Research Article

Received: 10 June 2011 Revised: 15 September 2011 Accepted article published: 16 September 2011 Published online in Wiley Online Library: 10 November 2011

(wileyonlinelibrary.com) DOI 10.1002/ps.2321

Comparative impact of an anthranilic diamide and other insecticidal chemistries on beneficial invertebrates and ecosystem services in turfgrass

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Abstract

BACKGROUND: Chlorantraniliprole, the first anthranilic diamide insecticide labeled for turf, combines strong selective activity against key pests with low vertebrate toxicity. The hypothesis that it is less disruptive to beneficial invertebrates and their ecosystem services than are other prevailing insecticide classes was tested. Plots in golf course settings were treated with chlorantraniliprole, or with a representative nicotinoid (clothianidin), pyethroid (bifenthrin) or a combination (clothianidin–bifenthrin) formulation. Non-target effects were assessed via pitfall traps (epigeal predators), Tullgren funnel extraction (soil microarthropods), hand sorting (earthworms), counting ant mounds and earthworm casts on tees and putting greens, assessing predation on sentinel pest eggs and comparing grass clipping decomposition in treated versus untreated turf.

RESULTS: Chlorantraniliprole had little or, in most cases, no impact on predatory or soil invertebrates, predation or decomposition. Each of the other insecticides temporarily reduced abundance and activity of one or more predator groups. Clothianidin and the clothianidin–bifenthrin combination retarded grass clipping decomposition, and the combination suppressed earthworms and casts more than did carbaryl, a toxic standard.

CONCLUSION: Chlorantraniliprole is compatible with conservation biocontrol and a good fit for industry initiatives to use relatively less toxic pesticides. One caveat is that its use on golf courses may require targeted management of ant mounds and earthworm casts that are suppressed as a side effect by some less selective insecticides.

Keywords: anthranilic diamide; chlorantraniliprole; clothianidin; ecotoxicology; golf course

1 INTRODUCTION

Chlorantraniliprole, the first anthranilic diamide insecticide labeled for turf, combines low use rates, 3–5 month residual activity in soil and very low toxicity to vertebrates.[1] Anthranilic diamides have a novel, specific mode of action, activating ryanodine receptors via stimulation of the release of calcium stores from the sarcoplasmic reticulum of muscle cells and causing impaired regulation and lethal paralysis in sensitive species.[1,2] Chlorantraniliprole has strong differential selectivity towards insect ryanodine receptors, which accounts for its low mammalian toxicity and favorable environmental profile and its receiving reduced risk status from the US Environmental Protection Agency for use on turf. It also has low intrinsic toxicity to honey bees, bumblebees and parasitoids,[3,4] and low potential of harmful systemic exposure via pollen and nectar.[5] Chlorantraniliprole is effective against the entire spectrum of turf-damaging scarab grubs, as well as billbug and other weevil larvae, grass-feeding caterpillars and invasive crane flies.[6]

Lawns, golf courses and sports fields are inhabited by a diverse community of beneficial invertebrates that contribute to pest suppression, decomposition of clippings and thatch, nutrient recycling and good soil tilth.[7,8] Although all classes of turf insecticides registered since 1993 have relatively low mammalian and avian toxicity,[6,9] some (e.g. pyrethroids, nicotinoids) are acutely toxic to pollinators,[10] and they may also impact sufficiently on natural enemies or earthworms to disrupt the positive ecosystem services of these organisms temporarily.[11–13] Conservation of non-target species, including beneficial invertebrates, is consistent, with industry-wide initiatives for environmental stewardship and more sustainable management of golf courses,[14–17] lawns and landscapes (National Wildlife Federation: http://www.nwf.org/Get-Outside/Outdoor-Activities/Garden-for-Wildlife.aspx). Target-selective insecticides offer potential for integrating chemical and conservation biological control.[6–9]

Post-patent marketing of mixtures of active ingredients is another industry trend. Typically, such products contain a pyrethroid, which binds to foliage and thatch and controls surface-active pests, and a nicotinoid, which is watered in to control

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root-feeding pests as well as certain stem feeders (e.g. billbug larvae) and foliage feeders by systemic action. Purported benefits of such combination products include broader, faster or longer-lasting control, savings in time, fuel and labor by reducing the number of applications and potential for target-site-based synergy.[18] The latter concept is controversial, although there is some laboratory evidence for greater than additive neurophysiological effects of imidacloprid + bifenthrin on mole crickets.[19] Such synergism, if it occurs, would logically also extend to exposure of non-target invertebrate species, although the authors are unaware of any previous field studies addressing that concept.

