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Authors: Silcox, D. E., Sorenson, C. E., and Brandenburg, R. L.

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QUANTIFYING EFFICACY AND AVOIDANCE BEHAVIOR BY TAWNY MOLE CRICKETS (ORTHOPTERA: GRYLLOTALPIDAE: *SCAPTERISCUS VICINUS*) TO THREE SYNTHETIC INSECTICIDES

D. E. SILCOX, C. E. SORENSON, AND R. L. BRANDENBURG

Department of Entomology, North Carolina State University, Campus Box 7613, Raleigh, NC 27695

ABSTRACT

Mole crickets (Orthoptera: Gryllotalpidae) are among the most economically important turfgrass insect pests in the southeastern United States. The tawny mole cricket *Scapteriscus vicinus* (Scudder) causes damage by feeding on the roots and shoots of turfgrass and by creating surface tunnels. Previous research on mole cricket control showed behavior modification, including reduced surface tunneling and avoidance of the treated soil, when a control agent was applied. The objectives of these studies were: a) to determine the mortality of 3 synthetic insecticides and their residues against small and large mole cricket nymphs in 2 bioassays and b) to monitor mole cricket behavioral responses to these insecticides. We used 3 synthetic insecticides (bifenthrin, chlorantraniliprole, and fipronil) to conduct 2 mortality bioassays and 2 behavioral studies, where we quantified surface tunneling, to determine the scope of this modified behavior. We found that, in general, the greater the efficacy of the product to mole crickets, the greater the likelihood of reduced surface tunneling and avoidance of the treated area. These studies confirm that mole crickets avoid an area treated with insecticide and emphasize the importance of appropriate timing of insecticides to achieve effective control of mole crickets.

Key Words: *Scapteriscus vicinus*, avoidance, insecticide control, management

RESUMEN

Los grillotopos (Orthoptera: Gryllotalpidae) se encuentran entre las plagas de insectos del césped de mayor importancia económica para el sureste de Estados Unidos. El grillotopo leonado, *Scapteriscus vicinus* (Scudder), causa daño al alimentarse de las raíces y los brotes de césped y por la creación de túneles en la superficie. En una investigación anterior sobre el control de grillotopos, se observó la modificación de conducta, incluyendo la reducción de túneles en la superficie y el evitar el suelo tratado, donde un agente de control fue aplicado. Utilizamos tres insecticidas sintéticos para realizar dos bioensayos y dos estudios de comportamiento para determinar el alcance de este comportamiento modificado. Hemos encontrado que, en general, cuanto mayor es la toxicidad aguda del producto a los grillotopos, mayor será la probabilidad de la repelencia y reducción de túneles en la superficie. Estos estudios confirman que los grillotopos evitan un área tratada con insecticida y hace hincapié en la importancia de la aplicación de insecticidas en un tiempo apropiado para lograr un control eficaz de los grillotopos.

The tawny mole cricket *Scapteriscus vicinus* (Scudder) is one of the most destructive soil-dwelling, turfgrass insect pests in the southeastern United States (Brandenburg 1997). Mole crickets cause damage by feeding on the roots and shoots of turfgrasses and through extensive soil tunneling (Brandenburg et al. 2000). The tunnel structure provides useful information about the behavior and ecology of this pest (Brandenburg et al. 2002). In North Carolina, the tawny mole cricket is univoltine, with the overwintering adults ovipositing in late Apr-May and egg hatch occurring in Jun and Jul. The newly-emerged nymphs begin feeding on the turfgrass; however damage is not readily visible early in the season, since the turfgrass is actively growing and the crickets are small. While the smaller crickets are

more susceptible to control agents, and their ability to tunnel is limited, control is often delayed because damage is not visually evident. As the nymphs grow and feed more extensively, damage becomes more apparent by mid-Aug. By the time damage is apparent, the mole crickets are more difficult to control than early instar nymphs. A full understanding of pest biology, including nymph behavior, could therefore enhance management (Brandenburg et al. 2000).

Mole crickets have been observed modifying their tunneling behavior in soil treated with either a conventional, biological insecticidal control agent, or naturally-occurring soil fungi (Brandenburg et al. 2000; Villani et al. 2002). The modified behaviors included reduced surface activity and avoidance of treated areas. Subsequent studies

using entomopathogenic fungal conidia and the synthetic insecticides bifenthrin (Thompson & Brandenburg 2005; Thompson et al. 2007) found similar changes in tunneling behavior. A study on fipronil degradation in soils found that mole crickets would escape containers treated with high rates of fipronil and remain in control containers (Cummings et al. 2006). Since changes in tunneling behavior could dramatically affect insect control and turf damage, additional research on mole cricket behavior was conducted using the synthetic insecticides bifenthrin, chlorantraniliprole, and fipronil in 2008 and 2009.

The objectives of these studies were: a) to determine the mortality of 3 synthetic insecticides and their residues against small and large mole cricket nymphs in 2 bioassays and b) to quantify mole cricket behavioral responses to these insecticides. These studies were conducted to quantify mole cricket avoidance behavior and surface tunneling in response to insecticide application and increase our knowledge of pest ecology.

MATERIALS AND METHODS

Insecticide bioassay and behavioral studies were conducted in North Carolina State University greenhouse facilities and at the Lake Wheeler Turfgrass Field Laboratory in Raleigh, NC, in 2008 and 2009. Mole crickets used in these studies were collected on golf course fairways and driving ranges using soapy water flushes (Short & Koehler 1979). All collected crickets were rinsed immediately in water and placed in a 68 liter storage box (Rubbermaid® Newell Rubbermaid Inc., Atlanta, Georgia) filled with native soil (Norfolk series, fine sandy loam, deep phase, HM = 0.66%, pH = 4.1, CEC = 3.2) from a mole cricket collection site (Olde Fort Golf Course, Brunswick Co., North Carolina) with no history of pesticide application. Native soil was used to keep the behavior of mole crickets in experiments as natural as possible. Mole crickets were collected no earlier than a week prior to use in any study. In 2008, mole crickets were collected from Emerald Golf Club in Craven Co., North Carolina, Scotch Meadows Country Club in Scotland Co., North Carolina, and Belvedere Country Club in Pender Co., North Carolina and in 2009 from Belvedere Country Club in Pender Co., North Carolina and Scotch Meadows Country Club in Scotland Co., North Carolina.

