Effect of Temperature on Efficacy of Insecticides to Differential Grasshopper (Orthoptera: Acrididae)

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ABSTRACT The effect of temperature on activity of insecticides for controlling grasshoppers in leafy green vegetables was evaluated. Insecticides evaluated had differing modes of action and included diflubenzuron, azadirachtin, *Beauveria bassiana*, spinosad, endosulfan, esfenvalerate, and naled. We evaluated these insecticides for efficacy to third instars of differential grasshopper, *Melanoplus differentialis* (Thomas), at temperatures ranging from 10 to 35°C. In the laboratory, treatment with esfenvalerate resulted in 100% mortality at temperatures of 10 to 35°C, and efficacy was not temperature dependent. Treatment with spinosad resulted in similar mortality as with esfenvalerate at all temperatures except 10°C. The activity of *B. bassiana* was greatest at 25°C and was adversely affected by high and low temperatures. Treatment with diflubenzuron resulted in increased mortality at high temperatures, and at 35°C its activity was similar to that of esfenvalerate and spinosad. The activity of azadirachtin ranged from 19 to 31% and was not influenced by temperature. In field studies, spinosad, diflubenzuron, and esfenvalerate provided differing levels of mortality both at application and when nymphs were exposed to 1-h-old residues. However, only spinosad and diflubenzuron provided similar levels of mortality when nymphs were exposed to 24-h-old residues. The residual activity of endosulfan, naled, esfenvalerate, and spinosad decreased with increasing time (0–24 h) after exposure to sunlight and high summer temperatures. Compared with other insecticides, naled had a short residual activity period and activity was dependent upon immediate contact with the nymphs or their substrate. *B. bassiana* was inactive under high temperatures and intense sunlight as occurs in summer.

KEY WORDS *Melanoplus differentialis*, temperature, insecticide efficacy

LEAFY GREEN VEGETABLES SUCH as kale, collards, mustard, turnips, and spinach are grown in the south central United States from September through May, for fresh and processing markets. Thus, the crops and insect pests encounter temperatures that vary between 5 and 35°C. The important insect pests of leafy green crops in the south central region include aphids, cucumber beetle species, seed maggots, and larvae of several species within the order Lepidoptera, including the diamondback moth, *Plutella xylostella* (L.), and armyworm, *Pseudaletia uinpunctata* (Haworth). In addition, grasshoppers have been identified as a significant contaminant problem in fields of leafy green vegetables (Gecan and Bandler 1990). Generally, grasshoppers do not reproduce within fields of leafy greens but rather move into them from adjacent rangeland, pastures, or other crops (Sweeden 1996). Grasshoppers do feed on leafy greens and may reduce yield. However, the main problem is associated with their large size and that they are a significant contaminant in the harvested product. Processed raw ingredients are subject to stringent regulatory standards set by the United States Food and Drug Administration (FDA). Due to strict FDA standards that limit insects, insect parts, and fecal material in processed vegetable crops, the management and control of grasshoppers is critical. The primary tool for managing grasshoppers is application of insecticides. Due to the variation of climatic conditions during the time when leafy greens are grown, both insecticides and insects are exposed to a broad range of temperatures.

Temperature is one of the most important factors affecting biological processes in all living organisms. Temperature influences metabolic rate, locomotion, rate of water loss, food consumption, growth, maturation, and habitat selection of grasshoppers (Chappell and Whitman 1990). Temperature is also a major factor affecting insecticide toxicity (DeVries 1978) and thus efficacy (Johnson 1990, Scott 1995). The effects of temperature on efficacy can be either positive or negative. The response relationship between temperature and efficacy has been found to vary depending on the mode of action of an insecticide, target

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species, method of application, and quantity of insecticide ingested or contacted (Johnson 1990).

Considerable research has been conducted to determine insecticide efficacy and residual activity for controlling grasshoppers on various crops. Due to implementation of the Food Quality Protection Act in 1996 several currently registered organophosphate, carbamate, and pyrethroid insecticides are under regulatory review and their registration may be lost. Registered insecticides that are currently under review by the Environmental Protection Agency (EPA) include diazinon, dimethoate, chlorpyrifos, malathion, methomyl, permethrin, carbaryl, and dibrom. Diazinon, dimethoate, methomyl, dibrom, and permethrin are effective, commonly used insecticides for controlling insect pests on leafy green crops (OSU Extension Agents' Handbook 2002). Possible replacements for these insecticides include spinosad, esfenvalerate, diflubenzuron, neem extracts, and biological insecticides based on the fungus *Beauveria bassiana.*

This study focused on the effects of temperature on efficacy of these various insecticides on differential grasshopper, *Melanoplus differentialis* (Thomas), populations. We also sought to determine the activity levels of possible alternative insecticides that may ultimately replace organophosphate and carbamate insecticides for controlling differential grasshoppers.