This study tested the hypothesis that chlorantraniliprole, a representative anthranilic diamide, is less disruptive to beneficial invertebrates and associated ecosystem services than are representatives of other widely used classes of turf insecticides. Stark *et al*.[20] emphasized that tests used to evaluate and predict pesticide hazards to beneficial species should be designed to simulate field conditions realistically, so the trials were done in golf course settings with rates and application methods similar to those used by turf managers. Predatory ants and earthworms, while beneficial in most turf settings,[21–23] can, however, become important pests when their mounds or casts disrupt the smoothness and uniformity of short-mowed playing surfaces.[24,25] Therefore, an ancillary hypothesis is that, because of its selectivity, the anthranilic diamide will also provide less suppression of these problems than occurs as a side effect of the use of certain other chemistries.

2 MATERIALS AND METHODS

The main trials comparing insecticide impacts on non-target invertebrates, predation on pest eggs and decomposition were conducted in untreated roughs at two central Kentucky golf courses. Although preventive treatments for scarab and weevil larvae are typically applied in spring, each of the insecticide classes may also be applied in late summer to control foliage-feeding pests, mound-building ants and scarab grubs, and sometimes for off-label suppression of earthworms and associated casts. Additional studies to evaluate impacts on populations of earthworms and other invertebrate decomposers, earthworm casting and ant mounding were conducted at other sites as noted below.

2.1 Impacts on predators and predation, August–September 2009

This trial was replicated across Kentucky bluegrass, *Poa pratensis* L., roughs at Idle Hour Golf Course, Lexington, Kentucky. Plots (6×6 m, with 2 m untreated borders) were arranged as a randomized complete block with six replicates and four treatments. Nine permanent pitfall traps were set in each plot, one in the center and the two others at 1 and 2 m from the center along diagonal transects toward each plot corner. Traps were plastic centrifuge tubes (50 mL, 3 cm diameter opening) inserted into cored-out holes. Each trap received 20 mL of ethylene glycol as a killing agent and preservative. Traps were operated for 1 week before treatment, and then the samples were collected and traps were removed before applying and watering in the insecticides.

The four treatments were chloranthraniliprole (Acelepryn, 18.4% active ingredient; Dupont, Wilmington, DE), clothianidin (Arena 50 WDG; Valent, Walnut Creek, CA), a combination product containing 24.7% clothianidin and 12.3% bifenthrin (Aloft; Arysta, Cary, NC), plus the untreated check. Chlorantraniliprole was applied at its high label rate for scarab grub control (0.23 kg AI ha^{-1}); the nicotinoid and nicotinoid–pyrethroid premix were applied at their single listed label rate for grubs, corresponding to 0.15 kg AI ha^{-1} clothianidin.

The applications were made with a portable CO_2 spray tank (R and D Sprayers, Opelousas, LA) equipped with a 1.8 m handheld boom with four Spraying System 8004 Tee Jet nozzles (Spraying Systems, Wheaton, IL) that delivered a pressure of 2109 g cm^{-2}. Spray volume was 468 L ha^{-1}, applied by making two passes in opposite directions over each plot. Separate spray bottles were used for each treatment. Treatments were applied on 20 August and were followed within 1 h by about 1.5 cm of irrigation from the golf course sprinkler system. Pitfall traps were replaced as soon as the residues had dried.

Traps were emptied weekly for 4 weeks after treatment. Within-plot samples were consolidated, and fresh preservative was added before replacing the traps. Weekly samples were sorted, and specimens belonging to predominantly predatory taxa, including Araneae (spiders), Formicidae (ants), Staphylinidae (rove beetles) and Carabidae (ground beetles), were counted.

Effects on predatory activity were evaluated by exposing groups of black cutworm, *Agrotis ipsilon* (Hufnagel), eggs in the plots. The eggs, from females in a lab colony, had been deposited on white muslin that was cut into pieces (2 cm^2), each with 20 one-day-old eggs. The cloth pieces with 20 eggs were taped 6 cm above the pointed end of wooden garden stakes (25.4 cm). Two such ‘egg sticks’ were placed in each plot at 1 and 3 weeks after treatment (i.e. 40 eggs per plot per date). The sticks were placed 1 m to either side of the central pitfall trap, equidistant between the traps on the diagonals, and pushed into the soil so that the cloth was flush with the base of the grass plants. Egg sticks were left in plots for 24 h and then pulled out and examined under a dissecting scope to determine the number of eggs that were missing or had otherwise been preyed upon. Eight additional sticks (160 total eggs) with sticky (Tanglefoot, Grand Rapids, MI) barriers above and below the cloth were placed in the border areas between plots during each trial as egg hatch controls.

