for *l*-menthol is 8,400 μg/g.

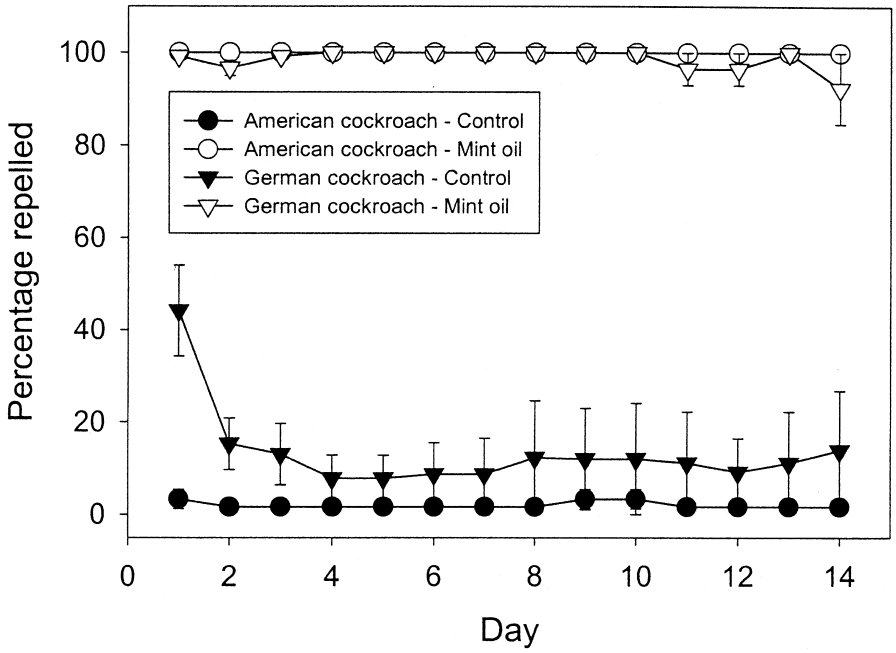

Fig. 1. Repellency of mint oil to American and German cockroaches determined in Ebeling choice boxes. Points represent means ± SE of six replicate boxes containing either 10 American or 20 German cockroaches.

TABLE 2. Contact toxicity of mint oil to adult male cockroaches exposed continuously in glass petri dishes.

Species	Concentration (%)	*n*	Slope ± SE	LT_{50}, min (95%CI)	χ^2	*P*
American	3	18	0.50 ± 0.28	469.89 (50.44–1.46 × 10^9)	3.12	0.0942
	10	18	5.68 ± 1.24	35.79 (28.23–45.45)	3.30	0.0271
	30	18	2.14 ± 0.79	10.41 (1.22–19.23)	2.05	0.0523
	100	18	0.98 ± 0.47	11.06 (1.89–84.46)	13.49	0.0003
German	3	60	5.06 ± 4.18	3,318 (1.99–7,845)	1.48	0.0463
	10	60	0.27 ± 0.11	20.65 (1.89–103.18)	313.66	0.0001
	30	60	2.18 ± 0.58	6.26 (1.71–10.12)	2.50	0.1451
	100	60	0.74 ± 0.51	1.03 (0.07–10.59)	1.05	0.2333

Mint oil vapor had fumigant effects against both American and German cockroaches. At a concentration of 46.45 μg cm^{-3}, the KT_{50} for both cockroach species was <10 h, and there was 100% mortality after 24 h of exposure. Menthol alone has a fumigant LC_{50} of 3.6 μg cm^{-3} to house flies after a 14-h exposure (Rice & Coats 1994). Although fumigation is rarely used for cockroach control (Cornwell 1976, Bennett et al. 1997), there are specialized situations such as in kitchen furniture and equipment, in filing cabinets, and even some parts of sewerage systems where fumigation with mint oil may be appropriate.

A variety of compounds, including commercial repellents (Bodenstein & Fales 1976), conventional insecticides (Ebeling et al. 1966; Appel 1990, 1992), desiccant dusts (Ebeling 1971), essential oils and terpenoids (Inazuka 1982, Steltenkamp et al. 1992), and experimental repellents (e.g., Hagenbuch et al. 1987, Steltenkamp et al. 1992), are repellent to cockroaches. Inazuka (1982) reported that oils of Japanese mint and Scotch spearmint were the most repellent of >80 essential oils tested against German cockroaches. Mint oil extract at the rate of 2 ml of mint oil to 457.5 cm^2 was extremely repellent (≈100%) to American and German cockroaches for 14 d (Fig. 1) in Ebeling choice box assays. Assuming complete volatilization and no adsorption to the walls of the choice box, a mint oil vapor concentration of 453.9 μg cm^{-3}, ≈10 times greater than in the fumigation experiments could be attained. Even after 14 d, the mint odor was readily detected in the choice boxes and a trace of the oil deposit remained on the foil-covered insert. Whether repellency was due to volatile odor perception or direct contact with the mint oil deposit (or adsorbed vapor) was not determined.

The toxicity and repellency of mint oil extract, together with its low mammalian toxicity and status as a natural insecticide, make it an ideal compound for use

TABLE 3. Fumigant activity of mint oil to adult male American and German cockroaches.

Species	*n*	Slope ± SE	KT_{50}, h (95%CI)	χ^2	*P*
American	36	6.96 ± 1.57	7.38 (6.84–8.40)	19.70	0.0001
German	60	3.96 ± 0.66	9.21 (8.05–11.71)	36.27	0.0001

in a comprehensive integrated pest management program against cockroaches. Mint oil has been formulated as an aerosol for direct spray onto cockroaches and other pest arthropods (Victor Poison-Free Ant & Roach Killer, Woodstream Corp., 4% mint oil). This formulation rapidly (<2 min) kills both American and German cockroaches on contact (Unpublished data). A controlled release formulation could be developed to be used as a long lasting repellent or fumigant in voids or other harborage areas.

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Organophosphate Resistance and Allelic Frequencies of Esterases in the Olive Fruit Fly *Bactrocera oleae* (Diptera: Tephritidae)[1]

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ABSTRACT The polymorphic esterase loci, Est A and Est B, of a major agricultural pest, the olive fruit fly, *Bactrocera oleae* (*Dacus oleae*), were studied using a standard laboratory population (Ls) that had never been subjected to insecticides, two "resistant" populations (Rs and RRs) constructed after exposure of larvae to sublethal doses of the organophosphate insecticide dimethoate, and a field population (Fs) subjected to long-term pesticide exposure. Data showed that dimethoate, as a selective agent, was responsible for the induction of the changes in the frequencies of alleles of the Est A locus, whereas it did not affect the Est B locus. Our results also showed that the changes in the frequencies of some active alleles of Est A locus, observed in resistant populations, were similar to those found in the field population.

KEY WORDS *Bactrocera oleae*, Tephritidae, esterases, resistance, organophosphate insecticide, dimethoate

Bactrocera oleae (Gmelin) (Drew 1989, White & Wang 1992) is a Tephritid species that is a major agricultural pest. It causes great economic losses in all of the olive-producing countries. Although there is considerable interest in biological control, control has been based on synthetic insecticides, especially organophosphates (OPs) (for review, see Roessler 1989 and ref. therein). Because of the extensive use of these insecticides since the early 1960s, *B. oleae* populations are unmanageable due to the development of insecticide resistance.

Insecticide-resistant populations of nearly all economically important pests have been reported (Georghiou 1994). Three enzyme systems are thought to be involved in the detoxification of insecticides: cytochrome P_{450} monooxydases, glutathione *S*-tranferases, and esterases. Esterases are hydrolytic enzymes responsible for insecticide resistance in many insect species (Soderlund & Bloomquist 1990, Scott 1995).

Previous reports on *B. oleae* (Zouros et al. 1968) indicated that esterases are controlled by two unlinked autosomal genes, A and B, which are highly polymorphic. Gene A is responsible for the synthesis of an acetylcholinesterase (Est A),

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which is a monomer, and gene B is responsible for the synthesis of a pseudocholinesterase or lipase (Est B), which is a dimer. The presence of more than 20 active alleles of Est A, and more than 15 alleles for Est B, as well as the existence of a silent allele in both loci has been reported (Zouros & Krimbas 1969, Krimbas & Tsakas 1971, Tsakas & Krimbas 1975, Tsakas & Zouros 1980). It was also reported that dimethoate selects against the silent allele of gene A, causing death preferentially to flies homozygous or heterozygous for this allele (Tsakas & Krimbas 1970). In the current study, in an attempt to clarify whether active alleles of esterase A and/or B loci are selected by the OP insecticide, dimethoate, we analyzed the electrophoretic patterns of esterases in different populations of *B. oleae.*

Materials and Methods

Insects. The following *B. oleae* populations were used: a standard *B. oleae* laboratory strain (Ls) obtained from the olive fruit fly colony of "Demokritos" Nuclear Research Center, Athens, Greece. This strain had no exposure to insecticides (Loukas et al. 1985) and has been in our laboratory for more than 10 years; two *B. oleae* populations "resistant" (Rs and RRs) to dimethoate (see below for their construction); and a field population (Fs) consisting of flies emerged from infested mature olive fruits. The olives were collected from a garden in Agia Paraskevi, Halkidiki, Greece. This garden was never directly subjected to OP insecticide sprays; however, these flies were thought to be under the influence of insecticides because all of the surrounding orchards have been sprayed since 1970. The standard population, as well as the two "resistant" populations, were reared (Tsitsipis 1977) at 25 ± 1°C with a photoperiod of 12:12 h (L:D) and 60% RH. Wild *B. oleae* flies were used after their emergence from olive fruits.

Chemicals. In the present study, dimethoate (Rogor) [*O,O*-dimethyl-*S*(*N*-methylcarbamoylmethyl) phosphorodi-thionate] 40.66% (active ingredient) was used (Isagro CAS No. 1480).

Insecticide bioassay. The experiments were carried out on *B. oleae* larvae of the Ls strain, as previously described by Franzios et al. (1997). Eggs were collected during a 4-h period, and 192 h later, groups of 30 larvae (5 d old) were transferred to individual Petri dishes (9 cm in diameter) containing Whatman (Clifton, NJ) 3-mm filter paper moistened with different concentrations of dimethoate. After preliminary experiments, four different concentrations (0.01, 0.05, 0.1, and 0.5 mg l^{-1}) that caused <100% mortality were used in our experiments. Dishes were kept at 25 ± 1°C and 60% RH for 18 h. After exposure, the larvae were transferred to new individual vials with food until adult emergence. Each experiment was repeated at least four times. For comparative analysis, a parallel experiment using only Ringer solution (Becker 1959) was carried out. Determining toxicity as the fraction of the adult flies that emerged from the treated larvae, the number of adults was counted in both control and test cultures (Franzios et al. 1997, Karpouhtsis et al. 1998). The mortality caused by dimethoate was corrected using the following equation: (x – y) 100/x, where x and y correspond to the number of the surviving adults in the control and test experiments, respectively.

Development of resistant populations. The Rs population was derived from the Ls strain by exposing 5-d-old *B. oleae* larvae to a dimethoate dose (0.25 mg l^{-1}) that inhibited emergence of 60% (LD_{60}) of adults. The flies that emerged

were considered to be resistant to the insecticide, and constituted the F_1 generation of the Rs strain. The RRs strain was constructed after exposure of 5-d-old larvae of the F_2 generation from the Rs population to a dimethoate dose of LD_{70}. The exposure of *B. oleae* larvae to a dimethoate dose of LD_{60} and LD_{70} follows the experimental procedure described above.

Gel electrophoresis. Phenotypes of Est A and Est B alleles were determined by horizontal starch gel electrophoresis (10%) (Poulik 1957) of the head homogenates of 3-d-old *B. oleae* adults. Electrophoresis was performed according to Tsakas & Krimbas (1970). Extracts from flies of the homozygous strain for both A and B esterases ($A_7A_7B_1B_1$) were used as the gel mobility standard. The homozygous strain was derived by single pair crosses.

Statistical analysis. Tests for Hardy-Weinberg equilibrium, genotypic differentiation, allele frequencies, and pairwise Fst values were performed using the GENEPOP package, version 3.1c (Raymond & Rousset 1995). A Bonferroni-type correction (Rice 1989) was applied to multiple tests.

Results

Toxicity of dimethoate. The toxicity of dimethoate on *B. oleae* was estimated after exposure of larvae to different concentrations of this compound. Taking into account the average survival in controls (80%), the insecticidal effects of dimethoate, expressed as the percentage of *B. oleae* adult that emerged after correction (see "Materials and Methods"), are given in Figure 1. From these curves, the LD_{50} and LD_{90} of dimethoate were found to be 0.1709 and 0.5 mg l^{-1}, respectively.

Gel electrophoresis. The bands of Est A and Est B alleles on starch gel electrophoresis were named according to their distance from the origin of the electrophoretic pattern. The representative positions and the names of the bands are given in Figure 2. The names do not correspond to the ones given by Zouros et al. (1968). Some Est A and Est B zones (e.g. A_9 and B_3) were found to be overlapping, and the differences in color intensity between A and B zones were very slight. Thus, for better gel screening, different concentrations of chemical compounds considered as esterase inhibitors, such as $MnCl_2$, $CuSO_4$, and $HgCl_2$ (Zouros et al. 1968), were tested (Table 1). A solution of $HgCl_2$ (0.02%, w/v) was found to inhibit strongly the Est B zones, and furthermore, to act as an enhancer of Est A zones. Thus, to visualize only Est A zones, the upper slice of each gel was incubated in 0.02% $HgCl_2$ (before staining), whereas the lower one was stained for both A and B esterases.

Esterase polymorphism. The electrophoretic pattern of 96, 88, 73, and 139 individuals of the Ls, Rs, RRs, and Fs populations, respectively, were analyzed. The proportions of flies belonging to the different phenotypes of Est A and Est B are given in Tables 2 and 3, respectively. The analysis revealed the presence of 47 different phenotypes for Est A (Table 2) and 5 for Est B (Table 3), indicating the existence of 11 and 3 active alleles for esterase A and B loci, respectively, as well as a silent one (As and Bs) for each locus. Because of the presence of the silent alleles, which do not synthesize active acetylcholinesterase, the estimation of allelic frequencies was problematic (Krimbas & Tsakas 1971) because genotypes AjAj and AjAs (where Aj is any active allele and As the silent one) were not

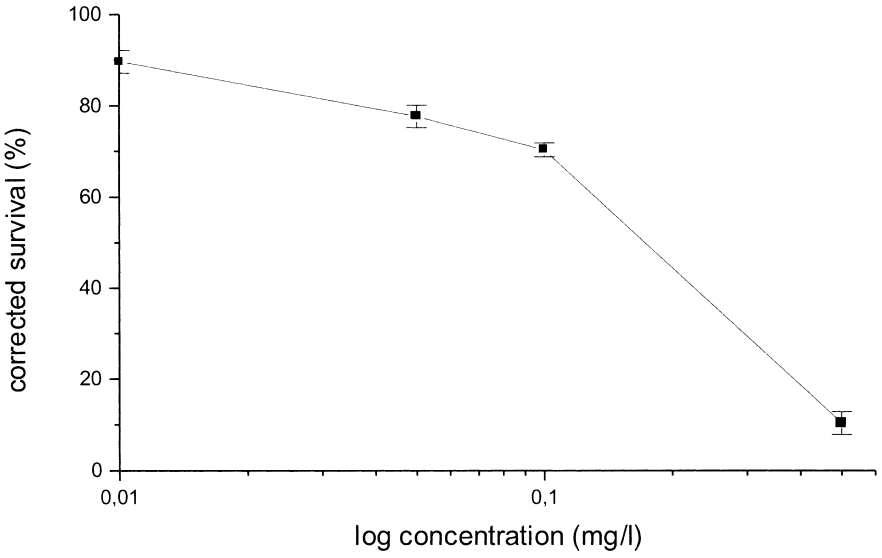

Fig. 1. Percentage of larvae surviving (mean ± SD) to adulthood after exposure to dimethoate.

distinguishable by electrophoresis. The frequencies of Est A and Est B alleles (including the silent ones) estimated by the GENEPOP package (Raymond & Rousset 1995) are given in Table 4. No significant differences (data not shown) were observed, however, when allelic frequencies were estimated according to Krimbas & Tsakas (1971).

Statistical analysis. Statistical analysis showed that there is a highly significant differentiation ($P < 0.01$) in the genotypic frequencies of the Est A locus between Ls and each of the other three (Rs, RRs, and Fs) populations, even after Bonferroni correction (Rice 1989), whereas no differentiation was found between the two resistant (Rs and RRs) populations ($P > 0.05$). Comparing resistant and wild populations, no significant differences were found after Bonferroni correction. Similar results can be deduced from pairwise Fst values (Table 5). Thus, higher interpopulation differences were observed when comparing Ls with Rs, RRs, and Fs populations, and less in comparing resistant populations with the field population. The results of the statistical analysis indicate that dimethoate, as a selective agent, is responsible for the induction of the observed changes in the Est A allele frequencies. For Est B locus, no genotypic differentiation was found in Ls, Rs, and RRs populations, a result confirmed by Fst values (Table 5) and one that indicates that dimethoate does not affect the Est B locus. However, the Hardy-Weinberg disequilibrium, found for both loci in all populations examined, may be due to the presence of silent alleles.

Discussion

The eradication and management of *B. oleae*, a major pest of the olive fruits, has been based mainly on the widely used OP insecticide, dimethoate, for up to

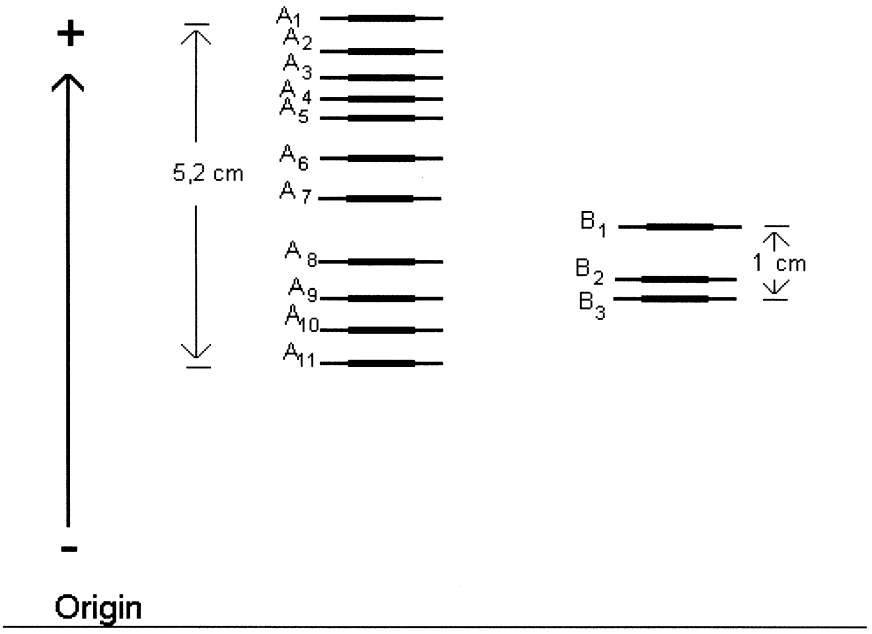

Fig. 2. Electrophoretic patterns of the allozymes of the alleles of Est A and Est B separated in a starch gel.

three decades. Despite the extensive use of this chemical compound, control of *B. oleae* flies remains problematic because of the development of resistant populations in nature. Among enzymatic systems, esterases are involved in the development of resistance of many pests to OP insecticides. Esterases have been reported as highly polymorphic enzymes in many insects (Burns & Johnson 1967, Prakash et al. 1969, McKechnie 1974, Triantaphyllidis et al. 1980, 1982, Yong 1986, 1992) and as the most polymorphic enzymes in *B. oleae* (Zouros & Loukas 1989).

In the present study, we aimed to detect possible relationships between OP resistance of *B. oleae* flies and the selection for any active allele of A and/or B esterases. Therefore, changes in the frequency of Est A and Est B alleles were examined using *B. oleae* flies that had never been subjected to dimethoate (Ls population), and flies that survived exposure to dimethoate and were considered to be resistant (Rs and RRs populations). Our data show that considerable changes occur in the frequencies of allozymes for the Est A locus (Tables 2 and 4) in these populations, indicating that this locus is selected by dimethoate. Thus, the frequencies of alleles A_1, A_4, A_5, and A_6 increased, whereas those of A_2, A_3, and A_7 decreased in both resistant strains. Furthermore, alleles A_9, A_{10}, and A_{11}, with very low frequencies in Ls, seemed to disappear in both resistant populations, whereas A_8 was absent only in RRs. On the contrary, with the exception of the B_2 allozyme (the frequency of which was extremely low in Ls and was not found in either resistant population), the frequencies of the active allozymes B_1 and B_3 were almost the same in all three populations (Tables 3 and 4), suggesting that dimethoate does not act as a selective agent on the Est B locus.

Table 1. Inhibition of A and B esterases.

Inhibitors	Concentration	Est A	Est B
Dimethoate	0.8132%	++++	+
$MgCl_2$	0.285%	+	–
$MgCl_2$	0.142%	–	–
$MgCl_2$	0.071%	–	–
$MgCl_2$	0.043%	–	–
$CuSO_4$	0.4%	+	+
$CuSO_4$	0.2%	–	+
$CuSO_4$	0.1%	–	+
$CuSO_4$	0.06%	–	+
$HgCl_2$	0.4%	++++	++++
$HgCl_2$	0.2%	++++	++++
$HgCl_2$	0.1%	++	++++
$HgCl_2$	0.06%	++	++++
$HgCl_2$	0.04%	+	++++
$HgCl_2$	0.02%	–	++++
$HgCl_2$	0.008%	–	+++
$HgCl_2$	0.004%	–	+++

The number of + signs indicates the intensity of inhibition, and a – sign indicates no inhibition. The concentrations are referred to as a percentage (% w/v) in incubation solution.

To determine whether the changes in the frequencies of the active alleles of the Est A locus caused by dimethoate exist under field conditions, a natural *B. oleae* population (Fs) was examined. This population had never been directly subjected to insecticides, but is considered to have been under long-term influence of dimethoate for about three decades. Data showed (Tables 2 and 4) that the changes in the frequencies of some alleles of Est A locus from the resistant populations (Rs and RRs) were also found in the field population. Indeed, the frequencies of alleles A_1, A_4, and A_6, which were relatively low in the laboratory strain and increased significantly after acute exposure to the insecticide, were also found to be elevated in the Fs population. Similarly, the frequencies of the most common alleles, A_3 and A_7, under laboratory conditions, which decreased drastically after acute exposure to dimethoate, were again found to be low after long-term exposure (Fs population). However, alleles A_2, which decreased, and A_5, which increased after exposure, were found to have similar frequencies in both the Ls and Fs populations. Moreover, the isoalleles A_8, A_9, A_{10}, and A_{11}, which were absent in the resistant populations, were found in very low frequencies in the Fs population, probably due to the larger sample size. Contrary to the Est A locus, no considerable changes were found in the allelic frequencies of Est B allozymes (Tables 3 and 4). The reappearance of the B_2 allele, which was present in Ls and disappeared in Rs and RRs, may be due to the larger sample of the Fs population.

It was previously reported that the mechanism of resistance in many cases is caused by elevated levels of esterases, either by gene amplification or by altered gene expression (Field et al. 1988, Mouches et al. 1990, Carlini et al. 1991, Ket-

Table 2. The proportions of individuals with different phenotypes of A esterases in the laboratory strain (Ls), the resistant strains (Rs and RRs), and the field population (Fs).

No. of flies examined	Ls 96	Rs 88	RRs 73	Fs 139
A_1^-	0.0208	0.0341	0.0411	0.0432
A_2^-	0.0208	0.0341	0.0411	0.0647
A_3^-	0.1354	0.0568	0.0274	0.0719
A_4^-	0.0208	0.1250	0.1096	0.1079
A_5^-	0.0625	0.2045	0.1233	0.0791
A_6^-	0.0417	0.1591	0.1781	0.0791
A_7^-	0.0833	0.0795	0.0548	0.0719
A_8^-	0.0000	0.0000	0.0000	0.0216
A_9^-	0.0000	0.0000	0.0000	0.0288
A_1A_2	0.0000	0.0227	0.0274	0.0072
A_1A_3	0.0104	0.0000	0.0000	0.0000
A_1A_4	0.0000	0.0000	0.0000	0.0072
A_1A_5	0.0000	0.0000	0.0000	0.0072
A_1A_6	0.0000	0.0000	0.0137	0.0072
A_1A_7	0.0104	0.0000	0.0137	0.0144
A_2A_3	0.0417	0.0114	0.0137	0.0072
A_2A_4	0.0000	0.0000	0.0000	0.0144
A_2A_5	0.0208	0.0000	0.0137	0.0000
A_2A_6	0.0000	0.0000	0.0000	0.0216
A_2A_7	0.0208	0.0000	0.0000	0.0216
A_2A_9	0.0104	0.0000	0.0000	0.0000
A_2A_{10}	0.0104	0.0000	0.0000	0.0000
A_3A_4	0.0208	0.0114	0.0000	0.0000
A_3A_5	0.0625	0.0114	0.0822	0.0072
A_3A_6	0.0313	0.0227	0.0411	0.0072
A_3A_7	0.0625	0.0000	0.0000	0.0000
A_3A_8	0.0000	0.0000	0.0000	0.0072
A_3A_9	0.0208	0.0000	0.0000	0.0144
A_3A_{10}	0.0208	0.0000	0.0000	0.0000
A_3A_{11}	0.0000	0.0000	0.0000	0.0072
A_4A_5	0.0104	0.0341	0.0000	0.0288
A_4A_6	0.0000	0.0227	0.0000	0.0288
A_4A_7	0.0729	0.0000	0.0411	0.0432
A_4A_9	0.0104	0.0000	0.0000	0.0000
A_4A_{10}	0.0000	0.0000	0.0000	0.0072
A_4A_{11}	0.0000	0.0000	0.0000	0.0072
A_5A_6	0.0104	0.0455	0.0274	0.0504
A_5A_7	0.0417	0.0227	0.1096	0.0144
A_5A_9	0.0000	0.0000	0.0000	0.0144
A_5A_{11}	0.0000	0.0000	0.0000	0.0072
A_6A_7	0.0208	0.0682	0.0274	0.0288
A_6A_8	0.0208	0.0114	0.0000	0.0000
A_6A_{10}	0.0000	0.0000	0.0000	0.0072
A_6A_{11}	0.0104	0.0000	0.0000	0.0000
A_7A_{10}	0.0208	0.0000	0.0000	0.0000
A_7A_{11}	0.0000	0.0000	0.0000	0.0072
A_sA_s*	0.0521	0.0227	0.0137	0.0360

*s indicates the silent allele.

Table 3. The proportions of individuals with different phenotypes of B esterases in the laboratory strain (Ls), the resistant strains (Rs and RRs), and the field population (Fs).

No. of flies examined	Ls 96	Rs 88	RRs 73	Fs 139
B_{1^-}	0.6148	0.6593	0.5479	0.5847
B_{2^-}	0.0000	0.0000	0.0000	0.0508
B_{3^-}	0.0738	0.0440	0.0548	0.1102
B_1B_3	0.2869	0.2857	0.3836	0.2203
B_1B_2	0.0164	0.0000	0.0000	0.0000
B_sB_s*	0.0082	0.0110	0.0137	0.0339

*s indicates the silent allele.

terman et al. 1992, Poirie et al. 1992, Cheikh & Pasteur 1993, Chen & Sun 1994, Raymond & Marquine 1994). In addition to amplification, mutation in esterase loci could produce structurally different enzymes that could metabolize insecticides more efficiently (Oppernooth 1982, Hama 1983, Byrne & Devonshire 1993, Berrada et al. 1994). In previous studies on *B. oleae,* a higher number of dupli-

Table 4. Allelic frequencies of Est A and Est B loci in the laboratory strain (Ls), the resistant strains (Rs and RRs), and the field population (Fs), estimated by GENEPOP package (Raymond & Rousset 1995).

Gene	Allele	Ls	Rs	RRs	Fs
Est A	A_1	0.0214	0.02918	0.04987	0.04513
	A_2	0.064	0.035	0.04987	0.07266
	A_3	0.2283	0.0593	0.08423	0.06546
	A_4	0.0693	0.10571	0.08343	0.13385
	A_5	0.1112	0.18236	0.1959	0.11182
	A_6	0.071	0.18291	0.16673	0.12326
	A_7	0.1799	0.09029	0.12908	0.107381
	A_8	0.0104	0.00568	0	0.01478
	A_9	0.0208	0	0	0.02973
	A_{10}	0.026	0	0	0.00725
	A_{11}	0.0052	0	0	0.01449
	As	0.1924	0.30957	0.24089	0.2742
Est B	B_1	0.70739	0.74321	0.69391	0.5151
	B_2	0.0082	0	0	0.02522
	B_3	0.20039	0.18023	0.23641	0.17452
	B_s	0.08402	0.07656	0.06968	0.28517

Table 5. Pairwise Fst values for the laboratory strain (Ls), the resistant strains (Rs and RRs), and the field population (Fs).