Materials and Methods

Insecticides. The following insecticides were obtained as formulated materials as noted: insect growth regulator (IGR) diflubenzuron (Dimilin 2L, Uniroyal Chemical Co., Inc., Middleburg, CT), 7.0 g [AI]/ha; botanically derived IGR azadirachtin (Neemix 4.5, Certis USA, Columbia, MD), 48 g [AI]/ha; microbial insecticide *Beauveria bassiana* GHA (Mycotrol, Mycotech Corp., Butte, MT), 179 g [AI]/ha; pyrethroid esfenvalerate (Asana XL, E. I. DuPont de Nemours & Co., Inc., Wilmington, DE), 55 g [AI]/ha; microbial-derived insecticide spinosad (Spintor 2 SC, Dow Agro Sciences LLC, Indianapolis, IN), 175 g [AI]/ha; cyclodiene endosulfan (Thiodan 3 EC FMC Corp., Philadelphia, PA), 1,118 g [AI]/ha; and organophosphate naled (Dibrom 8, Amvac Chemical Corp., Los Angeles, CA), 2102 g [AI]/ha. All insecticide solutions used in the laboratory and field studies were based on labeled application rates and mixed under the assumption that applications would be at the rate of 332 liters total spray solution per hectare.

Grasshoppers. In June and July 2001, and June 2002, third instars of *M. differentialis* were collected from a pasture in southeastern Oklahoma by using a 38-cm-diameter sweep net. The third instars were identified by the general color of pale yellow to tan, a body length of 9.4–12.4 mm, and a black stripe on the hind femur occupying the center of the medial area (Pfadt 1994). The Systematic Entomology Laboratory (USDA, Beltsville, MD) identified and verified the samples of the third instars and adults as *M. differentialis nigricans.* The collected grasshoppers were transported to the entomology laboratory at the Wes Watkins Agricultural Research and Extension Center (WWAREC), Lane, OK, and kept in aluminum wire mesh mosquito cages of 45 by 45 by 45 cm (Bioquip, Rancho Dominquez, CA). Cages were covered on the bottom with 3 cm of sand. Fresh collard leaves and water were provided each day. Cages were maintained under a 14-h photoperiod and a room temperature of 26.7 ± 0.5°C throughout the duration of the experiment.

Effect of Temperature. In May 2001, collard seeds of *Brassica oleraceae* L. (variety *acephala* 'Champion') were planted in plastic pots (10 cm in height and 11 cm in diameter) containing a soil-less medium (horticultural vermiculite [45%] and Canadian sphagnum moss [55%]) and 6 g of Osmocote (controlled release fertilizer, 14:14:14 N–P–K). They were grown in the greenhouse for 6 wk at 30°C.

In June 2001, individual leaves with a mean leaf area of 27.2 ± 1.5 cm^2 were selected from the plants grown in the greenhouse and were treated with the insecticides; *B. bassiana*, azadirachtin, diflubenzuron, and esfenvalerate. The concentration of each insecticide solution was calculated based on field-use labeled rates. Each insecticide was prepared as a 100-ml solution by using distilled water and stirred for 10 min by using an electrical stirrer. Individual leaves were dipped in the insecticides, removed, and dried at 25°C for 30 min. The plastic cages used for this experiment were made from 480-ml volume (16 oz), 115- and 90-mm top and bottom diameter clear plastic deli container cups with over snap caps (product nos. 9061 and 9065, Bio-Serve, Frenchtown, NJ). The bottom of the container was removed and replaced with a cloth sleeve, which was glued to the bottom. The lid was used to cover the top of the container and the sleeve on the bottom was secured with tie-on strip of material. The cut end of the leaf petioles were covered with moist cotton plugs and placed in plastic cages containing a third instar of *M. differentialis* that had been starved for 24 h. These containers were then transferred to controlled temperature chambers (model l-30VL, Percival Scientific Co., Boone, IA) and held at a photoperiod of 14:10 (L:D) h and a temperature of either 10, 15, 25, or 35°C. After 48 h, treated leaves were replaced with untreated leaves. Insecticide efficacy was determined based on mortality of grasshoppers over a 10-d observation period. Mortality was determined by either lack of movement, the insect lying on its side, or inability to respond to a slight shaking of the cage with a coordinated movement. The cadavers in cages containing leaves treated with *B. bassiana* were kept in an environmental temperature chamber at 25°C for 14 d for determination of mycelium growth. Mortality due to *B. bassiana* was determined when mycelia of *B. bassiana* were visible on the integument through a microscope. One cage, each with a third instar, was used per replicate. The experimental design was completely randomized with six replications of each insecticide and a water-treated control per temperature. In July 2001 and June 2002, the experiment was repeated with the addition of the insecticide spinosad (Spintor 2 SC) with five replica-

tions. The data for all three experiments (experiment 1, 2001; experiment 2, 2001; and experiment 3, 2002) were pooled (pooled data for spinosad were from experiments 2 and 3 only because spinosad was not included in experiment 1) and CATMOD procedure (SAS Institute 1999) was used to examine the effects of insecticide, temperature and time. PROC FREQ with Fisher's exact test was used to determine the effect of temperature on each treatment (SAS Institute 1999). We evaluated the efficacy of insecticides as insect mortality and used a $P < 0.1$ level for delineation of differences. Change in mortality over time due to each insecticide at each temperature was described using correlation analysis between cumulative mean mortality and days after treatments using PROC CORR procedure (SAS Institute 1999).