2.2 Effects on earthworm and soil microarthropods, 2009

This trial was conducted in a stand of ‘Penncross’ creeping bentgrass, *Agrostis stolonifera* L., on a Maury silt loam (fine, mixed, mesic Typic Paleudalf; pH = 6.0) at the University of Kentucky’s AJ Powell, Jr, Turfgrass Research Center (UKTRC), Spindletop Farm, near Lexington, KY. The turf, managed as for a golf course fairway, was mowed 3 times per week at 1.6 cm and irrigated as necessary to prevent drought stress, with fertilizer (urea: 46-0-0) applied in September, October and November at 0.48 kg N 100 m^{-1} per application. Plots (1.5×1.5 m, with 0.5 m untreated borders) were arranged in a randomized complete block with six replicates. The five treatments were the same as those used in the previous trial (Section 2.1), plus the carbamate insecticide carbaryl (Sevin SL, 43% AI, 9.16 kg AI ha^{-1}; Bayer, Research Triangle Park, NC), which was included as an earthworm-toxic standard.[23] The insecticides were applied on 1 October and watered in as described above.

Earthworms were sampled on 8 and 29 October, 1 and 3 weeks after treatment, by taking four soil cores (15.2 cm diameter, 15 cm deep) from each plot with a golf course cup cutter. The cores were broken apart by hand and examined in the field, and earthworms (mostly *Apporectodea* spp.) were collected into plastic bags with wet paper towels, brought to the lab, counted and weighed. The few nightcrawlers (*Lumbricus terrestris* L.) that were collected were excluded from analyses owing to their substantially greater individual mass.

The impacts of the insecticides on soil microarthropods were assessed by taking two soil cores (5.1 cm diameter, 7.6 cm deep) from each treated plot concurrently with the earthworm sampling. The cores were consolidated within the plot, quartered using a hacksaw, lightly crumbled by hand and placed in Tullgren funnels (Burkhardt Agronomic Instruments, Uxbridge, Middlesex, UK) equipped with 25 W bulbs for 48 h. Samples were stored in 70% ethanol until sorted. The predominant taxa, oribatid and mesostigmatid mites and Collembola, were counted with a dissecting microscope.

2.3 Impacts on predators, predation, decomposers and decomposition, 2010

The trials described in Sections 2.1 and 2.2 were repeated in 2010, with the following modifications. The study site was an untreated Kentucky bluegrass rough at the Lexington Country Club, Lexington, KY. Plots (10 × 10 m, with 2 m untreated borders) were arranged in a randomized complete block with six replicates and five treatments. The plots were treated at label rate with the same chemicals as in 2009, with the addition of bifenthrin (Talstar Select; 7.9% bifenthrin; FMC, Philadelphia, PA) at the label rate for surface-feeding pests (0.064 kg AI ha^{-1}) so that both active ingredients in the combination product were also individually represented. The insecticides were applied as above on 14 May, followed by irrigation (1.5 cm) ≤1 h after treatment. In 2010, larger pitfall traps (473 mL plastic cups; Solo, Lake Forest, IL) were used for better capture of ground beetles and other relatively large predators. Five traps were arranged in each plot, one central and the others at 1.5 m from the center diagonally towards each corner.

Traps were operated continuously for 3 days immediately before treatment, removed when the insecticides were applied and then replaced for additional 3 day trapping periods at 1, 3, 6 and 12 weeks after treatment. Within-plot samples were consolidated and sorted as before. Specimens of Formicidae, Staphylinidae, Carabidae and spiders, the four most abundant predominantly predatory taxa, were counted.

Impacts on predation were assessed by exposing cohorts of black cutworm and Japanese beetle, *Popillia japonica* Newman, eggs in treated and untreated plots. Egg sticks, each with 15 cutworm eggs, were prepared as described earlier. Two such sticks were placed in each plot, along the diagonals as in 2009, at 1, 3, 6 and 9 weeks after treatment (i.e. 30 eggs exposed per plot per date), left in the field for 48 h and then pulled out and examined. Hatching controls were included as above. Field-caught Japanese beetles were held in the laboratory in bins of moist soil with linden (*Tilia* sp.) leaves as food. Eggs (<2 days old) were collected from the soil. Ten eggs were placed in each of 90 petri dish bottoms (5 cm diameter) with a small amount of moist soil. Three dishes were implanted at 1 m distances around the central pitfall trap in each plot. This was done by removing three turf cores with a golf course cup cutter, cutting a slit about 2 cm below the thatch–soil interface into the side of each hole and sliding the open dish into the slit, after which the core was replaced. The dishes with eggs were implanted on 19 July, and remaining eggs were counted after 1 week.

Impacts of the insecticides on soil microarthropods were assessed by taking two soil cores (5.1 cm diameter, 7.6 cm deep) from each treated plot 3 weeks after application. Cores were consolidated by plot and quartered using a hacksaw. Specimens were extracted using Tullgren funnels as described earlier and stored in 70% ethanol, and the predominant taxa, including oribatid and mesostigmatid mites and Collembola, were counted.