The following formulated insecticides were used in all experiments: granular bifenthrin (Talstar EZ®, FMC Corp., Philadelphia, Pennsylvania), sprayable chlorantraniliprole (Acelepryn®, DuPont Professional Products, Wilmington, Delaware), or granular fipronil (Chipco Choice, Bayer Environmental Science, Montvale, New Jersey). All insecticides were applied at 2 rates: bifenthrin at 0.448 kg ai/ha (standard) or 0.896 kg ai/

ha (high); chlorantraniliprole at 0.351 kg ai/ha (standard) or 0.702 kg ai/ha (high); and fipronil at 0.0157 kg ai/ha (standard) or 0.0314 kg ai/ha (high). Treatments were scheduled 0, 30, 60, or 90 d prior to placement of crickets in the treated containers to provided post-treatment intervals that allowed for evaluation of mortality and behavior not only to the parent compound, but to any residues or metabolites of the compound. All treatment applications were made to bare soil. All soil was from the same location and containers were maintained in a similar manner so that behavior would not be biased due to soil conditions.

The granular formulations of bifenthrin and fipronil were weighed and evenly distributed across the soil surface using a shaker jar with a gloved hand. Care was taken to ensure uniform distribution. An aqueous preparation of chlorantraniliprole was applied using a Delta Orbital 360 Sprayer (Delta Industries, King of Prussia, Pennsylvania) delivering approximately 6,830 liters/ha for the standard rate and 13,661 liters/ha for the high rate applying 5 ml of solution for bioassay containers and 10 ml of solution for choice containers (Delta Industries, King of Prussia, Pennsylvania).

Insecticide Mortality Bioassay I: Small Nymphs

Bioassay I used small (1.27-2.5 cm) tawny mole cricket nymphs to determine mortality of the previously mentioned insecticides. For Bioassay I, one liter plastic Ziploc® containers (12.1 × 12.1 × 9.3 cm, SC Johnson, Racine, Wisconsin) with 5 drainage holes (0.5 cm diameter) were filled to a depth of 5.08 cm with native soil (Norfolk, fine sandy loam, deep phase, HM = 0.66%, pH = 4.1, CEC = 3.2) from the Olde Fort mole cricket collection site. Insecticide applications were made in 2009 on 16 Jul, 20 Jul (0 d), 6 Jun (30 d), 19 May (60 d), and 20 Apr (90 d) to produce desired intervals in post treatment exposure when mole crickets were exposed to treated soil (Tables 1 and 2). There were 5 replicates per treatment. Uncovered containers were placed outdoors immediately after soil treatments to expose them to ambient temperatures, rainfall, and sunlight, allowing for typical pesticide degradation on bare soil. The monthly average temperatures for the duration of this experiment were as follows: Apr: 15.83 °C, May: 18.77 °C, Jun: 24.88 °C, and Jul: 25.75 °C. The monthly total rainfall for the duration of this experiment was as follows: Apr: 2.77 cm, May: 9.60 cm, Jun: 17.22 cm, and Jul: 8.36 cm. To maintain relatively stable ambient conditions (23.8-26.6 °C) during the evaluation period, containers were brought into the laboratory at the Lake Wheeler Turfgrass Field Laboratory one d before cricket placement. A single, small nymph, collected on either 9 Jul or 15 Jul, was placed in each container at the onset of the evaluation pe-

riod and a lid perforated with five 0.635 cm holes was placed on top of the container. A 15.24 cm × 15.24 cm piece of fiberglass screen (Phifer Inc., Tuscaloosa, Alabama) was secured around the bottom of the container to prevent mole cricket escape through the drainage holes. To evaluate mortality, the contents of each container were sifted daily by using a spoon to excavate the soil for 3 d after initial cricket placement (17 Jul 2009–19 Jul 2009 and 21 Jul 2009–23 Jul 2009). Evaluation 1 pertains to mortality recorded 1 d after exposure (DAE) and evaluation 2 pertains to mortality recorded 3 DAE.

Insecticide Mortality Bioassay II: Large Nymphs

Bioassay II used large (2.5–3.8 cm) tawny mole cricket nymphs to determine the mortality of the previously mentioned insecticides. The bioassay was conducted with the same treatments and materials as the first bioassay of small nymphs. Treatment applications were made in 2009 on 10 Aug and 22 Aug (0 d), 6 Jul (30 d), 6 Jun (60 d), and 6 May (90 d) to produce the desired intervals in post treatment exposure when mole crickets were exposed to treated soil. The average temperature in Aug was 26.96 °C and the total rainfall was 5.46 cm (data for the other months were mentioned previously). A single large nymph collected either on 5 Aug, 9 Aug, 16 Aug, or 20 Aug was placed in each container at the onset of the evaluation period. The treatment and placement of crickets in the 0 d treatment of the standard rate containers was delayed 5 d from the 30, 60, and 90 d treatments of the standard rate containers due to limited cricket numbers. The contents of each container were sifted daily to evaluate mortality for 3 weeks after initial cricket placement (18 Aug 2009–08 Sep 2009, 23 Aug 2009–13 Sep 2009, and 11 Aug 2009–01 Sep 2009). Evaluation 1 pertains to mortality recorded 1 DAE and evaluation 2 pertains to mortality recorded 5 DAE.