	Est B			
	Ls	Rs	RRs	Fs
Est A				
Ls	–	–0.0028	–0.0039	0.0132
Rs	0.0441	–	0.0028	0.0225
RRs	0.0333	–0.0081	–	0.0059
Fs	0.0236	0.0086	0.0043	–

Below diagonal values are for Est A, and above are for Est B.

cations of the Est A locus have been reported in populations under continuous OP treatment than in untreated ones, indicating gene amplification (Tsakas & Krimbas 1970, Tsakas 1977). However, in our experiments, we did not detect differences in the number of apparent duplications because an average of 2% duplications per examined population was observed. We note that the detection of duplications is difficult, because only the electrophoretically different ones (showing three different allozyme bands) can be detected. It was also proposed (Tsakas & Krimbas 1970, Krimbas & Tsakas 1971) that dimethoate selects against the silent allele of gene A, As, an allele that does not synthesize an active acetylcholinesterase. Under our experimental conditions, no reduction of the As allele frequency was observed. In contrast, an increase in its frequency was found in both resistant and wild populations (Table 4). An explanation for this discrepancy may be due to differences in experimental procedures and the developmental stage of the treated *B. oleae* individuals. In the present study, the exposure to dimethoate was performed on larvae, whereas in previous studies, adults were subjected in a continuous flow of gaseous dimethoate. The developmental stage of the treated *B. oleae* individuals was found to be of great importance for selection, at least for the alcohol dehydrogenase locus (Cosmides et al. 1997). However, the frequency of the silent alleles can not be estimated directly, but rather as one minus the sum of the active alleles for each locus (Krimbas & Tsakas 1971).

Drastic changes in the environment may result in the extinction of a population or in genetic changes of the gene pool of the population. Data presented here show that exposure of the *B. oleae* Ls population to sublethal doses (LD_{60} and LD_{70}) of dimethoate resulted in extensive changes in the frequencies of active alleles of the Est A locus, whereas no changes were observed for the Est B locus. It seems that selection acts only on the Est A locus, a result that is in accordance with previous studies on this insect (for review, see Zouros & Loukas 1989). In addition, our data show that dimethoate selects for the quality rather than the quantity of esterases A, acting against some active allozymes (A_3 and A_7), and enhancing others (A_1, A_4, and A_6). However, in order to determine the "resistant" and non-"resistant" allozymes, homozygous strains for each active allele of Est A locus of *B. oleae* must be constructed, and mortality rests must be performed on these strains.

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Susceptibility of Natural Enemies to Infection by *Beauveria bassiana* and Impact of Insecticides on *Ipheseius degenerans* (Acari: Phytoseiidae)[1]

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ABSTRACT The direct and indirect effect of a commercial formulation of *Beauveria bassiana* strain JW-1 was evaluated against five biological control agents. *Orius insidiosus* (Say), *Phytoseiulus persimilis* Athias-Henriot, *Encarsia formosa* Gahan, and *Aphidius colemani* Viereck were evaluated under greenhouse cage and laboratory conditions, and *Ipheseius degenerans* (Berlese) was evaluated under greenhouse conditions. The natural enemies were highly susceptible to infection under laboratory conditions, and lower infection rates were observed in the greenhouse trials. *Aphidius colemani* was the most susceptible and *O. insidiosus* was the least susceptible of the five natural enemies evaluated. *Ipheseius degenerans* was evaluated against the insect pathogens *Beauveria bassiana* strain GHA, *Verticillium lecanii*, and *Metarhizium anisopliae* to determine direct and indirect infectivity under laboratory conditions. *Ipheseius degenerans* was least susceptible to *M. anisopliae* followed by *V. lecanii* and *B. bassiana.* Applications of soap, neem oil, azadirachtin, or water against *Ipheseius degenerans* were evaluated under laboratory conditions. Water and soap resulted in significantly lower mortalities than neem oil and azadirachtin. Results indicate that with proper timing, mycoinsecticides, azadirachtin, neem oil, and soap can be used in combination with the natural enemies evaluated.

KEY WORDS Biological control, ornamentals, *Orius insidiosus, Phytoseiulus persimilis, Encarsia formosa, Aphidius colemani, Metarhizium anisopliae, Verticilium lecanii*

Chemical pesticides are the primary method of pest control worldwide, with biopesticides only accounting for 1.5%–2% of the total pesticides used (Charnley 1997). Mycoinsecticides compose only a small component of the biopesticide market, but their use is gaining acceptance as an alternative to the use of traditional contact insecticides for greenhouse pest control. This is due to increased public concern over the use of traditional synthetic insecticides, the fact that there are fewer insecticides available, and the development of insecticide resistance. Advantages to the use of mycoinsecticides include short reentry intervals (often 4 h),

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low mammalian toxicity, and a decreased risk of resistance developing in the target pests. These factors make mycoinsecticides attractive for incorporation into integrated pest management programs.

As mycoinsecticides and natural enemies become more commercially available, it is important that we understand their interactive roles in pest management programs. Mycoinsecticides are often not species specific and often have a broad host range. As a result, their effect on nontarget organisms needs to be assessed if they are to be incorporated into integrated pest management programs (Jaronski et al. 1998). The interaction of mycoinsecticides and nontarget organisms has become an active research area (Magalhaes et al. 1988, Donegan & Lighthart 1989, James & Lighthart 1994, Jaronski et al. 1998, Askary & Brodeur 1999).

Research has been conducted to identify natural enemies for use in greenhouse pest control and, as a result, there are numerous species available for greenhouse pest control (Stoltz & Stern 1978, Gillespie 1989, Fransen & Tolsma 1992, Gillespie and Quiring 1992, Higgins 1992, van Houten & van Stratum 1993, Hoddle et al. 1997). The use of natural enemies and low mammalian-toxicity pesticides has increased in popularity in greenhouse production in recent years. Considerable research is being undertaken to evaluate the interactions between insecticides and natural enemies (Bethke & Redak 1997, Banken & Stark 1997, 1998, Stansly & Liu 1997, Shipp & Ferguson 2000).

Ipheseius degenerans (Berlese) has recently become commercially available and, as a result, its compatibility with mycoinsecticides or insecticides has not been studied as extensively as other predator species. This species has been studied under laboratory conditions as a predator of tetranychid mites (Takafuji & Chant 1976, Eveleigh & Chant 1981, Yao & Chant 1989) and has been used successfully as a thrips predator in greenhouse vegetable production (Ramakers 1995). It currently is being evaluated for use in ornamental production.

Our study had two objectives: to evaluate the direct and indirect effects of *Beauveria bassiana* on five natural enemies commonly used in ornamental pest control under greenhouse cage and laboratory conditions, and to evaluate *I. degenerans* mortality due to exposure to soap, neem oil, azadirachtin, and infection due to *B. bassiana, Verticillium lecanii*, and *Metarhizium anisopliae.*

Materials and Methods

***B. bassiana* susceptibility trials.** Greenhouse and laboratory trials were conducted to evaluate the direct and indirect effect of a commercial formulation of *B. bassiana* strain JW-1 (2.3×10^7 viable spores per milliliter of product) (Naturalis®-O, Troy BioScience, Phoenix, Arizona) on the beneficial organisms *Orius insidiosus* (Say), *Phytoseiulus persimilis* Athias-Henriot, *Encarsia formosa* Gahan, *Aphidius colemani* Viereck, and *I. degenerans.* The ability of the mycoinsecticide to infect the test organisms when placed into contact with fresh residues of *B. bassiana* will be referred to as a direct effect. The ability to infect the test organisms when placed into contact with the dried residues of *B. bassiana* will be referred to as an indirect effect.

Naturalis®-O was applied at 7.9 ml per liter (1.8×10^8 conidia per liter) in the laboratory and greenhouse studies. In the greenhouse studies, it was applied using a hand sprayer at 241 kPa to runoff. A sample was taken from each spray solution to determine the colony forming units (CFU). The CFU per millimeter of

test solution were determined using 10^{-5} and 10^{-6} serial dilutions. Dilutions were plated onto petri dishes containing sabouraud dextrose agar supplemented with gentamicin sulfate (SDA + G) and were incubated at 27°C for 3 d. The colonies were then marked and counted. The *O. insidiosus, P. persimilis*, *E. formosa*, and *A. colemani* were received by overnight shipment (Novartis, Greensboro, North Carolina). Experiments were initiated the day the natural enemies were received.

In the greenhouse trials, the direct effect of *B. bassiana* on beneficial organisms was determined by releasing adult beneficials on host plants immediately after the mycoinsecticide was applied. Natural enemy releases made prior to the insecticide application usually resulted in high mortality as a result of the high volume of water that is used to apply *B. bassiana.* Because we were interested in recapturing living organisms, we attempted to limit mortality due to the application technique. The indirect effect was evaluated by treating plants and allowing the spray to dry for approximately 4 h before releasing the test organisms. The control consisted of beneficial organisms released onto untreated plants.

Small chrysanthemum 'Dark Charm,' *Dendranthema* × *grandiflora* (Ramat.) Kitamura, plants were selected to facilitate recovery of the natural enemies, except for the *E. formosa* trials when poinsettia 'Red Freedom,' *Euphorbia pulcherrima* Willd., was used. Each replicate contained three plants placed into wooden cages with silk screen (110 mesh) sides and clear plastic tops measuring 1 × 1 × 1 m. Each treatment was replicated four times, with one replicate per cage. Within a trial, all reps and treatments were conducted at the same time. To keep cage conditions similar to the surrounding greenhouse, each cage had an air line attached, and a constant air flow was maintained. Temperature and humidity data loggers were used to record the environmental conditions in one cage. After 48 h, individual beneficials were collected from the plants using a camel-hair brush or aspirator and they were held in vials containing drops of honey until they died.

Individuals were washed with a 0.1% Triton®-X 100 (Aldrich Chemical Company, Milwaukee, Wisconsin) surfactant solution and were then rinsed twice with sterilized water. The individuals were then placed onto oatmeal dodine agar (ODA) plates. Plates were incubated at room temperature and evaluated for the presence of *B. bassiana* colonies after 7–10 d. Colonies were randomly selected and checked microscopically to ensure proper identification.

In the laboratory trials, the direct effect of *B. bassiana* on adult beneficial organisms was evaluated by dipping a 1.5-cm chrysanthemum leaf disk into the test solution, shaking off excess liquid, and then placing it into a 2-dram vial with two test individuals. The indirect effect was evaluated by placing individuals into vials with leaf disks that had been dipped into the *B. bassiana* solution and allowed to air dry for 4 h. In the water control, leaf disks were dipped into water, and individuals being evaluated were placed into vials with the leaf disk. Each replicate contained five vials, and the experiment was replicated four to five times. Vials were stored in a growth chamber (20°C, 14:10 h [L:D]). After 72 h, living individuals were washed, rinsed, and plated (as described above). Several individuals died during the experiment, probably due to manipulation, and sample size depended upon recapture; as a result, sample sizes were not consistent.

Insecticidal compatibility of *I. degenerans*. The following compounds were applied at the greenhouse ornamentals label rate: 1.2 ml/liter azadirachtin

(Azatin® XL, Olympic Horticulture Products, Mainland, Pennsylvania), 20 ml/liter neem oil (Triact® 90EC, Grace Biopesticides, Columbia, Maryland), and 20 ml/liter insecticidal soap (Safer Insecticidal Soap®, Safer Inc., Wellesley, Massachusetts) to determine their direct and indirect effects on *I. degenerans* mortality. To determine the direct effect, a chrysanthemum leaf disk was dipped into the test solution and was then placed into a 2-dram vial with one adult mite. In the indirect effect test, the leaf disks were allowed to air dry (for 4 h) before being placed into the vials with the mites. Vials were stored in a growth chamber (25°C, 14:10 h [L:D]). The vials were checked after 24 and 48 h to record mite mortality. The treatments were replicated seven times with five vials per replicate. The data were transformed using an arcsine transformation (Sokal and Rohlf 1995). The percentage of mortality data were subjected to analysis of variance (GLM procedure) (SAS Institute 1985). Means separation was accomplished using the least significant difference test (LSD) at the $P < 0.05$ level (SAS Institute 1985). All data are presented as original percentages.

Mycoinsecticide compatibility of *I. degenerans*. The direct and indirect effects of *B. bassiana* strain GHA (BotaniGard® ES, Mycotech, Butte, Montana), *V. lecanii* (MicroGermin® Plus, CHR Hansen BioSystems, Milwaukee, Wisconsin), and *M. anisopliae* strain ESC 1 (Bio-Blast®, EcoScience, East Brunswick, New Jersey) were evaluated to determine infection on *I. degenerans*. Spore levels for all three pathogens were standardized to be approximately 5.5×10^{10} spores per liter based on label information. To determine direct effects, a chrysanthemum leaf disk was dipped into the mycoinsecticide solution, excess water was shaken off, and it was then placed into a 2-dram vial with two adult mites. To determine indirect effects, leaf disks were allowed to air dry before being placed into the vials with the mites. Vials were stored in a growth chamber (25°C, 14:10 h [L:D]). After 48 h, individuals were washed with 0.01% Triton-X® 100 surfactant solution and were rinsed twice with sterilized water, then placed onto ODA plates and incubated at room temperature. Plates were visually evaluated for the presence of fungal colonies after 7–10 d to determine infectivity rates.

Results

***B. bassiana* susceptibility trials.** The average temperature and humidity recorded for each trial were similar to conditions found in a commercial greenhouse during the summer (Table 1). Samples taken from the spray solutions indicated that spore counts ranged from 1.4×10^7 to 1.7×10^7 viable spores per milliliter of product (Table 1).

Under greenhouse-caged conditions, *A. colemani* was highly susceptible to infection by *B. bassiana* (Table 2), with direct and indirect effect infection rates of 60% and 46.3%, respectively. The other four organisms tested had direct infection rates below 50%, with *P. persimilis* and *O. insidiosus* having infection rates of 7.9% and 4.9%, respectively. They also had indirect infection rates below 10%, with *I. degenerans* and *P. persimilis* having rates of 8.5% and 4.34%.

Under laboratory conditions, the natural enemies were highly susceptible to infection by *Beauveria* spores when exposed to the wet leaf, 68%–100% (Table 3). The infection rate varied widely when exposed to the dry residue. Rates ranged from 96% with *A. colemani*, to 42% with *P. persimilis* and 5.6 % for *O. insidiosus*.

Table 1. Environmental data and *Beauveria bassiana* spore count data from laboratory and greenhouse experiments evaluating compatibility of *B. bassiana* and natural enemies.

	Natural enemy	Trial	Spore count (spores/ml)	Humidity (% RH)	Temperature (°C)
Greenhouse	*Aphidius colemani*		1.6×10^7	76.2 ± 17.6	27.9 ± 5.7
	Encarsia formosa	1	1.6×10^7	76.2 ± 17.6	27.9 ± 5.7
		2	*	66.9 ± 17.0	23.6 ± 3.5
	Ipheseius degenerans		1.4×10^7	*	*
	Orius insidiosus	1	*	*	*
		2	1.5×10^7	66.9 ± 17.1	25.9 ± 5.1
	Phytoseiulus persimilis	1	1.6×10^7	76.2 ± 17.6	27.9 ± 5.7
		2	1.7×10^7	77.2 ± 17.6	29.1 ± 5.2
Laboratory	*Aphidius colemani*		1.7×10^7		
	Encarsia formosa		*		
	Orius insidiosus		1.5×10^7		
	Phytoseiulus persimilis		1.7×10^7		

[1]An asterisk indicates no data collected.

Table 2. *Beauveria bassiana* infection rates and number of plated individuals when five natural enemies were evaluated for compatibility with *B. bassiana* under greenhouse conditions.

	Direct effect			Indirect effect		
	Infected n[1] (range)	Infected n (range)	Mean infection rate	Infected n (range)	Infected n (range)	Mean infection rate
	1	2		1	2	
Aphidius colemani	60% 7.8 (5–11)		60.0%	46.3% 3.8 (1–5)		46.3%
Encarsia formosa	46.3% 4.3 (3–5)	37.5% 2.3 (1–5)	41.9%	55.5% 4.3 (3–7)	0.1% 3.5 (2–5)	27.8%
Ipheseius degenerans	42% a 4.5 (3–6)		42%	8.5% 5.8 (3–8)		8.5%
Orius insidiosus	6.3% 4.7 (3–7)	3.5% 5.5 (2–7)	4.9%	16.5% 5.3 (3–9)	7.5% 7.8 (5–10)	12.0%
Phytoseiulus persimilis	11.8% 3.5 (1–7)	4.0% b 3.7 (1–5)	7.9%	4.3% 4.5 (1–6)		4.3%

Mean infection rate was computed when more than one test was conducted.
[1]Average no. of individuals plated per replicate. Controls in all studies indicated no infection.

TABLE 3. *Beauveria bassiana* infection rates and number of plated individuals when four natural enemies were evaluated for compatibility with *B. bassiana* under laboratory conditions (20°C).

	Direct effect		Indirect effect		Control	
	Infected	n[1] (range)	Infected	n (range)	Infected	n (range)
Aphidius colemani	94.0%	8.8 (4–10)	96.0%	10.0	0.0%	10.0
Encarsia formosa	87.8%	8.0 (6–9)	9.6%	8.2 (8–9)	2.0%	9.4 (9–10)
Orius insidiosus	68.0%	6.8 (5–9)	5.6%	10.8 (10–13)	0.0%	9.8 (9–11)
Phytoseiulus persimilis	100%	8.8 (8–9)	42.0%	12.5 (12–14)	2.0%	12.5 (12–14)

[1]Mean no. of individuals plated per replicate.

Insecticidal compatibility of *I. degenerans*. In the direct effect trial, azadirachtin resulted in higher mortality rates ($P < 0.05$) than the other three treatments after 24 h (Table 4). After 48 h, azadirachtin and neem oil had significantly higher mortality rates ($P < 0.05$) than water and soap. In the indirect effect trial, neem oil mortality was higher ($P < 0.05$) than soap and azadirachtin after both 24 and 48 h, and was higher than water after 24 h (Table 4).

Mycoinsecticidal compatibility of *I. degenerans*. Infection rate trends among the three mycoinsecticides was consistent between the direct and indirect effect (Fig. 1). The highest infection rates were found for *B. bassiana* followed by *V. lecanii*, and then *M. anisopliae*.

Discussion

Our results indicate that beneficial organisms are susceptible to infection by *B. bassiana* by means of both direct and indirect exposure to this pathogen under laboratory and greenhouse conditions. Higher *B. bassiana* infection rates were observed in the laboratory study compared with the greenhouse study. Infectivity

TABLE 4. Percentage of indirect and direct mortality of *Ipheseius degenerans* after exposure to soap, water, azadirachtin, or neem oil under laboratory conditions (25°C).

	Indirect mortality[1]		Direct mortality[1]	
	24 h	48 h	24 h	48 h
Soap	0.0 a	11.4 a	28.0 a	34.3 a
Water	11.4 ab	20.0 a	20.0 a	25.7 a
Azadirachtin	5.7 a	11.4 a	54.3 b	65.7 b
Neem oil	25.7 b	42.9 b	31.4 a	62.9 b

[1]Means within columns with the same letter are not significantly different ($P > 0.05$, LSD).

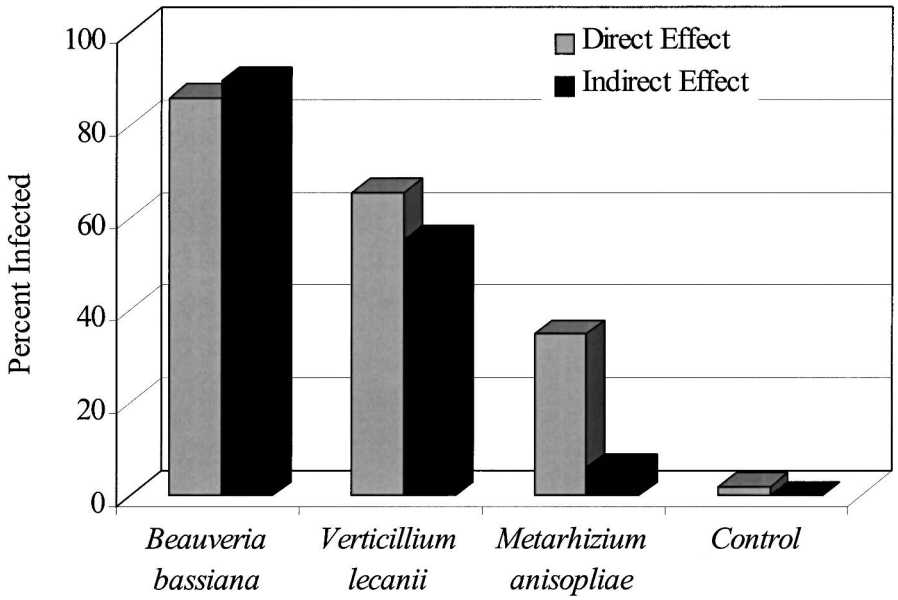

Fig. 1. *I. degenerans* direct and indirect infectivity rates when exposed to the insect pathogens *B. bassiana*, *V. lacanii*, and *M. anisopliae* under laboratory conditions.

of entomopathogenic fungi has been shown to increase under high humidity (Barson 1976). This may be due to the humid conditions that occur in a closed vial as a result of the spray solution evaporating and the leaf transpiring and desiccating.

Adult *A. colemani* was highly susceptible to *B. bassiana* in both greenhouse and laboratory studies. The mean infection rate in the greenhouse (60%) was the highest when this parasitoid was exposed to the spray (direct exposure). When exposed to plants after the spray dried (indirect exposure), the mean infection rate dropped one-half (33%). Under ideal conditions for sporulation in the laboratory, the *B. bassiana* infection rate was 94%. Despite the high infection rates, we would still recommend incorporating *B. bassiana* and *A. colemani* into a pest management program for aphids beacuse immatures appear to be protected from the spores by the aphid mummies. In another study, immature *A. colemani* were not infected by the microbial insecticide (D. O., unpublished data). It has been suggested that immature aphid parasitoids have evolved defensive means for preventing fungal infection (Askary & Brodeur 1999). Emerging adults are then capable of parasitizing any uninfected aphids. The indirect effect of the spores would have only a minor effect on the emerging adults.

Of the natural enemies evaluated, infection rates were lowest for *O. insidiosus* and *P. persimilis* (Table 2). Thus, *B. bassiana* used in combination with these species is likely to have minimal effects on their populations. Similar results have been reported in field studies. Jaronski et al. (1998) observed a 10% infection rate of *B. bassiana* in *Orius* sp. recovered from cotton fields treated with *B. bassiana.*

Insecticide compatibility trials involving *I. degenerans* demonstrated that insecticidal soap caused lower mortality than azadirachtin and neem oil (Table 4). Soap-induced mortality was not different than the water control, indicating that insecticidal soap had no direct effect on *I. degenerans*. Azadirachtin and neem oil caused the highest mortality to *I. degenerans* after 48 h. In addition to its acting as a contact insecticide, azadirachtin also acts as a growth regulator (Banken & Stark 1997). Because adult mites were evaluated, the effect of azadirachtin on the immature stages is not known. From visual observation, neem oil had a longer residual than the soap on the leaf disks, which may help explain its higher mortality. These results suggest that soap or azadirachtin can be used in conjunction with *I. degenerans* by releasing mites after an application. However, *I. degenerans* should not be released onto plants treated with neem oil until the residue has had a chance dissipate

Verticillium had similar direct and indirect infection rates (65.3% and 55.1%) as did *B. bassiana* (89.4% and 85.7%), respectively. *M. anisopliae* had a direct infection rate of 6.1% and indirect rate of 34.9%. The results indicate that all three mycoinsecticides are capable of infecting the predatory mite directly, when the solution is wet, or indirectly, after the solution has been allowed to dry. Results of the *B. bassiana* study indicate that mycoinsecticides can produce higher infection rates under laboratory conditions than greenhouse conditions. This suggests that under greenhouse conditions, infection due to *V. lecanii* and *M. anisopliae* should be minimal for *I. degenerans*. Further studies need to be conducted to evaluate the role of these pathogens on this predatory mite.

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Reproductive Success and Damage Potential of Tobacco Thrips and Western Flower Thrips on Cotton Seedlings in a Greenhouse Environment[1]

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ABSTRACT A greenhouse study was performed to assess the damage and reproductive potential of two thrips (Thysanoptera: Thripidae) species on cotton, *Gossypium hirsutum*, seedlings grown under relatively cool temperatures. The reproductive potential of tobacco thrips, *Frankliniella fusca* (Hinds), was greater than that of western flower thrips, *Frankliniella occidentalis* (Pergande), on two cotton varieties (Deltapine 436 RR® and Stoneville 474®). Plant biomass measurements and visual plant damage ratings performed on the last day of the study suggested that the damage potential of tobacco thrips was less than that of the western flower thrips. No differences in the reproductive potential of either thrips species were observed between the two cotton varieties used in the experiment. The results of this study confirm the importance of thrips identification because damage to cotton seedlings may be a function not only of population sizes, but also of the thrips species involved.

KEY WORDS Thysanoptera, Thripidae, *Frankliniella fusca*, *Frankliniella occidentalis*, *Gossypium hirsutum*, early-season injury

Thrips (Thysanoptera: Thripidae) are early season pests of cotton, *Gossypium hirsutum*, that typically infest seedlings immediately following emergence. Both adult and juvenile stages injure cotton seedlings by rasping the young, tender leaves and terminals, resulting in distortion of leaf shape, browning along leaf margins or over the entire leaf, and holes in the leaf margin. Thrips feeding injury to the cotyledon stage leaves is characterized by stippling that can ultimately result in loss of chlorophyll and, in severe cases, a torn, ragged appearance. Various thrips species may significantly impact both developmental and maturity parameters, including yields (Gaines 1934, Dunham & Clark 1937, Newsom et al. 1953, Herbert 1998, Van Duyn et al. 1998).

Numerous factors have been identified that contribute to variations in thrips damage to cotton seedlings observed annually. These factors include cotton variety, weather, and thrips density. Different cotton varieties have demonstrated varying levels of tolerance to thrips injury, and these variations have been attributed to plant characteristics such as trichome density, gossypol production,

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and thickness of the epidermal tissue (Gawaad & Soliman 1972, Rummel & Quisenberry 1979, Bowman & McCarty 1997). Faircloth et al. (1998) concluded that a combination of cool, wet weather, coupled with moderate to high thrips populations during the seedling stage of cotton resulted in heavily damaged cotton and reduced yields in eastern North Carolina.

Another factor that potentially impacts the extent of thrips damage is the species composition of thrips populations that colonize cotton seedlings. The importance of species composition as it relates to the extent of damage is not well understood. J. G. Watts (1937) suggested the importance of knowing the species composition of thrips as it relates to their control. Hightower (1958) later reported the damage potential of the tobacco thrips, *Frankliniella fusca* (Hinds), on seedling cotton in a greenhouse study to be greater than that of the eastern flower thrips, *Frankliniella tritici* (Fitch).

Two of as many as six species commonly found infesting cotton seedlings are western flower thrips, *Frankliniella occidentalis* (Pergande), and tobacco thrips. The most prevalent species inhabiting cotton seedlings in numerous states including North Carolina is the tobacco thrips (Newsom et al. 1953, Burris 1980, All et al. 1993, Van Duyn et al. 1998). Past studies conducted in North Carolina reported western flower thrips as composing only a small proportion of the species complex (Van Duyn et al. 1998, Faircloth et al. 1999); however, unusually high proportions of western flower thrips were reported on seedling cotton in certain areas of North Carolina in 1999 (Bacheler 1999). Increased proportions of western flower thrips in seedling cotton are of great concern for two reasons: western flower thrips are more difficult to control with certain conventional insecticides (Bacheler 1999), and they also may vary from other species of thrips in their reproductive and damage potentials.

One objective of this study was to compare the reproductive success of western flower thrips and tobacco thrips on seedlings of two cotton varieties under controlled environmental conditions in a greenhouse. Another objective of this study was to compare the damage potentials of western flower thrips and tobacco thrips on seedlings of two cotton varieties under controlled environmental conditions.

Materials and Methods

This study was conducted in a greenhouse at North Carolina State University in Raleigh, North Carolina. The two cotton varieties used were Deltapine 436 RR® and Stoneville 474® because these varieties are commonly grown in North Carolina. The test was initiated and seeds were sown on 10 October 1999. Seeds were sown in black plastic, 3.78-liter pots containing Scotts Metro Mix 200 potting soil. Cylindrical, insect-proof containers (15 cm diameter × 30 cm height) constructed of 5-mm, clear Vivak plastic (AIN Plastics Corp., Greensboro, North Carolina) covered with Bed Bug 110 fine-mesh screening (Green Thumb Group, Inc., Dawners Grove, Illinois) were placed inside the outer rim of the pots gently seated below the soil line. From 15 October to 27 October, mean daily temperatures of 28:16°C (L:D) and a photoperiod of 14:10 h (L:D) were recorded. Beginning 28 October and continuing through the remainder of the experiment, mean daily temperatures were increased and held at temperatures of 32:20°C (L:D) and a photoperiod of 14:10 h (L:D). Mean temperatures were increased in this experi-

ment to simulate the temperature typical at and shortly after planting cotton in eastern North Carolina in early to mid May.

