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IMPACT OF FIPRONIL RESIDUES ON MOLE CRICKET (ORTHOPTERA: GRYLLOTALPIDAE) BEHAVIOR AND MORTALITY IN BERMUDAGRASS

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ABSTRACT

In a greenhouse experiment, fipronil was applied at 0.014 kg ai/ha to bermudagrass, Cynodon dactylon L., in plastic 5-liter containers 120, 90, 60, 30, and 0 days before adding one tawny mole cricket nymph, Scapteriscus vicinus Scudder to the container. After the exposure period, soil in the containers was divided into depth increments of 0-4, 4-8, and 8-18 cm, and cricket status was recorded as dead, absent, or alive by thoroughly examining soil. Soil in the 0-4 cm-increment was analyzed for fipronil and four fipronil metabolite residues. Fipronil residue concentrations decreased with time (C = 0.00002 × 2 – 0.0053 × + 0.3675, R² = 0.9998 where C = fipronil concentration (µg/g of soil) and x = days after treatment). Concentrations of two metabolites, fipronil sulfone and fipronil sulfide, increased as fipronil residues decreased. Each treatment’s affect on late instar mole crickets was significantly different from the non-treated; however, there were no significant differences in nymph status among fipronil-treated containers. Fipronil and residues of its metabolites 120 days after application were 0.047 µg/g of soil and were high enough to kill or repel mole crickets to the same extent as the 0-day treatment, 0.368 µg/g of soil. Repellency of fipronil and its metabolites was significant as the majority of nymphs evacuated or died in the treated containers, but 35 of 37 nymphs were found alive in the non-treated containers.

Key Words: Scapteriscus vicinus, fipronil metabolite, fipronil sulfide, fipronil sulfone, repellency.

RESUMEN

En un experimento de invernadero, fipronil fue aplicado a razón de 0.014 kg ai/ha sobre pasto Bermuda, Cynodon dactylon L., en recipientes plasticos de 5-litros 120, 90, 60, 30, y 0 días antes de poner una ninfa del grillo topo, Scapteriscus vicinus Scudder en el recipiente. Después del período de exposición, el suelo en los recipientes fue dividido en incrementos de 0-4, 4-8, y 8-18 cm de profundidad, y el estado del grillo fue registrado como muerto, ausente, o vivo por la examinación completa del suelo. El incremento de suelo de 0-4 cm fue analizado por residuos de fipronil y cuatro metabolitos de fipronil. La concentración de fipronil en los residuos disminuyeron con el tiempo (C = 0.00002 × 2 – 0.0053 × + 0.3675, R² = 0.9998 donde C= la concentración de fipronil (µg/g de suelo) y x = días de tratamiento). Las concentraciones de dos metabolitos, sulfona de fipronil y sulfito de fipronil, aumentaron con la diminución de residuos de fipronil. El efecto de cada tratamiento sobre los mayores estadios de grillo topo fue significativamente diferente que en las pruebas no tratadas; sin embargo, no habían diferencias significativas en el estado de las ninfas entre los recipientes tratados con fipronil. Los residuos de fipronil y sus metabolitos 120 días después de la aplicación, 0.047 µg/g de suelo, fueron suficientemente altos para matar o repeler los grillos topos al mismo grado que en el tratamiento de 0-días, 0.368 µg/g de suelo. La repelencia de fipronil y sus metabolitos fue significativa, ya que la mayoría de las ninfas emigraron o morían en los recipientes tratados, pero 35 de las 37 ninfas se encontraron vivas en los recipientes no tratados.
Fipronil (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfonyl]-1H-pyrazole-3-carboxylic acid), fipronil sulfide (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfonyl]-1H-pyrazole-3-carbonitrile), a member of the phenyl pyrazole class of pesticides, is an insecticide that provides excellent mole cricket control. Fipronil and its metabolites effectively control home pests, termites, fire ants, mole crickets, water rice weevil, and field corn pest (USGS 2003). In turf, fipronil is applied as a granular product at very low use rates (g ai/ha). Fipronil has four major metabolites: desulfanyl fipronil (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-trifluoromethyl-1H-pyrazole-3-carbonitrile), fipronil amide (5-amino-1-[2,6-dichloro-4-[(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfonyl]-1H-Pyrazole-3-carboxylic acid), fipronil sulfone (5-amino-1-[2,6-dichloro-4-[(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfonyl]-1H-Pyrazole-3-carbonitrile) and fipronil sulfoxide (5-amino-1-[2,6-dichloro-4-[(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfonyl]-1H-Pyrazole-3-carbonitrile). Desulfanyl fipronil is formed through photodegradation in water and on soil. Fipronil amide is the product of alkaline hydrolysis in water and soil. Fipronil sulfone forms slowly during anaerobic metabolism. Fipronil sulfone is the product of oxidation in soil.