Behavior Study I: No-Choice Test

Mole cricket behavioral responses to selected insecticides were monitored in 68-liter storage boxes (60.7 × 40.4 × 41.9 cm, Rubbermaid Inc., Atlanta, Georgia) in 2008 and 15-liter storage boxes (42.2 × 29.8 × 17.8 cm, Sterilite Corp., Townsend, Massachusetts) in 2009. Drainage holes drilled into the bottom of each storage box reduced excess moisture. Fiberglass screen (Phifer Inc., Tuscaloosa, Alabama) was secured to the bottom of the storage box with glue to prevent mole cricket escape. In 2008, the 68 liter storage boxes were filled to a depth of 30.5 cm with native soil (Norfolk, fine sandy loam deep phase, HM = 0.66%, pH = 4.1, CEC = 3.2) from the Olde Fort Golf Course mole cricket collection site. In 2009, the 15-liter storage boxes were filled to a depth

of 12.7 cm with soil from the same location. In 2008, soil was treated on 23 Oct (0 d), 19 Sep (30 d), and 19 Aug (60 d), prior to cricket placement and in 2009, treatments were made on 11 Sep (0 d), 7 Aug (30 d), 8 Jul (60 d), and 9 Jun (90 d). The aqueous preparation of chlorantraniliprole was applied using a Delta Orbital 360 Sprayer (Delta Industries, King of Prussia, Pennsylvania) delivering approximately 708 liters/ha in 2008 and 795 liters/ha in 2009. The spray volume was different due to the change in container size. In 2008, there were no control treatments for the 30 and 60 d applications. Storage boxes placed outdoors were exposed to ambient temperatures, rainfall, and sunlight to allow for typical pesticide degradation on bare soil. In 2008, the monthly average temperatures for the duration of this experiment were as follows: Aug: 24.86 °C, Sep: 22.11 °C, and Oct: 14.41 °C. The monthly total rainfall for the duration of this experiment was as follows: Aug: 8.48 cm, Sep: 17.55 cm, and Oct: 3.45 cm. In 2009, the Sep average temperature was 20.11 °C and the total rainfall was 8.25 cm. To maintain relatively stable ambient conditions (23.8–26.6 °C) during the evaluation period storage boxes were brought into the greenhouse before cricket placement in 2008, and shade cloth (PC Pools, St. Paul, MN) was placed over the storage boxes during the evaluation period in 2009. In 2008, the experiments were conducted later in the fall (Oct–Nov) than in 2009, so the tubs were brought into the greenhouse to reduce mole cricket exposure to cold temperatures. In 2009, experiments were conducted earlier in the fall (Sep), so the tubs remained outside; a shade cloth, allowing 50% of sunlight transmission, was placed over top of the containers to reduce mole cricket exposure to high temperatures.

A single large nymph or adult tawny mole cricket (2.5–3.8 cm) collected on 21 Oct 2008 or on 10 Sep 2009 was placed in the center of each storage box at the onset of the evaluation period. A lid was placed over the top of the storage box to prevent cricket escape. In 2008, the center of each 68 liter storage box lid was removed, leaving a 2.54 cm border, and fiberglass screen (Phifer Inc., Tuscaloosa, Alabama) was secured to the border with glue. In 2009 ten drainage holes were drilled into each 15 liter storage box lid to allow water accumulated on the top to drain. A grid system similar to that used by Cobb & Mack (1989) was used to assess surface tunneling in the storage boxes over both years. In 2008, a 49.53 cm × 34.29 cm grid was made using metal bar as the frame and monofilament fishing line attached to the frame to create the 54 internal grid squares. In 2009, a 40.64 cm × 29.21 cm grid was made using a lid from one of the 15 liter storage boxes and Lehigh #18 gold mason line (The Lehigh Group, Macungie, PA) to create the 35 internal grid squares. The change to gold string in 2009 made the grid easier

to see when against the soil background. In both years the grid was placed over the top of the storage box, but not touching the soil and the number of squares that contained mole cricket surface tunneling was counted. The number of squares that contained evidence of tunneling was divided by the total number of grid squares to determine the percent tunneling for each storage box. Ratings were taken at 5, 11, and 15 d (referred to hereafter as evaluation 1, 2, and 3 respectively) after cricket placement in 2008 and 1, 3, and 7 d (referred to hereafter as evaluation 1, 2, and 3 respectively) after cricket placement in 2009.

Behavior Study II: Choice Test

Behavior study II used the same containers and same preparation methods as behavior study I. Each storage box was marked along the top edge along the long dimension to define the halfway point. A field stake was placed on top of the soil to demarcate each half and prevent overspray of untreated side. One half of the soil in each box was then treated with the appropriate insecticide, while the other side remained untreated. There was no physical barrier between the treated and untreated soil for the duration of the experiment. The hand-held sprayer produced coarse droplets, thus eliminating the concern of pesticide drift. Care was taken to ensure the soil surface was level before application so water infiltration would be downwards and not contaminate the untreated soil. The boxes were rotated so that the orientation of the treated and untreated sides in the boxes within a replicate was randomized. In 2008, soil was treated on 13 Nov (0 d), 3 Oct (30 d), and 30 Aug or 2 Sep (60 d) days prior to cricket placement and in 2009 treatments were made on 27 Sep (0 d), 17 Aug (30 d), 15 Jul (60 d), and 15 Jun (90 d). Insecticides were applied to the treated halves using the same techniques at the standard rates previously listed. Storage boxes were placed outdoors and exposed to ambient temperatures, rainfall, and sunlight, to allow for typical pesticide degradation on bare soil. To maintain relatively stable ambient conditions (23.8-26.6 °C) during the evaluation period, storage boxes were brought into the greenhouse before cricket placement (in 2008), or shade cloth (PC Pools, St. Paul, MN) was placed over the storage boxes during the evaluation period (in 2009), as previously described.

A single large nymph or adult tawny mole cricket (2.5-3.8 cm) collected in 2008 on 21 Oct, 6 Nov, or in 2009 on 24 Sep, or 6 Oct was placed on the soil at the border between treated and untreated sides of each container at the onset of the evaluation period. The 60 d chlorantraniliprole treated tubs in 2008 had crickets placed on soil and data collected 22 d prior to the rest of the tubs due to limited cricket numbers. A lid was

placed over the top of the storage box to prevent cricket escape as previously described. Tunneling ratings were taken using the same grids used in previous experiment, except that in this study, a separate tunnel rating was taken for the treated and untreated halves of each box. The grid was placed on top of the storage box, but not touching the soil, and the same mark used to divide the storage box in half during treatment was used to determine the middle of the grid. The number of squares that had surface tunneling in them was counted separately for each side. The number of squares that had tunneling in them was divided by the total number of grid squares for that side to determine the percent tunneling for each side of each storage box. Ratings were taken at 7, 13, and 20 d for the 60 d chlorantraniliprole tubs in 2008, 5, 11, and 28 d for the rest of the tubs in 2008 [referred to herein as evaluation 1 (d 7 and 5 respectively after cricket placement) evaluation 2 (d 13 and 11 respectively after cricket placement) and evaluation 3 (d 20 and 28 respectively after cricket placement)] and 1, 3, and 7 d (referred to hereafter as evaluation 1, 2, and 3 respectively) intervals after cricket placement in 2009.