Newly emerged seedlings of both varieties were individually infested with 10 newly eclosed adult thrips per plant on 15 October. Both thrips species used in the experiment were obtained from a laboratory colony maintained on green bean pods (*Phaseolus vulgaris* L.) in an enclosed chamber held at 24°C, 65% RH, and a photoperiod of 14:10 (L:D) h. Approximately 24–48 h following enclosure, adults were aspirated into 2.5-ml disposable Pasteur® pipettes (10 thrips/pipette), and the ends of the pipettes were sealed with Parafilm® to prevent escape.

The seedlings remained infested for 21 d, representing the period of time that thrips commonly infest North Carolina cotton seedlings at damaging population levels. This period of time was also selected to maximize thrips recovery based on documented life cycles (Robb 1988, Lewis 1997). To compare the reproductive success of the two species, the exclusion cages were lifted from each pot on 4 November while simultaneously severing the seedlings directly below the cotyledonary nodes. A plant washing procedure similar to that described by Irwin & Yeargan (1980) was used. Seedlings were placed in labeled mason jars containing a 0.1% detergent solution and were subsequently emptied over a #100 U.S.A. Standard Testing Sieve (150 μm). All residue remaining in the sieve was further rinsed into a 20-ml scintillation vial containing 70% EtOH. Each vial was later individually emptied into a petri dish under a dissecting scope where the number of juvenile thrips and adult thrips were counted and recorded.

On 29 October and 4 November, the cotton seedlings were visually rated to assess the relative damage potential of the two species of thrips. Ratings were based on the severity of thrips feeding damage to the newly emerging true leaves. The signs of thrips damage considered included distortion, discoloration, and tears or missing areas in the true leaves. The rating scale used ranged from 1 to 5 and is described as follows: 1, leaves with no visible damage; 2, slight damage (crinkling or browning of leaf with less than 25% leaf loss); 3, moderate damage (crinkling and browning with less than 50% leaf loss); 4, severe damage (leaf loss greater than 50% yet remains of the leaf present); and 5, complete loss of true leaves.

Immediately following thrips recovery on 4 November, the seedlings from each jar were dried for 24 h in a Thelco, Model 17 (Precision Scientific, Winchester, Virginia) drying oven at 65°C. At the end of the drying period, the plants were removed from the oven and weighed. Because thrips damage on cotton seedlings is usually more intense on the true leaves, and the cotyledons appeared to comprise a significant amount of the total seedling weight, cotyledons were then removed and all plants were reweighed. All weights were taken using a Precision Standard (OHAUS, Florham Park, New Jersey) scale.

The four treatments consisted of two cotton varieties (Stoneville 474® and Deltapine 436®) and two species of thrips (tobacco and western flower thrips) in all combinations. This two by two factorial design was set within a randomized complete block with three replicates. Each replicate was contained on a different bench within the greenhouse. Populations of thrips recovered were log transformed prior to analysis to normalize variance. The analysis of variance was performed using Pesticide Research Manager Software (Gyllings Data Management, Inc., Brooking, South Dakota). Reported means in tables and figures are all

back-transformed. Data from the plant measurements were analyzed using the same analysis of variance but without transformation.

Results and Discussion

Thrips reproduction. Both thrips species used in these experiments reproduced successfully under the greenhouse environments utilized. Mean numbers of juvenile thrips per plant were 36.90 and 23.97 for tobacco thrips and western flower thrips, respectively, after 21 d, and averaged over both cotton varieties (Table 1). Populations of tobacco thrips juveniles were significantly higher (F = 501.8, df = 1 and 6, P = 0.059) than that of western flower thrips and there was not a significant thrips species by cotton variety interaction (F = 0.550, df = 1 and 6, P = 0.4861), suggesting that the reproductive potential was higher for tobacco thrips on both plant varieties. Adult numbers were relatively low upon termination of the test at 2.52 tobacco thrips per plant and 4.27 western flower thrips per plant. No significant differences (F = 4.628, df = 1 and 6, P = 0.0750) in adult counts were observed between thrips species averaged over both cotton varieties or with the thrips species by cotton variety interaction (F = 0.638, df = 1 and 6, P = 0.4547) (Table 1). Cotton variety did not appear to have a significant effect on reproduction in either thrips species as there were no significant main effects regarding juvenile (F = 0.459, df = 1 and 6, P = 0.5234) or adult (F = 0.353, df = 1 and 6, P = 0.5741) thrips, averaging over thrips species (Table 2).

Successful reproduction of both thrips species confirmed the validity of the greenhouse techniques employed to compare damage potential on seedling cotton. Furthermore, the adult-to-juvenile thrips ratios approximate those observed in field experiments (Faircloth et al. 1998).

Plant damage evaluation. The 29 October damage rating (14 d postinfestation) averaged 2.20 and 3.68 over both varieties for tobacco thrips and western flower thrips, respectively, implying that western flower thrips populations imparted significantly greater damage (F = 274.14, df = 1 and 6, P = 0.0001) (Table 1). Likewise, on 4 November (20 d postinfestation), seedlings infested with western flower thrips again averaged a significantly higher damage rating (F = 91.125, df = 1 and 6, P = 0.0001) of 3.7 compared with 2.8 for tobacco thrips populations. There were no significant effects of cotton variety averaged over thrips species on 29 October (F = 1.69, df = 1 and 6, P = 2.413) or on 4 November (F = 1.125, df = 1 and 6, P = 0.3297). The thrips species by cotton variety interaction was not significant on 29 October (F = 0.034, df = 1 and 6, P = 0.8588) or on 4 November (F = 1.125, df = 1 and 6, P = 0.3297). Damage did not increase proportionally between 29 October and 4 November, perhaps as a result of greenhouse temperatures being elevated during the experiment to simulate temperature increases typical of field conditions. Thus, cotton seedling growth and seedling tolerance to thrips infestations may have increased over the course of the experiment.

Dry weights of whole cotton seedlings after 21 d of western flower thrips infestation were significantly reduced relative to dry weights of whole cotton seedlings after 21 d of tobacco thrips infestation (F = 64.483, df = 1 and 6, P = 0.0002) (Table 1). Significant differences were also found regarding cotton variety averaged over thrips species (F = 0.0258, df = 1 and 6, P = 12.53) as Stoneville

TABLE 1. Mean number (± SEM) of adult and juvenile thrips, rating scale values, and seedling dry weights per plant at 21 d after infestation, averaged over two cotton varieties.

Thrips species	Juvenile thrips	Adult thrips	Rating scale value[a] (Oct. 29)	Rating scale value (Nov. 4)	Dry weight	Dry weight[b]
F. fusca	36.90 ± 4.85	2.52 ± 0.59	2.20 ± 0.11	2.80 ± 0.07	196.22 ± 13.6	78.17 ± 10.16
F. occidentalis	23.97 ± 2.44	4.27 ± 1.33	3.68 ± 0.16	3.70 ± 0.12	155.10 ± 14.62	40.97 ± 9.87

[a]Rating scale values ranged from 1 to 5 and is described as follows: 1, leaves with no visible damage; 2, slight damage (crinkling or browning of leaf with less than 25% leaf loss); 3, moderate damage (crinkling and browning with less than 50% leaf loss); 4, severe damage (leaf loss greater than 50% yet remains of the leaf present); and 5, complete loss of true leaves.
[b]Dry weight taken without cotyledons attached in grams.

TABLE 2. Mean number (± SEM) of adult and juvenile thrips, rating scale values, and seedling dry weights per plant at 21 d after infestation, averaged over two thrips species.

Cotton variety	Juvenile thrips	Adult thrips	Rating scale value[a] (Oct. 29)	Rating scale value (Nov. 4)	Dry weight	Dry weight[b]
Stoneville 474	29.38 ± 5.6	3.63 ± 1.35	2.88 ± 0.26	3.30 ± 0.49	183.2 ± 14.05	57.87 ± 12.94
Deltapine 436	31.48 ± 5.54	3.15 ± 0.77	3.00 ± 0.33	3.20 ± 0.48	168.12 ± 22.22	61.27 ± 17.54

[a]Rating scale values ranged from 1 to 5 and is described as follows: 1, leaves with no visible damage; 2, slight damage (crinkling or browning of leaf with less than 25% leaf loss); 3, moderate damage (crinkling and browning with less than 50% leaf loss); 4, severe damage (leaf loss greater than 50% yet remains of the leaf present); and 5, complete loss of true leaves.
[b]Dry weights taken without cotyledons attached in grams.

474® whole seedling weight was reduced less than that of Deltapine 436 RR® seedlings. There was not a significant thrips species by cotton variety interaction ($F = 5.786$, df = 1 and 6, $P = 0.0529$) with respect to the mean dry weights of whole cotton seedlings. By visual inspection, it appeared that the cotyledonary leaves of the seedlings sustained less damage relative to the terminals of the plants while the cotyledons comprised over 75% of the total biomass. Therefore, the seedlings were reweighed after removing the cotyledonary leaves. The effect of thrips species on seedling weight after removal of cotyledon leaves averaged across varieties was significant ($F = 143.102$, df = 1 and 6, $P = 0.0001$) (Table 1). Cotton variety averaged across thrips species had no significant effect on the seedling weights after removal of cotyledons ($F = 1.195$, df = 1 and 6, $P = 0.3162$) (Table 2), and there was not a significant thrips species by cotton variety interaction ($F = 3.243$, df = 1 and 6, $P = 1218$).

In summary, the reproductive potential of tobacco thrips was greater than that of western flower thrips on cotton seedlings in this greenhouse study; however, the damage potential of western flower thrips was greater than that of tobacco thrips. Thus, although population size of thrips infesting cotton is important, the species composition of thrips populations may also markedly impact damage potential. The results of this study confirmed the importance of thrips species identification in studies that evaluate thrips management tactics and strategies.

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Tolerance to Cry1Ac in Populations of *Helicoverpa zea* and *Heliothis virescens* (Lepidoptera: Noctuidae): Three-Year Summary[1]

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ABSTRACT Field populations of *Helicoverpa zea* (Boddie) and *Heliothis virescens* F. from the eastern one-half of the United States cotton belt were monitored from 1996 to 1998 for tolerance to the *Bacillus thuringiensis* toxin Cry1Ac. Spray chamber analyses of *H. zea* from the Mississippi Delta revealed a slight decrease in susceptibility to Cry1Ac during the 3-year period. Areas producing the greatest change in tolerance to Cry1Ac had a greater percentage of their acreage planted in *Bt* cotton. In general, tolerances of *H. virescens* populations did not change, with the single exception being the third generation of *H. virescens* collected from the Mississippi Delta. The small changes in tolerance in *H. zea* reported herein suggested that even though populations were slightly more tolerant of Cry1Ac, they were not at a level to cause control failures in the field.

KEY WORDS *Helicoverpa zea*, *Heliothis virescens*, *Bacillus thuringiensis*, resistance monitoring, Cry1Ac, transgenic cotton

The conservation of susceptibility to endotoxin proteins of *Bacillus thuringiensis* (*Bt*) in field populations of insect pests of cotton has received considerable interest. Resistance to *Bt* proteins has been documented in laboratory and field populations of several insect pests (Stone et al. 1989, Tabashnik et al. 1990, 1997, 2000, Gould et al. 1992, 1995, Moar et al. 1995). In addition, the recent registration and deployment of transgenic cotton expressing the *Bt* protein, Cry1Ac, and the development of resistance to many conventional insecticides (most recently, pyrethroids) by lepidopteran pests of cotton have made the preservation of susceptibility to Cry1Ac an important goal of pest management in cotton-growing areas (Hardee et al. 2001). To manage resistance effectively, cotton insect pests must be monitored for changes in their tolerances to Cry1Ac (Gould et al. 1997, Andow & Alstad 1998).

For three growing seasons (1996 to 1998), we monitored tolerances of Cry1Ac in field populations of *Heliothis virescens* F. and *Helicoverpa zea* (Boddie). Our objectives were to develop a baseline of tolerances for field populations and at-

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Prod. #

tempt to detect any decline in susceptibility to the Cry1Ac toxin. We present the status of our monitoring efforts for those years in this paper.

Methods and Materials

Entomologists and consultants from 10 states within the United States cotton belt collected eggs or larvae of field populations of *H. virescens* and *H. zea* (Fig. 1). The collected individuals (F_1's) were shipped to USDA-ARS, Stoneville, Mississippi, and were reared to pupation on artificial diet at 29 ± 3°C, 55%–60% RH, with a 14:10 (L:D)-h photoperiod. The (F_2) offspring were evaluated for tolerance to Cry1Ac. Field populations producing fewer than 50 F_1 adults were discarded to diminish the likelihood of inbreeding effects. Each field colony was considered a replicate.

A spray chamber bioassay was used during 1996 to evaluate all field populations and laboratory (USDA-ARS, Stoneville, Mississippi) strains of *H. virescens* and *H. zea*. The spray chamber bioassay did not allow large numbers of larvae to be efficiently tested for the many populations submitted during 1997 and 1998. Therefore, during 1997 and 1998, an artificial diet bioassay was begun in order to more efficiently test large samples of larvae and colonies. Only populations from

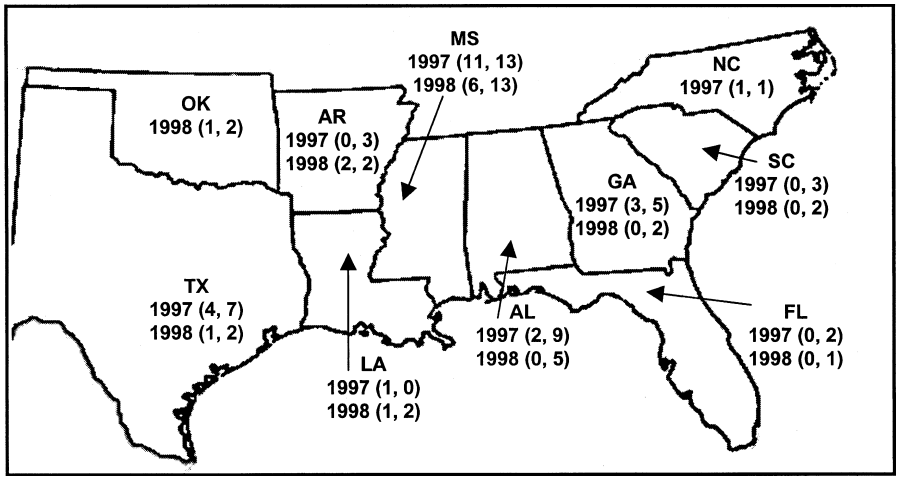

Fig. 1. Numbers of submitted populations for each state during 1997 and 1998 [Number of populations: ("# of *H. virescens* populations," "# of *H. zea* populations")]. The following individuals submitted populations ([1] = 1996, [2] = 1997, [3] = 1998): AL: D. Barden[2], A. Barron[2], S. Clarke[2], G. Jones[2], B. Kimbrell[2], W. Moar[2], R. Smith[2,3], S. Smith[2], R. Weeks[2], J. Zorn[2]; AR: C. Allen[1,2,3]; FL: M. Braxton[2], W. Moar[2], S. Smith[2,3]; GA: M Braxton[2], R. Higdon[2], R. McDaniel[3], P. Roberts[2,3]; LA:- R. Leonard[2,3]; MS: C. Adams[3], R. Ford[1,2,3], B. Layton[1,2], B. Kimbrell[2], J. Rusco[2], J. Shuford[1], S. Stewart[2,3], A. Wier[2], W. Worley[2,3]; NC: J. Bachelor[2], D. Pitts[2]; OK: M. Karner[1,3]; SC: J. Durant[3], D. Pitts[2], M. Sullivan[2,3]; and TX: J. Benedict[12,3], J. Mann[2], S. Nemec[1,2].

Washington County, Mississippi, were evaluated via the spray chamber bioassay during 1997 and 1998 in order to make comparisons over a 3-year period. The methods and materials used in the spray chamber bioassays are reported in Elzen et al. (1990). In these bioassays, MVPII, the biological insecticide closest in properties to the Cry1Ac protein expressed in transgenic cotton, was applied to cotton terminals placed in florist's water wicks at a rate of 3 pt/acre. A single 3rd instar (30 ± 3 mg) was placed on each cotton terminal 30 min after the application of MVPII. Numbers of moribund and dead larvae were determined after 72 h.

All field and laboratory populations of *H. virescens* and *H. zea* were evaluated for tolerance to Cry1Ac during 1997 and 1998 using an agar overlay containing a freeze-dried formulation of MVPII powder applied to an artificial diet base (J. T. G., unpublished data). One neonate was added to each well containing the agar overlay of Cry1Ac and was allowed to feed on the toxin for 5 d at 29 ± 3°C, 55%–60% RH, with a 14:10 (L:D)-h photoperiod. For each test, larvae from each colony were assayed on the Bt toxin (mean number of larvae tested: 69.6 larvae from 21 *H. virescens* colonies during 1997, and 128 larvae from 10 colonies during 1998; 68.8 larvae from 39 *H. zea* colonies during 1997, and 128 larvae from 30 colonies during 1998), as well as nontoxic diet (69.4 *H. virescens* larvae during 1997, and 128 larvae during 1998; 70.6 *H. zea* larvae during 1997, and 128 larvae during 1998). Concentrations of Cry1Ac in the agar overlay were 0.05 and 5.0 μg/ml for *H. virescens* and *H. zea*, respectively. The concentrations were based on EC98 data for the two species (Sims et al. 1996). Larvae were scored for tolerance of Cry1Ac based on their length, which was an assessment of their developmental rate. Larvae > one-quarter of an inch and < one-half of an inch were scored as tolerant larvae. These larvae represented late 2nd to early 3rd instar larvae. Larvae from the USDA-ARS, Stoneville, Mississippi, laboratory colonies of *H. virescens* and *H. zea* were used in each test as a control for diet and growing conditions (same as mentioned above).

Statistical analyses. *G* tests were used to compare years for percentage mortality of field populations of *H. virescens* and *H. zea* when evaluated within the spray chamber. Pairwise comparisons between years were made via unplanned tests of the homogeneity of replicates (years) tested for goodness of fit when significant heterogeneity was found with the *G* tests (Sokal & Rohlf 1995). This technique minimizes the experiment-wise error rate. For the MVPII overlay tests, comparisons between the percentage of tolerance of field colonies and the laboratory strain were made for each species via paired *t* tests during 1997 and 1998. All percentages were arcsin-square root-transformed before analyses were performed, and all analyses were performed separately for toxic and nontoxic diets. During these tests, the laboratory colony was tested each time a field colony was tested. Thus, because a laboratory colony was paired with a field colony, we used a paired *t* test with replication resulting from the pairs. In addition, the mean difference between the transformed tolerances of field colonies and the laboratory strain (i.e., transformed percentage of tolerance of field colony – transformed percentage of tolerance of laboratory colony) in 1997 and 1998 were compared via *t* tests for two samples assuming unequal variances. To better compare the absolute performances of field and laboratory tolerances in 1997 and 1998, a one-way ANOVA (PROC MIXED, Littell et al. 1996) was performed on transformed percentages of tolerances. Differences between population groups designated as Field 1997, Field 1998, Laboratory 1997, and Laboratory 1998 were

separated using least-squared means with differences considered significant when $P < 0.05$.

To look for geographic patterns of tolerance to Cry1Ac, we pooled populations that were within a 50-km radius of each other where sampling occurred in both 1997 and 1998. Because only the Mississippi Delta was intensively sampled during the first and second generations, we limited our comparisons to only the third and fourth generations. For *H. zea*, we were left with seven regions that met the above criteria: (1) area surrounding Corpus Christi, Texas (TX); (2) Arkansas Delta (AR); (3) Mississippi Delta (Delta); (4) central Alabama (AL); (5) southern Alabama/Florida panhandle (AL/FL); (6) area around Tifton, Georgia (GA); and (7) area around Blackville, South Carolina (SC). The relative acreage planted to *Bt* cotton ranged from >80% (AL/FL) to very a small portion of the acreage (<5%, TX). Only three areas of *H. virescens* collections met our criteria: (1) TX; (2) LA; and (3) Delta. *G* tests were used to compare percentages of tolerances between years, among regions, and to determine if differences among geographic locations were dependent on the year of collection. Pairwise comparisons between year × region treatments were made via unplanned tests of the homogeneity of replicates (year × region treatments) tested for goodness of fit when significant heterogeneity was found with the *G* tests (Sokal & Rohlf 1995).

We also investigated whether host plant/generation affected the tolerance of *H. zea* populations. Because we had intensively sampled populations surrounding the USDA-ARS research station in Stoneville, Mississippi, we compared populations collected on different host plants. Because of the sequence of host plant use by *H. zea*, generation effects are confounded by host plant effects and vice versa. *G* tests were used to compare percentage of tolerances between years, among host plants, and to determine if differences among host plants were dependent on the year of collection. Pairwise comparisons between year × host plant treatments were made via unplanned tests of the homogeneity of replicates (year × host plant treatments) tested for goodness of fit when significant heterogeneity was found with the *G* tests (Sokal & Rohlf 1995). This technique minimizes the experimentwise error rate.

Results

Spray chamber assays. In all spray chamber bioassays, the MVPII killed significantly more larvae than the water-control treatment. The mortality of *H. zea* populations from Washington County, Mississippi, treated with MVPII varied among years (Fig. 2; $G = 11.012$; df = 2; $P = 0.004$). *H. zea* larvae were more tolerant of MVPII in 1998 relative to 1996, and each year the percentage of mortality decreased. Unlike populations of *H. zea*, there were no significant differences among years for mortality of *H. virescens* populations from Washington County (Fig. 2; $G = 1.682$; df = 2; $P = 0.431$). However, only 24.4% of *H. virescens* larvae from the third generation of 1998 were killed by the MVPII treatment (see below).

MVPII overlay studies

H. zea. There were no significant differences among the field populations and tests of the laboratory strain of *H. zea* in their performance on nontoxic diet during 1997 and 1998 (1997: $0_{\text{Difference}} = 1.89\%, t = 0.246$, df = 37, $P = 0.807$; 1998: $0_{\text{Difference}} = 0.13\%, t = 0.593$, df = 19, $P = 0.560$). Furthermore, there were

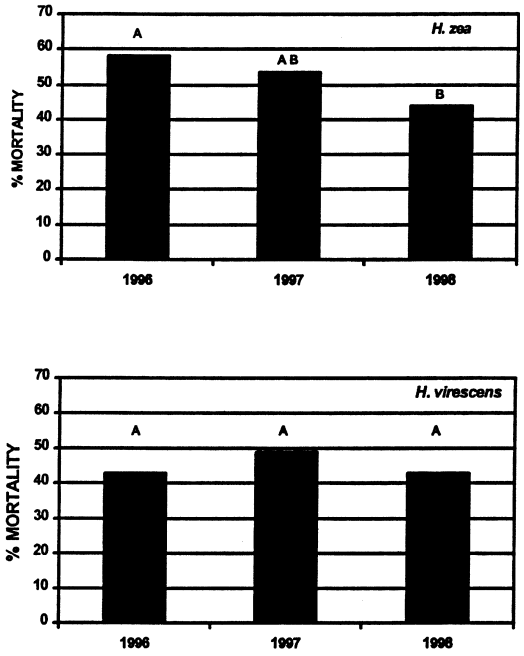

Fig. 2. Percentage mortality of *H. zea* (top) and *H. virescens* (bottom) from Washington County, Mississippi, when treated with spray chamber applications of MVPII.

no significant differences for percentages of tolerance on nontoxic diet for tests involving larvae from laboratory 1997, laboratory 1998, field 1997, and field 1998 (Table 1). As described above, the replication was in the pairing of a laboratory-colony replication with each test of the field colony; thus, a paired t test was used.

Differences among field populations in tolerance of MVPII were not related to their general vigor because the performance of field populations on the nontoxic diet was not significantly correlated with their performance on the toxic diet in either year ($r_{1997} = -0.045$, df = 37, $P = 0.779$, and $r_{1998} = 0.183$, df = 19, $P = 0.324$). When fed diet containing Cry1Ac, significantly more larvae from *H. zea* field colonies were tolerant than those from the laboratory control strain [paired t test; 1997: $0_{\text{Difference}}$ (± SE) = 1.50% ± 0.29%, $t = 5.199$, df = 38, $P < 0.0001$; 1998: $0_{\text{Difference}} = 6.20\% \pm 1.71\%$, $t = 4.590$, df = 19, $P = 0.0002$]. ANOVA revealed significant differences among the performances of field and laboratory populations during 1997 and 1998 (Table 1). Field populations collected during 1998 were significantly more tolerant of MVPII than the field populations of 1997 and the tests of the laboratory colony during 1997 and 1998 (Fig. 3). Tolerances of field populations collected during 1997 were significantly greater than 1997 tests involving the laboratory colony. However, the laboratory colony was slightly more tolerant of MVPII in 1998 than during 1997. Because of the greater tolerance of the laboratory colony during 1998, we compared the 1997 and 1998 differences between field and laboratory strains to determine if field colonies were

TABLE 1. Comparison of mean tolerances for tests involving field and laboratory colonies on nontoxic and toxic diet.

Diet	df	*F*	*P*	% Tolerance[a] Laboratory 1997	Field 1997	Laboratory 1998	Field 1998
H. zea							
Nontoxic	3, 96	1.18	0.322	92.33a	92.17a	90.49a	92.84a
Cry1Ac	3, 96	19.43	<0.0001	0.24a	1.76b	2.15b	7.66c
H. virescens							
Nontoxic	3, 59	0.21	0.892	91.94a	90.88a	90.76a	91.72a
Cry1Ac	3, 59	4.07	0.011	0.29a	2.09b	0.78ab	2.73b

[a]Mean tolerances in a row followed by the same letter are not significantly different as determined by comparisons of least-squared means for data that were arcsin-square root-transformed before analysis.

more tolerant of Cry1Ac during 1998 relative to 1997. The differences between field strains and the control strain were significantly greater in 1998 than 1997 (t test assuming unequal variances: $t = 2.703$, df = 30, $P = 0.0111$).

To better compare the results of 1997 and 1998, we pooled populations from the regions sampled in both years. Only the Mississippi Delta was sampled in both years during the first and second generations. Therefore, to control for host plant/generation effects, we only compared the tolerances among regions and between years for the third and fourth generations. We found significant differences

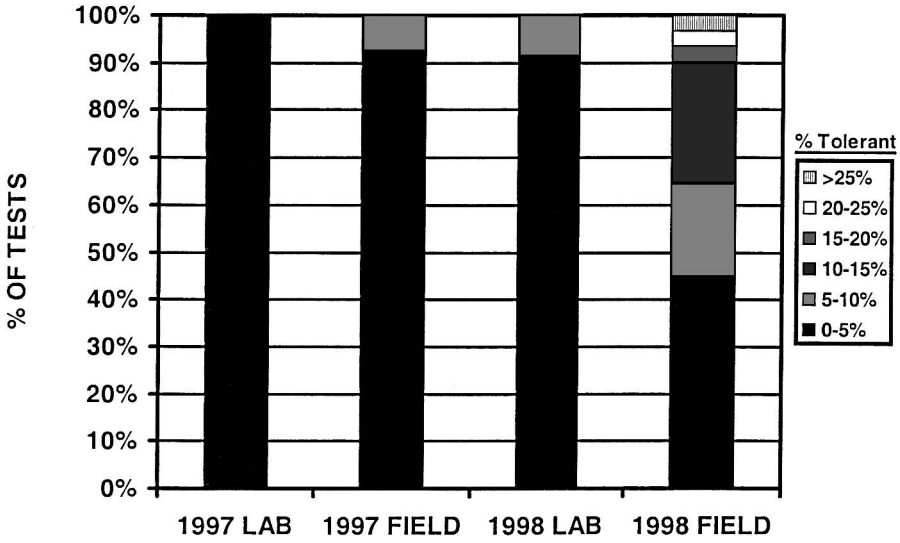

Fig. 3. Distribution of percentage of tolerance of Cry1Ac for tests of field and lab colonies of *H. zea* during 1997 and 1998.

among regions for the percentage of tolerant larvae (range = 1.71%–13.37%, G = 94.398, df = 6, $P < 0.0001$; Fig. 4). There were also significantly more tolerant larvae in 1998 (9.50%) than 1997 (1.85%) ($G = 162.317$, df = 1, $P < 0.0001$). Differences among regions were dependent on year. During 1997, there were no significant differences among regions for the percentage of tolerant larvae (G = 9.687, df = 6, $P = 0.138$). However, significant differences among regions in the tolerance of larvae to Cry1Ac were found during 1998 (G = 78.569, df = 6, $P <$ 0.0001). 1997 populations and populations collected in Texas and Arkansas during 1998 did not significantly differ in their percentage of tolerant larvae (Fig. 4). 1998 populations from southern Alabama and the panhandle of Florida (AL/FL) were significantly more tolerant than all other regions (Fig. 4).