Litter bags with grass clippings were buried to assess insecticide impacts on rates of decomposition. Pouches (7.5 × 10.5 cm) were constructed of plastic mesh screening (2 × 2 mm openings) with heat-sealed seams. A preweighed (3 g) sample of oven-dried non-endophytic tall fescue (*Lolium aurundinaceum* Schreb.) clippings (about 4 cm long) was placed in each bag. Two such bags were buried just under the soil–thatch interface (3–4 cm deep) on 14 May 2010, the morning before the insecticides were applied. This was done by cutting a shallow diagonal slit with a flat-blade spade, inserting the bags and tamping down the turf and soil. Each litter bag had a numbered aluminium tag so that it could be found using a metal detector. The implanted bags were left in place during the spray applications, and then separate sets were recovered at 2 or 4 months after treatment. Remaining grass clippings were oven dried and weighed.

2.4 Effects on ant mounding and earthworm casting, 2010

Trials were conducted to test the hypothesis that selective insecticides might result in less suppression of nuisance ant mounds and earthworm casts than occurs as a side effect when broader-spectrum chemistries are applied. The first two trials compared suppression of mounding by the ant *Lasius neoniger* (Emery) by chlorantraniliprole versus bifenthrin, a standard for controlling cutworms and other surface-active insect pests on golf courses.[6] Eighteen plots (3.7 × 3.7 m), were marked on creeping bentgrass tees with abundant ant mounds at Champion Trace Golf Course, Nicholasville, KY. Most plots were on separate tees; a few large tees had two plots separated by at least 12 m. Treatments, including the untreated checks, were blocked by pretreatment counts taken on 10 June 2010 by centering a PVC pipe frame (2.44 × 2.44 m) in each plot and counting the total mounds within the frame. Plots were treated on 11 June at the same rates and in the same manner as those described in Section 2.3. The tees were irrigated (1.5 cm) from the golf course sprinkler system on the night of 11–12 June and maintained under the normal mowing and watering regime. No additional insecticides were applied. Treatments were evaluated on 17 June and 1 July by placing the aforementioned frame in the central portion of each plot and counting the total number of ant mounds therein. The trial was repeated on the same golf course in 2011, using different plots but the same methods, treatments and rates as in 2010. The insecticides were applied on 20 April, and mounds were counted 1 and 3 weeks later.

The final trial compared effects of chlorantraniliprole, clothianidin, the clothianidin + bifenthrin combination and carbaryl on earthworm casting activity. The study site was a stand (29 × 29 m) of creeping bentgrass and annual bluegrass, *Poa annua* L. (species distribution by visual estimation 75 and 25% respectively) established in 1978 and managed as a push-up golf putting green at the UKTRC. The soil was homogeneous and original, and no sand topdressing had been applied. The turf was mowed 5 times per week (4.0 mm height of cut) and irrigated from a permanent sprinkler system to prevent visible stress. The site had a history of high numbers of earthworms (mostly *Apporectodea* spp.[25]), and there was active casting in the weeks before and during the trial. Plots (1.5 × 1.5 m, six replicates, with 0.5 m untreated borders) were marked, and casts were counted in the inner 1 m^2 of each plot on 16 April. Treatments were blocked by these counts and applied on the same day. Insecticides and rates were the same as in Section 2.2. Earthworm casts in each plot were counted on

3 and 19 May. The stand was mowed every 2–3 days, but fresh casts were allowed to accumulate for 1 day and night before each sample date. On 25 May, all plots were treated with tea seed pellets (1.83 kg 100 m^{-2}), a saponin-rich byproduct of *Camellia* oil manufacture that irritates and causes earthworms to come to the surface.[25] The tea seed treatment was watered in (1.5 cm of irrigation), and earthworms surfacing in each plot were counted.

2.5 Statistical analyses

Data were analyzed within each trial by two-way analysis of variance (ANOVA) with means separation by Fisher's protected least significant difference (LSD) test ($P = 0.05$) when the ANOVA indicated a significant ($P < 0.05$) treatment effect. Log or square-root transformations were applied when needed to correct for heterogeneity of variance in count data, whereas the arcsine square-root transformation was applied to percentages. Analyses were done with Statistix 8.0.[26] Data are presented as original means ± standard error (SE).