The objectives of this study were to determine the length of influence of fipronil and its metabolites on tawny mole cricket nymphs in bermudagrass and to measure degradation of fipronil and appearance of fipronil metabolites.

**MATERIALS AND METHODS**

**Bermudagrass Cultivation**

Dormant ‘Tifway’ bermudagrass from the Sandhills Research Station, Jackson Springs, NC was sectioned with a shovel into semi-cube shapes 15-cm wide and 8-cm deep on 22 Mar., 2003. The 150 bermudagrass cubes were placed in plastic 5-liter containers (19 cm in diameter x 18 cm deep (Classic 400, Nursery Supplies, Inc., Chambersburg, PA)). The bottom five cm of each container was fitted with fiberglass screen (1.4-mm (New York Wire, Mt. Wolf, PA)) to prevent nymph escape through the drain holes as nymphs have the ability to tunnel deep in the container.

The containers of soil and bermudagrass were placed in a greenhouse with a supplemental photoperiod of 14:10 (L:D). Sufficient top soil from the research station (Candor sand (sandy, siliceous, thermic, Arenic Paleudult)) was added to the bottom of each container to ensure that the screen was correctly positioned over the drain holes and to make certain that when the bermudagrass cube was added, the surface of the cube would be 2.0 cm below the rim of the container. The bermudagrass was maintained at 2.0 cm mowing height with battery-powered, reciprocating shears. Turf in the containers was cut twice weekly. Containers were turned ¼ turn after each mowing and rotated across the bench once a week. Automatic overhead irrigation provided 0.6 cm water/day (2x annual mean). Peters ® 20-20-20 fertilizer was applied at 24 kg N/ha on 4 Apr, 30 Apr, 23 May, 25 Jun, and 18 Aug 2003.

Fipronil (Chico Choice 0.1 granular (G), Bayer Environmental Science, Research Triangle Park, NC) was applied with a saltshaker to the surface of the bermudagrass containers at 0.014 kg ai/ha and aged120, 90, 60, 30, or 0 days before adding one late-instar tawny mole cricket to each container. All mole crickets were added on the same day in both experiments. The five treatments were arranged in a randomized complete block with 11 replicates, and the experiment was conducted twice. The number of replicates in the untreated checks varied but was greater than 11. Fipronil was applied in Experiment 1 on 7 May, 6 Jun, 8 Jul, 5 Aug, and 5 Sep 2003 to bermudagrass in unique containers. For Experiment 2, fipronil was applied 21 May, 25 Jun, 22 Jul, 19 Aug, and 19 Sep 2003. Immediately following each fipronil application, 0.3 cm of irrigation water was applied. Experiment 1 included 11 non-treated containers, and Experiment 2 included 26 non-treated containers (total of 147 containers with one mole cricket nymph in each). Only one cricket was added to each test container because tawny mole crickets are cannibalistic.

Tawny mole cricket nymphs were collected from Brunswick County in southeastern NC on 14 Aug 2003 from the Oyster Bay Golf Links (33° 52.91' N 78° 30.10' W) for Experiment 1 and on 2 Sep 2003 from Sea Trails Golf Links (33° 54.28' N 78° 30.60' W) for Experiment 2. Nymphs were brought to the soil surface by applying a 0.4% solution of lemon Joy® brand liquid dishwashing detergent in six liters of water/m² of turf (Short & Koehler 1979). The collected nymphs were rinsed...
in lake water to remove the soapy residue and placed in a 19-liter bucket half filled with moist soil from the surrounding area. The day after collection, each nymph was placed in its own 473-ml plastic container half filled with moist soil and half an earthworm (Lumbricus ssp.) as a food source. Nymphs were monitored for at least two weeks before being added to the fipronil-treated containers to ensure that the nymphs had recovered from the stresses of collection.

One nymph was placed in a 3-4 cm deep hole made by a rod 1 cm in diameter in the thatch layer of the bermudagrass in the center of each container on 5 and 19 Sep 2003 for Experiments 1 and 2, respectively. Nymphs were added to the containers three h after “watering in” the 0-day treatment. Nymphs were added to containers in the order of expected lowest fipronil concentration to highest (non-treated, 120, 90, 60, 30, or 0 days). For Experiment 1, on 15 Sep, 10 days after adding the nymphs, the containers were divided into three sections (0-4, 4-8, 8-18 cm). The thatch layer was approximately 0-4 cm thick. The status of the nymphs was recorded as live, dead, or absent. Nymphs found alive were placed in individual 473-ml plastic container with moist soil and monitored for four days to ensure the recorded status was accurate because fipronil has been observed to require several days to control mole crickets in the field (R. Brandenburg, Department of Entomology, North Carolina State University, personal communication 20 Jul 2004). The depth increment where the nymph was located was noted. Because more nymphs were recorded as absent in Experiment 1 than expected, containers in Experiment 2 were processed four d after adding nymphs on 23 Sep 2003. The decreased nymph residence time in treated containers facilitated locating dead nymphs. In addition, the number of non-treated containers was increased from 11 to 26.