Because *H. zea* larvae were collected during all four generations in Washington County, Mississippi, we used larvae from these populations to compare the host plant effects on the tolerance of *H. zea* larvae during 1997 and 1998. The tolerances of collected *H. zea* larvae during 1998 were significantly greater than the tolerances of larvae collected during 1997 (Fig. 5; G = 52.887, df = 1, $P < 0.0001$). Host plant also significantly affected the tolerances of collected larvae (G = 33.138, df = 3, $P < 0.0001$). However, the effect of host plant was dependent on the year of collection (Fig. 4). No significant host plant effects were observed during 1997 (G = 3.585, df = 2, $P = 0.167$). The host plant from which *H. zea* larvae were collected significantly influenced tolerance to Cry1Ac during 1998 (G = 42.523, df = 3, $P < 0.0001$). Populations of *H. zea* collected from *Bt* cotton during 1998 were significantly more tolerant of Cry1Ac than all than all other groups. Larvae collected from corn during 1998 were also significantly more tolerant of the Cry1Ac than 1997 larvae and 1998 larvae collected from wild hosts and non-*Bt* cotton.

H. virescens. There were no significant differences among the field and laboratory populations of *H. virescens* in their performance on nontoxic diet during

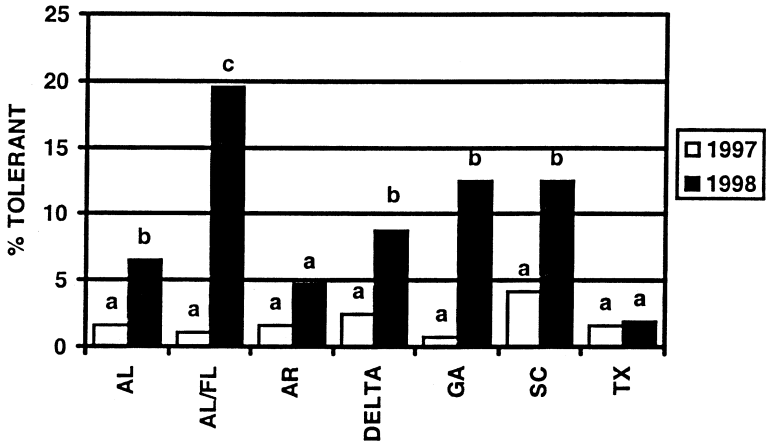

Fig. 4. Percentage of *H. zea* larvae scored as tolerant after 5 days of feeding on diet containing Cry1Ac. Percentages denoted by the same letter represent regions that are not significantly different as determined by tests of homogeneity.

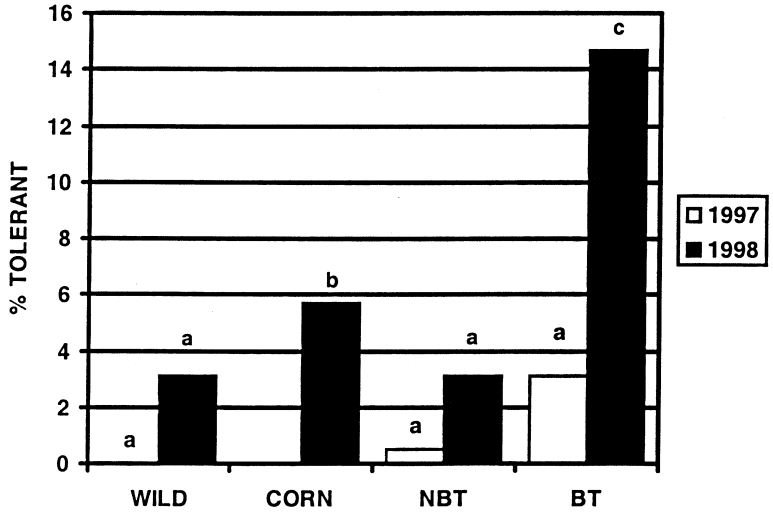

Fig. 5. Percentage of *H. zea* larvae from Washington County, Mississippi, scored as tolerant after 5 days of feeding on diet containing Cry1Ac. Percentages denoted by the same letter are not significantly different as determined by tests of homogeneity.

1997 and 1998 (1997: $0_{\text{Difference}} = 2.28\%$, $t = 1.391$, df = 22, $P = 0.178$; 1998: $0_{\text{Difference}} = 0.55\%$, $t = 0.217$, df = 9, $P = 0.833$). Furthermore, there were no significant differences for percentage of tolerance on nontoxic diet for tests involving larvae from laboratory 1997, laboratory 1998, field 1997, and field 1998 (Table 1; $F = 0.21$, df = 3 and 59, $P = 0.892$).

Differences among field populations in tolerance of MVPII were not related to their general vigor during 1998 because the performance of field populations on the nontoxic diet was not correlated with their performance on the toxic diet in either year ($r_{1998} = -0.112$, $P = 0.748$). However, the performance of field populations on nontoxic and toxic diet was significantly correlated during 1997 ($r_{1997} = 0.480$, $P = 0.015$). When compared with the laboratory strain, field populations of *H. virescens* were more tolerant of Cry1Ac during 1997 and not different in 1998 (1997: $0_{\text{Difference}}$ (± SE) = 1.46% ± 0.49%, $t = 2.974$, df = 22, $P = 0.007$; 1998: $0_{\text{Difference}} = 1.82\% \pm 1.27\%$, $t = 1.421$, df = 9, $P = 0.189$). ANOVA revealed significant differences among the performances of field and laboratory populations during 1997 and 1998 (Table 1). Comparisons of the least-squared means for tolerances found a significant difference between the field colonies and tests of the laboratory colony during 1997 (Table 1). Tolerances of field populations collected during 1998 did not differ from 1998 tests of the laboratory colony. The performance of the laboratory colony did not differ in 1997 and 1998. In addition, the performance of field colonies did not differ in 1997 and 1998. The average differences between the tolerances of field and laboratory colonies in 1998 were not significantly different from those in 1997 [$P = 0.833$, $t = 0.213$, df = 33, range (percentage of tolerance): 1997, 0%–6.25%; 1998, 0%–13.4%].

For regions where *H. virescens* larvae were collected during 1997 and 1998, there were significant differences in tolerances of Cry1Ac among regions (Fig. 6; $G = 9.544$, df = 2, $P = 0.009$). The differences among regions were a consequence of the higher tolerances of third generation populations of *H. virescens* from the Mississippi Delta (Fig. 6). *H. virescens* larvae from this population were the most tolerant of Cry1Ac in both overlay (13.4% tolerant) and spray chamber (24% mortality) bioassays.

Discussion

In order for insecticide resistance to evolve, it is necessary for at least some of the tolerant individuals to survive exposure to the new insecticide. The magnitude of change in the mean phenotype of the population that will allow some survival of tolerant individuals will influence the genetic architecture of the resistance trait and, therefore, how the population responds to selection (Macnair 1991). Previous research found populations of *H. virescens* considerably more susceptible to Cry1Ac than populations of *H. zea* (Stone & Sims 1993, Luttrell et al. 1999). Because the barrier for populations of *H. zea* to adapt to Cry1Ac is not as great as for *H. virescens*, it has been speculated that populations of *H. zea* may respond more quickly to selection exerted by transgenic cotton expressing Cry1Ac, primarily via quantitative gene action (Gould & Tabashnik 1998).

The data presented above suggest that current field populations of *H. zea* are slightly more tolerant of Cry1Ac than populations at the beginning of the study. Our results do indicate that factors improving tolerance may already be increasing in the field. Southern Alabama and the panhandle of Florida are areas where the most *Bt* cotton was grown. It is of interest that *H. zea* populations collected from this region show the greatest change in tolerance to Cry1Ac. Furthermore,

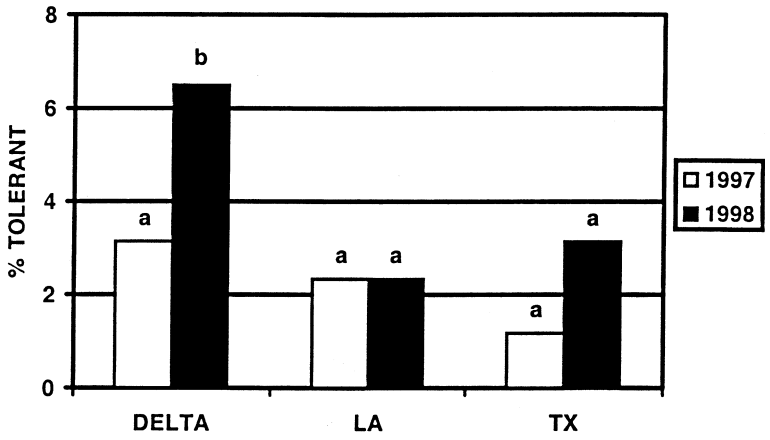

Fig. 6. Percentage of *H. virescens* larvae scored as tolerant after 5 days of feeding on diet containing Cry1Ac. Percentages denoted by the same letter represent regions that are not significantly different as determined by tests of homogeneity.

populations collected from *Bt* cotton in the Mississippi Delta exhibited the greatest tolerances relative to neighboring populations collected from non-Bt cotton.

Our results support previous research that has shown more variability in tolerance of Cry1Ac among populations of *H. zea* than *H. virescens* (Stone & Sims 1993, Luttrell et al. 1999). Because *H. zea* is more intrinsically tolerant of Cry1Ac, the small changes observed in their tolerance of Cry1Ac is not an unexpected result. Small changes in the tolerance of *H. virescens* may be less likely to be detected with the overlay assay, considering the recessive inheritance of Cry1Ac resistance (Gould et al. 1997). We are currently developing other methods to complement the overlay bioassay.

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Spider Composition in a Renovated and Non-Renovated Strawberry Field[1]

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ABSTRACT This study reports biodiversity of spiders in a renovated and a non-renovated pesticide-free strawberry plot in southern Quebec. On average 1,174 and 1,079 spiders were trapped and captured in the non-renovated and renovated plots, respectively. In the non-renovated plot, 16 families were identified, whereas in the renovated plot, only 12 families were found. Nevertheless, the ratio of web-building spider families to hunter families remained 1:1. The dominant families in the non-renovated plot were Theridiidae (30.9%), Linyphiidae (24.1%), and Lycosidae (17.3%). In the renovated plot, they were Araneidae (38.9%), Theridiidae (22.4%), Philodromidae (16.4%), and Salticidae (12.4%).

KEY WORDS strawberries, renovation, spider abundance

In Canada, the importance of spiders as predators of injurious arthropods in fruit crops was first recognized by Pickett et al. (1946). Since then, several studies addressing different aspects of their importance in agricultural ecosystems have been carried out. Thus, McCaffrey & Horsburgh (1980) and Costello & Daane (1995) have reported on spider abundance. Sengonca & Klein (1988), Hagley & Allen (1989), and Lang & Klarenberg (1997) have discussed the biology of different species. Mansour & Whitecomb (1986), Bogya (1995), and & Prokopy (1997) have reported on the role of spiders in biological control. Bostanian et al. (1984), Mansour (1987), and Pekar (1999) have elucidated the effects of pesticides on spider communities. Costello & Daane (1998) have shown the effect of ground cover on spider populations in Californian vineyards. With the exception of the last reference, all the other studies have been carried out in orchards. Nevertheless, progress, albeit on a reduced scale, has also been made with small fruit crops.

In strawberries, a total of 21 species of spiders were collected from 1968 to 1969 in southern Norway, and 54% of the species were identified as *Theridium ovatum* (Clerck) (Taksdal 1973). In Berlin, Germany, Broen (1977) collected 30 species from a strawberry bed and reported briefly on the biology of some of the more important species. In Sweden, Almquist (1981) found 42 species from 11 spider families. *Enoplognatha ovata* (Clerck), a Theridiidae, was the dominant species as it represented 56.5% of the total catch. He also reported that the edges of the field had more individuals than the central section of the field and spider num-

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bers decreased significantly following a fenitrothion treatment. In England, pirimicarb and heptonophos had no effect on spiders in strawberry fields (Easterbrook 1997). Outside of Europe, 28 genera from 14 families were identified near Pretoria, South Africa. Lycosids made up 70% of the catch, with *Pardosa crassipalpis* making up 80% of the lycosids (Dippenaar-Schoeman 1979).

In Quebec, spiders have only been studied in apple orchards (Dondale et al. 1979, Bostanian et al. 1984). This study reports the composition of spiders in an untreated strawberry plot and elucidates the effects of summer renovation to that composition in an adjacent plot.

Materials and Methods

The study was conducted for 3 years (1989–1991) in two pesticide-free strawberry plots, A and B (24.20 × 24.20 m), of cv Glooscap. The plots were only replicated in time because of their size, and the sum of spiders captured by pitfall traps and D-Vac sampling for each family for the 3 years (July to September) was considered a replication. The two plots were at the Agriculture and Agri-Food Experimental Farm at Frelighsburg, Quebec. In each plot, the rows were 1.1 m apart and the plants were 50 cm apart. Throughout the study, the strawberry plants were 3 years old and in their 2nd year of production. Plot A was not renovated and it was sampled from May to September. Plot B was renovated in July and it was sampled from July to September. The two plots were separated by a hedge. Renovation consisted of cutting the strawberry plants at ground level. Each plot was sampled with pitfall traps and a D-Vac (D. Vac Co., Riverside, California). The first and last rows served as guard rows and they were not sampled. The remaining 20 rows had one pitfall trap each and were also sampled with a D-Vac (100 samples each of 1 sq. ft. of ground). The pitfall traps were emptied twice a week. D-Vac sampling was carried out only once a week between 9 am and 11 am.

The pitfall trap was a 1-liter plastic yogurt container with its top. The sidewalls had five openings, each 1.5 cm high and 5.7 cm long (Fig. 1A). The pitfall traps were inserted into plywood frames (25 × 25 cm) such that the lower edge of the openings were flush with the surface of the frames. Holes were dug in the soil and the pitfall traps were placed in the holes whereby the frames covered the holes and were flush with the ground (Fig. 1B). The pitfall traps were then filled with 300 mL of preservative. The preservative consisted of 25 parts 95% ethanol, 70 parts water, and 5 parts glacial acetic acid. All spiders were identified to the family. Because the bulk of the captured spiders were juveniles, identification to the species was not possible.

Two unpaired t tests were carried out. The first unpaired t test compared the mean of spider captures (pitfall plus D-Vac) for 3 years in the non-renovated plot with the renovated plot. A replicate consisted of the total captures for a year (July to September). The second unpaired t test compared the composition (pitfall plus D-Vac captures) of the different families in the non-renovated with the renovated plot. The data were transformed according to for the unpaired t tests.

Results and Discussion

Over the 3 years, the combined catch of spiders in the non-renovated plot by the two techniques together averaged 1,174.3 spiders per season (July to Sep-

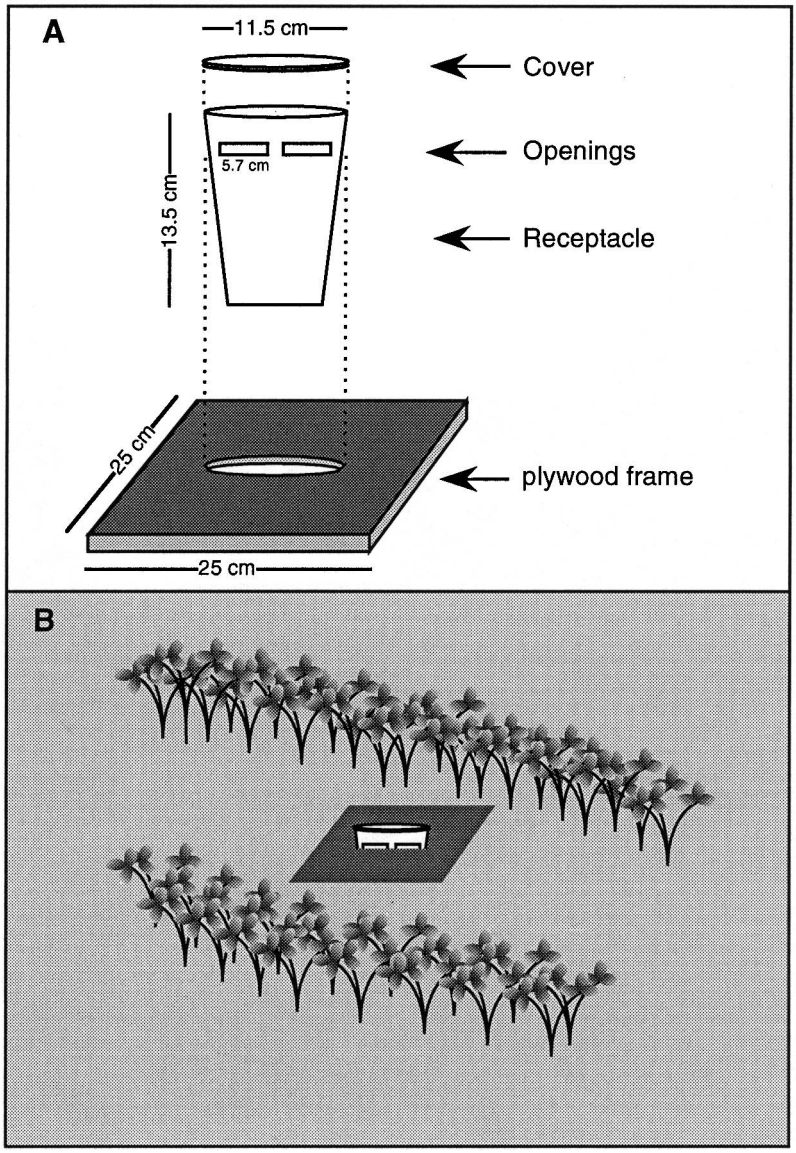

Fig. 1. Pitfall traps used to capture spiders. A, Trap components. B, A trap in the plot. Note that the frame is flush with the ground.

tember). In the renovated plot, an average of 1,078.7 specimens were captured. There was no significant difference in spider abundance between the two plots for the entire season (unpaired t test, $P > 0.05$). This indicated that the spider numbers rebounded quickly by immigration from adjacent fields, and renovation had no adverse effects on spider abundance. It also showed that in the non-renovated

plot, spider-abundance reached a peak and it did not increase indefinitely in numbers even when it was not disturbed.

Table 1 reports the average percentage of different spider families captured over a 3-year period in the two plots. Sixteen families comprised the catch in the non-renovated plot, whereas only 12 families comprised the catch in the renovated plot. This represented a 25% reduction in family diversity. Nevertheless, the ratio of web-builder spider families to hunter families in these plots remained 1:1. Closer examination of Table 1 shows that the Theridiidae, Linyphiidae, and Lycosidae dominated the catch in non-renovated plots. Taksdal (1973) in Norway and Almquist (1981) in Sweden reported that the Theridiidae comprised 54% and 56.5% of the total catch. Following renovation of the plot, only the Theridiidae maintained their pre-renovation eminence, whereas the percentage of Linyphiidae and Lycosidae were reduced to insignificant numbers and were replaced by the Araneidae, Philodromidae, and Salticidae (Table 1).

Figure 2 reports the average densities of spider families on a monthly basis for the 3 years in plots A and B. Only families that exceeded 10% of the seasonal captures for the 3 years were examined and six families met the criterion. The Theridiidae were at maximum abundance in August and September in the non-renovated plot, whereas the Linyphidae and Lycosidae appeared to be evenly

Table 1. Average percentage of spiders captured from July to September by pitfall traps plus D-Vac samples in a renovated and a non-renovated strawberry plot in Frelighsburg, Quebec, for 1989–1991.

		Strawberry fields	
Groups	Spider families	Non-renovated (Plot A)	Renovated (Plot B)
Hunters	Lycosidae	17.3a	0.1b
Hunters	Thomisidae	4.0a	4.2a
Hunters	Clubionidae	2.9a	0.1b
Hunters	Salticidae	1.1a	12.4b
Hunters	Philodromidae	1.0a	16.4b
Hunters	Liocranidae	0.8[a]	0.0[a]
Hunters	Gnaphosidae	0.7[a]	0.0[a]
Hunters	Selenopidae	0.1a	0.1a
Web-builders	Theridiidae	30.9a	22.4a
Web-builders	Linyphiidae	24.1a	1.1b
Web-builders	Dictynidae	6.0a	1.6b
Web-builders	Erigonidae	4.4a	0.9b
Web-builders	Araneidae	4.1a	38.9b
Web-builders	Tetragnathidae	1.8a	1.9a
Web-builders	Agelenidae	0.8[a]	0.0[a]
Web-builders	Hahniidae	0.2*	0.0*

Note: Values within a row followed by different letter are significantly different {unpaired t test, $P < 0.05$, performed on the [capture (%) + 1]$^{0.5}$}.
[a]Insufficient data.

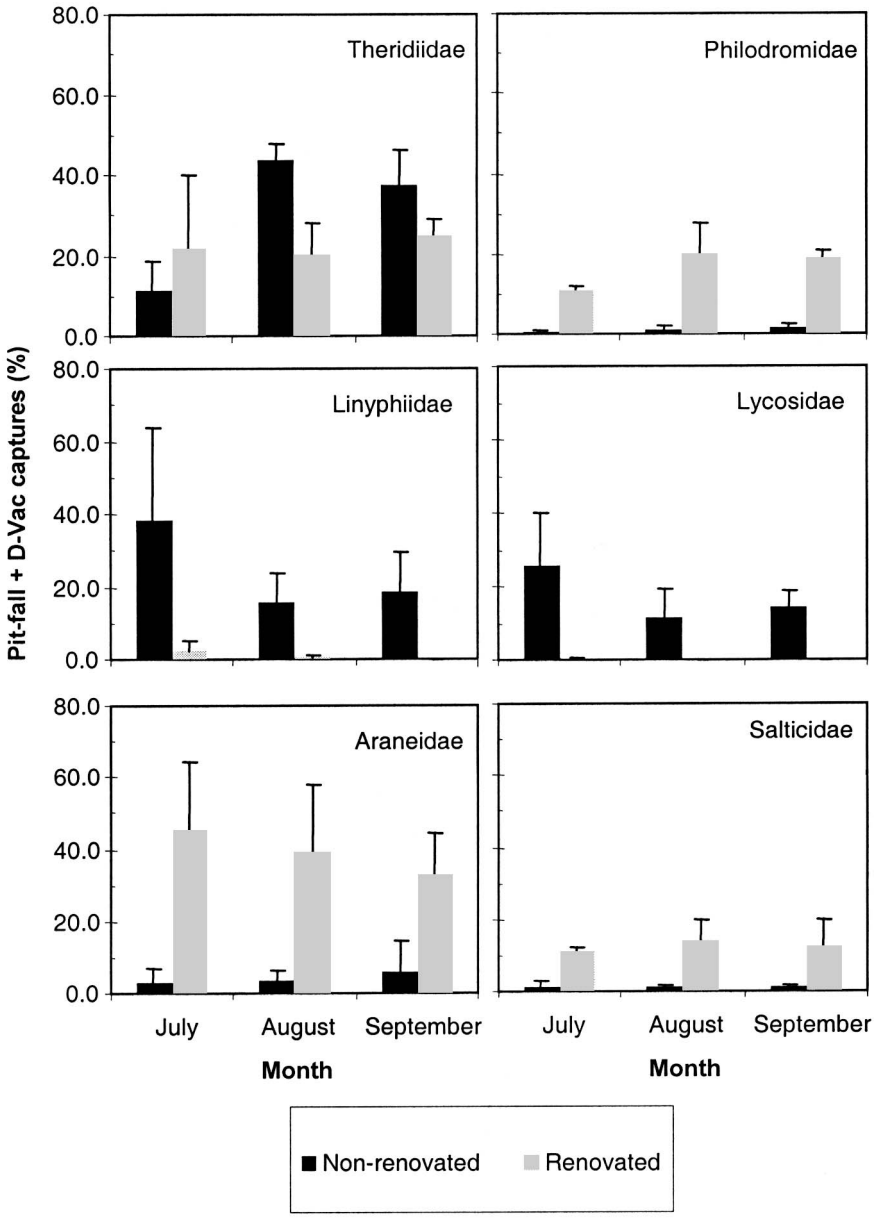

Fig. 2. Mean densities (percentage of pitfall plus D-Vac captures) of spiders representing monthly captures in a non-renovated and renovated strawberry plot (Frelighsburg, Quebec, 1989–1991).

distributed throughout the 3 months. The large variations (approximately 20%) in the mean data from year to year did not permit us to be more precise with the distribution of these two families. The remaining three families, the Aranaidae, Philodromidae, and Salticidae, were all less than 5% of the total captures per season and were evenly distributed (Fig. 2). In the renovated plot, the Theridiidae maintained their eminence, but they were evenly distributed throughout the season. The Linyphiidae and Lycosidae never recovered the renovation shock and remained for the remainder of the season in exceedingly low numbers. Meanwhile, the Araneidae, Philodromidae, and Salticidae benefited from the absence of the Linyphidiidae and Lycosidae and reached unprecedented numbers (Table 1). Again, the large variation among the means from year to year did not permit us to identify the month(s) that each family reached its maximum abundance. Figure 3 summarizes mean captures of all spider families tallied together per season for the non-renovated plot and it shows that spiders were at maximum abundance in August. A similar observation was reported for spider abundance in apple orchards (Bostanian et al. 1984).

In synopsis, the study reports the dominant families of spiders in a renovated and a non-renovated strawberry plot. It also shows that despite an environmental shock such as cutting the plants at soil level, spiders maintain their numbers because of the availability of prey. The ratio of web-builder families to hunters is also constant. Nevertheless, there are subtle differences in composition of spider families captured in a field following renovation. Only the Theridiidae maintain their numbers after the shock of renovation. The Linyphiidae and Lycosidae dwindle in numbers and their ecological niche is occupied by Araneidae, Philodromidae, and Salticidae, immigrants from nearby fields.

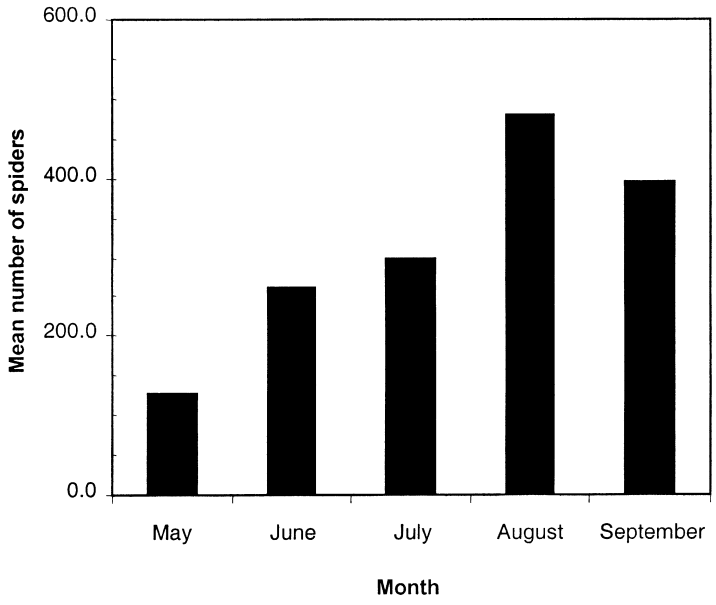

Fig. 3. Mean captures (pitfall plus D-Vac) of spiders for 3 years in a non-renovated strawberry plot (Frelighsburg, Quebec, 1989–1991).

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Effect of the Chemical Inducer Actigard™ in Inducing Resistance to Bean Leaf Beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae), Feeding in Soybean[1]

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ABSTRACT Induced resistance in soybean was investigated in the greenhouse using different concentrations of the chemical inducer Actigard™ to determine the influence on feeding preference of bean leaf beetle adults. Treatments of 0 (control), 15, 25, 40, 60, and 80 ppm Actigard™, and artificial defoliation followed by 25 ppm Actigard™ were applied to V1 and V6 stages of soybean plants. Dual-choice feeding preference tests with the bean leaf beetle were used to assess induced resistance. The adult beetles were collected from soybean fields in east central Nebraska 2 to 5 days prior to the feeding preference tests. Pair-wise comparisons of leaflets from Actigard™-treated plants and control plant leaflets demonstrated that Actigard™ at 25 ppm concentration showed lower preference index (PI) when plants were treated at V1 stage. At V6, feeding preferences were not significantly different; however, all PI values for both V1 and V6 plants indicated that the bean leaf beetles preferred the untreated plants over the Actigard™-treated plants.