3 RESULTS

3.1 Impacts on predators and predation, late summer 2009

The three most abundant taxa of predominantly predatory invertebrates captured in pitfall traps were Formicidae [mostly *L. neoniger* and *Solenopsis molesta* (Say)], Araneae (mostly Linyphiidae, Erigonidae and Lycosidae) and Staphylinidae [mostly *Platydracus mysticus* (Erichson), *Philonthus cognatus* Stephens and *Philonthus carbonarius* (Gyllenhal)]. Pretreatment counts did not differ for any of above groups ($F_{3,15} \geq 0.45$, $P \geq 0.58$) (Fig. 1), but there were significant post-treatment effects on ants ($F_{3,15} = 6.2$, 6.6, 5.4 and 6.1 at 1, 2, 3 and 4 weeks respectively; $P < 0.01$) and spiders ($F_{3,15} = 3.2$, 4.6 and 6.6 at 1, 2, and 4 weeks respectively; $P < 0.05$). Chlorantraniliprole did not significantly affect captures of any of the aforementioned groups compared with the untreated check (Fig. 1). There were no treatment effects on staphylinids, but the clothianidin–bifenthrin combination suppressed spiders on two of the four sample dates (Fig. 1). Ant captures were elevated, relative to the untreated check, on multiple sample dates after treating the turf with the combination insecticide or clothianidin alone (Fig. 1). Too few carabids (ten total specimens) were captured for meaningful analysis, probably because of the small (3 cm) diameter of the pitfall traps used in 2009.

Predators consumed 73–81% of the sentinel black cutworm eggs in the untreated plots within 48 h (Table 1). Neither chlorantraniliprole nor clothianidin affected egg predation. In contrast, the combination insecticide significantly reduced egg predation by 37–47% relative to the untreated checks (Table 1). There was no loss of eggs from the hatch checks.

3.2 Effects on earthworm and soil microarthropods, 2009

Earthworm numbers ($F = 12.8$, 14.7; $P < 0.001$) and biomass ($F_{4,12} = 3.8$, 4.9; $P < 0.05$) were reduced by some treatments on both sample dates. Chlorantraniliprole had no apparent impact, but the clothianidin–bifenthrin combination had reduced earthworm numbers and biomass, by 33 and 49% respectively after 1 week, and by 35 and 50% after 3 weeks (Fig. 2). Clothianindin alone reduced earthworm numbers and biomass by 32 and 39% respectively after 1 week. Carbaryl, the toxic standard, had even greater short-term impact (Fig. 2).

Oribatid mites showed no response to the treatments on either sample date ($F_{4,12} \leq 0.85$, $P \geq 0.51$), but predatory mites

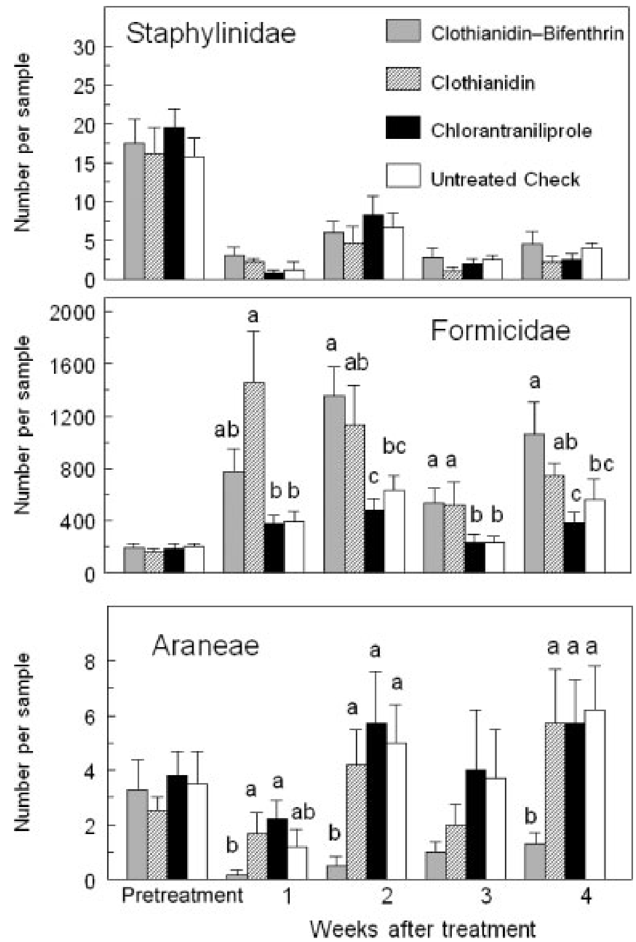

Figure 1. Pitfall captures of predatory invertebrates in golf course roughs during 1 week intervals before and after plots were treated with representatives of different insecticide classes on 20 August 2009. Data are means (± SE) per plot. ANOVA results are shown above those sets of bars for which the within-date treatment effect was significant ($P \leq 0.05$). Within these sample intervals, bars not marked with the same letter differ significantly (LSD, $P < 0.05$).

($F_{4,12} = 9.5$, 5.5; $P < 0.01$) and Collembola ($F_{4,12} = 7.8$, 18.2; $P < 0.001$) were significantly impacted at 1 week and for at least 3 weeks after treatment (Fig. 3). Chlorantraniliprole had no measurable effects, but clothianidin alone suppressed Collembola for at least 3 weeks, and the clothianidin–bifenthrin combination reduced numbers of predatory mites by 52–66% and the numbers of Collembola by 89–92% compared with populations in untreated turf. Carbaryl, too, suppressed both of these groups (Fig. 3).

