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Source: Florida Entomologist, 89(3) : 293-298

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/0015-4040(2006)89[293:IOFROM]2.0.CO;2

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 ($C = 0.00002x^2 - 0.0053x + 0.3675$, $R^2 = 0.9998$ 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 ia/ha sobre pasto Bermuda, *Cynodon dactylon* L., en recipientes plasticos de 5-litros 120, 90, 60, 30, y 0 dias antes de poner una ninfa del grillo topo, *Scapteriscus vicinus* Scudder en el recipiente. Después del periodo 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 diminuyeron con el tiempo ($C = 0.00002x^2 - 0.0053x + 0.3675$, $R^2 = 0.9998$ donde C= la concentración de fipronil (µg/g de suelo) y x = dias de tratamiento). Las concentraciones de dos metabolitos, sulfona de fipronil y sulfito de fipronil, aumentaron con la diminuiación de residuos de fipronil. El efecto de cada tratamiento sobre los mayores estadios de grillo topos fue significativamente diferente que en las pruebas no tratadas; sin embargo, no habian diferencias significativas en el estado de las ninfas entre los recipientes tratados con fipronil. Los residuos de fipronil y sus metabolitos 120 dias 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-dias, 0.368 µg/g de suelo. La repelencia de fipronil y sus metabolitos fue significativa, ya que la mayoria de las ninfas emigraron o morian en los recipientes tratados, pero 35 de las 37 ninfas se encuentraron vivas en los recipientes no tratados.

Tawny mole crickets, *Scapteriscus vicinus* Scudder (Orthoptera: Gryllotalpidae), are one of the most injurious turfgrass pests of bermudagrass (*Cynodon dactylon* L.), zoysiagrass or Korean lawngrass *(Zoysia japonica* Steud.), centipedegrass (Eremochloa ophiuroides (Munro) Hack.), St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze), bahiagrass (*Paspalum no-*

tatum Fluegge), and pasture grasses on the Coastal Plain regions of the US. From North Carolina to Texas injury to the turf and cost of control are high (Frank & Parkman 1999). Mole crickets spend most of their lives tunneling through soil feeding on turfgrass roots and soil organisms. As a consequence, they uproot turf plants which dry out and die (Leslie 1994). Young nymphs are difficult to monitor because of their subterranean nature. As a result, mole crickets are among the most difficult turfgrass insect to control and account for hundreds of millions of dollars in damage and control each year (Potter 1998). Very severe turf damage may require reseeding or resodding; and reinfestation is a common problem (Frank & Parkman 1999). The turf may be further damaged by predators trying to dig up the mole crickets (Frank & Parkman 1999). Furthermore, thinned turf may be colonized by opportunistic weeds (Frank & Parkman 1999).

Fipronil (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfinyl]-1*H*-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 (active ingredient)/ha). Fipronil has four major metabolites: desulfinylfipronil (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-trifluoromethyl-1-*H*-pyrazole-3-carbonitrile), fipronil amide (5-amino-1-[2,6-dichloro-4-[(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfonyl]-1-*H*-Pyrazole-3-carboxylic acid), fipronil sulfide (5-amino-1-[2,6-dichloro-4-[(trifluoromethyl)phenyl]-4-[(trifluoromethyl)thio]-1-*H*-pyrazole-3-carbonitrile), and fipronil sulfone (5-amino-1-[2,6-dichloro-4-[(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfonyl]-1-*H*-pyrazole-3-carbonitrile). Desulfinylfipronil is formed through photodegradation in water and on soil. Fipronil amide is the product of alkaline hydrolysis in water and soil. Fipronil sulfide 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 (2× 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 (Chipco 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 applied 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° 32.10' 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/m^2 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 (*Lumbriculus* 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 containers where nymphs were found absent or dead, soil samples from the 0-4 cm section (thatch layer) from each of two replicates where the nymphs were reported as absent and where the nymphs were reported as dead at the end of the experiment were randomly selected for analysis from each treatment in both Experiments 1 and 2. Because only four nymphs were found alive in all fipronil-treated containers, only two samples (120-day and 0-day) were analyzed where nymphs were found alive.

Soil samples were extracted by sonication (EPA Method 525.2 modified as described below). Ten g of soil and 150 ml of acetone and *n*-hexane (1:1) were placed in a 250-ml beaker and sonicated (Branson® Sonifier 450, Branson Ultrasonics, Danbury, CT) for five min at 450 watts and 40 min duty cycle. The supernatant was filtered through anhydrous sodium sulfate and glass wool into a 500-ml boiling flask. Another 150 ml of extraction solvent was added to the beaker and sonicated for three min. The supernatant was filtered into the same boiling flask. The volume was reduced to 2.0 ml by rotary evaporation under vacuum at 35°C; then transferred quantitatively to a 10-ml test tube by using *n*-hexane for rinsing. In order to remove the acetone from the solution, the rinsate was reduced to 0.5 ml with a stream of dry nitrogen and diluted to 8.0 ml with *n*-hexane. The volume was reduced to 0.2 ml with dry nitrogen, and then diluted to 3.0 ml with *n*-hexane. A florisil solid phase extraction (SPE) Sep-Pak cartridge (Waters, Milford, MA) was prepared with 10 ml of hexane. The fipronil in 3.0 ml of hexane was then injected into the cartridge very slowly. The elutent was concentrated and analyzed to make certain all fipronil was retained in the cartridge. The fipronil and its metabolites were removed from the cartridge with 5.0 ml of acetone mixed with 5.0 ml of *n*-hexane. This elutent was reduced to 1.0 ml with dry nitrogen and transferred to a 2-ml GC auto sampler vial.