KEY WORDS soybean, bean leaf beetle, induced resistance, plant-insect interactions, chemical inducer, Actigard™

The induced response to various biotic and abiotic or chemical elicitors**,** attributed to the synthesis of defensive phytochemicals (Kogan & Paxton 1983) and plant pathogenesis-related proteins (Ebel & Cosio 1994) in tissues away from the site of prior damage, has been reported in a variety of host-pest systems (Kogan & Fischer 1991, Bodnaryk & Rymerson 1994, Hammerschmidt & Dann 1997, Stout et al. 1998). Resistance in soybean has been induced by different types of injuries involving insects such as soybean looper, *Pseudoplusia includens* (Walker), and Mexican bean beetle, *Epilachna varivestis* Mulsant (Smith 1985, Fischer et al. 1990a,b, Lin & Kogan 1990, Lin et al. 1990b, Kogan & Fischer 1991).

Induction of resistance using abiotic elicitors such as sodium azide (Chakraborty & Purkayastha 1987), ethylene (Yoshikawa et al. 1990), ozone (Lin et al. 1990a), and antibiotics (Purkayastha & Banerjee 1990) also has been reported. Specifically, the chemical inducer 2,6-dichloroisonicotinic acid (INA) has been demonstrated to induce resistance against pathogens of pear (*Pyrus* sp.),

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tobacco (*Nicotiana tabacum* L.), rice (*Oryza sativa* L.), and green bean (*Phaseolus vulgaris* L.) (Me'traux et al. 1991, Dann & Deverall 1996). Another chemical inducer, benzo (1,2,3)-thidiazole-7-carbothioic *S*-methyl ester (Actigard™, Novartis Crop Protection Inc., Greensboro, North Carolina), was reported as the first synthetic chemical that induced resistance to pathogens in cereals and tobacco (Ruess et al. 1996). It was reported to induce resistance against pathogens in wheat (*Triticum aestivum* L.), tomato (*Lycopersicon esculentum* Mill.), tobacco (*N. tabacum*), soybean (*Glycine max* [L.] Merrill), and cucumber (*Cucumis sativus* L.) (Kessmann et al. 1993, Friedrich et al. 1996, Gorlach et al.1996, Lawton et al. 1996, Benhamou & Belanger 1998, Dann et al. 1998). Neither INA nor Actigard™ exhibit any direct antimicrobial activity, but they do activate genes responsible for induced responses (Friedrich et al. 1996, Lawton et al. 1996). This has led to the conclusion that they are chemical inducers activating the plant's own defense system (Morris et al. 1998). Actigard™ was used by Inbar et al. (1998) to induce resistance in tomato, thereby reducing the incidence of bacterial spot (*Xanthomonas campestris* pv. v*esicatoria*), early blight (*Alternaria solani*), and also leading to lower larval leafminer (*Liriomyza* sp.) densities. These authors also reported reduction of whiteflies (*Bemisia argentifolii* Bellows & Perring) and powdery mildew (*Oidium* sp.).

The bean leaf beetle, *Cerotoma trifurcata* (Forster), has been a sporadic pest of soybean in Nebraska and the midwestern region of the United States, but in recent times has increased in importance. Previous research by Srinivas et al. (2001) showed that Actigard™ induced resistance against bean leaf beetle, *C. trifurcata*, in soybean. The objectives of this study were to determine if applications of Actigard™ can induce resistance to *C. trifurcata* feeding, and to determine the optimum concentration of Actigard™ that induces resistance to *C. trifurcata* feeding in two early season growth stages (V1 and V6) of soybean.

Materials and Methods

This study was conducted using soybean, *G. max* (L.) Merrill, plant introduction 227687, at V1 and V6 growth stages (Fehr et al. 1971). This plant introduction exhibited induced resistance to *P. includens* and *E. varivestis* in earlier studies (Smith 1985, Chiang et al. 1987, Lin et al. 1990a,b, Kogan & Fischer 1991). Seeds were planted in 15-cm plastic pots with a sterile (soil) mixture of (3:2:1, top soil:sand:vermiculite) and were grown in a greenhouse maintained at 30°C ± 5°C, 70% ± 10% RH, and a 14:10 (L:D)photoperiod with supplementary metal halide illumination.

Adult beetles were collected 2 to 4 days prior to feeding preference tests from fields at University of Nebraska-Lincoln (UNL)-ARDC in Saunders County, Nebraska, and from fields at the UNL East Campus and Havelock Farms in Lancaster County, Nebraska. Actigard™ 50 WG [benzo(1,2,3-thiadiazole-7-carbothioic acid (*S*)-methyl ester] was obtained from Novartis Crop Protection Inc.

The experimental design was completely randomized with seven treatments of 0 (control), 15, 25, 40, 60, and 80 ppm Actigard™, and artificial defoliation (25%) by tearing leaflets with no. 1 insect pins followed by 25 ppm Actigard™. There were 14 replications for the study done on V1 stage plants and 12 replications on V6 stage plants. Each caged plant was considered an experimental unit. All the potted plants, including the control plants, were covered with cylindrical cages

(15.24 cm wide × 60.96 cm tall) constructed of clear lexan plastic fitted with vents for aeration to avoid insect damage. The apical trifoliates were used for dual-choice feeding preference tests 2 weeks after plants were treated. This procedure eliminated the possibility of Actigard™ directly affecting bean leaf beetle feeding because the apical trifoliates used in the preference tests emerged after the application of Actigard™. In these tests, the adult beetles were starved for 24 h prior to the tests and were supplied with water.

Dual-choice tests were conducted to assess bean leaf beetle feeding preferences in this study. Each test arena consisted of six excised leaflet disks from both untreated or control (C) and treated (T) experimental plants that were arranged in an alternating pattern around the bottom of a petri dish. Similar dual-choice feeding preference tests were used by Srinivas et al. (2001) and Lin et al. (1990b) in induced resistance studies in soybean. Four starved adult beetles were released into each petri dish, and were allowed to feed for 4 to 6 h. Leaflet disk areas were measured before and after feeding using a LICOR-3000 area meter (LICOR, Lincoln, Nebraska). The proportion of consumed disk area of T and C leaflets were used to calculate the feeding preference index (PI), where PI = 2T/(T + C) (Kogan & Goeden 1970, Kogan 1972). The PI value ranges from 0 to 2, with a PI of 1 indicating no feeding preference for either C or T disks, a PI >1 indicating preference for T disks, and a PI <1 indicating preference for C leaflet disks.

The PI data were analyzed using a general linear model procedure (SAS Institute 1997) for completely randomized design and means were compared using the least significance difference (LSD) computed at $\alpha = 0.05$.

Results and Discussion

Results of this experiment confirmed the report of Srinivas et al. (2001) that Actigard™ could be used as a chemical elicitor in inducing resistance against bean leaf beetle adult damage. All PI values were less than 1.0, suggesting that all concentrations of Actigard™ tested elicited an induced response, which affected subsequent *C. trifurcata* feeding in both V1 and V6 stages of soybean (Table 1). The differences in feeding preference of leaflets from treated and untreated plants were considered as the effect of different concentrations of Actigard™ treatment on *C. trifurcata* feeding.

The average PI of *C. trifurcata* feeding varied with the concentration of Actigard™ used, and induced responses were significantly different for V1 stage plants ($F = 5.62$; df = 5, 78; $P = 0.0002$). The response induced by 25 ppm (PI = 0.30) was similar to the induced response produced using artificial defoliation plus 25 ppm (PI = 0.32). The PI (= 0.50) for 60 ppm concentration of Actigard™ spray was statistically similar to resistance induced at 40 ppm (PI = 0.44), 15 ppm (PI = 0.43), and 80 ppm (PI = 0.41) Actigard™ concentrations. The results from tests with V6 stage soybean were not statistically significant ($F = 1.64$; df = 5, 66; $P = 0.3060$) for the Actigard™ concentrations; however, the preference indices were less than 1.0, suggesting an elicitation of induced response and indicating that the beetles preferred the untreated (C) leaflet disks in this study.

Results of our experiment suggest that different concentrations of Actigard™ can be used to induce resistance to *C. trifurcata* feeding in soybean. The highest level of induction against *C. trifurcata* feeding was produced by 25 ppm and artificial defoliation plus 25 ppm applications made at V1 stage of soybean

Table 1. Mean feeding preference index (PI) ± SE values for bean leaf beetles fed soybean plant introduction 227687 leaflets treated with various concentrations of Actigard™.

Actigard™ treatment (ppm)	Mean PI index[a]	
	V1 stage	V6 stage
15	0.432 ± 0.012 a	0.543 ± 0.035 a
25	0.302 ± 0.014 c	0.470 ± 0.035 a
40	0.437 ± 0.019 a	0.454 ± 0.041 a
60	0.496 ± 0.022 a	0.596 ± 0.028 a
80	0.410 ± 0.020 ab	0.606 ± 0.047 a
AD + 25	0.324 ± 0.015 bc	0.479 ± 0.045 a
P value[b]	0.0002	0.3060

[a]Means followed by the same letter are not significantly different ($P < 0.05$) by LSD.
[b]Probability values for the *F* test of treatments in each test soybean. AD, artificial defoliation.

growth. The induction of resistance with Actigard™ demonstrates that it can be used as a chemical elicitor to stimulate a defensive mechanism against *C. trifurcata* feeding in soybean.

Inbar et al. (1998) used three foliar applications of Actigard™ to induce resistance in tomato against leafminer larvae and whiteflies. Dann et al. (1998) showed that multiple applications of Actigard™ were necessary in field and greenhouse soybean to decrease fungal disease incidence and severity. However, our study demonstrates that a single application of Actigard™ is sufficient to induce resistance in soybean to *C. trifurcata* feeding at 2 weeks post-application (samples for feeding preference were collected 2 weeks after treatment). This seems to be in corroboration with results from Gorlach et al. (1996) that showed that a single application of Actigard™ provided long lasting protection in wheat and rice against fungal diseases.

The PIs of different concentrations of Actigard™ were not significantly different at V6 growth stage, but all PIs being <1.0 indicates *C. trifurcata*'s preference for untreated versus Actigard™-treated plants. However, the response at V1 stage was quite pronounced and statistically significant. These results seem to agree with the findings of Alarcon & Malone (1995), who demonstrated induced resistance most pronounced in young tomato seedlings in the form of higher amounts of proteinase inhibitors. These endogenous proteinase inhibitors produced as a result of wound response have been found to be effective against insect pests (Hilder et al. 1987, Ryan 1990). We believe that further investigation should occur at the biochemical and molecular levels to understand the level of induced gene expression and pathways involved in induced responses to pests when Actigard™ is used. Future research should also focus on studying the optimization of Actigard™ dosage in the field against soybean insect pests and also the possibilities of using chemical elicitors like Actigard™ in combination with insecticides for insect pest management in soybean.

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Cytochrome P450 Monooxygenases in the Cotton Bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae): *In Vivo* Effects of Deltamethrin Exposure[1]

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ABSTRACT The *in vivo* effects of deltamethrin on *Helicoverpa armigera* cytochrome 450s were investigated by the measurements of cytochrome P450 content and two enzymatic activities. Deltamethrin showed dose-, tissue-, and exposure way-specific effects on cytochrome P450 monooxygenases, ranging from induction to repression. Topical deltamethrin (0.1 and 1.0 μg insect^{-1}) significantly decreased cytochrome P450 contents with no changes in the examined activities with the exception of an increased *p*-nitroanisole *O*-demethylation in the midgut at low deltamethrin dose. However, topical deltamethrin exposure did not cause any changes in cytochrome P450 content and the examined monooxygenase activities in the fatbody microsomes. Feeding cotton bollworm larvae a diet containing deltamethrin (5 and 20 mg kg^{-1}) for 2 days significantly increased total cytochrome P450s in the midgut and fatbody microsomes and enhanced *p*-nitroanisole *O*-demethylation in the midgut microsomes. Low-dose dietary deltamethrin slightly increased the activity of aldrin epoxidase in the midgut and fatbody. The differential effect of microsomal P450 monooxygenases by deltamethrin suggested the multiplicity of cytochrome P450 in this species. A comparison of the two exposure methods revealed that dietary deltamethrin exposure, especially at low dose, was more effective in the induction of the P450 system.

KEY WORDS *Helicoverpa armigera*, deltamethrin, cytochrome P450, aldrin epoxidase, *p*-nitroanisole *O*-demethylase

Pyrethroids have emerged as a major class of highly active insecticides due to their bioefficacy and relatively low toxicity to mammals (Casida et al. 1983). In China, pyrethroids such as deltamethrin have been used widely to control *Helicoverpa armigera*, which has been a major pest of cotton since 1983 (Ru et al 1998). The extensive use of pyrethroids has resulted in the development of insecticide resistance in field populations. The resistance to pyrethroid insecticides has been detected in a number of regions in China (Shen et al. 1992, Wu et al. 1997, Ru et al 1998). Previous physiological and biochemical studies with pyrethriod-resistant and -susceptible populations of *H. armigera* showed that resistance mechanisms include reduced penetration, enhanced metabolism, and knockdown

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resistance (Ru et al. 1998, Head et al 1998). Increases in P450 monooxygenase activities are regarded as the major mechanism of metabolism resistance to pyrethroids in *H. armigera* (McCaffery 1998).

P450 monooxygenase activity is known to be changed by exposure to certain types of chemicals (Scott et al. 1996). These compounds probably alter the metabolism kinetics of themselves or other insecticides by changing the P450 activity, resulting in different toxicological consequences to insects, ultimately affecting the efficiency of this protective strategy (Yu & Hsu 1993, Tan & Guo 1996). Therefore, revealing the xenobiotic-induced alterations of P450 monooxygenases will facilitate the production of effective management strategies.

It was demonstrated that the activity of P450 monooxygenase in *H. armigera* was increased after feeding of certain host plants, resulting in an induced resistance to deltamethrin (Tan & Guo 1996). The expression of specific P450 genes of *H. armigera* was increased by exposure to allechemicals or pyrethroids (Wang & Hobbs 1995, Ranasinhe et al 1997). Previous studies in our laboratory suggested that P450 monooxygenase activity was increased by phenobarbital sodium salt (Qiu & Leng 1999). However, we know little about the *in vivo* effect of deltamethrin, the widely used pyrethriod insecticide, on P450 monooxygenase activities in this insect. The tissue-specific difference in the sensitivity of P450s to deltamethrin still remains to be investigated. In this investigation, attempts have been made to study the effects of deltamethrin on P450 monooxygenases in the midgut and fatbody, the major tissues involved in xenobiotic metabolism in *H. armigera* (Qiu & Leng 2000). Moreover, the differences in the effectiveness of deltamethrin administered by different exposure routes on P450s were also examined.

Materials and Methods

Chemicals. Aldrin and dieldrin (>99%) were obtained from Riedel-de Haen AG (Seelze-Hannoveh, Seelze, Switzerland), bovine serum albumin (BSA, 98%–99%) was obtained from Sigma Chemical Company (St. Louis, Missouri), phenylmethyl sulfonyfluoride (PMSF, >99%) was obtained from Serva (Feinbiochemica, Heidelberg), NADPH (tetrasodium salt, 98%) and 1,4-dithiothreitol (DTT, >97%) was obtained from Boehringer Mannheim (Mannheim, Germany), and Coomassie Brilliant Blue G-250 was obtained from Fluka (Buchs, Switzerland). IR-Deltamethrin (99%) was donated by Roussel Uclaf (Romainville, France). Other chemicals of the analytical grade were purchased from the Beijing Chemical Factory (Beijing, China).

Insects. *H. armigera* was collected from a cotton field in Dingxing county of Hebei Province of China in 1997. This population has been reared according to the method of Wu & Gong (1997) in an environmental chamber maintained at 26°C with a 14:10 (L:D) photoperiodic regime for 21 generations.

Treatment. *Topical treatment.* A colony of healthy, evenly sized larvae in the second day of the 6th instar were divided into three groups of 35 to 40 insects each. Animals of each group were individually exposed to deltamethrin in 1 μL of acetone by topical application to the dorsal thorax at doses of 0 (control), 0.1, and 1.0 μg per insect, respectively. Twenty-four hours after treatment, the animals of each group were weighed, and the numbers of dead or wrinkled individuals were recorded. The wrinkled insects referred to in this paper were sluggish, occasionally feeding, shrunken individuals.

Dietary treatment. Newly molted 6th-instar larvae were divided into three groups of 40 to 45 insects each. Animals of each group were respectively provided with diet into which deltamethrin (0, 5, and 20 mg kg^{-1}) was incorporated when the diet was prepared. Forty-eight hours after treatment, the insects were weighed, and their excretion were collected and weighed. The numbers of dead or wrinkled insects were recorded.

The above doses of deltamethrin were selected according to the results of preliminary experiments addressing the toxicity of deltamethrin to the target animals. The survived insects were dissected in 1.15 % KCl solution on ice. The midgut and fatbody were removed and processed for isolation of microsomes.

Microsome isolation. Microsomes were isolated following Lee & Scott (1989). The clean tissues from the animals of the same groups were pooled and then transferred into a glass homogenizer. Each tissue group (1–2 individual equivalents per milliliter of buffer) was homogenized in the ice-cold homogenization buffer (0.1 M sodium phosphate buffer, pH 7.5, containing 10% [v/v] glycerol, 1 mM ethylenediaminetetraacetic acid [EDTA], 0.1 mM DTT, 1mM 1-phenyl-2-thiourea [PTU], and 1 mM PMSF) with 30 up and down strokes. The homogenate was filtered through one layer of nylon (80–100 meshes) and was centrifuged at 10,000 × *g* for 15 min in a J2-MC centrifuge (Beckman, Fullerton, CA) equipped with the JA-20 fixed-angle rotor. The supernatant was filtered through one layer of nylon again and was centrifuged at 100,000 × *g* for 1 h in a Himac CP70G (Hitachi, Koki, Japan) ultracentrifuge equipped with an RPS50-2 swing-out rotor. The microsomal pellets were resuspended in resuspension buffer (0.1 M sodium phosphate buffer, pH 7.5, containing 20% [v/v] glycerol, 1 mM EDTA, 0.1 mM DTT, and 1 mM PMSF). Microsomes were then diluted to 2 mg/mL in resuspension buffer and were stored at −80°C until use. Microsomes prepared in this manner were stable for 6 months without loss of enzyme activity (data not shown).

Enzyme assays. Cytochrome P450 content was determined according to the method of Omura & Sato (1964) using a DU-650 spectrophotometer (Beckman). Two determinations were made per microsome sample.

The activity of aldrin epoxidase (AE) was assayed according to the method of Lee & Scott (1992). One milliliter of reaction mixture contained 0.1 mL of enzyme preparation, 0.1 M sodium phosphate buffer (pH 7.8), and 0.36 mM NADPH. Five microliters of aldrin solution (4 mg mL^{-1} in ethanol) was added to the mixture to initiate the reaction. After 10 to 15 min of shaking in a water bath at 25°C, the activity was terminated by the addition of ice-cold acetone (0.8 mL). The reaction product (dieldrin) was extracted with petroleum ether (60–90°C, 2 mL) by vortexing the mixture for 1 min. Aliquots were analyzed by gas chromatography after drying over anhydrous sodium sulfate. Analyses were performed on a HP5890 series II gas chromatograph (Hewlett-Packard Company, Palo Alto, CA) equipped with an electron capture detector. The column used was BPX50 (SGE China Service, Beijing, China). Injector, column, and detector temperatures were 250, 230, and 300°C, respectively. Under these conditions, the retention time for dieldrin was 6.3 min.

The activity of *p*-nitroanisole *O*-demethylase (ODM) was determined by the methods of Hansen & Hodgson (1971), Shang & Soderlund (1984), and Qiu & Leng (1999). The standard incubation mixture in a total volume of 2 mL contained the 0.5 mL of enzyme preparation, 0.1 M sodium phosphate (pH 7.8), and 0.36 mM

NADPH. Reactions were initiated by the addition of *p*-nitroanisole in 30 μL of ethanol to give a final concentration of 3 mM, and they were incubated in a water bath at 25°C for 30 min with constant shaking. The reaction was terminated by the addition of 0.5 mL of 1 M HCl. The product, *p*-nitrophenol, was extracted with $CHCl_3$, and was then centrifuged to two fractions. The $CHCl_3$ fraction was back-extracted with 0.5 M NaOH. The absorption of the NaOH solution at 400 nm was recorded to quantify the product using an experimentally determined standard curve.

Protein determination. Protein was determined by the method of Bradford (1976) using Coomassie Brilliant Blue G-250 with BSA as a standard.

Statistical analysis. Data are means ± standard deviation of three separate experiments. Student's *t* test for paired samples was employed to calculate the significance between control and deltamethrin-treated groups. $P < 0.05$ was considered to be significant.

Results

Effect of deltamethrin on insect growth. Deltamethrin exposure inhibited the growth of the larvae. The inhibitory effect was dose dependent as shown in Table 1. At the dose of 0.1μg insect^{-1}, all treated insects appeared to be as healthy and active as the control group. At the dose of 1.0 μg insect^{-1}, one-half of the treated insects showed toxic symptoms with 1.8% ± 1.5% mortality and 48% ± 3% wrinkled individuals.

The dietary deltamethrin also showed inhibitory effects on the growth of the cotton bollworms (Table 2). When the diet contained deltamethrin at a concentration of 5 mg kg^{-1}, no obvious toxic symptoms were observed. However, at 20 mg kg^{-1}, the treated insects showed reduced live mass and excretion weight; 43% of the treated individuals were wrinkled, although no mortality was recorded.

Effect of deltamethrin on P450 monooxygenases. After topical exposure to deltamethrin at either dose for 24 h, treated larvae had significantly decreased microsomal cytochrome P450 content of midgut tissue compared with the control (Table 3). Larvae treated with 0.1 μg insect^{-1} deltamethrin showed slightly increased activity of ODM, whereas topical deltamethrin had no significant effect on AE activity in the midgut. Topical deltamethrin administration did not cause significant changes in P450 content or in monooxygenase activities in the fatbody microsomes.

Feeding cotton bollworm larvae a diet containing deltamethrin for 2 days significantly increased (1.2- to 1.5-fold) total cytochrome P450s in the two examined tissues (Table 4). Dietary deltamethrin induced ODM activity in the midgut microsomes but did not alter ODM activity in the fatbody microsomes. Low deltamethrin treatment also slightly enhanced the activity of AE in the midgut and fatbody, whereas no significant induction was obtained in the larvae treated at high deltamethrin concentrations (Table 4).

Discussion

The induction and repression effect of P450 monooxygenases by deltamethrin. It is believed that insecticides are generally unlikely to cause significant induction of P450 monooxygenases under normal conditions due to their

Table 1. Effects of topical deltamethrin on growth and mortality of the cotton bollworm, *H. armigera*.

Topical deltamethrin dose	Fresh body weight (mg insect^{-1})	Mortality (%)	Wrinkled insects (%)
0 μg insect^{-1} (Control)	389 ± 24	0	0
0.1 μg insect^{-1}	373 ± 19	0	0
1.0 μg insect^{-1}	290 ± 36[a]	1.85 ± 1.5	48 ± 3

[a]Mean weight was significantly lower than control, t = 5.22, df = 2, P = 0.035.

toxicity (Scott et al. 1996, Ranasinghe et al. 1997). Terriere & Yu (1974) suggested that the failure of induction of P450 with insecticides in insects resulted from inadequate doses or exposure periods, and dose limitations would be severe when the inducers were toxic insecticides. In contrast, our data indicated that deltamethrin could induce P450 monooxygenase activities. Dietary deltamethrin increased the total levels of cytochrome P450 and activities of AE, ODM in the midgut, and P450 and AE activity in the fatbody at a dosage rate of 5 mg kg^{-1} deltamethrin, at which no toxic symptoms were observed. Our results also showed that insects topically treated with 0.1 μg of deltamethrin per individual had enhanced ODM activity. Similarly, other reports have suggested that pyrethroid insecticides could induce P450 monooxygenase (Carlson & Schoenig 1980, Huang & Leng 1992, Dayal et al. 1999). Microsomal cytochrome P450 content of female oriental house fly abdomens was significantly induced by topical treatment deltamethrin (Huang & Leng 1992). A low level of permethrin (0.001% of the diet), which allowed most of the larvae to survive, still caused an increase in a P450 mRNA (CYP6B2) of the cotton bollworm *in vivo* (Wang & Hobbs 1995). Consistent with the previous studies (Carlson & Schoenig 1980, Dayal et al. 1999), the present study has also demonstrated that pyrethriods are a weak inducer of P450 monooxygenases.

Topical deltamethrin decreased the total P450 content in the midguts of the cotton bollworms (Table 3). The repression effects of deltamethrin were also ob-

Table 2. Effects of dietary deltamethrin on growth and mortality of the cotton bollworm, *H. armigera*.

Dietary deltamethrin concentrate	Fresh body weight (mg insect^{-1})	Excretion weight (mg insect^{-1})	Mortality (%)	Wrinkled insects (%)
0 mg kg^{-1} (Control)	383 ± 15	679 ± 66	0	0
5 mg kg^{-1}	363 ± 7	525 ± 66	0	0
20 mg kg^{-1}	298 ± 10[a]	212 ± 21[b]	0	43 ± 5

[a]Mean weight was significantly lower, t = 7.385, df = 2, P = 0.018.
[b]Mean weight was significantly lower, t = 15.40, df = 2, P = 0.004.

Table 3. Effects of topical deltamethrin on the P450 monooxygenases in the cotton bollworm, *H. armigera*.

	Midgut			Fatbody		
	Control	0.1 μg insect^{-1}	1.0 μg insect^{-1}	Control	0.1 μg insect^{-1}	1.0 μg insect^{-1}
P450[a]	0.98 ± 0.08	0.66 ± 0.06[c]	0.65 ± 0.21[d]	0.61 ± 0.10	0.59 ± 0.05	0.45 ± 0.12
ODM[b]	4.65 ± 0.56	5.48 ± 0.64[e]	4.28 ± 0.04	2.82 ± 0.53	2.80 ± 0.62	2.27 ± 0.32
AE[b]	4.04 ± 0.99	5.10 ± 1.03	4.03 ± 0.47	2.34 ± 0.72	2.31 ± 0.22	2.05 ± 0.44

[a]Cytochrome P450, nanomoles per milligram of protein.
[b]ODM, *p*-nitroanisole *O*-demethylase activity; AE, aldrin epoxidase activity; nanomoles of product formed per milligram of protein per minute.
[c]The P450 content of insects treated with 0.1 μg insect^{-1} deltamethrin was significantly lower than that of control, t = 5.382, df = 2, $P = 0.033$.
[d]The P450 content of insects treated with 1.0 μg insect^{-1} deltamethrin was significantly lower than that of control, t = 4.321, df = 2, $P = 0.049$.
[e]The ODM of midgut microsome of insects treated with 0.1 μg insect^{-1} deltamethrin was significantly higher than that of control, t = −7.592, df = 2, $P = 0.017$.

served in rats (Tang et al. 1987, Huang & Leng 1993) and in carp (Deer et al. 1996, Banka et al. 1997). Tang et al. (1987) presumed that the inhibitory effect of deltamethrin on cytochrome P450 and aniline hydroxylase was associated with the disruption of synthesis of microsomal protein. Deer et al. (1996) suggested that deltamethrin at higher concentrations inhibited the P450 activities due to the damaging effect of deltamethrin on the enzyme systems of fish. In the case of *H. armigera*, considering that a decline of total P450 content did not result in the decrease of the examined enzyme activities, we suggest that the declined synthe-

Table 4. Effects of dietary deltamethrin on the P450 monooxygenases in the cotton bollworm, *H. armigera*.

	Midgut			Fatbody		
	Control	5 mg kg^{-1}	20 mg kg^{-1}	Control	5 mg kg^{-1}	20 mg kg^{-1}
P450[a]	0.85 ± 0.02	1.16 ± 0.11[c]	1.04 ± 0.05[c]	0.70 ± 0.05	0.92 ± 0.09[c]	1.07 ± 0.07[c]
		t = −4.371	t = −5.492		t = −7.660	t = −5.827
		$P = 0.048$	$P = 0.032$		$P = 0.017$	$P = 0.028$
ODM[b]	5.21 ± 1.64	6.21 ± 1.39[c]	5.69 ± 1.61[c]	4.95 ± 1.21	5.22 ± 1.28	5.07 ± 0.53
		t = −6.876	t = −4.649		t = −1.092	t = −0.231
		$P = 0.021$	$P = 0.043$		$P = 0.389$	$P = 0.839$
AE[b]	6.19 ± 1.69	7.13 ± 1.63[c]	6.45 ± 1.82	2.46 ± 0.48	3.50 ± 0.84[c]	3.17 ± 0.50
		t = −17.285	t = −1.028		t = −4.337	t = −2.835
		$P = 0.003$	$P = 0.412$		$P = 0.049$	$P = 0.105$

[a]Cytochrome P450, nanomoles per milligram of protein.
[b]ODM, *p*-nitroanisole *O*-demethylase activity; AE, aldrin epoxidase activity; nanomoles of product formed per milligram of protein per minute.
[c]Mean was significantly higher when compared with control.

sis of some P450 isozymes was one of the factors that resulted in the decrease of total P450s.