3.3 Impacts on predators, predation, decomposers and decomposition, 2010

Chlorantraniliprole slightly suppressed pitfall captures of staphylinids (3 week samples) and spiders (3 and 6 weeks) but otherwise did not measurably impact on predators compared with the untreated checks (Fig. 4). Impacts of the other insecticides were greatest at 3 weeks after application ($F_{3,15} = 8.1$, 12.0, 2.70; $P < 0.001$, 0.001, 0.05 for Staphylinidae, Araneae and Carabidae respectively). Bifenthrin and the combination insecticide reduced 1 and 3 week spider captures by >80% compared with the untreated check; the combination product also reduced 3 week captures of staphylinids and carabids by 67 and 83% respectively

Table 1. Predation on cohorts of black cutworm, *A. ipsilon*, eggs exposed in plots in a golf course rough at 1 or 3 weeks after treatment with representatives of different insecticide classes in August 2009

	Mean ($\pm$ SE) percentage of eggs taken in 48 h[a]	
Treatment	1 week after treatment	3 weeks after treatment
Clothianidin–bifenthrin	45.8 ± 8.7 b	43.3 ± 8.9b
Clothianidin	67.5 ± 13.0 ab	77.5 ± 4.9a
Chlorantraniliprole	88.8 ± 6.3 a	71.3 ± 13.1a
Untreated check	72.5 ± 11.7 a	81.3 ± 9.3a

[a] Two-way ANOVA. 1 week: $F = 4.15$; df = 3,15; $P = 0.025$. 3 weeks: $F_{3,15} = 3.12$; $P = 0.058$. Within columns, means not followed by the same letter are significantly different (LSD, $P < 0.05$).

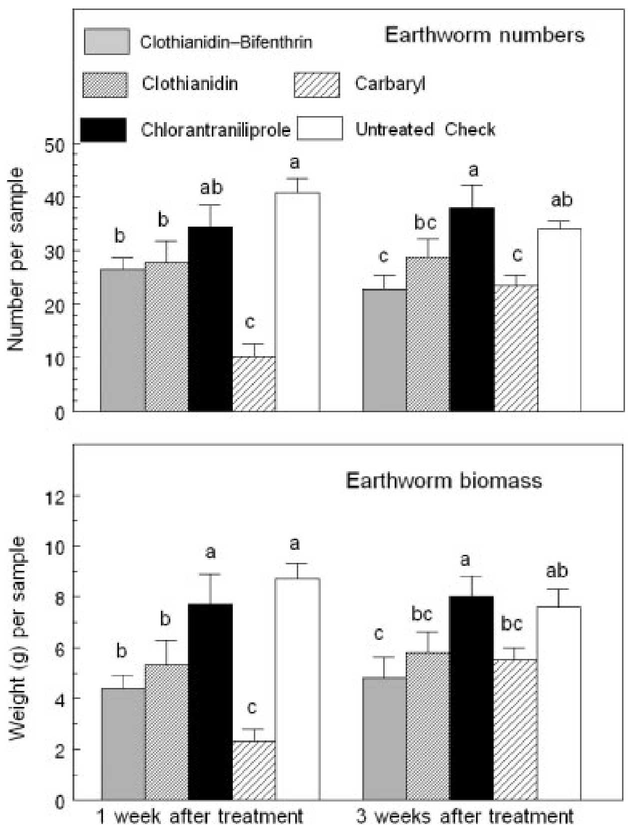

Figure 2. Earthworm (mainly *Apporectodea* spp.) numbers and biomass in fairway-height creeping bentgrass treated with representatives of different insecticide classes on 1 October 2009. Statistics are as in Fig. 1.