Field Efficacy. The experimental site was a research field at the WWAREC. Before planting, fertilizer was applied to the soil at the rate of 112.08 kg/ha of 17:17:17 N–P–K. The preemergent herbicide trifluralin was applied to the soil 3 d before planting at the rate of 0.84 kg/ha. Collards of *B. oleraceae* L. (variety *acephala*, 'Champion') seeds were seeded directly into raised beds, 1.8 m in width, two rows spaced 0.9 m apart on 16 May 2001 by using a tractor-mounted planter. Within the rows, seeds were spaced 2.5 cm apart. Irrigation was provided through an overhead sprinkler system. The experimental design was completely randomized with five replications. Each plot was one bed wide and 6 m in length, bordered on each side by an untreated bed and on each end by a 4.5-m-wide fallow strip.

Forty third instars of *M. differentialis* nymphs were selected from the rearing cages. Nymphs of similar size were isolated in groups and starved for 24 h in the laboratory at a temperature of 26.7 ± 0.5°C. Each was placed in an aluminum wire mesh cage 30 cm in length and 45 cm in perimeter. Cloth sleeves were glued to each end to facilitate access to the cage from both ends. The sleeves were secured with a tie-on strip of material.

After the 24-h starvation period, cages with third instars were sealed from both sides and transferred to plots. A plant was selected from each plot and one leaf from the upper plant canopy with a mean leaf area of 265.1 ± 8.5 cm^2 was carefully inserted into each cage by opening one end of the cloth sleeve and tied closed. One cage each with a third instar was used per replicate. Each insecticide treatment (azadirachtin, diflubenzuron, spinosad, esfenvalerate, endosulfan, and naled), except *B. bassiana*, was sprayed on 6 July 2001 (experiment 1) by using a tractor-mounted sprayer with six hollow cone nozzles per bed applying 332 liters/ha solution. *B. bassiana* was sprayed on the evening of 5 July 2001 to avoid exposure to high temperature and sunlight soon after treatment.

After 24 h, each cage was checked daily for insect mortality for a 10-d period. Mortality of grasshoppers was determined as mentioned previously. Cadavers of the nymphs from the *B. bassiana* treatments were assessed for mycelial growth, and mortality was determined as described above. To assess residual activity, a cage containing one starved (24-h) third instar of *M. differentialis* was placed in each plot 24 h after the initial treatment. Grasshopper mortality in this study was recorded at 24 h intervals over a 10-d period. This experiment was repeated on 19 July 2001 (experiment 2) and on 20 June 2002 (experiment 3). For experiment 2, the cages were placed in the field 1 h and 24 h after spraying. For experiment 3, the plots were direct seeded on 26 April 2002 and insecticide treatments were applied on 20 June 2002, and the cages were placed in plots before treatment, 1 h after treatment and 24 h after treatment. Mortality of grasshoppers was recorded as indicated previously.

The data for each individual time of placing the cages in the field (before treatment, 1 h after treatment, and 24 h after treatment) were pooled and the CATMOD procedure was used to examine the effects of insecticide and time (SAS Institute 1999). The PROC FREQ procedure with Fisher's exact test was used to determine differences among the insecticide treatment effects (SAS Institute 1999). The PROC CORR procedure was used to determine correlation (Pearson correlation coefficient and significance probability of correlation) between mortality of *M. differentialis* nymphs and number of days after exposure to insecticide treatments at differing time (hours) periods after field spray application (SAS Institute 1999).

Table 1. Maximum likelihood analysis of variance (CATMOD procedure) by using pooled data from three laboratory experiments

Source	df	χ^2	Pr>χ^2
Day	5	3.30	0.6538
Temperature	3	8.74	0.0330
Day*temperature	15	13.39	0.5719
Treatment	5	198.91	<0.0001
Day*treatment	25	49.00	0.0028
Temperature*treatment	15	29.72	0.0130
Day*temperature*treatment	75	34.25	1.0000
Likelihood ratio	74	452.16	<0.0001

Results

Effect of Temperature. Results from the maximum likelihood analysis of variance (ANOVA) (CATMOD procedure) indicated that there were insecticide and treatment effects (Table 1). There was also a significant insecticide × temperature interaction. In addition, the results of the PROC FREQ analysis for the mortality data for treatment effects for each temperature indicated that there were significant interactions for these effects for several insecticide–temperature combinations. No mortality of grasshopper nymphs was observed in the water control at any temperature.