Sample Preparation

All soil sections were bagged individually in polyethylene bags, weighed, and stored at -18°C. All sections from containers with the same fipronil treatment were simultaneously thawed, sub sampled to determine percent moisture by weight, and placed in 30 × 25 × 6-cm disposable aluminum pans for two days to air dry at room temperature. Then, the soil sections were sieved (1.4 mm) and sub sampled. In addition to preparing the soil for fipronil and its metabolites extraction, sieving was used to locate fragments of nymphs in Experiment 1 before recording them as absent.

Extraction Procedure

To determine if the concentrations of fipronil and fipronil metabolites were different in container
sample was injected into a mass spectrometer to verify the concentration and molecule.

Data Analysis

The status of the nymphs was analyzed by Pearson Chi Square analysis and by logistic regression fitting experiment and treatment effects with the GENMOD procedure of SAS version 8.0 (SAS Institute 2001). GENMOD requires a binary response; thus to accommodate this procedure, numbers of absent and dead nymphs (impacted by fipronil or metabolite) were combined to compare against the number of live nymphs. Likelihood ratio chi square-tests ($P \leq 0.05$) were used to determine significant differences. The most appropriate degradation model for fipronil and fipronil metabolites residual concentrations was determined with SAS.

RESULTS AND DISCUSSION

Only four nymphs of 147 were recorded as live in fipronil-treated containers at the end of both experiments even after 120 d of summer degradation in a greenhouse. The numbers of dead and absent nymphs in the 110 fipronil-treated containers were 51 and 55, respectively. The data suggest that fipronil and its metabolites modified the behavior of the nymphs and indicate repellency (Table 1). Of 37 nymphs in the non-treated containers, 35 (94.6%) were found alive; one was absent and one was dead. Therefore, the experimental apparatus was not inherently lethal or repellant to the nymphs. When fipronil concentrations were greatest (0-day), the number of absent nymphs was greatest. As fipronil concentrations decreased and fipronil metabolite (fipronil sulfone and fipronil sulfide) concentrations increased, nymph mortality also tended to increase and repellency tended to decrease (Fig. 1). Therefore, fipronil or its metabolites were impacting the mole crickets by either causing mortality or avoidance behavior.

After noting the higher than expected number of absent nymphs in Experiment 1, the nymph residence time in Experiment 2 was reduced from 10 to four d. In Experiment 1, the absence of a nymph in a container was attributed to quick death followed by rapid mole cricket decomposi-
tion. While field observations have suggested nymph avoidance of treated areas, fipronil and its metabolites have not been documented to cause nymph repellency. Villani et al. (2002) noted that nymphs avoided biocontrol control agents (pathogenic fungi) in the treated layer of soil at the surface by remaining deep in the soil. If nymphs were to detect the fipronil and its metabolites, they were not expected to pass through the treated layer at the surface and walk off the edge of the container, but rather that they would tunnel deeper into the soil (Villani et al. 2002). However, Villani et al. (2002) conducted experiments in containers with high side walls and lids where mole crickets could not leave the system; tunneling deeper was the only option for avoidance.

Fipronil targets the γ-aminobutyric acid type A (GABA) receptor system which disrupts nerve function in insects by blocking the GABA-gated chloride channels of neurons (California Environmental Protection Agency (CEPA) 2001). Thus at sufficient doses, fipronil causes excessive neuronal excitation. At the conclusion of the 4-d nymph exposure period of Experiment 2, 13 nymphs were found alive. Nine of these nymphs were supine with rapid movement of the legs. All nine of these nymphs died within four d and were recorded as dead. Therefore, based on observation of these nymphs and the mode of action of fipronil, it is likely that some of the nymphs reported as absent, received a high enough dose to be affected by fipronil and its metabolites. The affected nymphs may have walked over the edge of the container. Seven nymphs were found dead below the containers, but could not be assigned to a specific container. Perhaps some of the nymphs, which were absent but not found, were consumed by rodents known to inhabit the greenhouse.

When Experiments 1 and 2 are combined, the number of nymphs recorded as absent is similar to the number of dead (Table 1). During the statistical analysis, the numbers of dead and absent nymphs (impacted nymphs) were combined for comparison against the number found alive. All fipronil treatment timings were different from the non-treated (Chi-square = 124, $P = 0.001$). When the results of the non-treated containers were removed from the statistical analysis, there were no significant differences.