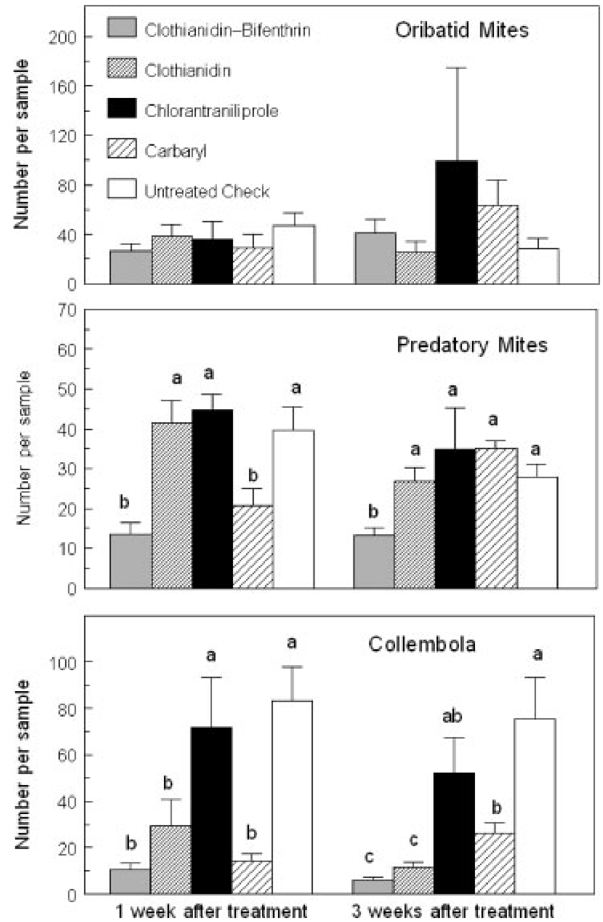

Figure 3. Abundance of soil microarthropods in samples fairway-height creeping bentgrass treated with representatives of different insecticide classes on 1 October 2009. Statistics are as in Fig. 1.

(Fig. 4). By 12 weeks post-treatment, however, pitfall samples indicated no remaining differences in predator abundance and activity between treated and untreated plots ($F_{4,20} \leq 1.9$; $P \geq 0.15$; data not shown). More carabids (predominantly *Harpalus pennsylvanicus* DeGeer and *Amara* spp.) were captured in 2010 than in 2009, probably because of the larger-sized pitfall traps that were used. Ant captures were not reduced by any treatment; rather, as in 2009, they tended to increase after clothianidin (1 week sample, $F_{4,20} = 2.46$, $P = 0.08$) or the combination product (6 week sample, $F_{4,20} = 3.82$, $P < 0.05$) was applied (Fig. 4).

Rates of predation on Japanese beetle eggs ranged from 65 to 83% and were not significantly affected by any treatment (Table 2). Except for the combination insecticide, which reduced predation at 3 weeks after treatment, none of the other insecticides influenced the percentage of black cutworm eggs taken by predators (Table 2). There was no eclosion from the egg clusters that had been protected from predation as hatch checks.

None of the treatments significantly reduced the abundance of oribatid mites, predatory mites or Collembola relative to the untreated checks (Fig. 5). Although there were significant differences among treatments for predatory mites after 3 weeks ($F_{4,20} = 2.83$, $P = 0.05$) and for Collembola after 9 weeks ($F_{4,20} = 4.13$, $P = 0.05$), they reflect the lesser abundance of these groups in plots where clothianindin–bifenthrin or carbaryl, as opposed to chlorantraniliprole, had been applied (Fig. 5).

The clothianidin–bifenthrin combination, and to a lesser extent the clothianidin alone, retarded decomposition of grass clippings in the buried litter bags (Table 3). The dry weight of grass remaining in these treatments was 4.0 and 3.3-fold higher than in untreated plots after 2 months, and 9.7 and 6.9-fold higher respectively at 4 months after treatment.

3.4 Effects on ant mounding and earthworm casting

In the 2010 trial, chlorantraniliprole had not reduced the numbers of ant (*L. neoniger*) mounds on treated golf course tees after 1 week,