Insecticide bioassay data (% mortality) were transformed (*binary*) prior to analysis for insecticide and residue effect using Proc LOGISTIC. Data were analyzed through use of Statistical Analysis System version 9.1 program (SAS Institute 2003). No-choice behavior data were analyzed for insecticide and residue effect using Proc GLIMMIX and adjusted using a Bonferroni correction for multiple comparisons. Data were analyzed through use of Statistical Analysis System version 9.2 program (SAS Institute 2008). Choice-test behavior data were transformed (*arcsine*) prior to analysis for insecticide and residue effect using Proc MIXED. Data were analyzed through use of Statistical Analysis System version 9.1 program (SAS Institute 2003). For all analyses of variance, $\alpha = 0.05$.

RESULTS

Insecticide Bioassay I: Small Nymphs

There were significant differences in mortality among treatments for the 0 d residues at the standard and high rates at both evaluations (Tables 1 and 2). There were significant differences in mortality among treatments for the 30 d residues at the standard rate on evaluation 2 and the high rate on evaluations 1 and 2 (Tables 1 and 2). There were significant differences in mortality among treatments for the 60 d residues at the standard and high rates on evaluation 2 (Tables 1 and 2). There were significant differences in mortality among treatments for the 90 d residues at the standard rate on both evaluations and at

TABLE 1. AVERAGE PERCENT MORTALITY OF SMALL NYMPHS ON EVALUATION 1 AND 2 TO TUBS TREATED WITH 3 INSECTICIDES AT THE STANDARD RATE^a FOR APPLICATIONS MADE 0, 30, 60, AND 90 D PRIOR TO CRICKET INTRODUCTION^b.

Treatment	% Mortality							
	Evaluation 1				Evaluation 2			
	0 d	30 d	60 d	90 d	0 d	30 d	60 d	90 d
Untreated	0	0	20	0	0	20	20	0
Bifenthrin	60	20	40	60	100	80	60	80
Chlorantraniliprole	0	0	0	20	0	0	0	20
Fipronil	80	20	0	0	100	40	0	0
χ^2	14.16	2.99	5.17	8.28	27.72	9.16	8.28	12.49
<i>P</i>	0.0027	0.3924	0.1595	0.0405	<0.0001	0.0272	0.0405	0.0059

^aRates: bifenthrin = 0.448 kg ai/ha; chlorantraniliprole = 0.351 kg ai/ha; fipronil = 0.0157 kg ai/ha.

^bData were transformed (binary) prior to analysis for treatment effect using Proc LOGISTIC, in all cases df = 3.

TABLE 2. AVERAGE PERCENT MORTALITY OF SMALL NYMPHS ON EVALUATION 1 AND 2 TO TUBS TREATED WITH 3 INSECTICIDES AT HIGH RATE^a FOR APPLICATIONS MADE 0, 30, 60, AND 90 D PRIOR TO CRICKET INTRODUCTION^b.

Treatment	% Mortality							
	Evaluation 1				Evaluation 2			
	0 d	30 d	60 d	90 d	0 d	30 d	60 d	90 d
Untreated	0	0	0	0	0	0	0	0
Bifenthrin	60	60	40	20	100	100	100	100
Chlorantraniliprole	0	0	0	0	0	0	0	0
Fipronil	80	0	0	0	100	20	0	0
χ^2	14.16	10.18	6.27	2.94	27.72	19.43	22.49	22.49
<i>P</i>	0.0027	0.0171	0.0991	0.4015	<0.0001	0.0002	<0.0001	<0.0001

^aRates: bifenthrin = 0.896 kg ai/ha; chlorantraniliprole = 0.702 kg ai/ha; fipronil = 0.0314 kg ai/ha

^bData were transformed (binary) prior to analysis for treatment effect using Proc LOGISTIC, in all cases df = 3.

the high rate on evaluation 2 (Tables 1 and 2). There were no significant differences in mortality among treatments for the 30 and 60 d residues, at standard rate on evaluation 1 (Table 1) and for the 60 and 90 d residues, at standard rate on evaluation 1 (Table 2).

Insecticide Bioassay II: Large Nymphs

There were significant differences in mortality among treatments for the 0 d residues at the standard and high rates at both evaluations (Tables 3 and 4). There were significant differences in mortality among treatments for the 30 d residues at the standard and high rates on evaluation 2 (Tables 3 and 4). There was no analysis run for the 30 d residues at the standard or high rate at evaluation 1 as there was no cricket mortality in any treatment (Tables 3 and 4). There were significant differences in mortality among treatments for the 60 d residues at the high rate on evaluation 2 (Table 4). There were no significant differences in mortality among treatments for the 90 d residues at either rate on both evaluation d (Table 3 and 4). There was no analysis run for the 90 d

residues at the standard or high rates on evaluation 1 as there was no cricket mortality in any treatment (Table 3 and 4). There were no significant differences in mortality among treatments for the 60 d residues at the standard rate on both evaluation d (Table 3) and for the 60 d residues at the high rate on evaluation 1 (Table 4).

Behavior Study I: No-Choice Test

There were significant differences in the amount of surface tunneling between treatments for the 0, 30, and 60 d residue at all evaluations for the 2008 storage boxes-entire surface treated behavior study (Table 5). Results from the no-choice behavior study in 2008 indicate that for all 0 d residue treatments there was a significant difference in the amount of surface tunneling between the untreated control and the 3 insecticide treatments on all evaluations (Table 6). For all residue treatments there was also a significant difference in the amount of surface tunneling between the bifenthrin and chlorantraniliprole treatments as well as marginally significant between the chlorantraniliprole and fipronil treatments on all

TABLE 3. AVERAGE PERCENT MORTALITY OF LARGE NYMPHS ON EVALUATION 1 AND 2 TO TUBS TREATED WITH 3 INSECTICIDES AT THE STANDARD RATE^a FOR APPLICATIONS MADE 0, 30, 60, AND 90 D PRIOR TO CRICKET INTRODUCTION^b.