At 10°C and 1 d after treatment, exposure to esfenvalerate resulted in 100% mortality of nymphs and exposure to spinosad resulted in 40% mortality (Table 2). Mortality from both insecticides was greater than that obtained from the water control. Cumulative mortality increased with time for each insecticide except esfenvalerate (Table 3). At 10 d, mortality from

Table 2. Mean (± SEM) percentage of mortality of *M. differentialis* nymphs due to temperature over time by insecticide treatments using pooled data from three laboratory experiments

Insecticide	Temp (°C)	Days after treatment					
		1	2	4	6	8	10
Water control	10	0.0 ± 0.0A	0.0 ± 0.0A	0.0 ± 0.0A	0.0 ± 0.0A	0.0 ± 0.0A	0.0 ± 0.0A
	15	0.0 ± 0.0A	0.0 ± 0.0A	0.0 ± 0.0A	0.0 ± 0.0A	0.0 ± 0.0A	0.0 ± 0.0A
	25	0.0 ± 0.0A	0.0 ± 0.0A	0.0 ± 0.0A	0.0 ± 0.0A	0.0 ± 0.0A	0.0 ± 0.0A
	35	0.0 ± 0.0A	0.0 ± 0.0A	0.0 ± 0.0A	0.0 ± 0.0A	0.0 ± 0.0A	0.0 ± 0.0A
B. bassiana	10	0.0 ± 0.0A	0.0 ± 0.0A	12.5 ± 8.5abA	12.5 ± 8.5aA	12.5 ± 8.5abAB	18.75 ± 10.1aAB
	15	6.3 ± 6.3AB	6.3 ± 6.3AB	12.5 ± 8.5abA	18.8 ± 10.1aA	25.0 ± 11.2bcB	50.0 ± 12.9bC
	25	0.0 ± 0.0A	18.8 ± 10.1A	31.3 ± 11.9bC	50.0 ± 12.9bB	50.0 ± 12.9cB	62.5 ± 12.5bC
	35	0.0 ± 0.0A	0.0 ± 0.0A	0.0 ± 0.0aA	0.0 ± 0.0aA	0.0 ± 0.0aA	6.25 ± 6.3aA
Spinosad	10	40.0 ± 16.3aB	50.0 ± 16.7aB	50.0 ± 16.7aB	50.0 ± 16.7aB	60.0 ± 16.3aC	60.0 ± 16.3aC
	15	30.0 ± 15.3aB	30.0 ± 15.3aB	70.0 ± 15.3abB	80.0 ± 13.3abB	80.0 ± 13.3abC	90.0 ± 10.0abD
	25	60.0 ± 16.3abB	60.0 ± 16.3abB	90.0 ± 10.0bcD	100.0 ± 0.0bC	100.0 ± 0.0bC	100.0 ± 0.0bD
	35	80.0 ± 13.3bB	90.0 ± 10.0bC	100.0 ± 0.0cC	100.0 ± 0.0bD	100.0 ± 0.0bC	100.0 ± 0.0bC
Diflubenzuron	10	0.0 ± 0.0A	0.0 ± 0.0aA	12.5 ± 8.5aA	18.75 ± 10.1aA	25.0 ± 11.2aB	31.25 ± 11.9aBC
	15	6.3 ± 6.3AB	6.3 ± 6.3abAB	18.8 ± 10.1aA	18.8 ± 10.1aA	25.0 ± 11.2aB	25.0 ± 11.2aBC
	25	12.5 ± 8.5A	12.5 ± 8.5abA	25.0 ± 11.2abBC	25.0 ± 11.2aB	31.25 ± 11.9aB	37.5 ± 12.5aBC
	35	6.25 ± 6.3A	25.0 ± 11.2bB	50.0 ± 12.9bB	62.5 ± 12.5bC	87.5 ± 8.5bC	93.75 ± 6.3bC
Azadirachtin	10	0.0 ± 0.0A	0.0 ± 0.0A	0.0 ± 0.0A	12.5 ± 8.5A	12.5 ± 8.5AB	18.75 ± 10.1AB
	15	0.0 ± 0.0A	6.3 ± 6.3AB	12.5 ± 8.5A	18.8 ± 10.1A	18.75 ± 10.1AB	18.75 ± 10.1AB
	25	0.0 ± 0.0A	0.0 ± 0.0A	6.25 ± 6.3AB	25.0 ± 11.2B	25.0 ± 11.2B	31.25 ± 11.9B
	35	0.0 ± 0.0A	0.0 ± 0.0A	12.5 ± 8.5A	31.25 ± 11.9B	37.5 ± 12.5B	37.5 ± 12.5B
Esfenvalarate	10	100.0 ± 0.0C	100.0 ± 0.0C	100.0 ± 0.0C	100.0 ± 0.0C	100.0 ± 0.0D	100.0 ± 0.0D
	15	100.0 ± 0.0C	100.0 ± 0.0C	100.0 ± 0.0C	100.0 ± 0.0B	100.0 ± 0.0C	100.0 ± 0.0D
	25	81.3 ± 10.1B	93.8 ± 6.3C	100.0 ± 0.0D	100.0 ± 0.0C	100.0 ± 0.0C	100.0 ± 0.0D
	35	93.8 ± 6.3B	100.0 ± 0.0C	100.0 ± 0.0C	100.0 ± 0.0D	100.0 ± 0.0C	100.0 ± 0.0C