![Table 1: Tawny Mole Cricket Nymph Status After Exposure for 10 and Four D Combining Experiments 1 and 2 When Containers Were Treated with 0.014 kg AI/HA Fipronil Either 0, 30, 60, 90, or 120 D Before Adding One Nymph to Each Container (11 Replicates, 2 Experiments; Not-Treated Had 11 Replicates in Experiment 1 and 26 in Experiment 2).](https://biblionline.org/journals/Florida-Entomologist/89(3)/296)

| Fipronil Application Time | Live (%) (number) | Dead (%) (number) | Absent (%) (number) |
|---------------------------|-----------------|-----------------|-------------------|
| 0 Day                     | 9.1 (2)*        | 27.3 (6)        | 63.6 (14)         |
| 30 Day                    | 4.5 (1)*        | 54.5 (12)       | 40.9 (9)          |
| 60 Day                    | 0 (0)*          | 54.5 (10)       | 45.5 (12)         |
| 90 Day                    | 4.5 (1)*        | 45.5 (10)       | 50 (11)           |
| 120 Day                   | 0 (0)*          | 59.1 (13)       | 40.1 (9)          |
| Not Treated               | 94.6 (35)*      | 2.7 (1)         | 2.7 (1)           |

*Application Time 0, 30, 60, 90 and 120 d are not different with regard the number of live insects recovered ($P = 0.15$).

*Application times 0, 30 60, 90 and 120 d are all different from the Not Treated (Chi-Square $P = <0.001$).
significant differences among fipronil treatment timings. Therefore, the 120-d application had the same effect as the 0-d application in a greenhouse during the summer where two times the normal annual rainfall was applied (Chi-square $\geq 2.095$ with $P \geq 0.15$). Fipronil was expected to degrade more rapidly as its reported disappearance time in turf is 12-15 d vs. 33-75 d in bare soil (CEPA 2001). In this experiment, the half-life of fipronil was approximately 40 d.

The mean 0-d-treatment concentration for Experiments 1 and 2 for fipronil in the soil in the 0-4-increment was 0.368 µg/g of soil, and mean 120-d-treatment concentration was 0.047 µg/g of soil (Fig. 1). The degradation of fipronil is best described by the quadratic equation $C = 0.00002x^2 - 0.0053x + 0.3672$, $R^2 = 0.9998$ where $C = \text{fipronil concentration (µg/g of soil)}$ and $x = \text{days after treatment}$. As the concentration of fipronil decreased, the concentrations of fipronil sulfone and fipronil sulfide increased. Fipronil sulfone is the major fipronil metabolite detected in this study. Fipronil sulfone is formed through aerobic soil metabolism and was expected to be the major degradation product. The appearance of fipronil sulfone is best described by the quadratic equation $C = -0.00003x^2 + 0.0039x + 0.1228$, $R^2 = 0.8629$ where $C = \text{fipronil sulfone concentration (µg/g of soil)}$ and $x = \text{days after treatment}$. The concentration of fipronil sulfone was greatest in the 60-d-soil samples which indicates that this molecule is also degrading. Fipronil sulfide concentrations also increased as fipronil concentrations decreased with a maximum concentration reported in the 60-d-samples. Fipronil sulfide is formed through degradation in soil and water under anaerobic conditions. The heavy irrigation rate and screen in front of the drain holes may have allowed anaerobic conditions to form temporarily. There were no significant differences in fipronil concentrations in containers where nymphs were dead or absent at the end of the experiment. The same relationship is true for fipronil sulfone and fipronil sulfide. Metabolite residues were detected in the 0-d-samples because nymphs were exposed for 10 and four d after application for Experiments 1 and 2, respectively.

Desulfinylfipronil and fipronil amide were detected in all fipronil treated containers at minor concentrations (data not shown). The mean concentrations were similar across treatments and did not show a relation to fipronil residue concentrations. Desulfinylfipronil is formed during photolysis and was not expected to be present in high concentrations.
Concentrations due to shading of the soil surface by the bermudagrass canopy. Fipronil amide is formed during alkaline hydrolysis; the conditions necessary for fipronil amide to be produced were not expected (USGS 2003).

CONCLUSIONS

Fipronil at a mean concentration of 0.047 µg/g of soil in the 120-d-treatment impacted tawny mole cricket nymphs as much as fipronil at a mean concentration of 0.368 µg/g of soil in the 0-d-treatment (0-4 cm). Fipronil or fipronil metabolites impacted the nymphs similarly by either causing death or repellency. As fipronil concentration decreased, fipronil sulfone and fipronil sulfide concentrations increased. Fipronil and its metabolites are highly effective tawny mole cricket nymph management tools and have the potential to provide season-long control or prevent reinvasion in the same season (120 d).

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