Treatment	% Mortality							
	Evaluation 1				Evaluation 2			
	0 d	30 d	60 d	90 d	0 d	30 d	60 d	90 d
Untreated	0	0	0	0	0	0	0	20
Bifenthrin	60	0	20	0	100	100	40	40
Chlorantraniliprole	0	0	20	0	0	0	20	0
Fipronil	20	0	0	0	80	0	20	0
χ^2	8.28	NA ^c	2.99	NA ^c	22.52	22.49	3.28	5.17
<i>P</i>	0.0405	NA	0.3924	NA	<0.0001	<0.0001	0.3507	0.1595

^aRates: bifenthrin = 0.448 kg ai/ha; chlorantraniliprole = 0.351 kg ai/ha; fipronil = 0.0157 kg ai/ha.

^bData were transformed (binary) prior to analysis for treatment effect using Proc LOGISTIC, in all cases df = 3.

^cComparisons not made due to 100% mole cricket survival among treatments.

TABLE 4. AVERAGE PERCENT MORTALITY OF LARGE NYMPHS ON EVALUATION 1 AND 2 TO TUBS TREATED WITH 3 INSECTICIDES AT THE HIGH RATE^a FOR APPLICATIONS MADE 0, 30, 60, AND 90 D PRIOR TO CRICKET INTRODUCTION^b.

Treatment	% Mortality							
	Evaluation 1				Evaluation 2			
	0 d	30 d	60 d	90 d	0 d	30 d	60 d	90 d
Untreated	0	0	20	0	20	0	40	0
Bifenthrin	20	0	40	0	100	80	100	40
Chlorantraniliprole	0	0	0	0	20	0	20	0
Fipronil	80	0	0	0	100	0	0	0
χ^2	12.49	NA ^c	5.17	NA ^c	16.91	15.01	15.19	6.27
<i>P</i>	0.0059	NA	0.1595	NA	0.0007	0.0018	0.0017	0.0991

^aRates: bifenthrin = 0.896 kg ai/ha; chlorantraniliprole = 0.702 kg ai/ha; fipronil = 0.0314 kg ai/ha.

^bData were transformed (binary) prior to analysis for treatment effect using Proc LOGISTIC, in all cases df = 3.

^cComparisons not made due to 100% mole cricket survival among treatments.

TABLE 5. AVERAGE PERCENT SURFACE TUNNELING OF MOLE CRICKETS ON EVALUATION 1, 2, AND 3 OF NO-CHOICE TESTS WITH 3 INSECTICIDES AT STANDARD RATE FOR APPLICATIONS MADE 0, 30, AND 60 D PRIOR TO CRICKET INTRODUCTION IN 2008^a.

Treatment	Average Surface Tunneling								
	Evaluation 1			Evaluation 2			Evaluation 3		
	0 d	30 d	60 d	0 d	30 d	60 d	0 d	30 d	60 d
Untreated	0.51	NA ^b	NA	0.55	NA	NA	0.61	NA	NA
Bifenthrin	0.14	0.11	0.11	0.14	0.11	0.11	0.14	0.11	0.11
Chlorantraniliprole	0.33	0.27	0.33	0.35	0.37	0.37	0.38	0.40	0.47
Fipronil	0.16	0.14	0.17	0.20	0.18	0.20	0.22	0.18	0.22
<i>F</i>	19.07	7.23	9.98	20.21	14.12	12.03	24.70	16.93	21.60
df	3	2	2	3	2	2	3	2	2
<i>P</i>	<0.0001	0.0027	0.0005	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	<0.0001

^aData were analyzed for treatment effect using Proc GLIMMIX.

^bData not available due to lack of control treatments for 30 and 60 d applications.

TABLE 6. NO-CHOICE TESTS PAIR-WISE COMPARISONS OF THE AVERAGE PERCENT SURFACE TUNNELING OF MOLE CRICKETS ON EVALUATION 1, 2, AND 3 ON TUBS TREATED WITH 3 INSECTICIDES FOR APPLICATIONS MADE 0, 30, AND 60 D PRIOR TO CRICKET INTRODUCTION IN 2008 ^a

Treatment	P value ^b								
	Evaluation 1			Evaluation 2			Evaluation 3		
	0 d	30 d	60 d	0 d	30 d	60 d	0 d	30 d	60 d
Unt vs Bifenthrin	<0.0001	NA ^c	NA	<0.0001	NA	NA	<0.0001	NA	NA
Unt vs Chlorantraniliprole	0.0235	NA	NA	0.0135	NA	NA	0.0030	NA	NA
Unt vs Fipronil	<0.0001	NA	NA	<0.0001	NA	NA	<0.0001	NA	NA
Bifenthrin vs Chlorantraniliprole	0.0049	0.0057	0.0007	0.0020	<0.0001	0.0002	0.0006	<0.0001	<0.0001
Bifenthrin vs Fipronil	1.0000	1.0000	0.5536	0.9757	0.3031	0.1822	0.4390	0.3031	0.0747
Chlorantraniliprole vs Fipronil	0.0227	0.0283	0.0164	0.0555	0.0039	0.0154	0.0478	0.0011	0.0005

^aData were analyzed using Proc GLIMMIX; in all cases df = 30.

^bP values were adjusted with a Bonferroni correction.

^cData not available due to lack of control treatments for 30 and 60 d applications.

evaluations (Table 6). For all residue treatments there was no significant difference between the amount of surface tunneling between the bifenthrin and fipronil treatments on all evaluations (Table 6).

In the 2009 no-choice behavior study, there were significant differences in the amount of surface tunneling between treatments for the 0, 30, 60, and 90 d residues at all evaluations (Table 7). Results from the no-choice behavior study in 2009 were not consistent with the results seen in 2008 in the amount of surface tunneling between the untreated control and the 3 insecticide treatments (Table 8). Chlorantraniliprole had the greatest number (10) of significant differences in amount of surface tunneling compared to the amount of surface tunneling for the untreated controls (Table 8). There was also variability in the amount of surface tunneling between the 3 insecticides when compared to each other (Table 8). Bifenthrin and chlorantraniliprole treatments had the greatest number (10) of significant differences in the amount of surface tunneling between the two treatments. Chlorantraniliprole and fipronil treatments had the second greatest number of differences between the 2 treatments.