Means within columns and for each insecticide followed by same lowercase letter, and means within columns for different insecticides at the same temperature are not different ($P > 0.1$, Fisher's exact test).

treatment with spinosad, diflubenzuron, and esfenvalerate was greater than that in the water control (Table 2). Mortality ranged from 100% with esfenvalerate to 19% with *B. bassiana* and azadirachtin. Efficacy of the latter two insecticides was not different from that obtained from the water control (Table 2).

At 15°C and 1 d after treatment, 100% mortality was observed in nymphs exposed to esfenvalerate, and exposure to spinosad resulted in 30% mortality (Table 2). Mortality from both insecticides was greater than that obtained from the water control. Exposure to both *B. bassiana* and diflubenzuron resulted in mortality of 6%, which was not different from the levels obtained from the water control. Cumulative mortality increased with time for each insecticide except esfenvalerate (Table 3). At 10 d, each treatment resulted in mortality of nymphs ranging from 100% with esfenvalerate to 19% with azadirachtin. Mortality from azadirachtin was not different from the water control. Similar to results obtained at 10°C, esfenvalerate treatment resulted in the greatest mortality followed by treatment with spinosad.

At 25°C and 1 d after treatment, exposure to esfenvalerate resulted in 81% mortality of nymphs and exposure to spinosad resulted in 60% mortality (Table 2). Mortality from both insecticides was greater than levels with the water only treatments. Exposure to diflubenzuron resulted in 13% mortality, but this was not different from the water control. Cumulative mortality increased with time for each insecticide (Table 3). At 10 d, mortality ranged from 100% with esfenvalerate and spinosad to 31% with azadirachtin, which were greater than levels obtained from the water control. Exposure to *B. bassiana* resulted in 63% mortality.

At 35°C and 1 d after treatment, exposure to esfenvalerate resulted in nearly 94% mortality of nymphs and spinosad resulted in 80% mortality (Table 2). Mortality from both insecticides was greater than the

Table 3. Correlation (Pearson correlation coefficient and significance probability of correlation) between mortality of *M. differentialis* nymphs and number of days after exposure to insecticide treatments in laboratory temperature cabinets

Insecticide	Temperature (°C)							
	10		15		25		35	
	Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value
Azadirachtin	0.94	0.005	0.91	0.01	0.96	0.002	0.96	0.002
B. bassiana	0.91	0.01	0.93	0.007	0.96	0.002	0.68	0.14
Diflubenzuron	0.99	0.0001	0.94	0.005	0.97	0.001	0.98	0.0004
Esfenvalarate	NC	NC	NC	NC	0.74	0.09	0.56	0.22
Spinosad	0.90	0.01	0.92	0.01	0.89	0.02	0.79	0.06

NC, no correlation.

Table 4. Maximum likelihood analysis of variance (CATMOD procedure) for days after exposure to insecticides at differing times (hours) after field spray application

Source	No. hours after spray at which insects were exposed								
	0			1			24		
	df	χ^2	Pr>χ^2	df	χ^2	Pr>χ^2	df	χ^2	Pr>χ^2
Day	5	4.50	0.4793	5	5.31	0.3798	5	12.01	0.0346
Treatment	7	37.54	<0.0001	7	23.92	0.0012	7	61.86	<0.0001
Day*treatment	35	7.51	1.0000	35	11.51	0.9999	35	12.01	0.9999
Likelihood ratio	33	139.68	<0.0001	37	93.23	<0.0001	26	221.59	<0.0001

levels obtained from the water control. Exposure to diflubenzuron resulted in 6% mortality, which was not different from the water control. Cumulative mortality increased with time for each treatment except *B. bassiana* and esfenvalerate (Table 3). At 10 d, the treatments with esfenvalerate and spinosad resulted in 100% mortality and provided greater mortality than that obtained from the water control. Treatment with diflubenzuron resulted in 94% mortality, whereas azadirachtin resulted in 38% mortality of nymphs and these were greater than the levels obtained from the water control.