Tissue difference in responsiveness to insecticides and its relation to exposure routes. Data presented here indicate that differences exist in responsiveness to insecticide between the two tissues. The midgut seems to be more sensitive to deltamethrin than the fatbody. Topical administration of deltamethrin decreased the levels of cytochrome P450 and induced the ODM activity at 0.1 μg insect^{-1} in the midgut, whereas no change was found in the fatbody. Dietary deltamethrin increased the ODM activity in the midgut, but not in the fatbody, although the effects of deltamethrin on cytochrome P450 and AE were similar in both tissues. Our results were in agreement with previous findings showing that monooxygenase activities in the southern armyworm, *Spodoptera eridania*, were more inducible by dietary chemicals in the midgut than in the fatbody, with the exception of methoxyresorufin *O*-demethylation (Brattsten et al. 1980). Similarly, a study focusing on a single cytochrome P450 mRNA suggested that the addition of peppermint oil to diet caused substantial differences in the degree of induction of the mRNA among different tissues of *H. armigera*, with the greatest seen in the midgut (Ranasinghe et al. 1997). Interestingly, under *in vitro* conditions, a different induction pattern was observed in the cotton bollworm (Ranasinghe & Hobbs 1999). Ranasinghe & Hobbs (1999) used an organ culture system derived from *H. armigera* to study the expression of cytochrome P450 mRNAs and found that the CYP6B7 mRNA could only be induced in the fatbody, whereas no induction was observed in either the midgut or the integument.

It has been suggested that compounds entering the insect via the digestive tract may be metabolized by the gut enzymes, whereas those effecting direct cuticular penetration may be detoxified mainly by the enzymes in the fatbody or Malpighian tubules (Brattsten & Wilkinson 1973). Thus, the relative degree of microsomal enzyme induction in different tissues might be expected to depend on the route of administration of the agents (Brattsten & Wilkinson 1973), i.e. the fat body would be more sensitive to chemicals entering the body by penetration and the midgut would be more sensitive to dietary intake. This hypothesis was not validated by our results. In our investigation, taking the fatbody as an example, no change in all tested activities was observed following topical administration of deltamethrin, but dietary treatment elevated the level of cytochrome P450 and enhanced certain monooxygenase activity.

The phenomenon that dietary deltamethrin induced P450s and topical administration inhibited P450s caught our attention. We presumed that induction or repression was related to the insect's response to deltamethrin application. In the case of dietary treatment, insects could regulate intake and adapt themselves to the changed food. As presented in Table 2, the excretion weight of the cotton bollworm decreased as the concentration of deltamethrin in diets increased, which reflected the anti-feeding effect of deltamethrin and also probably implied a behavioral response, i.e. the more deltamethrin the diet contained, the less the cotton bollworm ate. An increase of cytochrome P450 in the treated larvae might be one of its adaptation mechanisms in order to reduce the toxic consequences to a lower level. Insects with induced enzymes are often more tolerant of insecticides (Yu & Hsu 1993, Tan & Guo 1996) and, thus, eliminating enzyme induction would reduce insecticide application rates (Yu & Hsu 1993). The fact that dietary deltamethrin induced P450 whereas topical administration inhibited P450 indicated

that applying insecticides directly to an insects body would reduce the metabolism of some insecticides, and the residues of insecticides on host plants would probably enhance the development of insecticide resistance.

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Carpenter Ant (Hymenoptera: Formicidae) Fauna of South Carolina[1]

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ABSTRACT In a statewide survey using four different methods, 10 species of carpenter ants were recorded from South Carolina. *Camponotus pennsylvanicus* (DeGeer) and *Camponotus decipiens* Emery were encountered most frequently. They were recorded in 19 of the 46 South Carolina counties. *Camponotus americanus* Mayr, *Camponotus chromaiodes* (Bolton), and *Camponotus nearcticus* Emery were recorded in 12, 11, and 9 counties, respectively. *Camponotus floridanus* (Buckley) was found in only four counties, all of which were along the southern coastal area of South Carolina. *Camponotus castaneus* (Latreille) was found in three counties, showing no proclivity toward any one area of the state. *Camponotus caryae* (Fitch), *Camponotus discolor* (Buckley), and *Camponotus snellingi* Bolton each were found in one county.

KEY WORDS *Camponotus*, distribution, new records

The genus *Camponotus* (Formicidae) is a large, widespread, and important group of ants (Klotz et al.1996, Hedges 1997). Several species are serious structural and nuisance pests in the United States (Klotz et al. 1996). One commonly encountered and wide-spread species in the eastern United States is the black carpenter ant, *Camponotus pennsylvanicus* (DeGeer) (Hedges 1997). Its range extends from North Dakota to Quebec and Ontario and southward to Texas and Florida (Smith 1965). *Camponotus pennsylvanicus* can nest in live or dead trees, rotting logs and stumps, buildings, telephone poles, and other wooden structures (Smith 1965).

Another economically important species found in the eastern United States is the Florida carpenter ant, *Camponotus floridanus* (Buckley). Klotz et al. (1995) reported that *C. floridanus* was the fourth most encountered ant by pest management professionals (PMPs) in Florida, and the most encountered carpenter ant. It is established from Alabama and Horn Island, Mississippi eastward to Florida and north to North Carolina (Smith 1965).

Other less economically important, but still prevalent *Camponotus* species also are found in the eastern United States. One commonly encountered ant is *Camponotus decipiens* Emery. Currently, *C. decipiens* is known from Georgia and northern Florida west to western Texas. It has also been recorded in the Mexican

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states of Nuevo Leon and Tamaulipas (Snelling 1988). The red carpenter ant, *Camponotus chromaiodes* Bolton, ranges from Nebraska to New York, and south to Georgia (Smith 1965). The American carpenter ant, *Camponotus americanus* Mayr, ranges from New England and southern Ontario south to the Gulf Coast and west to Iowa, Missouri, Oklahoma, and Texas. *Camponotus castaneus* (Latreille) ranges from Iowa to New York and south to Texas and Florida (Smith 1965). *Camponotus nearcticus* Emery has a discontinuous distribution (Creighton 1950) and is recorded from Colorado to North Dakota, east to Ontario, and south to Florida (Smith 1965).

From our experience, there is confusion among some PMPs regarding the distribution and number of *Camponotus* species in South Carolina. One problem is misidentification of other ant species, particularly in the genera *Formica* and *Acanthomyops*, as carpenter ants by PMPs. Proper identification of carpenter ant species and knowledge of their distribution is key for implementing management programs. In South Carolina, a statewide faunal survey for carpenter ants has never been conducted. The objectives of this study were to document carpenter ant species in South Carolina and determine their distribution by county. All county records reported here are new.

Materials and Methods

The distribution survey of carpenter ants in South Carolina was conducted using four methods: examining specimens in the Clemson University Arthropod Collection (CUAC); examining records from the Clemson University Plant Problem Clinic (CUPPC); conducting a mail survey of state pest management companies; and field collecting. Voucher specimens for field collections were deposited with the CUAC.

Records from CUAC. The current CUAC was established in 1926 and houses over 212,000 specimens. It supports teaching, research, and extension activities at the University and aids in the identification of samples submitted in by individuals each year. Voucher specimens from past research are housed there. The CUAC *Camponotus* specimens were reviewed for new county records.

Records from CUPPC. The CUPPC is part of the Cooperative Extension Service. Personnel identify and provide control recommendations for insects submitted by county Extension Agents and the public. Taxonomists and Extension Specialists at the CUPPC verify identifications. Records from 1985–1999, the extent of the computer database on file, were examined for new county records for *Camponotus* species.

Mail survey. All 153 member companies of the South Carolina Pest Control Association (SCPCA) were sent survey materials on 22 February 1999. A package consisting of a survey letter, two 2-dram vials filled with 95% ethanol, collection data sheets, and a pre-stamped padded envelope was sent to companies. Instructions requested PMPs to send carpenter ant samples encountered on the job to Clemson University along with specific collection data. A reminder postcard was sent on 27 May 1999. The survey was concluded on 01 September 1999.

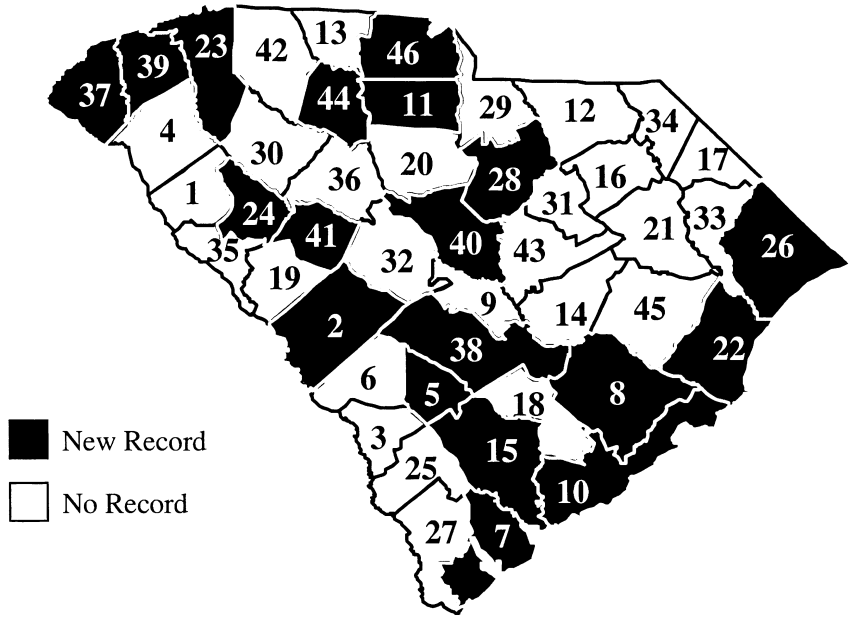
Field collection. The original method for selecting collection sites in South Carolina involved placing a grid over a map of the state, with 20% of the grids randomly selected for sampling. However, this method was discontinued due to the difficulty of sampling urban areas and private property. Ultimately, field

collecting consisted of travelling to counties for which records of a given carpenter ant species did not exist, and searching suitable locations such as live and dead trees, stumps, logs, wood piles, general debris piles, and areas around structures. Night- and daytime sampling was conducted from May to October 1999 and 2000. Specimens were returned to Clemson University and were identified using keys by L. D. Hansen (L. D. H., unpublished data) or Snelling (1988).

Results

Distribution maps for carpenter ant species recorded or collected in four or more counties of South Carolina were developed (Figs. 1–6). Ten species of carpenter ants were recorded.

Records from CUAC. Prior to this study, the CUAC had records for eight carpenter ant species for South Carolina. They included *C. americanus*, *C. castaneus, C. chromaiodes*, *C. floridanus*, *C. nearcticus*, and *C. pennsylvanicus*. The CUAC also contained county records for *C. rasilis* and *C. sayi* (Emery). However, based on Snelling's (1988) revised key, both of these species are now identified as



1 Abbeville	9 Calhoun	17 Dillon	25 Hampton	33 Marion	41 Saluda
2 Aiken	10 Charleston	18 Dorchester	26 Horry	34 Marlboro	42 Spartanburg
3 Allendale	11 Chester	19 Edgefield	27 Jasper	35 McCormick	43 Sumter
4 Anderson	12 Chesterfield	20 Fairfield	28 Kershaw	36 Newberry	44 Union
5 Bamberg	13 Cherokee	21 Florence	29 Lancaster	37 Oconee	45 Williamsburg
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. 1. Distribution of *C. pennsylvanicus* (DeGeer) in South Carolina by county.

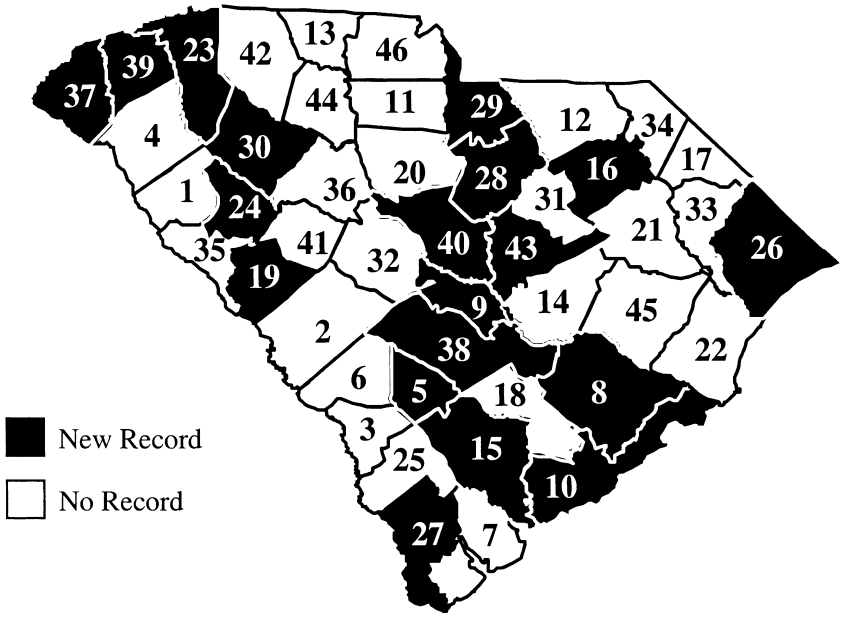


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Fig. 2. Distribution of *C. decipiens* Emery in South Carolina by county.

C. decipiens. *Camponotus sayi* is an accepted species, however, it only occurs in the western United States (Snelling 1988).

Examination of collection data in the CAUC showed that the American carpenter ant, *C. americanus*, has been found in Charleston, Pickens, and Oconee Counties. *Camponotus castaneus* has been found in Pickens, Orangeburg, and Sumter Counties. *C. chromaiodes* has been found in Allendale, Greenwood, Lancaster, and Pickens Counties, and *C. decipiens* has been found in Kershaw, Pickens, Bamburg, and Richland Counties. In addition, *C. sayi* specimens, reidentified as *C. decipiens,* have been collected in 11 counties throughout the state, making 15 county records for *C. decipiens*. *Camponotus floridanus* specimens in the CAUC were from Beaufort, Charleston, Colleton, and Jasper Counties. *Camponotus nearcticus* was found in Jasper, Pickens, Chesterfield, Union, and Darlington Counties. *Camponotus pennsylvanicus* specimens have been recorded in nine counties throughout the state, including Bamberg, Charleston, Colleton, Greenville, Greenwood, Horry, Oconee, Pickens, and Saluda Counties.

Records from CUPPC. CUPPC records included nine species from South Carolina. However, keys before the revision by Snelling (1988) were used for

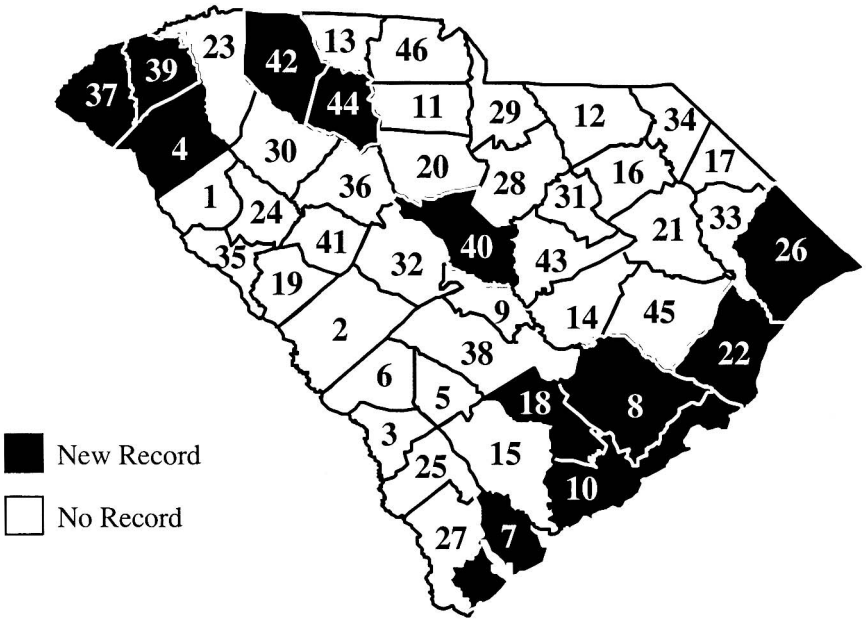

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Fig. 3. Distribution of *C. americanus* Mayr in South Carolina by county.

many of the identifications. For example, *C. sayi* was reported to be in nine South Carolina counties. Re-identified as *C. decipiens*, specimens were recorded in Berkeley, Charleston, Colleton, Edgefield, Kershaw, Pickens, Oconee, Orangeburg, and Richland Counties. In addition, *C. rasilis* was reported in 10 counties. Snelling (1988) also included *C. rasilis* as a conspecific of *C. decipiens*. This expands the *C. decipiens* records to include Calhoun, Darlington, Greenwood, Greenville, Lancaster, and Sumter Counties. *Camponotus chromaiodes*, recorded with the homonym of *C. ferrugineus*, was reported in two counties: Lexington and Pickens.

Camponotus pennsylvanicus was recorded in Beaufort, Charleston, Chester, Colleton, Greenville, Oconee, Pickens, Richland, Saluda, Union, and York Counties. *C. nearcticus* was recorded in Anderson, Chester, Chesterfield, Colleton, Greenville, Sumter, and Union Counties. *Camponotus castaneus* and *C. floridanus* were reported from two counties each, Beaufort and Greenville, and Beaufort and Charleston Counties, respectively. *C. caryae* was reported only in Chester County. *Camponotus caryae discolor*, which is now its own species, *C. discolor* (Snelling 1988), was reported in Charleston County.

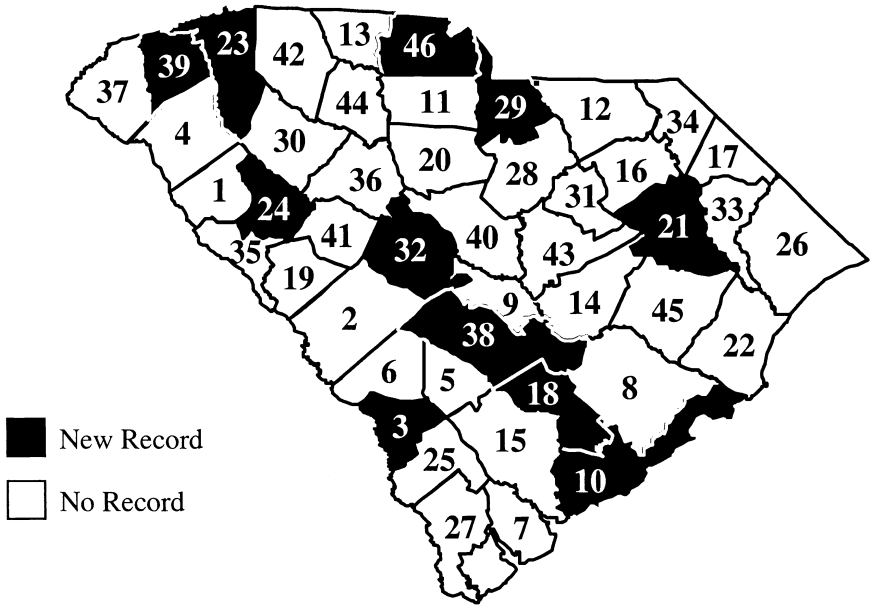

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Fig. 4. Distribution of *C. chromaiodes* (Bolton) in South Carolina by county.

Mail survey. A total of 25 samples were received from PMPs. Seventeen companies, or 11% of the South Carolina Pest Control Association responded. Of the 25 samples, five specimens were unidentifiable ant alates, two specimens were in other ant genera, and one specimen was a coleopteran. Five carpenter ant species were identified from the samples: *C. chromaiodes* from Lexington and York Counties, *C. decipiens* from Greenville and Laurens Counties, *C. floridanus* from Beaufort and Charleston Counties, *C. snellingi* from Beaufort County, and *C. pennsylvanicus* from Beaufort and Union Counties.

Field collection. Twenty-three of the 46 counties in South Carolina were sampled for carpenter ants (Table 1). *Camponotus americanus* was collected most frequently and was found in Anderson, Beaufort, Berkeley, Dorchester, Georgetown, Horry, Oconee, Richland, Spartanburg, and Union Counties. *Camponotus pennsylvanicus* was found in Aiken, Beaufort, Berkeley, Georgetown, Horry, Kershaw, Orangeburg, and Richland Counties. *Camponotus chromaiodes* was collected in Charleston, Dorchester, Florence, Greenville, and Orangeburg Counties. *Camponotus decipiens* was collected in Jasper and Horry Counties, and *C. floridanus* was collected in Beaufort, Charleston, Colleton, and Jasper Counties.

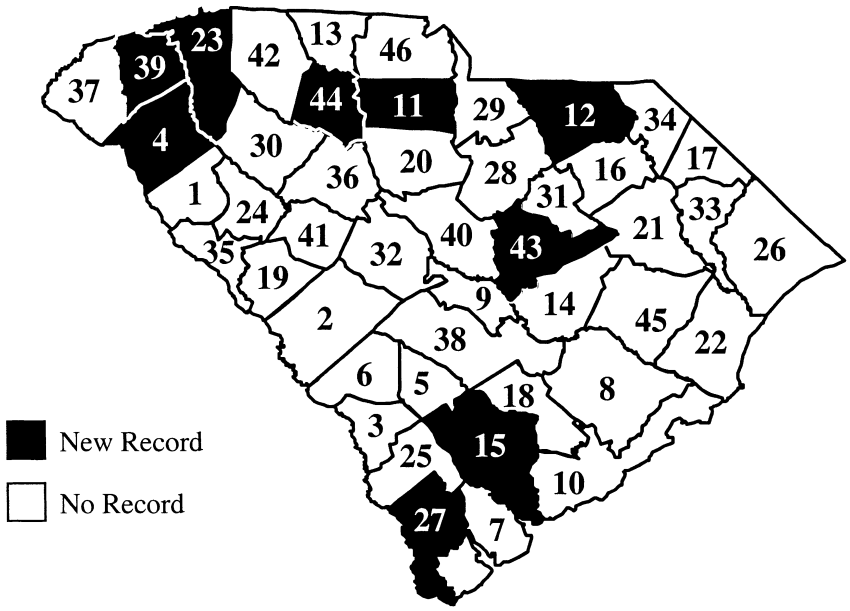

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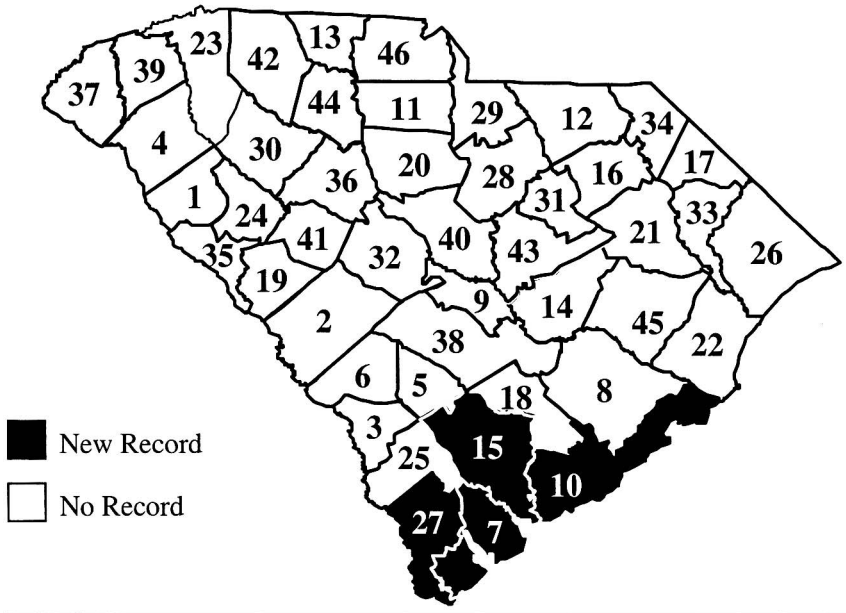
Fig. 5. Distribution of *C. nearcticus* Emery in South Carolina by county.

Discussion

The 10 *Camponotus* species documented in this study represent four subgenera of the genus: *Camponotus* Mayr, *Myrmentoma* Forel, *Myrmothrix* Forel, and *Tanaemyrmex* Ashmead. The subgenus *Camponotus* contains *C. americanus*, *C. chromaiodes*, and *C. pennsylvanicus*. *Camponotus floridanus* is the only species in South Carolina in the subgenera *Myrmothrix,* and *C. castaneus* is the only species in South Carolina in the subgenus *Tanaemyrmex*. The subgenus *Myrmentoma* contains *C. caryae*, *C. decipiens*, *C. discolor*, *C. nearcticus*, and *C. snellingi*.

Camponotus pennsylvanicus and *C. decipiens* have now been recorded from 19 South Carolina counties. They are considered both a nuisance and structural pest because of their prevalence in and around structures. *C. americanus* was recorded from 12 counties and *C. chromaiodes* was recorded from 11 counties. Geographically, *C. chromaiodes* has a distribution very similar to *C. pennsylvanicus*. This ant also can be a pest, but usually not to the level of *C. pennsylvanicus* (Smith 1965). *Camponotus nearcticus* is one of the most widely distributed and common carpenter ants in the subgenus *Myrmentoma* and was found in nine counties



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6 Barnwell	14 Clarendon	22 Georgetown	30 Laurens	38 Orangeburg	46 York
7 Beaufort	15 Colleto	23 Greenville	31 Lee	39 Pickens	
8 Berkeley	16 Darlington	24 Greenwood	32 Lexington	40 Richland	

Fig. 6. Distribution of *C. floridanus* (Buckley) in South Carolina by county.

throughout the state. *Camponotus nearcticus* colonies generally are smaller than other species of carpenter ants, ranging from less than 100 to several hundred members (Smith 1965). They are known to nest in structures, and usually are associated with moist wood (Smith 1965). We speculate that if more extensive sampling was conducted, *C. pennsylvanicus, C. decipiens, C. americanus, C. chromaiodes*, and *C. nearcticus* would be found throughout the state. In our collections, their distribution was not confined to any particular region of South Carolina.

Camponotus floridanus was recorded in four counties, all of which are located along the southeastern coast of the state. During field collections, these ants were often located in areas of new construction. The prevalence of *C. floridanus* in these areas and their tendency to construct large nests and actively forage makes them a potentially serious urban pest.

Camponotus castaneus, found in three counties, is not known to be a major household pest. It prefers to nest in rotten logs and stumps, in exposed or covered soil, or in a combined log and soil matrix (Smith 1965). Also not considered to be major pests in South Carolina are *C. caryae*, recorded in Chester county, *C.*

Table 1. *Camponotus* species collected by county in South Carolina. Of the 46 counties in the state, 23 were sampled in 1999 and 2000.

South Carolina County	*Camponotus* species
Anderson	*C. americanus*
Aiken	*C. pennsylvanicus*
Allendale	None
Beaufort	*C. americanus, C. floridanus,* and *C. pennsylvanicus*
Berkeley	*C. americanus* and *C. pennsylvanicus*
Calhoun	None
Charleston	*C. chromaiodes* and *C. floridanus*
Colleton	*C. floridanus*
Dorchester	*C. americanus* and *C. chromaiodes*
Florence	*C. chromaiodes*
Georgetown	*C. americanus* and *C. pennsylvanicus*
Greenville	*C. chromaiodes*
Hampton	None
Horry	*C. americanus, C. decipiens,* and *C. pennsylvanicus*
Jasper	*C. decipiens* and *C. floridanus*
Kershaw	*C. pennsylvanicus*
Lexington	None
Oconee	*C. americanus*
Orangeburg	*C. chromaiodes* and *C. pennsylvanicus*
Pickens	None
Spartanburg	*C. americanus*
Richland	*C. americanus* and *C. pennsylvanicus*
Union	*C. americanus*

discolor recorded in Charleston county, and *C. snellingi*, recorded in Beaufort County.

When considering control options, it is important to know the species of carpenter ant and its distribution in a state where PMPs operate. During this study, *C. pennsylvanicus*, *C. decipiens*, *C. americanus*, *C. chromaiodes*, and *C. nearcticus* were found throughout South Carolina. Each could be a pest problem where encountered. *Camponotus floridanus* was found only in the four southeastern coastal counties. Ants in the genera *Formica* and *Acanthomyops* may be misidentified as *Camponotus.* If these genera are in a pest situation, they may require either different or no control measures. In addition, many of the less encountered nuisance and benign species of carpenter ants such as *C. castaneus*, *C. caryae*, *C. discolor*, and *C. snellingi* generally require little or no control. It is important to preserve these non-pest ant species because they occupy habitats and compete for resources with pest species in both natural and urban ecosystems.