Behavior Study II: Choice Test

In the 2008 choice test behavior study there was no significant difference in the amount of surface tunneling between the treated and untreated sides of the insecticide treated tubs ($F = 1.71$, $df = 1$, $P = 0.2026$), between insecticide treatments ($F = 0.81$, $df = 2$, $P = 0.4544$), or between residue (0, 30, 60, 90 d) treatments ($F = 0.50$, $df = 2$, $P = 0.6133$ for evaluation 1 (Table 9). There was no significant difference in the amount of surface tunneling between the treated and untreated sides of the insecticide treated tubs ($F = 0.00$, $df = 1$, $P = 0.9854$), between insecticide treatments ($F = 1.19$, $df = 2$, $P = 0.3186$), or between residue treatments ($F = 1.59$, $df = 2$, $P = 0.2222$ for evaluation 2 (Table 10). There was no significant difference in the amount of surface tunneling between the treated and untreated sides of the insecticide treated tubs ($F = 0.00$, $df = 1$, $P = 0.9710$), between insecticide treatments ($F = 1.82$, $df = 2$, $P = 0.1810$), or between residue treatments ($F = 2.16$, $df = 2$, $P = 0.1353$) for evaluation 3 (Table 11). Overall the choice test behavior study in 2008 indicated bifenthrin and fipronil had the numerically lowest average surface tunneling grid ratings compared to the average tunneling on the untreated side (Tables 9-11). The chlorantraniliprole treated soil had the numerically highest average surface tunneling grid ratings compared to the average tunneling on the untreated side (Tables 9-11). The control storage boxes, in which neither half was treated, had numerically moderate surface tunneling compared to the average tunneling on

TABLE 7. AVERAGE PERCENT SURFACE TUNNELING OF MOLE CRICKETS ON EVALUATION 1, 2, AND 3 ON NO-CHOICE TESTS WITH 3 INSECTICIDES AT STANDARD RATE FOR APPLICATIONS MADE 0, 30, 60, AND 90 D PRIOR TO CRICKET INTRODUCTION IN 2009^a.

Treatment	Average Surface Tunneling											
	Evaluation 1				Evaluation 2				Evaluation 3			
	0 d	30 d	60 d	90 d	0 d	30 d	60 d	90 d	0 d	30 d	60 d	90 d
Untreated	0.33	0.47	0.38	0.24	0.41	0.69	0.50	0.42	0.49	0.87	0.62	0.55
Bifenthrin	0.51	0.23	0.37	0.23	0.54	0.26	0.45	0.41	0.54	0.29	0.49	0.52
Chlorantraniliprole	0.50	0.38	0.53	0.47	0.66	0.53	0.69	0.78	0.67	0.79	0.91	0.85
Fipronil	0.41	0.29	0.31	0.14	0.48	0.44	0.45	0.27	0.50	0.62	0.54	0.33
<i>F</i>	5.60	9.79	7.50	18.95	9.13	25.16	10.47	35.42	6.10	51.40	25.81	33.85
<i>P</i>	0.0015	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0009	<0.0001	<0.0001	<0.0001

^aData were analyzed for treatment effect using Proc GLIMMIX, in all cases df = 3.

TABLE 8. NO-CHOICE TESTS PAIR-WISE COMPARISONS OF THE AVERAGE SURFACE TUNNELING OF MOLE CRICKETS ON EVALUATION 1, 2, AND 3 ON TUBS TREATED WITH 3 INSECTICIDES FOR APPLICATIONS MADE 0, 30, 60, AND 90 D PRIOR TO CRICKET INTRODUCTION IN 2009^a.

Treatment	<i>P</i> value ^b											
	Evaluation 1				Evaluation 2				Evaluation 3			
	0 d	30 d	60 d	90 d	0 d	30 d	60 d	90 d	0 d	30 d	60 d	90 d
Unt vs Bifenthrin	0.0029	<0.0001	1.0000	1.0000	0.0612	<0.0001	1.0000	1.0000	1.0000	<0.0001	0.0456	1.0000
Unt vs Chlorantraniliprole	0.0100	0.3870	0.0200	<0.0001	<0.0001	0.0067	0.0010	<0.0001	0.0015	0.1970	<0.0001	<0.0001
Unt vs Fipronil	0.8023	0.0024	0.7689	0.0956	1.0000	<0.0001	1.0000	0.0071	1.0000	<0.0001	0.5837	0.0001
Bifenthrin vs Chlorantraniliprole	1.0000	0.0096	0.0081	<0.0001	0.0913	<0.0001	<0.0001	<0.0001	0.0402	<0.0001	<0.0001	<0.0001
Bifenthrin vs Fipronil	0.2071	0.9153	1.0000	0.1266	1.0000	0.0020	1.0000	0.0129	1.0000	<0.0001	1.0000	0.0016
Chlorantraniliprole vs Fipronil	0.4893	0.4000	0.0001	<0.0001	0.0022	0.4057	<0.0001	<0.0001	0.0029	0.0033	<0.0001	<0.0001

^aData were analyzed using Proc GLIMMIX, in all cases df = 80.

^b*P* values were adjusted with a Bonferroni correction.

the untreated sides of the half insecticide treated storage boxes (Tables 9-11).

In the 2009, choice test behavior study there was a significant difference in the amount of surface tunneling between the treated sides and untreated side of the insecticides treated tubs ($F = 20.91$, $df = 1$, $P < 0.0001$), but the treatment effect did not vary between insecticides ($F = 0.21$, $df = 2$, $P = 0.8091$) for evaluation 1 (Table 12).

There was a significant difference in the amount of surface tunneling between residue treatments ($F = 3.03$, $df = 3$, $P = 0.0360$) for evaluation 1 (Table 12). There was a significant difference in the amount of surface tunneling between the treated and untreated sides of the insecticide treated tubs ($F = 21.40$, $df = 1$, $P < 0.0001$), but the treatment effect did not vary between insecticides ($F = 0.43$, $df = 2$, $P = 0.6496$) for evaluation 1.