Samples were analyzed for fipronil, desulfinylfipronil, fipronil amide, fipronil sulfide, and fipronil sulfone with an HP 6890 GC coupled to an HP 5973 MSD and a ZB 50 (Phenomenex, Torrance, CA) column (30 m × 0.32 mm × 0.25µm film thickness) with the following temperature program: Injection port: 175°C, initial temp: 80°C, initial hold: 1.0 min, ramp rate: 20°C/min to 250°C, ramp rate 6°C/min to 287°C and hold 1.0 min, ramp 25°C/min to 300°C and hold 5.0 min. The electron capture detector (ECD) temperature was 300°C. Helium was used as the carrier gas at a mean flow of 1.0 ml/min. Nitrogen was used as the detector makeup gas. One µl of sample was injected. Calibration standards at concentrations of 100, 10, and 5.0 µg/ml were used for quantification. Separate soil samples treated with known concentrations of fipronil and its metabolites were run after every six samples. Every other

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 repellent 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 decomposition. 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 choride 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 live. 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

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).

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

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

[b]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 ≥ 2.095 with P ≥ 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 $C = 0.00002\times^2 - 0.0053\times + 0.3672$, $R^2 = 0.9998$ where C = fipronil concentration (µg/g of soil) and × = 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 $C = -0.00003\times^2 + 0.0039\times + 0.1228$, $R^2 = 0.8629$ where C = fipronil sulfone concentration (µg/g of soil) and × = 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

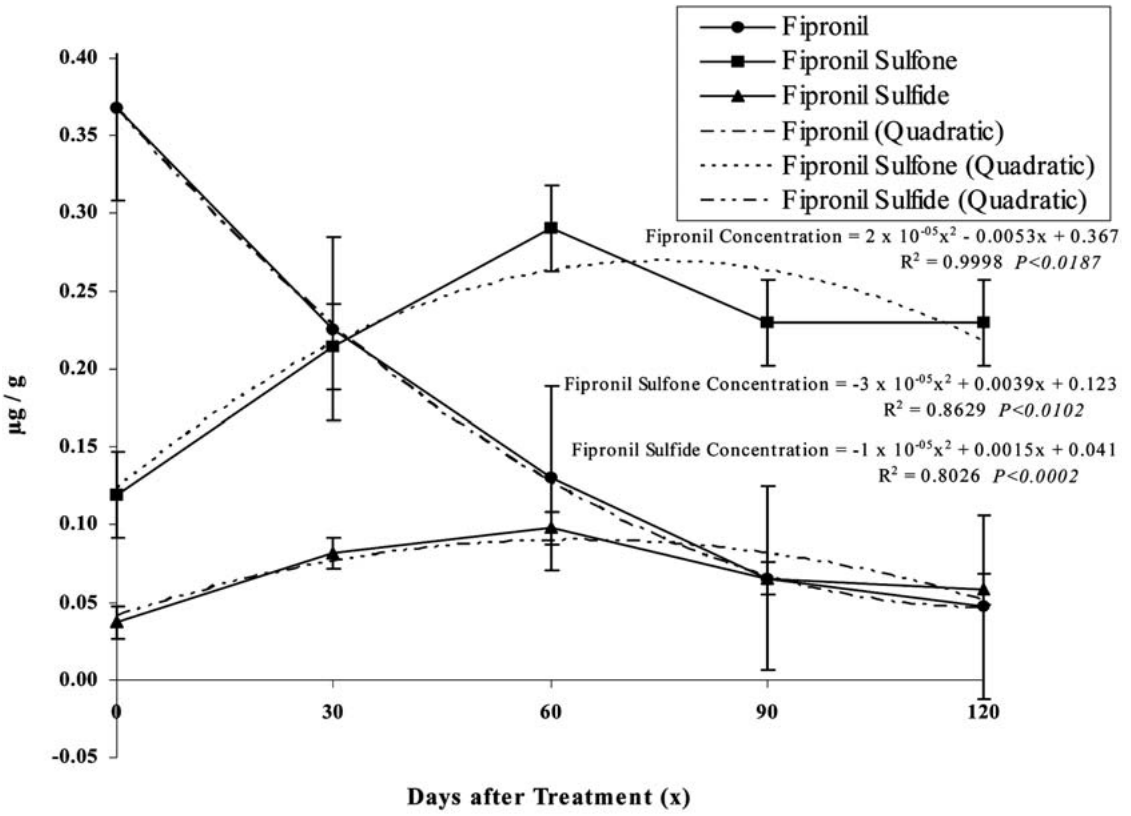

Fig. 1. Degradation of parent material and appearance of metabolites with time.

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).

ACKNOWLEDGMENTS

We acknowledge Bayer Environmental Science for providing products; S. R. Thompson, R. M. Heltsley, and Dr. G. E. Mahnken for technical assistance; North Carolina Turfgrass Council and Center for Turfgrass Environmental Research and Education for financial support; and Dr. Cavell Brownie for assistance with statistical analysis. This work was completed as part of a post graduate degree requirement.

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