Acknowledgment

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the identifications and species confirmations of *Camponotus* specimens in the subgenus *Myrmentoma* by R. Snelling of the Los Angeles County Natural History Museum. This publication is Technical Contribution number 4695 of the South Carolina Agriculture and Forestry Research System.

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Orientation of Sorghum Midge, *Stenodiplosis sorghicola*, Females (Diptera: Cecidomyiidae) to Color and Host-Odor Stimuli[1]

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ABSTRACT Sorghum midge, *Stenodiplosis sorghicola* (Coquillett), is one of the most important pests of grain sorghum worldwide. Sorghum midge adults emerge in the morning, mate at or near the site of emergence, and then the females proceed in search of sorghum crop at flowering for oviposition, and some visual and odor stimuli play an important role in host finding and oviposition process. We used a glass apparatus with two (Y-tube) arms to study the orientation of sorghum midge females to visual and odor stimuli under laboratory conditions. Most sorghum midge females were attracted to yellow (30%), followed by green (26%), red (23%), and blue (10%). Sorghum midge females responded more quickly to yellow, followed by red, green, and blue. However, under dual-choice conditions, differences in numbers of sorghum midge females attracted to yellow versus green, red versus blue, and blue versus green were not significant. More sorghum midge females were attracted to sorghum panicle odors plus red (47%) or yellow (40%) colors than to host odors alone (31%). Information on the color preference of sorghum midge females could be exploited for developing suitable traps to monitor its abundance in combination with kairomones or pheromones.

KEY WORDS sorghum midge, *Stenodiplosis sorghicola*, color stimuli, host odor, plant resistance, attraction, *Sorghum bicolor*

Sorghum, *Sorghum bicolor* (L.) Moench, is one of the most important cereal crops in Africa, Asia, Australia, and the Americas. Nearly 150 species of insects have been recorded as pests of sorghum (Jotwani et al. 1980), of which sorghum midge, *Stenodiplosis sorghicola* (Coquillett), is the most important pest worldwide (Harris 1976). Sorghum midge adults emerge in the morning from infested sorghum panicles at the milk to dough stages of development, mate at or near the site of emergence, and then the females search for flowering sorghums for oviposition. The males hover around the site of emergence, mate with the newly emerged females, and die soon after (Harris 1976). Sorghum midge females lay eggs in flowering sorghum spikelets during the morning hours, and usually die

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within 4 to 6 h. Thus, sorghum midge females have a limited time to locate a flowering sorghum crop for oviposition. Odors emanating from flowering sorghum panicles attract sorghum midge females (Sharma et al. 1990). Yellow- and white-colored traps are more attractive than blue or black traps to sorghum midge females in the field (Wiseman et al. 1972, Sharma et al. 1990). However, there is no information on the relative attraction of different colors alone and in combination with sorghum odors to sorghum midge females. Therefore, we used dual-, multi-, and no-choice tests to study the attraction of sorghum midge females to different colors alone and in combination with sorghum odors to gain an understanding of the host-finding behavior of this insect.

Materials and Methods

Sorghum midge females were obtained from sorghum panicles collected from farmers' fields in Darling Dawns, Queensland, Australia, and were kept in 30 × 45-cm brown paper bags at 27°C ± 2°C, 60% ± 5% RH, and a 12:12-h (L:D) photoperiod in the laboratory. An inverted transparent plastic jar (21.5-cm long × 10.5-cm in diameter) with three wire mesh-screened windows (4-cm diameter), two on the sides and one at the top, was placed over the paper bag and tied to it by a 1-cm-wide rubber band. The rubber band was twisted at the rim of the jar and pulled onto the upper end of the handle of the jar to keep the jar upright without support. Upon emergence, sorghum midges moved upward into the plastic jar because of their positive phototactic behavior. Sorghum midges were retained in the plastic jar until 1,000 h, where they presumably mated during this period. Each jar containing sorghum midges was covered on the sides with a sheet of black polyethylene. Sorghum midges were collected in a 20-mL glass vial attached to an opening (2.5 cm in diameter) in the lid of the jar. A small piece of clay was used to hold the vial to the lid. The sorghum midges moved into the glass vial as a result of attraction to light. The vial containing 40 to 50 sorghum midges was removed from the jar and was replaced with a new one. Twenty sorghum midge females were collected in each 20-mL glass vial. Attraction of these sorghum midge females to different color and odor stimuli was studied under laboratory conditions.

Attraction of sorghum midge females to color and odor stimuli under dual-choice conditions was studied in a Y-shaped glass olfactometer with an insect-holding chamber (6-cm diameter × 23-cm length; Fig. 1). One end of the glass chamber was blocked with sintered glass followed by an 18-cm-long glass joint tapering into a 2-cm-diameter tube. A plastic tube (1.8-cm diameter × 15-cm length) was connected to the glass tube. The other end of the plastic tube was connected by a T-joint to an airtight vacuum chamber (17-cm diameter × 15-cm height). A plastic hose (1.5-cm diameter × 3-m length) was connected to the vacuum chamber at one end and to a vacuum pipe inlet (connected to the central vacuum system) at the other. The knob of the vacuum inlet was adjusted carefully to create a steady airflow (nearly 1 cm sec^{-1}) through the glass apparatus. A bifurcated T-joint to which two glass arms (3.5-cm diameter, 30-cm length) were attached was connected to the insect-holding chamber at the other end. The glass arms were blocked with sintered glass at 18 cm, leaving an 11-cm portion to hold flowering rachis branches of sorghum panicle (the stage at which sorghum midge females lay eggs in sorghum panicles). Five rachis branches from a flowering

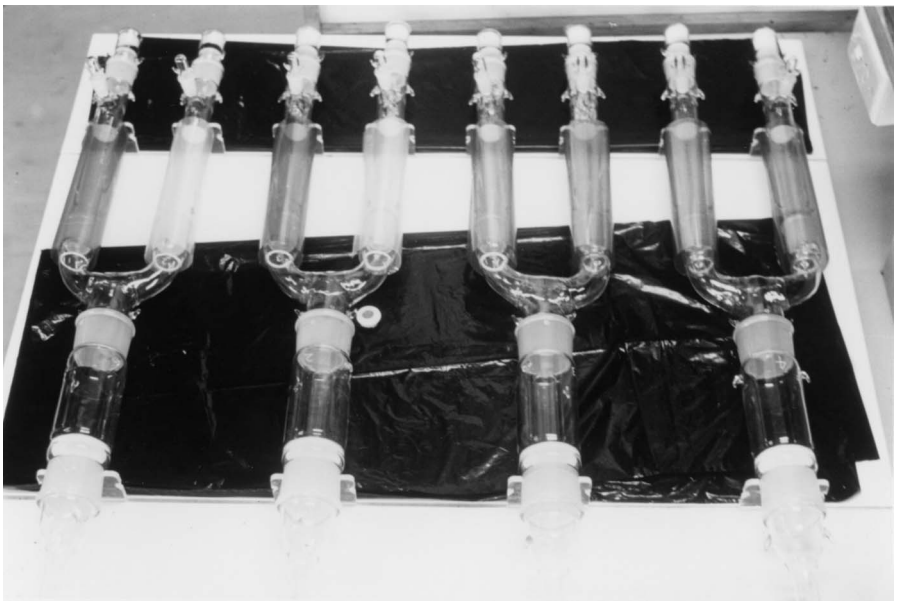

Fig. 1. Glass apparatus used to assess response of sorghum midge females to different colors and odor stimuli.

sorghum panicle were placed in this section and were used to measure the attraction of sorghum midge females to odors alone or in combination with visual stimuli. A 9-cm glass tube containing charcoal and cotton wool to filter the incoming air was attached at the end of this section. Four glass units (Y-tubes) were placed horizontally on a wooden board in the laboratory. The four glass units were used for comparing combinations of color and odor stimuli. The light intensity was uniform on all sides of the glass apparatus. A black polyethylene sheet was placed under the glass apparatus to avoid reflection of light from the white wooden board. Twenty sorghum midge females were released into the holding chamber, and the glass apparatus were joined together immediately. The insect-holding chamber and 5-cm portions of the glass arms were covered with a black polyethylene sheet to provide directional visual stimuli to the sorghum midge females. The glass apparatus was cleaned with soap, rinsed with alcohol, and dried at 105°C after each experiment.

Movement of sorghum midge females towards light and sorghum odors. Movement of sorghum midge females towards the two arms of the Y-tube from the holding chamber of the glass olfactometer was studied by covering the entire glass apparatus with a black polyethylene sheet, by covering the holding chamber and 5-cm portion of the Y-tube arms with a black sheet to provide a directional light stimuli, and by leaving the entire glass apparatus uncovered. Five flowering rachis branches of sorghum cultivar 'ICSV 197' were placed in one arm, and a cotton swab soaked in 2 mL of water was kept in the other arm as a blank. Twenty sorghum midge females were released into the holding chamber. The numbers of sorghum midge females moving to two arms of the Y-tube were

recorded after 30 min. The experiment was repeated four times, and the three treatments (flowering rachis branches and water in combination with covered, partially covered, and uncovered glass apparatus) were compared in a completely randomized design. The data were subjected to analysis of variance (ANOVA), and significance of differences between the treatments was judged by paired t test at $P = 0.05$.

Attraction of sorghum midge females to different colors in relation to normal light. In this experiment, one arm of the dual-choice Y-tube was wrapped with a green, red, blue, or yellow transparent plastic sheet (30 × 30 cm), whereas the other arm was left uncovered (normal light). This provided a choice to the sorghum midge females between normal light and light passing through colored transparent plastic sheets (green, red, blue, or yellow). Twenty freshly emerged sorghum midge females were released in the holding chamber, and the holding chamber and 5-cm portion of the Y-tube were covered with a black sheet. Numbers of sorghum midges moving to the two arms of the Y-tube were recorded after 30 min. The insects were discarded after each test. The treatments were tested in pairs, and there were eight replications over time for each comparison. The significance of differences between the treatments was compared by paired t test at $P = 0.05$ for each comparison.

Relative attraction of sorghum midge females to different colors under dual-choice conditions. Relative attraction of different colors to sorghum midge females was studied in all possible combinations (yellow versus blue, yellow versus green, yellow versus red, red versus green, red versus blue, and blue versus green). The two arms of the Y-tube were covered with transparent sheets of the two colors being compared. The holding chamber and 5-cm portion of the glass arms were covered with a black polyethylene sheet. This provided the sorghum midge females a dual-choice between the colors being compared. Twenty sorghum midge females were released into the holding chamber, and the numbers of sorghum midge females that had moved to the glass arms were recorded 30 min later. Different colors were tested in pairs as described above, and there were 10 replications over time for each comparison. The treatment means were compared with a paired t test at $P = 0.05$ for each comparison.

Relative attraction of sorghum midge females to different colors under no-choice conditions. In this experiment, both arms of the Y-tube of the glass olfactometer were wrapped with transparent plastic sheets of the same color. The holding chamber and 5-cm portion of the glass arms were covered with a black polyethylene sheet as described before. Twenty sorghum midge females were provided only one choice, i.e. to respond to the same color in both the arms of the olfactometer. Numbers of sorghum midge females moving to the glass arms were recorded at 5, 10, 15, and 30 min after initiating the experiment to get an idea of the speed at which sorghum midge females respond to different colors. The numbers of sorghum midge females moving to both the arms of the glass apparatus at different time intervals was used as a measure of the attractiveness of a particular color to sorghum midge females. The experiment was repeated three times, and the four-color treatments were arranged in a randomized complete block design. The data were subjected to ANOVA using GENSTAT 5.0. The significance of differences between the treatments was judged by F test, and the treatment means were compared by least significant difference (LSD) at $P = 0.05$.

Relative attraction of sorghum midge females to different colors under multi-choice conditions. Relative attraction of sorghum midge females to red, yellow, green, and blue transparent plastic sheets wrapped on the four sides of the cage (30 × 30 × 30 cm) was also studied under multi-choice conditions, i.e. the sorghum midge females were offered a choice between the four colors being compared at the same time. The cage frame was made of aluminum, and the four sides and top were covered with a thin, transparent polyethylene sheet. Red, yellow, green, and blue transparent plastic sheets (30 × 30 cm) were placed on the four sides of the cage. Twenty sorghum midge females were released in the center of the cage, and the numbers of sorghum midge females that settled on the four sides of the cage with different colors were recorded at 15, 30, 45, and 60 min after initiating the experiment. The position of colors was changed after each test to avoid the directional effect, if any, of the normal light. The treatments were arranged in a completely randomized design and there were three replications. The data were subjected to ANOVA using GENSTAT 5.0. The significance of differences between the treatments was judged by F test, and the treatment means were compared by LSD at $P = 0.05$.

Relative attraction of sorghum midge females to different colors plus odor stimuli. Dual-choice conditions were used to study the attraction of sorghum midge females to odors alone and in combination with yellow, green, or red colors. Five rachis branches from flowering panicles of the sorghum genotypes 'QL 12' or 'QL 39' were placed at the ends of the arms of the glass Y-tube. Both arms of the Y-tube were covered with yellow, red, or green transparent plastic sheets. Twenty sorghum midge females were released into the holding chamber. The holding chamber and 5-cm portions of the glass arms were covered with a black polyethylene sheet. The Y-tube then was connected to the vacuum chamber. This resulted in a steady stream of air passing over the sorghum rachis branches placed at the ends of the glass arms. The air moved from the ends of the glass arms toward the holding chamber, where the sorghum midge females were released. Numbers of sorghum midge females moving to the arms of the Y-tube were recorded after 15, 30, and 60 min after initiating the experiment. The experiment was repeated three times, and the treatments were arranged in a completely randomized design. The data were subjected to ANOVA using GENSTAT 5.0. The significance of differences between the treatments was judged by F test, and the treatment means were compared by LSD at $P = 0.05$.

Results and Discussion

Attraction to normal light and odor stimuli. More sorghum midge females (60%) moved into the arms of the Y-tube having a light and odor stimuli (HC + O) than to the arms providing only light stimulus (HC; 30%) when the holding chamber and 5-cm portion of the glass arms was covered with a black polyethylene sheet (which provided a directional light stimulus to the sorghum midge females; Fig. 2). The differences between these treatments were not statistically significant ($P > 0.05$) when the glass apparatus was fully covered with a black polythene sheet, indicating that visual stimuli are important in the orientation behavior of this insect. When the entire glass apparatus was left uncovered, there were no significant differences in the numbers of sorghum midge females that moved into the arms of the Y-tube in response to light and odor

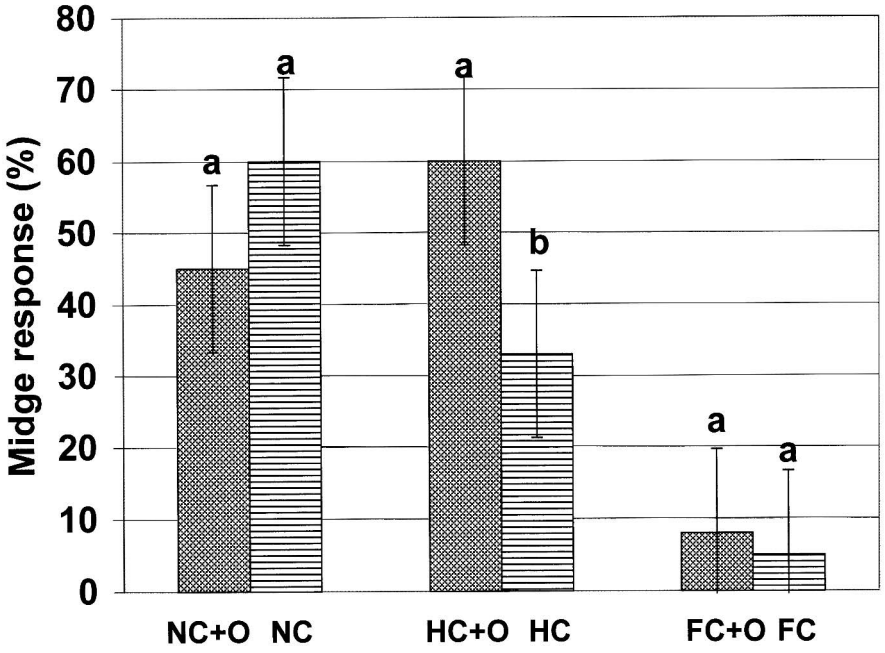

Fig. 2. Attraction of sorghum midge females to normal light and odor from the panicles of sorghum cultivar 'ICSV 197.' NC, No black polythene cover on the glass apparatus; O, odors from the sorghum panicle; HC, one-half of the glass apparatus covered with a black polythene sheet; FC, glass apparatus fully covered with a black polythene sheet. A pair of bars with the similar letters are not significantly different at $P = 0.05$ (df = 15) based on paired t test.

stimuli. Covering the insect-holding chamber and 5-cm portions of the glass arms with a black polyethylene sheet resulted in maximal response of sorghum midge females in the Y-tube, and this was used as a standard procedure to study the response of sorghum midge females to different colors.

Attraction of sorghum midge females to different colors in relation to normal light. When the sorghum midge females were offered a choice between normal light and the light passing through different colored polythene sheets, significantly (df = 7, $P \leq 0.05$) more sorghum midge females responded to yellow and blue colors than to the normal light. Maximum numbers of midges responded to yellow (62%), followed by blue (43%), green (30%), and red (26%; Fig. 3). Differences in numbers of sorghum midge females attracted to red and green versus normal light (blank) were not significant at $P = 0.05$ based on paired t test.

Relative attraction of sorghum midge females to different colors. When sorghum midge females were offered a choice between the light passing through different colored transparent polyethylene sheets in a dual-choice assay, significantly (df = 9; $P \leq 0.05$) more sorghum midges responded to yellow than to blue (38% versus 22%) or red (48% versus 24%) colors (Fig. 4). Also, more sorghum

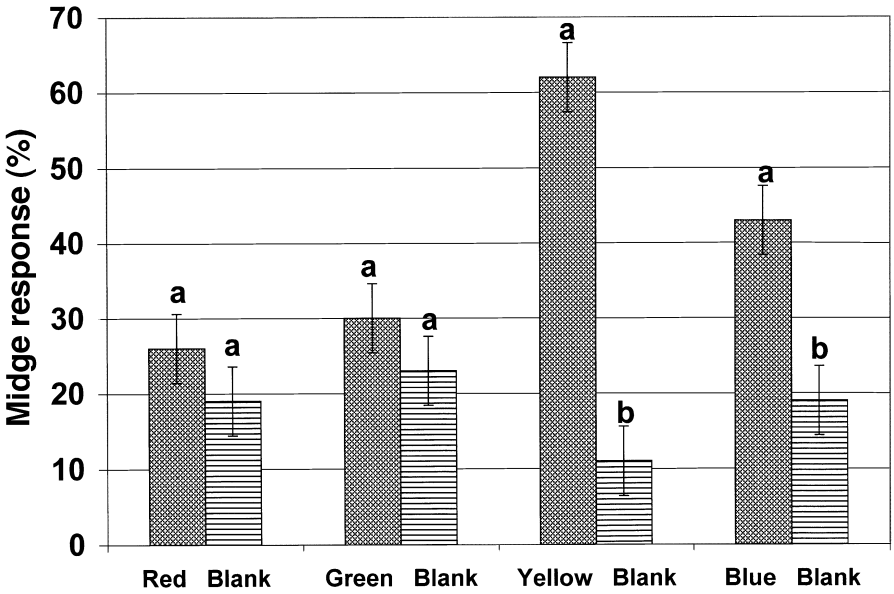

Fig. 3. Attraction of sorghum midge females to different colors versus normal light (Blank) under dual-choice conditions. A pair of bars with similar letters are not significantly different at $P = 0.05$ based on paired t test.

midges were attracted to red (39%) than to blue (25%). Differences in attraction of sorghum midge females to yellow versus green, red versus green, and to blue versus green were not significant at $P = 0.05$ based on paired t test. In general, the sorghum midge females preferred yellow over the other colors tested, and red to green and blue colors.

Relative attraction of sorghum midge females to different colors under no-choice conditions. Attraction of sorghum midge females to different colors under no-choice conditions was used as a measure of the promptness with which sorghum midge females respond to different colors. Sorghum midge females moved fastest toward yellow, followed by movement toward red, green, or blue (Fig. 5). Five minutes after initiating the experiment, significantly (df = 7, $P = 0.06$) more midges responded to yellow and red colors (80% and 85%, respectively) than to green and blue colors (65% and 67%, respectively) based on LSD comparison. Ten minutes after initiating the experiment, 88% of the midge females responded to yellow, 83% to green, 80% to red, and 77% to blue, although the difference in midge response to different colors were not significant ($P > 0.05$). The movement of sorghum midge females was relatively slower towards blue and green colors initially, but was similar to red and yellow colors at 10 to 30 min after initiating the experiment, and there were no significant differences in midge response to different colors at 30 min.

Relative attraction of sorghum midge females to different colors under multi-choice conditions. Under multi-choice cage conditions, more sorghum midge females responded to yellow (40% to 53%) than to the other colors

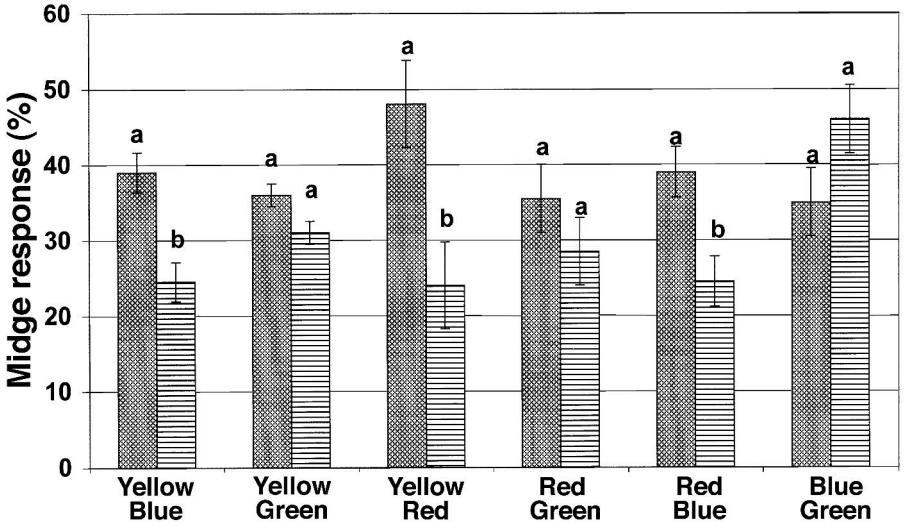

Fig. 4. Relative attraction of sorghum midge females to different colors in dual-choice tests under laboratory conditions. A pair of bars with similar letters are not significantly different at $P = 0.05$ based on paired t test.

tested (10% to 28%; Fig. 6). However, based on F test and LSD comparison, the differences in midge response to different colors were significant (df = 6) only at $P = 0.09$. At 15 min after initiating the experiment, more sorghum midge females were attracted to yellow (40%), followed by red (25%), green (13%), and blue (12%) colors. Throughout the experiment, most sorghum midge females were attracted to yellow, followed by red, green, and blue. Maximum response of the sorghum midge females to different colors was observed 45 min after initiating the experiment.

Attraction of sorghum midge females to color plus odor stimuli. More sorghum midge females were attracted to yellow color plus odor (34%) 15 min after initiating the experiment than to red (25%) or green (33%) plus odor or to odor alone (26%; df = 10; P 0.07; Fig. 7). However, differences in midge response to different colors plus odor, and odor alone were not significant at $P = 0.05$. Similar trends in midge response were also observed at 30 and 60 min after initiating the experiment. Thus, color plus odor stimuli seemed to be slightly more attractive to sorghum midge females than host odor alone.

Color, contact, and odor stimuli influence host selection behavior of insects. However, we do not understand fully how insects detect their host plants. Some insects have strong color preferences (Prokopy & Owens 1983, Harris et al. 1993, Barker et al. 1997). In general, phytophagous insects prefer yellow, and darker colors such as blue and black, are least preferred. Both hue and intensity influence insect response to different colors. Traps with fluorescent yellow paint are more attractive than traps with non-fluorescent yellow paint that reflects the same wavelength (550 nm; Meyerdirk et al. 1979).

Detailed information on the role of visual stimuli in host selection behavior is available only for a few insect species, whereas the influence of chemical stimuli

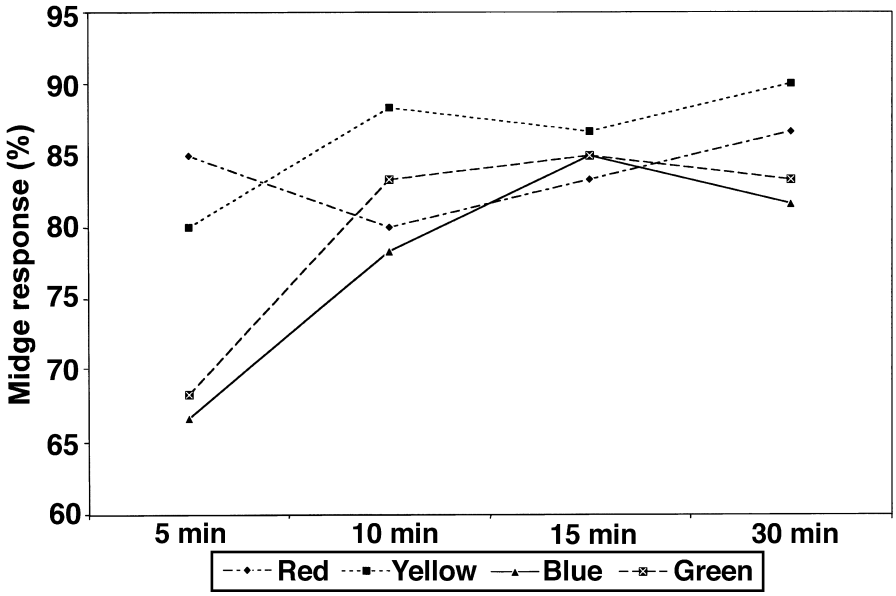

Fig. 5. Attraction of sorghum midge females over time to different colors in a glass olfactometer under no-choice conditions. The SEM for comparing mean midge response to different colors is 4.3, 5.1, 4.2, and 3.1 at 5, 10, 15, and 30 min after initiating the experiment, respectively.

in host plant selection has been studied in considerable detail (Harris et al. 1993). Visual cues and odor stimuli together elicit greater response from Hessian fly, *Mayetiola destructor* (Say), than do visual stimuli alone (Harris et al. 1993), and egg-laying response is stimulated by the hue rather than intensity of colors (Harris & Rose 1990).

Spectral quality and patterns of individual plants or their components serve as visual cues to insects (Prokopy & Owens 1983). Spectral transmission curves of foliage under different light conditions are consistent for several plant species because of absorption properties of chlorophyll (hue of 500 to 580 nm). Light reflectance is affected by plant surface characteristics such as glossiness or glume hairiness (Sharma & Nwanze 1997), high cellular water content, or lack of chlorophyll. Most of these characteristics result in greater total reflectance between 350 to 650 nm (Prokopy & Owens 1983). Spectral characteristics of foliage can enable insects to distinguish between living plants and other objects. In many plants, spectral quality seems to be the principal stimulus eliciting alightment (Kennedy et al. 1961, Coombe 1981), and intensity of reflected or transmitted light by foliage is a more variable parameter than spectral composition (Prokopy & Owens 1983). The diversity of insect species that are known to respond positively to yellow color (Kennedy et al. 1961, Walker 1974, Cross et al. 1976, Coombe 1981) has led to the speculation that for many insects, yellow constitutes a supernormal foliage stimulus emitting energy in the same band-width as that of the insect vision spectrum.

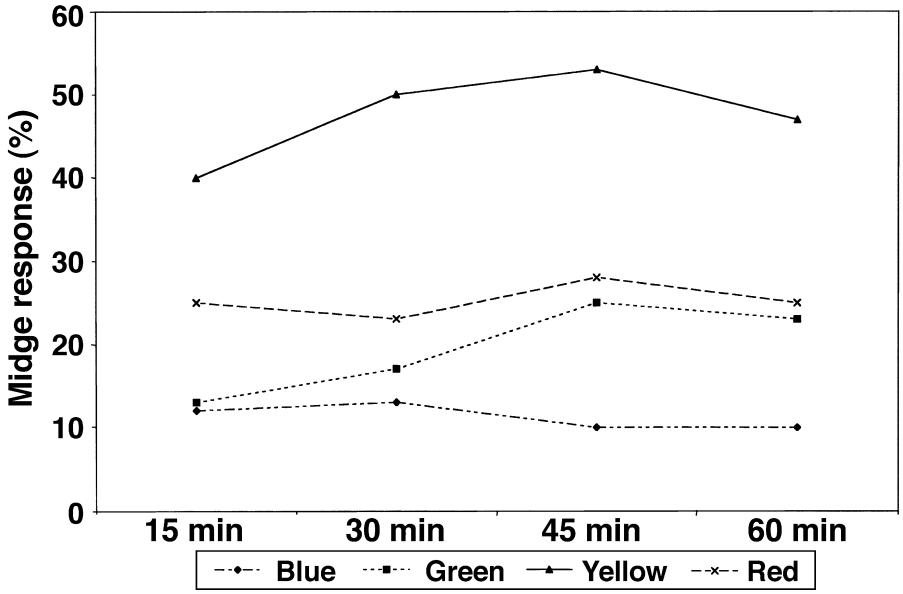

Fig. 6. Attraction of sorghum midge females to different colors over time in a cage (multi-choice conditions). The SEM for comparing the mean midge response to different colors is 12.9, 14.9, 11.9, and 11.6 at 15, 30, 45, and 60 min after initiating the experiment, respectively.