There was no difference in mortality levels among the temperatures for *B. bassiana* at 1 d after treatment, but at 10 d after treatment, mortality levels at 15 and 25°C were greater than mortality levels at 10 and 35°C (Table 2). At 35°C, mortality resulting from spinosad treatment at 1 d after treatment was greater than mortality resulting from spinosad treatment at 10 and 15°C, and mortality levels at both of these temperatures were not different from mortality levels at 25°C (Table 2). At 10 d after treatment, the percentage of mortality resulting from treatment with spinosad at 25 and 35°C was greater than mortality levels at 10°C, but it was not different from that at 15°C. There were no differences among the mortality levels at the four temperatures for diflubenzuron treatments at 1 d after treatment but at 10 d after treatment, mortality at 35°C was greater than mortality levels at the other three temperatures. There was no difference among the mortality levels resulting from azadirachtin and esfenvalerate treatments at the four temperatures both at 1 and 10 d after treatment (Table 2).

Field Efficacy. Results from maximum likelihood ANOVA (CATMOD procedure) indicated that there was an insecticide effect for the cages placed in plots before treatment, 1 h after treatment and 24 h after treatment (Table 4). Results of the PROC FREQ analysis for the mortality data indicated that there were treatment effects for several insecticides for the different time intervals.

Daily maximum temperatures during the field experiments ranged from 26 to 37°C (mean 34.73 ± 0.42°C) in 2001 and from 27 to 32°C (30.78°C ± 0.28) in 2002 in Lane, OK (Oklahoma Climatological Survey 2001, 2002). Daily average temperatures for July 2001 and June 2002 were 28.62 ± 0.32 and 24.91 ± 0.29°C, respectively. The maximum temperatures in the field were similar to the 25 and 35°C treatments in the temperature chambers. For cages placed on plants before treatment, there was no mortality of nymphs in plots treated with the water control (Table 5). Treatment of plants with naled resulted in 100% mortality of nymphs at 1 d after treatment. Endosulfan treatment resulted in 80% mortality, whereas treatment with esfenvalerate and spinosad resulted in 70% mortality. Cumulative mortality increased over time in the diflubenzuron-, azadirachtin-, and *B. bassiana*-treated plots (Table 6). At 10 d, mortality ranged from 100% in plots treated with naled to 20% in plots treated with *B. bassiana*, but the latter was not different from the water control (Table 5). Results indicate that spinosad, endosulfan, naled, and esfenvalerate applications resulted in greater mortality than the water control at 1 d and that azadirachtin and diflubenzuron eventually resulted in mortality levels in excess of those in the water control at 10 d.

For cages placed on plants 1 h after treatment, there was no mortality of nymphs on plants treated with the water control (Table 7). At 1 d after treatment, 40% of the nymphs died on plants treated with spinosad and 30% died on plants treated with naled. During the

Table 5. Mean (± SEM) percentage of mortality of *M. differentialis* nymphs over time for insecticide treatments when insects were placed on plants before application in the field

Insecticide	Days after treatment					
	1	2	4	6	8	10
Water control	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
Spinosad	70.0 ± 15.3bc	90.0 ± 10.0b	90.0 ± 10.0c	90.0 ± 10.0ef	90.0 ± 10.0ef	90.0 ± 10.0ef
Diflubenzuron	0.0 ± 0.0a	10.0 ± 10.0a	30.0 ± 15.3b	30.0 ± 15.3bc	30.0 ± 15.3bc	60.0 ± 16.3cde
Azadirachtin	0.0 ± 0.0a	10.0 ± 10.0a	20.0 ± 13.3ab	40.0 ± 16.3c	40.0 ± 16.3c	40.0 ± 16.3bc
Endosulfan	80.0 ± 13.3cd	80.0 ± 13.3b	80.0 ± 13.3c	80.0 ± 13.3def	80.0 ± 13.3def	80.0 ± 13.3def
Naled	100.0 ± 0.0d	100.0 ± 0.0b	100.0 ± 0.0c	100.0 ± 0.0f	100.0 ± 0.0f	100.0 ± 0.0f
Esfenvalarate	70.0 ± 15.3bc	80.0 ± 13.3b	80.0 ± 13.3c	80.0 ± 13.3def	80.0 ± 13.3def	80.0 ± 13.3def
B. bassiana	0.0 ± 0.0a	10.0 ± 10.0a	10.0 ± 10.0a	10.0 ± 10.0ac	10.0 ± 10.0ac	20.0 ± 13.3ab

Means within columns followed by the same letter are not different ($P > 0.1$, Fisher's exact test).