TABLE 9. AVERAGE SURFACE TUNNELING OF MOLE CRICKETS ON EVALUATION 1 OF CHOICE TESTS TREATED WITH 3 INSECTICIDES AT STANDARD RATE OF APPLICATIONS MADE, 0, 30, AND 60 DAYS PRIOR TO CRICKET INTRODUCTION IN 2008^a

Treatments	Average % Surface Tunneling					
	0 d		30 d		60 d	
	Treated Side	Untreated Side	Treated Side	Untreated Side	Treated Side	Untreated Side
Untreated	N/A	60.65	N/A	N/A	N/A	N/A
Bifenthrin	53.33	62.30	21.33	34.58	12.72	30.83
Chlorantraniliprole	71.67	73.75	30.83	41.67	51.67	50.83
Fipronil	16.46	10.83	50.21	43.54	21.04	25.42

^aData transformed (log) prior to analysis for treatment effect using Proc MIXED.

^bData not available due to lack of control treatments for 30 and 60 day applications and the treated sides.

TABLE 10. AVERAGE SURFACE TUNNELING OF MOLE CRICKETS ON EVALUATION 2 OF CHOICE TESTS TREATED WITH 3 INSECTICIDES AT STANDARD RATE OF APPLICATIONS MADE, 0, 30, AND 60 D PRIOR TO CRICKET INTRODUCTION IN 2008^a.

Treatments	Average % Surface Tunneling					
	0 d		30 d		60 d	
	Treated Side	Untreated Side	Treated Side	Untreated Side	Treated Side	Untreated Side
Untreated	N/A	60.65	N/A	N/A	N/A	N/A
Bifenthrin	53.33	62.30	21.33	34.58	17.29	30.83
Chlorantraniliprole	86.88	82.71	30.83	41.67	51.67	50.83
Fipronil	26.46	18.33	50.21	43.54	21.04	25.42

^aData transformed (log) prior to analysis for treatment effect using Proc MIXED.

^bData not available due to lack of control treatments for 30 and 60 d applications and the treated sides.

TABLE 11. AVERAGE SURFACE TUNNELING OF MOLE CRICKETS ON EVALUATION 3 OF CHOICE TESTS TREATED WITH 3 INSECTICIDES AT STANDARD RATE OF APPLICATIONS MADE, 0, 30, AND 60 D PRIOR TO CRICKET INTRODUCTION IN 2008^a.

Treatments	Average % Surface Tunneling					
	0 d		30 d		60 d	
	Treated Side	Untreated Side	Treated Side	Untreated Side	Treated Side	Untreated Side
Untreated	N/A	60.65	N/A	N/A	N/A	N/A
Bifenthrin	53.33	62.30	21.33	34.58	17.29	30.83
Chlorantraniliprole	86.88	82.71	30.83	41.67	51.67	50.83
Fipronil	26.46	18.33	50.21	43.54	21.04	25.42

^aData transformed (log) prior to analysis for treatment effect using Proc MIXED.

^bData not available due to lack of control treatments for 30 and 60 d applications and the treated sides.

TABLE 12. AVERAGE SURFACE TUNNELING OF MOLE CRICKETS ON EVALUATION 1 OF CHOICE TESTS TREATED WITH 3 INSECTICIDES AT STANDARD RATE OF APPLICATIONS MADE, 0, 30, 60, AND 90 DAYS PRIOR TO CRICKET INTRODUCTION IN 2009^a.

Treatments	Average % Surface Tunneling							
	0 d		30 d		60 d		90 D	
	Treated Side	Untreated Side	Treated Side	Untreated Side	Treated Side	Untreated Side	Treated Side	Untreated Side
Untreated	N/A	31.43	N/A	59.52	N/A	27.62	N/A	28.57
Bifenthrin	25.83	35.84	23.33	43.88	19.15	51.11	15.60	39.40
Chlorantranilprole	10.28	35.28	30.83	44.17	29.72	28.33	14.72	60.56
Fipronil	31.39	28.88	36.11	26.67	13.88	42.50	13.61	49.72

^aData transformed (log) prior to analysis for treatment effect using Proc MIXED.

^bData not available due to lack of control treatments for the treated sides.

TABLE 13. AVERAGE SURFACE TUNNELING OF MOLE CRICKETS ON EVALUATION 2 OF CHOICE TESTS TREATED WITH 3 INSECTICIDES AT STANDARD RATE OF APPLICATIONS MADE, 0, 30, 60, AND 90 D PRIOR TO CRICKET INTRODUCTION IN 2009^a.

Treatments	Average % Surface Tunneling							
	0 d		30 d		60 d		90 D	
	Treated Side	Untreated Side	Treated Side	Untreated Side	Treated Side	Untreated Side	Treated Side	Untreated Side
Untreated	N/A	48.00	N/A	74.28	N/A	42.38	N/A	38.09
Bifenthrin	47.78	53.61	50.83	55.55	37.14	54.17	46.11	73.88
Chlorantranilprole	17.34	56.00	36.38	55.83	45.00	33.33	31.11	71.38
Fipronil	45.83	42.50	58.33	53.33	16.11	52.22	30.56	54.72

^aData transformed (log) prior to analysis for treatment effect using Proc MIXED.

^bData not available due to lack of control treatments for the treated sides.

tion 2 (Table 13). There was no significant difference in the amount of surface tunneling between residue treatments ($F = 2.44$, $df = 3$, $P = 0.0733$) for evaluation 2 (Table 13). There was a significant difference in the amount of surface tunneling between the treated and untreated sides of the insecticide treated tubs ($F = 10.26$, $df = 1$, $P = 0.0022$), but the treatment effect did not vary between insecticides ($F = 0.22$, $df = 2$, $P = 0.8066$) for evaluation 3 (Table 14). There was a significant difference in the amount of surface tunneling between residue treatments ($F = 3.00$, $df = 3$, $P = 0.0375$) for evaluation 3 (Table 14).

Results from the choice test behavior study in 2009 indicate the treated sides had an overall numerically lower average surface tunneling grid ratings compared to the average tunneling on the untreated side (Tables 12-14). Bifenthrin-treated soil had the numerically lowest overall average surface tunneling grid ratings compared to the average tunneling on the untreated side (Tables 12-14). The fipronil-treated and chlorantranilprole-treated sides had variation between which halves (treated or untreated); the lower amount of surface tunneling was observed in the fipronil and chlorantranilprole treatments (Tables 12-14).