Genotypic variation may be as great as phenotypic variation in composition of the spectral light reflected from the plants at the intraspecific level. Individuals of a plant species may vary greatly morphologically between genotypes: e.g. sorghum genotypes have different intensities of trichomes, waxiness, glossiness, chlorophyll content, and hairiness of the glumes (Sharma & Nwanze 1997). These characteristics may influence the quality and intensity of light reflected from the sorghum plant, and ultimately influences the host-plant selection by the sorghum midge females. Sorghum midge-resistant and sorghum midge-susceptible genotypes also differ in the intensity of light reflected at the flowering stage of plant growth (H. C. S., unpublished data), and this may influence the host plant selection by sorghum midge females.

Sorghum midge females are attracted to yellow sticky traps (Wiseman et al. 1972, Sharma et al. 1990) and lay fewer eggs in spikelets of sorghum panicles covered with yellow or white cloth bags than in panicles covered with blue or black cloth bags (Sharma et al. 1988, 1990, Sharma & Vidyasagar 1994). In the present studies, sorghum midge females showed greater preference to light passing through yellow than to light passing through red, green, or blue colors. More sorghum midge females were attracted to odors from a sorghum panicle in combination with red or yellow than to host odors and the normal light. Thus, both color and odor stimuli are important in host finding by the sorghum midge females. Many insects use combined visual and chemical information when selecting their host plants (Prokopy & Owens 1983). Visual cues have a greater effect

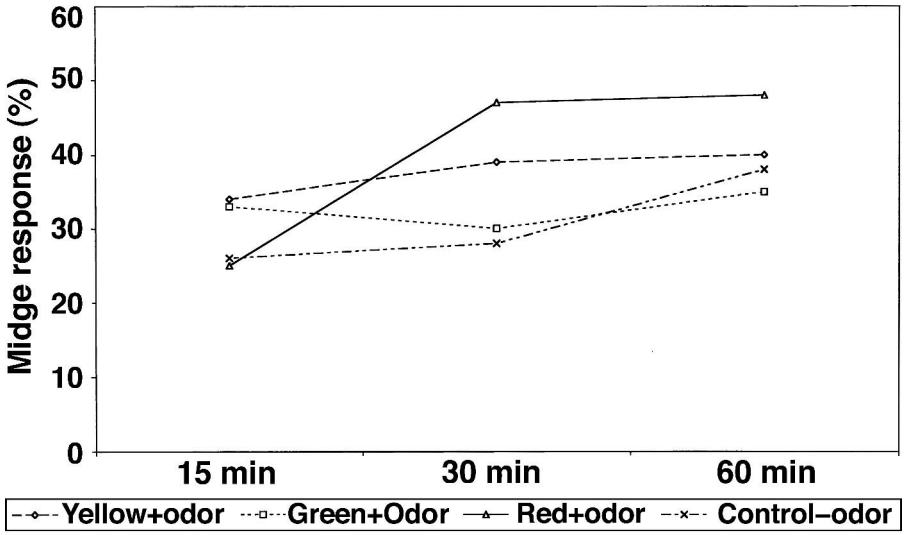

Fig. 7. Attraction of sorghum midge females to sorghum panicle odor alone and in combination with color stimuli (df = 10; P = 0.07). The SEM for comparing the midge response to different treatments is 5.1, 8.1, and 11.1 at 15, 30, and 60 min after initiating the experiment, respectively.

on flight orientation responses than plant odors for the Hessian fly (Harris et al. 1993). Similar responses were observed with sorghum midge, where different colors in combination with plant odors elicited a greater response than the plant odors alone.

Information on the attraction of sorghum midge females to visual and chemical stimuli may be useful for designing appropriate techniques to study population dynamics under field conditions. Monitoring sorghum midge density by visual counts is time consuming, must be carried out during the morning hours, and often is difficult with the unaided eye. However, the color preference of sorghum midge females could be exploited for developing suitable traps for monitoring its abundance in combination with sex pheromones or other odor stimuli. Development of suitable color traps to monitor sorghum midge abundance in the field could play an important role in understanding the behavior and population dynamics of this insect.

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The Non-Indigenous Ant, *Solenopsis invicta*, Reduces Loggerhead Shrike and Native Insect Abundance[1]

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ABSTRACT The red imported fire ant (*Solenopsis invicta* Buren) is an aggressive, non-indigenous species that is a threat to native biota in the southeastern United States. We determined the effect of *S. invicta* on loggerhead shrike (*Lanius ludovicianus* L.) abundance and investigated a possible mechanism of impact, which is a reduction in insect prey availability. We used a fire ant bait (hydramethylnon) to reduce fire ant populations on one randomly chosen member of each of five pairs of 202-ha study areas in the Texas coastal Bend region, and also measured shrike relative abundance and a volumetric index of insect biomass on the study areas. Loggerhead shrike relative abundance was assessed at five counting stations established along 3.2-km transects through prairie habitat on each study area during the fall of 1992 and 1993. We sampled non-*S. invicta* invertebrates with 13.3-L capacity UV light traps and found that insect volume, species richness, and diversity were greater on treated sites. More shrikes were observed on areas where *S. invicta* populations had been reduced. Both insect biomass and shrike abundance were negatively correlated to the level of *S. invicta* infestation. Our data suggest that shrikes may avoid areas on wintering habitats that have been invaded by *S. invicta* and that this avoidance may result from reduced insect availability.

KEY WORDS endangered species, Formicidae, Hymenoptera, invasive species, Laniidae, *Lanius ludovicianus*, Passeriformes

Ants are important in ecological systems, acting as keystone species in many instances (Risch & Carroll 1982). Ants provide key and irreplaceable ecosystem services such as pollination, nutrient turnover, energy flow, and seed dispersal (Handel et al. 1981). As invasive or introduced species, ants also may have a large impact by affecting native invertebrate (Porter & Savignano 1990, Cole et al.

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1992) and vertebrate species (Allen et al. 1998), as well as ecological processes (Bond & Slingsby 1984, Zettler et al. 2001).

The red imported fire ant (*Solenopsis invicta* Buren) is native to the Pantanal flood plain of South America and was introduced into the United States in the vicinity of Mobile, Alabama, in the 1930s (Vinson & Sorensen 1986). It spread throughout the southeastern United States and continues to expand its range and populations at both local (Wojcik 1994) and regional scales (Cokendolpher & Phillips 1989). *S. invicta* is of interest to ecologists and invasion biologists because of its rapid spread and increasing evidence of negative impacts on diverse native fauna, both vertebrate and invertebrate.

In its native range, and historically in the United States, *S. invicta* are predominately monogynous, with a single fertile queen in each colony, and are intraspecifically territorial. Polygyny was unknown in *S. invicta* until the discovery of multiple-queen colonies in Mississippi (Glancey et al. 1973). Competition among monogynous colonies generally maintains colony densities at <99/ha (Wojcik 1983). Polygynous colonies are characterized by high mound densities (>100 mounds/ha) associated with an apparent failure of the nest-mate recognition system and subsequent loss of territoriality (Mirenda & Vinson 1982). Polygynous colonies are now common in some infested regions, most notably in Texas where >50% of all colonies are polygynous (Porter et al. 1991). The sustained high population densities associated with polygyne *S. invicta* have increased the potential for negative impacts on native invertebrate and vertebrate species and communities.

Impacts on vertebrates, and in particular on avian species, include decreased fledgling success (Sikes & Arnold 1986) and population reductions (Allen et al. 1995). *S. invicta* may prey on pipping young (Allen et al. 1994, Drees 1994) and have indirect impacts on behavior (Pedersen et al. 1996), survival, and weight gain (Guiliano et al. 1996, Allen et al. 1997). Presumably, decreases in insects such as orthopterans and lepidopteran larvae and overall decreases in arthropod abundance (Porter & Savignano 1990) have indirect negative influences on insectivorous birds (and other animals) by decreasing food availability.

Loggerhead shrikes (*Lanius ludovicianus* L.) and *S. invicta* forage on many of the same insect species, and shrike-cached insects may be consumed by *S. invicta*. We hypothesized that fire ants may reduce insect availability for wintering shrikes, thereby reducing habitat quality for shrikes on wintering grounds. To test this hypothesis, we reduced fire ant populations and measured the effect on a volumetric index of available invertebrate biomass and loggerhead shrike relative abundance.

Materials and Methods

We conducted our research on three private ranches in the Texas Coastal Prairie vegetational area of Texas (Gould, 1975). We selected 10 202-ha study areas (five pairs: R1, R2, V1, VC1, and VC2) during the fall of 1990 and paired them based on similarities in degree of *S. invicta* infestation, vegetation, soils, and pretreatment wildlife indices. A detailed description of the study areas appears in Allen et al. (1995).

Red imported fire ant populations were reduced on one randomly chosen member of each study area pair with aerial applications (1.67 kg/ha) of Hydramethylnon (Amdro®) fire ant bait (American Cyanamid Co., Wayne, New Jersey), a

delayed-action toxicant. Hydramethylnon was applied in April and October 1991, and May 1992. Fire ant populations and the efficacy *of S. invicta* population reductions were assessed with 10 0.10-ha, non-overlapping circle counts near the center of each study area. All *S. invicta* mounds within each 0.10-ha circle were counted and assigned a value corresponding to mound size and presence or absence of worker brood (Lofgren & Williams 1982). This index weights colonies with worker brood more heavily than colonies without, because the lack of worker brood indicates the absence of a fertile queen and, thus, a moribund colony. Red imported fire ants were surveyed prior to treatments (April 1991) and seven times post-treatment. Efficacy of treatments to reduce *S. invicta* populations were analyzed with randomized design block analysis of variance using pretreatment fire ant indices as a covariate (Allen et al. 1995).

Six of the 10 available 202-ha study areas in three pairs (R1, V1, and VC1; Allen et al. 1995) were used to investigate *S. invicta* impact on invertebrate biomass measured indirectly via collected volume. We sampled (non-*S. invicta*) invertebrates with 13.3-L capacity UV light traps (Universal Black Light Trap 2851A, BioQuip Products, Gardena, California) using one trap-night per study area during each sampling period. Light traps were placed at ground level >100 m from study area boundaries approximately 30 min before sunset, and they were collected and emptied approximately 30 min after sunrise. Placement of traps on the ground and well within study area boundaries on our brush-prairie sites limited visibility so that our sampling was restricted as much as possible to invertebrates residing in our study areas. Members of a study area pair were sampled concurrently during three nights of consecutive sampling. The contents of light traps were transferred to graduated cylinders and were settled by tapping prior to the measurement of volume. Recovered Carabidae, Scarabidae, orthopterans (Tettigoniidae, Acrididae, Tetrigidae, Gryllotalpidae, and Gryllidae), and Dictyoptera (roaches) were identified to species. We compared species richness and diversity using the Shannon Diversity Index (Magurran 1988) among treated and untreated sites with Wilcoxon signed rank tests because the data were not normally distributed ($P < 0.04$). Data from light traps (cm^3 of insects per study area) were analyzed with randomized block design analysis of variance (SAS Institute 1989). Each sampling period was analyzed separately because species turnover was high between sampling periods and thus represented independent samples. Voucher specimens are maintained at the U.S. Department of Agriculture Imported Fire Ant Station (Gulfport, Mississippi).

Pretreatment data were collected in April 1991, and treatment data were collected in June, July, and October 1991, and again in January (*S. invicta* indices only), March, May (biomass only), June, August, and October (biomass only) of 1992. In June and August 1993, sampling documented *S. invicta* populations and insect biomass >1 year after the cessation of treatments. Pearson correlation analysis was used to test for a relationship between *S. invicta* indices and insect biomass for those periods where both measurements were collected. To decrease the chance of Type II error, we considered $P < 0.10$ significant (Tacha et al. 1982).

We measured loggerhead shrike relative abundance on 3.2-km transects (Allen et al. 1995) through representative habitat on each study area. Five regularly spaced counting stations were placed on each transect. Transects were walked within 3 h of sunrise/sunset, and 3 min were spent at each station counting all shrikes seen within 50 m of the station. Stations were surveyed 12 times on each

study area between 2 September and 2 November 1992 for a total of 60 counts on each study area. Between 1 September and 13 November 1993, stations were surveyed an average of 17 times (range 13–26) for a total of 85 counts on each study area. Members of paired study areas were counted simultaneously. Shrike abundance was expressed as the average number of shrikes observed on each five-station transect. Loggerhead shrike abundance was analyzed with randomized block design analysis of variance, and we calculated both linear and nonlinear regressions to determine if shrike abundance was affected by fire ant abundance as measured by indices (SAS Institute 1989).

Results

Fire ants in our study areas were primarily polygynous (Allen et al. 1995). Prior to treatment, *S. invicta* indices averaged 435 per 0.10-ha plot on treated areas and 297 per 0.10-ha plot on untreated areas, roughly corresponding to average mound densities of 290 and 198 mounds/ha, respectively. Following treatments, *S. invicta* population indices were reduced on treated sites (see Allen et al. 1995), remaining below untreated indices for the remainder of 1991 and throughout 1992. *S. invicta* indices after treatment averaged 18% of pretreatment levels on treated sites, whereas they remained virtually unchanged on untreated sites (averaging 105% of pretreatment level). In August 1992, immediately prior to the collection of shrike data, fire ant population levels on treated areas averaged 10% of untreated area indices. In 1993, a year after the last treatment to reduce fire ants, fire ant populations were similar on treated and untreated study areas (Allen et al. 1995).

Before treatments, insect volume was less ($P = 0.08$) on those areas randomly selected to be treated than on untreated areas (Table 1). After treatments, recovered insect volume was greater on treated sites and averaged more than double that of untreated areas. Significant differences were detected in June ($P = 0.02$) and October 1991 ($P = 0.03$) and May 1992 ($P < 0.10$), and approached significance in March ($P = 0.15$) and June 1992 ($P = 0.16$, Table 1). In August 1992, immediately prior to the collection of shrike data, insect volume on untreated sites was 43% of the volume recovered on treated sites. Invertebrate taxa recovered from light traps were predominately Scarabaeidae, Trogidae, Carabidae, Tettigoniidae, Acrididae, and Gryllidae, as well as Lepidoptera, Homoptera, and Hemiptera. A significant negative correlation existed between *S. invicta* indices and recovered invertebrate volume ($r^2 = -0.281$, df = 12, $P = 0.07$). One year after the cessation of treatments, recovered insect volume from treated and untreated areas were similar and not significantly different ($P > 0.10$, Table 1).

Scarabidae, Carabidae, Orthoptera (Tettigoniidae, Acrididae, Tetrigidae, Gryllotalpidae, and Gryllidae), and Dictyoptera were identified to species. Species richness was higher on treated sites ($W = 17$, df = 7, $P = 0.094$; Table 2). One hundred forty-four species were collected, 124 from treated sites and 100 from untreated sites (Table 2). The median Shannon Diversity index for treated sites (1.577) was greater than median diversity (1.386) on untreated sites ($W = 19$, df = 5, $P = 0.063$; Table 2).

We observed 57 shrikes during 600 3-min observation periods. Significantly more shrikes were observed on areas where *S. invicta* populations had been

Table 1. Insect volume (cc) (± one SE) collected from light traps on three pairs of treated and untreated study areas in the Texas Coastal Prairie, 1991–1992.

	Treated	Untreated		
Date	X ± SE	X ± SE	*F* value[a]	*P* level
Pretreatment (April 1991)	164 ± 18	248 ± 39	10.5	0.083
June 1991	2,405 ± 244	960 ± 215	60.1	0.016
July	487 ± 105	323 ± 87	3.2	0.213
October	43 ± 3	21 ± 6	33.8	0.028
March 1992	9 ± 4	4 ± 2	5.2	0.151
May	162 ± 90	126 ± 81	8.9	0.096
June	1,007 ± 314	467 ± 91	4.6	0.164
August	670 ± 371	290 ± 98	1.8	0.316
October	135 ± 87	87 ± 57	2.4	0.259
S. invicta recovery period				
June 1993	170 ± 98	177 ± 111	0.3	0.662
August	246 ± 47	8 ± 70	3.1	0.220

[a]2 degrees of freedom in all comparisons.

reduced ($F = 11.58$, df = 4, $P = 0.027$; Table 3). An average of 0.72 shrikes per five-station transect was observed on treated areas, and an average of 0.25 per five-station transect on untreated areas. Linear regressions of the relationship between fall shrike abundance and the abundance of *S. invicta* were significant as measured in both June ($r^2 = 0.650$, df = 9, $P < 0.005$) and August ($r^2 = 0.394$, df = 9, $P < 0.052$; Fig. 1) 1992. However, non-linear models provided a better fit,

Table 2. Species richness (totals) and diversity (means; Shannon Diversity Index) of selected invertebrate families captured in light traps on three pairs of treated and untreated study areas in the Texas Coastal Prairie, 1991–1992.

	Treated		Untreated	
	Richness	Diversity	Richness	Diversity
Carabidae	52	2.886	52	2.963
Tettigoniidae	12	1.367	11	1.107
Acrididae	14	1.832	9	1.665
Tetrigidae	0	–	1	–
Gryllidae	3	0.489	3	0.303
Gryllotalpidae	1	–	0	–
Dictyoptera	3	0.902	1	0
Scarabeidae	39	1.786	23	1.671
Totals or means	124	1.544	100	1.285

Table 3. Loggerhead shrike abundance (± one SE) on five pairs of treated and untreated study areas in the Texas Coastal Prairie, 2 September–2 November 1992.

Study area pair	Treated	Untreated
R1	0.92 ± 0.31	0.08 ± 0.08
R2	0.83 ± 0.21	0.75 ± 0.22
V1	0.67 ± 0.43	0.17 ± 0.17
VC1	0.42 ± 0.15	0.17 ± 0.11
VC2	0.75 ± 0.25	0.08 ± 0.08
Mean ± SE	0.72 ± 0.09	0.25 ± 0.13

explaining more variance (June $r^2 = 0.823$, df = 9, $P = 0.011$, rational four-parameter model; June $r^2 = 0.71$, df = 9, $P = 0.0124$, inverse 2nd order polynomial; Fig. 2). In 1993, less than 1 year after the cessation of treatments to reduce fire ants, shrike populations were similar on treated and untreated study areas ($W = 5$, df = 4, $P = .625$; Table 4).

Discussion

We quantified an increase in overall non-*S. invicta* invertebrate volume, richness, and diversity on areas treated to reduce *S. invicta* populations. Treatments were unlikely to have any direct effect on non-*S. invicta* insects (Apperson et al. 1984). *S. invicta* dominates baits and, even at relatively low density, are the first species to recruit to >95% of baits and to eventually exploit 100% of baits (Baroni Urbani & Kannowski 1974). *S. invicta* may remove >90% of bait in less than 2 h. Furthermore, hydramethylnon decomposes rapidly with exposure to sunlight (Vander Meer et al. 1982) and leaves no detectable residues after 24 h (Apperson et al. 1984). We conclude that fire ants negatively affected overall insect biomass, richness, and diversity.

Shrike abundance was higher on sites where *S. invicta* populations were reduced. A mechanism responsible for lower shrike abundance on sites with higher fire ant populations may be the deterioration of habitat quality due to a decrease in the availability of insects. The better fit of non-linear models of the relationship between shrike abundance and fire ants suggests that there may be a threshold infestation level that occurs at fairly modest fire ant densities (Fig. 2). Food resource abundance affects territory quality, with sites supporting fewer insects also supporting lower densities of shrikes (Seki & Takano 1998). Shrikes largely feed on arthropods (Craig 1978) such as grasshoppers, crickets, and beetles (Lymn & Temple 1991). Red imported fire ants also feed heavily on arthropods (Hays & Hays 1959), and on our study sites we detected community-level negative effects on native insects. The indirect influence of *S. invicta* on insect availability also has been suggested as a mechanism of negative impact on quail populations (Allen et al. 1995). More direct resource co-option also may affect the quality of shrike habitat. Food caching behavior by the loggerhead shrike is not fully understood (Fraser & Luukkonen 1986), but does influence reproductive success

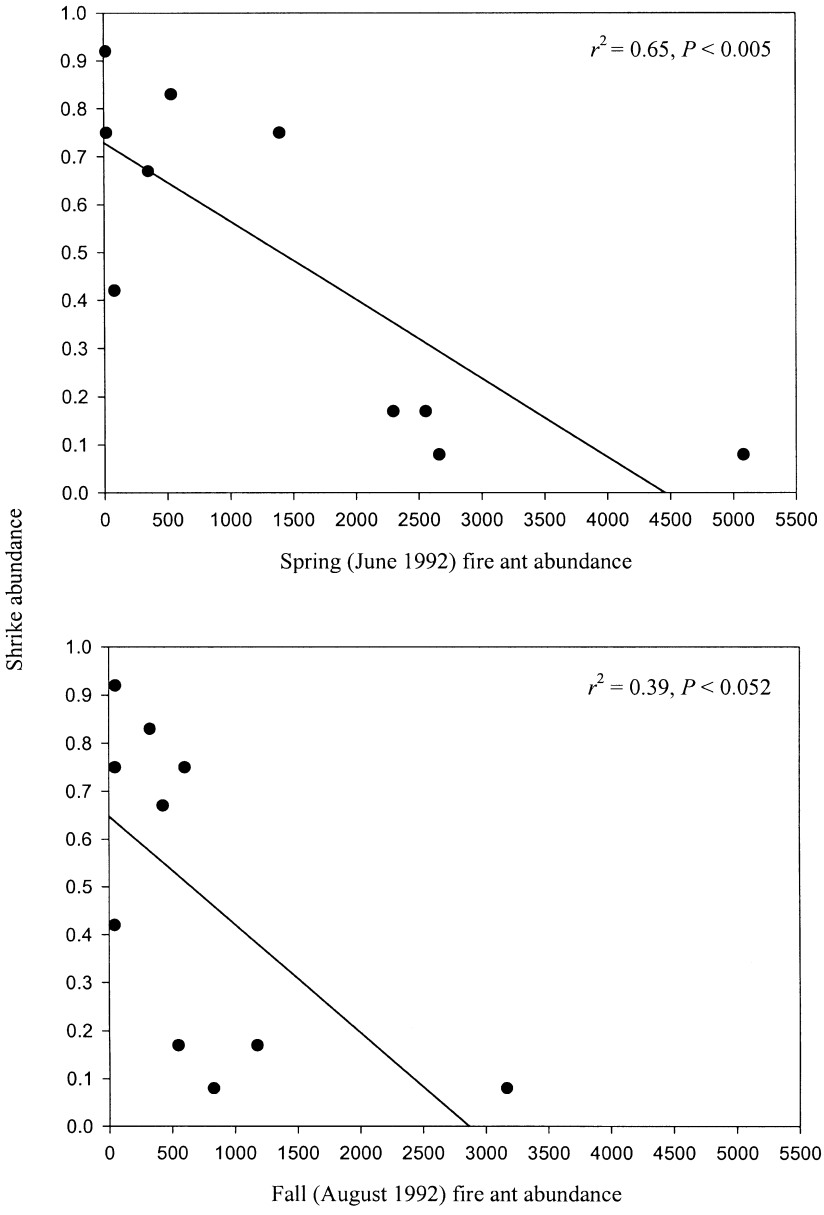

Fig. 1. Linear relationship between fall abundance of loggerhead shrikes and spring and fall fire ant abundance, 1992.

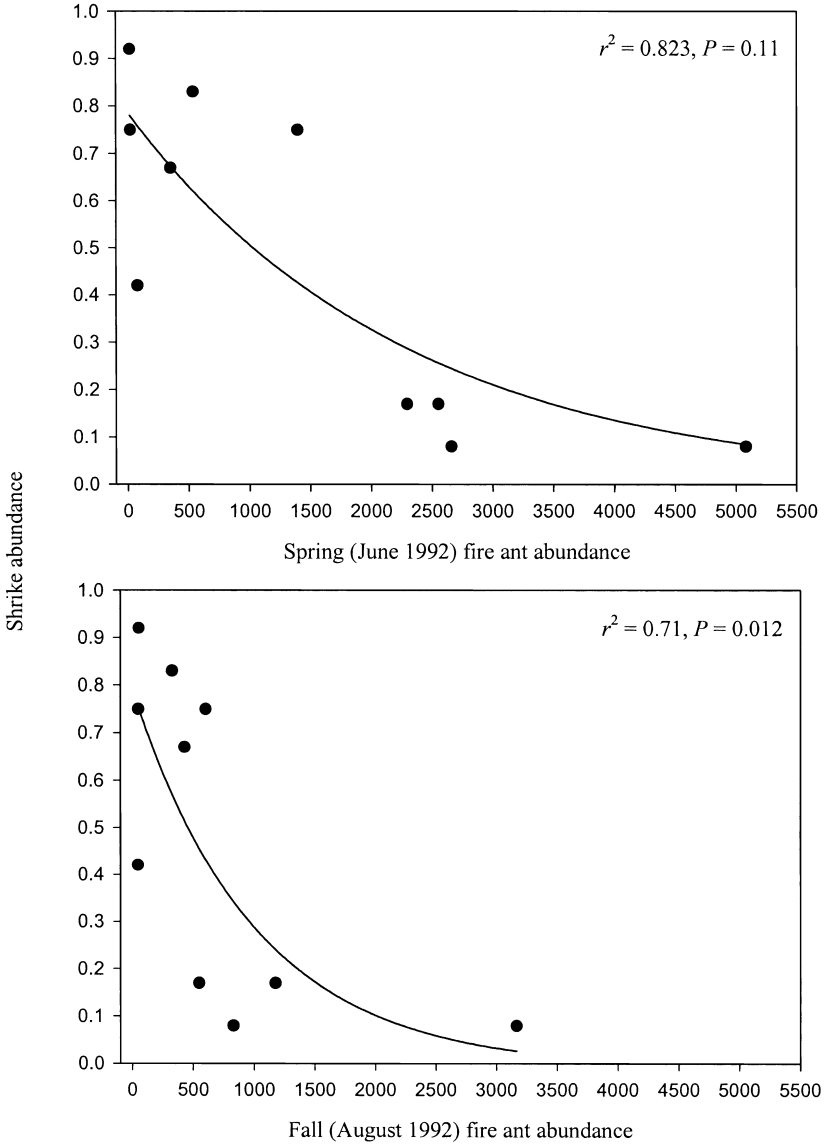

Fig. 2. Non-linear relationship between fall abundance of loggerhead shrikes and spring and fall fire ant abundance, 1992.

(Yosef & Pinshow 1989). In 1992, a loggerhead shrike was observed caching a freshly impaled grasshopper on a fence post and, minutes later, this grasshopper was dismembered and carried away by foraging *S. invicta* (C. R. A., personal observation). This observation and the speed of recruitment by *S. invicta* to food sources suggests that prey cached in areas heavily infested by *S. invicta* are likely

Table 4. Loggerhead shrike abundance (± one SE) on five pairs of treated and untreated study areas in the Texas Coastal Prairie >1 year after the cessation of treatments, 1 September–13 November 1993.

Study area pair	Treated	Untreated
R1	0.44 ± 0.13	0.42 ± 0.10
R2	0.29 ± 0.13	0.16 ± 0.09
V1	0.13 ± 0.09	0.07 ± 0.07
VC1	0.39 ± 0.14	0.75 ± 0.11
VC2	0.18 ± 0.10	0.05 ± 0.05
Mean ± SE	0.29 ± 0.06	0.29 ± 0.13

to be exploited by fire ants. If caching by male shrikes is important in the reproductive energetics (Applegate 1977) and success (Yosef & Pinshow 1989) of females, or as a reserve food source for shrikes in times of prey scarcity, this may be an additional mode of impact in the loggerhead shrike.

Conservation of native species often requires understanding the effect of invasive species upon native species, the mechanisms of impacts, and the factors leading to invasion. Predaceous invasive invertebrate species can have profound negative effects on native insect communities (Simberloff 1981), both by preying upon native species and by competing with them. The impact of *S. invicta* on native species diversity and abundance is becoming more clearly understood (Allen et al. 1998), yet more remains unknown than known concerning the ecological impacts of fire ants. However, it is clear that fire ants are a threat to native invertebrate and vertebrate communities, especially in areas where polygynous fire ants are established.

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