Table 6. Correlation (Pearson correlation coefficient and significance probability of correlation) between mortality of *M. differentialis* nymphs and number of days after exposure to insecticide treatments at differing times (hours) after field spray application

Insecticide	No. hours after spray at which insects were exposed					
	0		1		24	
	Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value
Azadirachtin	0.93	0.008	0.98	0.0008	0.96	0.002
B. bassiana	0.82	0.05	0.93	0.008	NC	NC
Diflubenzuron	0.93	0.008	0.96	0.002	0.99	0.0001
Esfenvalarate	0.59	0.22	0.73	0.10	NC	NC
Endosulfan	NC	NC	0.56	0.22	0.59	0.22
Naled	NC	NC	0.59	0.22	NC	NC
Spinosad	0.59	0.22	0.79	0.06	0.92	0.009

NC, no correlation.

same time period, 20% of the nymphs died after exposure to treatments with endosulfan and esfenvalerate, but these levels were not different from those obtained from the water control. Cumulative mortality increased with time for esfenvalerate, spinosad, *B. bassiana*, diflubenzuron, and azadirachtin (Table 6). At 10 d, mortality ranged from 80% on plants treated with spinosad to 20% on collards treated with *B. bassiana*. Mortality of grasshoppers on plants treated with *B. bassiana* was not different from that obtained from the water control. Results at 10 d after treatment indicated that residues (1 h old) of spinosad, esfenvalerate, diflubenzuron, naled, endosulfan, and azadirachtin resulted in greater levels of mortality than that from the water control.

When cages with nymphs were placed on plants 24 h after treatment, there was no difference in mortality levels for any of the insecticide applications at 1 d after treatment (Table 8). Cumulative mortality increased with time for spinosad, azadirachtin, and diflubenzuron (Table 6). At 10 d, 60% of the grasshoppers died on plants treated with diflubenzuron, 47% died on plants treated with spinosad, and 27% died on plants treated with azadirachtin. The mortality levels obtained for these insecticides were different from the water control. Results from exposure of grasshoppers to residues (24 h old) indicated that treatment of plants with spinosad, diflubenzuron, and azadiractin resulted in differing levels of mortality compared with grasshoppers exposed to plants receiving the water control.

The two insecticides included in the field trials, but not in the laboratory trials, naled (organophosphate insecticide) and endosulfan (cyclodiene insecticide), provided differing levels of mortality when nymphs were exposed to direct applications or to residues that were 1 h old. However, no different levels of mortality resulting from treatment with these two insecticides were noted when grasshoppers were exposed to residues 24 h after application.

Discussion

Results of laboratory studies conducted under controlled temperature conditions indicated that temperature affects efficacy of some commonly used and alternative insecticides when applied for control of grasshopper nymphs. At labeled use rates, the pyrethroid insecticide esfenvalerate resulted in 100% mortality of nymphs at temperatures ranging from 10 to 35°C, and at each temperature it resulted in differences in mortality within 24 h of exposure. Spinosad also provided differing levels of mortality at each temperature, but at 10°C, mortality occurred more slowly over time, indicating that activity is negatively affected by reduced temperatures (Johnson 1990).

The IGR insecticide diflubenzuron did not result in different levels of mortality until 8 d at low temperatures of 10 and 15°C. Diflubenzuron caused mortality rapidly at higher temperatures and was more efficient at the highest temperature (35°C). This was probably due to the fact that this IGR is only effective when the

Table 7. Mean (± SEM) percentage of mortality of *M. differentialis* nymphs over time for insecticides when the insects were placed on plants 1 h after application in the field

Insecticide	Days after treatment					
	1	2	4	6	8	10
Water control	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
Spinosad	40.0 ± 16.3c	60.0 ± 16.3e	80.0 ± 13.3e	80.0 ± 13.3e	80.0 ± 13.3f	80.0 ± 13.3f
Diflubenzuron	0.0 ± 0.0a	10.0 ± 10.0abc	20.0 ± 13.3abcd	30.0 ± 15.3bcd	70.0 ± 15.3ef	70.0 ± 15.3ef
Azadirachtin	0.0 ± 0.0a	0.0 ± 0.0a	10.0 ± 10.0abc	20.0 ± 13.3abcd	30.0 ± 15.3bcd	30.0 ± 15.3bcd
Endosulfan	20.0 ± 13.3abc	30.0 ± 15.3bcde	30.0 ± 15.3bcd	30.0 ± 15.3bcd	30.0 ± 15.3bcd	30.0 ± 15.3bcd
Naled	30.0 ± 15.3bc	40.0 ± 16.3cde	40.0 ± 16.3cd	40.0 ± 16.3cd	40.0 ± 16.3cde	40.0 ± 16.3cde
Esfenvalarate	20.0 ± 13.3abc	50.0 ± 16.7de	50.0 ± 16.7de	50.0 ± 16.7de	50.0 ± 16.7def	60.0 ± 16.3def
B. bassiana	0.0 ± 0.0a	0.0 ± 0.0a	10.0 ± 10.0abc	10.0 ± 10.0abc	10.0 ± 10.0abc	20.0 ± 13.3abc

Means within columns followed by the same letter are not different ($P > 0.1$, Fisher's exact test).