DISCUSSION

Across all 4 bioassays, bifenthrin and fipronil had larger effect on mortality compared to chlorantranilprole and the control. This is consistent with the relative mortality to mole crickets of these 3 insecticides observed in other studies (Potter 1998; Brandenburg et al. 2004). In general, bifenthrin treatments had higher mortality for the first evaluation than fipronil. This is expected; fipronil and its metabolites are slower acting than bifenthrin (Brandenburg et al. 2004). The low mortality seen in the chlorantranilprole treatments reflects the apparent low toxicity of the compound against mole crickets (RLB unpublished data).

The results seen in the 2008 no-choice studies are consistent with the mortality results of the bioassays. The bifenthrin and fipronil treatments had the highest efficacy against mole crickets and reduced surface tunneling by the mole crickets to a greater extent than chlorantranilprole. The chlorantranilprole had the lowest efficacy against mole crickets and had little impact on reducing surface tunneling. In 2009, the trends were similar, but there was greater variability among treatments that had lower surface tunnel-

TABLE 14. AVERAGE SURFACE TUNNELING OF MOLE CRICKETS ON EVALUATION 3 OF CHOICE TESTS TREATED WITH 3 INSECTICIDES AT STANDARD RATE OF APPLICATIONS MADE, 0, 30, 60, AND 90 D PRIOR TO CRICKET INTRODUCTION IN 2009^a.

Treatments	Average % Surface Tunneling							
	0 d		30 d		60 d		90 d	
	Treated Side	Untreated Side	Treated Side	Untreated Side	Treated Side	Untreated Side	Treated Side	Untreated Side
Untreated	N/A	75.72	N/A	91.43	N/A	65.24	N/A	45.72
Bifenthrin	78.75	70.80	66.60	71.70	32.20	64.40	54.72	76.11
Chlorantraniliprole	75.66	79.00	51.33	72.67	53.37	51.33	52.50	73.88
Fipronil	35.55	33.33	61.11	60.83	48.61	77.50	41.67	65.28

^aData transformed (log) prior to analysis for treatment effect using Proc MIXED.

^bData not available due to lack of control treatments for the treated side.

ing. This could be partly due to the change in the size of the tub. The smaller tubs in 2009 could have increased the concentration of the volatiles or other residues from the insecticides the crickets came in contact with. Bifenthrin is more volatile than fipronil, which is more volatile than chlorantraniliprole (Fecko 1999; Connelly 2001; Australian Pesticides and Veterinary Medicines Authority 2008). The differences in volatility may help explain the behavioral changes rather than mortality alone. The mole crickets could have been trying to escape the treatment, but in a smaller container this may have actually increased surface tunneling. In previous research, the behavioral response of mole crickets was influenced by the inherent mortality caused by the product; the more effective (toxic) the product, the greater the impact on mole cricket avoidance behavior (Thompson & Brandenburg 2005 and Thompson et al. 2007). The differences in mode of action of each insecticide could also explain the behavioral changes rather than mortality alone. Bifenthrin and fipronil primarily work through contact with the insect, while chlorantraniliprole primarily works through ingestion by the insect. Pyrethroids (bifenthrin in this study) are known to cause repellency in other arthropods (Desneux et al. 2007). Our results confirm the findings of previous studies (Villani et al. 2002; Thompson & Brandenburg 2005) using entomopathogenic fungi and bifenthrin.

The results of the choice studies were in general similar to the results of the no-choice containers. There was less statistical significance in the choice tests; however, in the majority of the assays, the average surface tunneling for the bifenthrin-treated sides was lower than the average surface tunneling for the untreated sides. The average amount of surface tunneling on the chlorantraniliprole and fipronil treated sides varied from lower to higher compared to the untreated sides among residue treatments. The relatively low average percent surface tunneling seen in the chlorantraniliprole treated sides versus untreated

sides could be related to the variable mortality seen in the bioassays. Chlorantraniliprole caused minimal mole cricket mortality, so they did not avoid the treatment, but some subsequently died from natural causes. The variability in the fipronil-treated sides could be related to the high mortality of fipronil for the 0 day residue in the bioassays, but the lower mortality of fipronil for the 30, 60, and 90 day residues in the bioassays. The mole crickets could have tried to escape the toxic treatment, but the small container resulted in tunneling in the treated side.

In both behavior studies, mortality was only recorded if the cricket was on the soil surface dead. Mortality below the soil surface during the course of the assay could not be measured with this study design. As a result, percent surface tunneling was recorded for every tub on all evaluations. Soil analysis (Silcox 2011) was conducted by private laboratories which confirmed that the insecticides were breaking down as expected on bare soil and insecticide residues were detected.

This study allowed us to associate mortality caused by an insecticide, with surface tunneling observed in behavioral experiments. If a treatment produced high mortality in the bioassay we would expect reduced surface tunneling in the containers with similar treated soils due to mole cricket avoidance and/or mortality. However, in the case of the no-choice tests they could not tunnel at the surface without exposure to the insecticide. It was difficult to separate out whether the reduced surface tunneling recorded was due to mortality or avoidance of that treated area. The reduction of surface tunneling was seen immediately in bifenthrin and fipronil treatments in the no-choice experiments; however the mortality recorded in the bioassays did not occur as quickly. The choice tests provided an opportunity for the crickets to tunnel at the surface while avoiding treated soil. The reduced surface tunneling seen on the treated sides of tubs versus the untreated sides, although not significant, provides insight

into avoidance to treated tubs as compared to untreated tubs.

In general, we found that the greater the mortality of the insecticide the greater the reduction of surface tunneling. These studies confirm previous management recommendations that timing of insecticides is very important in achieving effective control of mole crickets (Potter 1998; Brandenburg et al. 2004) and confirmed reported avoidance observed in previous studies (Villani et al. 2002; Thompson et al. 2007). Insecticides should be applied when the nymphs are small (less than 1.27 cm) to minimize the consequences of behavioral responses of large nymphs (greater than 2.54 cm). Large nymphs are able to tunnel as far as 70 cm in the ground (Brandenburg et al. 2002) and would be able to modify their behavior in such a way that the control measure could not be effective. This requires monitoring the infested areas for surface tunneling as well as soapy water flushing to determine egg hatch and nymph size. Proper timing, complete coverage and appropriate rates of the insecticides are the keys for maximizing effectiveness (Xia and Brandenburg 2000).

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