Table 8. Mean (± SEM) percentage of mortality of *M. differentialis* nymphs over time for insecticides when the insects were placed on plants 24 h after application in the field

Insecticide	Days after treatment					
	1	2	4	6	8	10
Water control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0a	0.0 ± 0.0 a	0.0 ± 0.0a
Spinosad	0.0 ± 0.0	13.3 ± 9.1	26.7 ± 11.8b	46.7 ± 13.3c	46.7 ± 13.3cd	46.7 ± 13.3cd
Diflubenzuron	0.0 ± 0.0	6.7 ± 6.7	20.0 ± 10.7ab	33.3 ± 12.6bc	53.3 ± 13.3d	60.0 ± 13.1d
Azadirachtin	0.0 ± 0.0	6.7 ± 6.7	6.7 ± 6.7ab	20.0 ± 10.7abc	26.7 ± 11.8bcd	26.7 ± 11.8bc
Endosulfan	0.0 ± 0.0	6.7 ± 6.7	6.7 ± 6.7ab	6.7 ± 6.7a	6.7 ± 6.7ab	6.7 ± 6.7ab
Naled	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
Esfenvalarate	6.7 ± 6.7	6.7 ± 6.7	6.7 ± 6.7ab	6.7 ± 6.7a	6.7 ± 6.7ab	6.7 ± 6.7ab
B. bassiana	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a

Means within columns followed by the same letter are not different ($P > 0.1$, Fisher's exact test).

insect molts (Ware 2000) and the insect growth rate and thus molting rate increase at higher temperatures (Lactin and Johnson 1995, United Nations Environment Program 1996). The botanically derived IGR azadirachtin provided only moderate levels of mortality at temperatures of 25 and 35°C and only after 6 d of exposure. The biological insecticide *B. bassiana* resulted in different levels of mortality only at the moderate temperatures of 15 and 25°C and was not active at a low temperature of 10°C or at a high temperature of 35°C. This insecticide's activity is based on the ability of a living fungus to infect and propagate within the insect. The fungus has been previously shown to be inactive at low and high temperatures (Inglis et al. 1999).

Results of the field experiments that were conducted at high temperatures (July 2001 and June 2002, mean daily maximum temperature 34.73 ± 0.42 and 30.78 ± 0.28°C, respectively; Oklahoma Climatological Survey 2001, 2002) validated the controlled laboratory studies. Esfenvalerate and spinosad applications resulted in different levels of mortality when nymphs were exposed to direct contact to insecticides (cages placed on plant before application) and when nymphs were exposed to 1-h-old residues. Diflubenzuron applications resulted in similar levels of mortality; however, only after 8 d due to the slower activity of the IGR. The botanically derived IGR azadirachtin provided only moderate levels of mortality and only after 6 to 8 d of exposure. *B. bassiana* was not active at the high temperatures incurred during the experiment in the field trials as was predicted based on results from the laboratory trials.

Results from the laboratory and/or field trials indicate that esfenvalerate, spinosad, diflubenzuron, naled, and endosulfan can provide significant levels of mortality of nymphs. Under field conditions with high temperatures, which occur in late spring or early fall in the south central United States, only diflubenzuron and spinosad applications provided high levels (nearly 50 and 60%, respectively) of mortality when grasshoppers were exposed to residues that were 24 h old. Results from laboratory trials indicate that esfenvalerate and spinosad should provide significant levels of mortality under moderate temperature conditions (15 and 25°C) as occur during most of the production season for leafy greens in the south central United States during October through May. However, under low temperature conditions as may occur in December through March, only esfenvalerate and spinosad may provide levels of mortality (>60%) sufficient for successful control of grasshopper nymphs.

If registration of some older pesticides, such as naled and endosulfan, is canceled after EPA review, then current grasshopper management recommendations will need to be changed. Based on this research, esfenvalerate or spinosad may be an alternative for immediate control of grasshopper nymph infestations on leafy green crops, especially just before harvest when the immediate knockdown of infestations is required. However, when nymphs are present under moderate to high temperatures (i.e., early to late spring), applications of diflubenzuron to the crop and adjacent areas may aid in reducing grasshopper populations. Results indicate that diflubenzuron will provide significant mortality with time and thus population reductions, and this insecticide can be expected to remain active over a longer time than either esfenvalerate or spinosad. Results from the laboratory trials indicate that *B. bassiana* can provide levels of mortality of 50–60% at moderate temperatures (15 and 25°C). However, efficacy of *B. bassiana* under field conditions at these temperatures should be further investigated. Based on results from our tests, the biologically based insecticides formulated from the fungus *B. bassiana* should not be used at temperatures <15°C or >25°C.

Adult grasshoppers generally lay eggs in fall (September and October) in the south central United States, and the eggs hatch in mid-to-late-spring, April to May. Nymphs emerge, feed, and molt and mature into adults by midsummer. Thus, nymphs occur in fields of leafy greens primarily during the spring and early summer production period, from April through June. Based on the results of this study, we can predict that esfenvalerate and spinosad will provide effective control at low to high temperatures as occur during this time. Additionally, under moderate to high temperatures, diflubenzuron may be used to reduce populations around and in leafy green production fields and may be used up to 10 d before harvest and be expected to provide significant levels of population reduction.

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