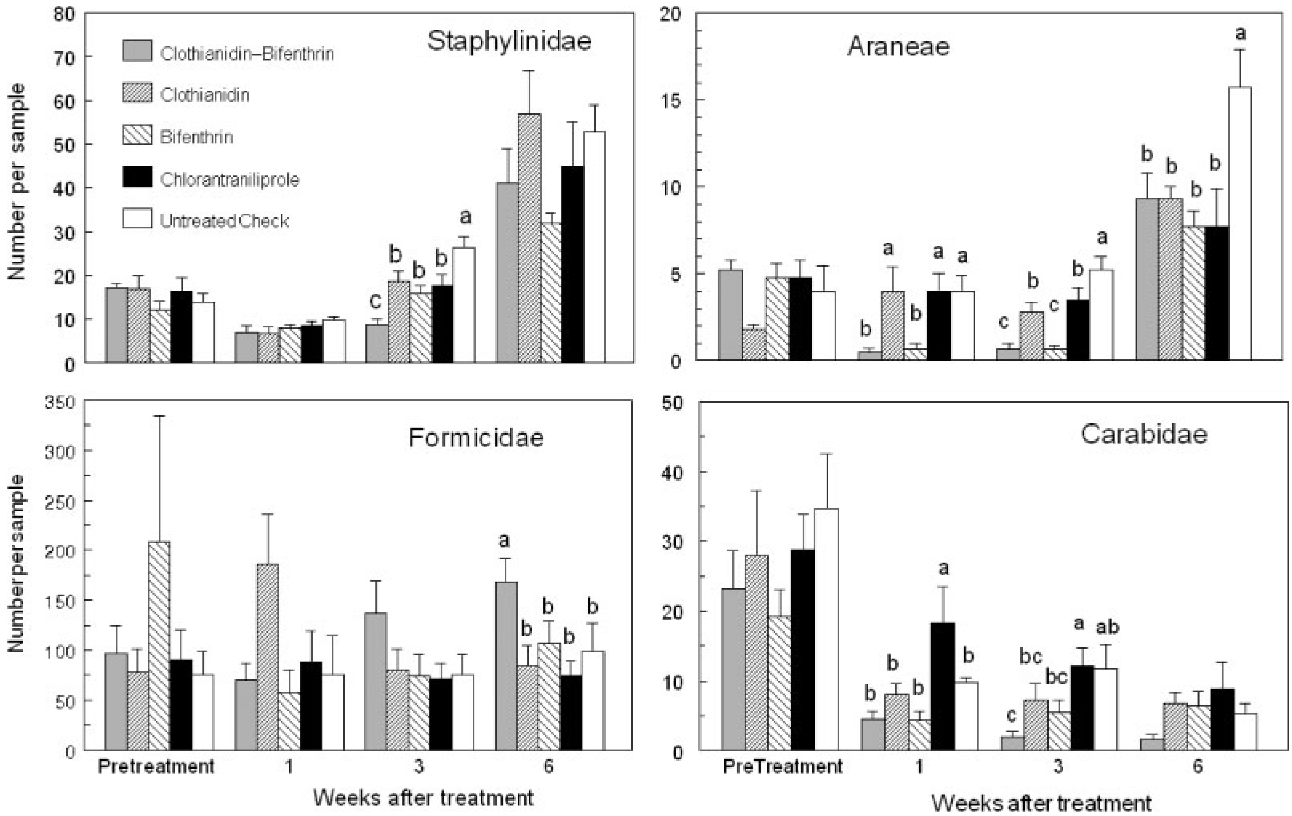

Figure 4. Pitfall captures of predatory invertebrates in golf course roughs during 3 day trapping periods before and after plots were treated with representatives of different insecticide classes on 14 May 2010. There were no significant treatment effects at the 12 week sample date (data not shown). Statistics are as in Fig. 1.

Table 2. Predation on sentinel black cutworm eggs exposed for 24 h and Japanese beetle eggs buried for 7 days in plots on a golf rough at specified weeks after treatment (WAT) with representatives of different insecticide classes, 2010

	Mean (± SE) percentage of eggs taken[a]			
	A. ipsilon			*P. japonica*
Treatment	1 WAT	3 WAT	6 WAT	9 WAT
Clothianidin–bifenthrin	14.3 ± 4.6	27.7 ± 6.1	91.0 ± 2.2	73.0 ± 10.6
Clothianidin	40.0 ± 13.9	44.0 ± 11.4	95.0 ± 1.1	65.3 ± 7.1
Bifenthrin	37.6 ± 9.1	35.6 ± 9.3	94.0 ± 3.0	67.3 ± 4.3
Chlorantraniliprole	32.3 ± 6.9	41.0 ± 14.0	97.7 ± 1.1	70.0 ± 2.1
Untreated control	26.7 ± 8.1	56.7 ± 9.8	95.7 ± 1.1	83.3 ± 6.8

[a] For *A. ipsilon*, $F_{4,20} = 1.32, 1.41, 1.52$ and $P = 0.30, 0.26, 0.23$ for separate cohorts of 40 eggs at 1, 3 and 6 WAT respectively. For *P. japonica*, $F_{4,16} = 1.91$ and $P = 0.16$.

although there was some suppression after 3 weeks compared with the numbers of mounds on untreated tees (Table 4). Bifenthrin suppressed mounding activity by 90 and 65% at 1 and 3 weeks after treatment respectively (Table 4). Repetition of the trial in 2011 supported the hypothesis that, compared with bifenthrin, chlorantraniliprole provides little or no suppression of *L. neoniger* mounding (Table 4).

Chlorantraniliprole also did not reduce numbers of earthworms or casts compared with the untreated checks (Table 5). Carbaryl, as expected, significantly suppressed earthworm casting at 3 weeks after treatment, but the clothianidin–bifenthrin combination had even greater impact, reducing casts by 71 and 81% after 3 and 5 weeks respectively, and earthworm abundance by 78% for at least 5 weeks (Table 5). There was also a trend ($P = 0.07$) on both sample dates for cast reduction following treatment with clothianidin alone.

4 DISCUSSION

Insecticide selectivity can be physiological, reflecting differences in uptake, detoxification or excretion, or ecological, arising from differential exposure.[27,28] Assessment of pesticide hazards to beneficial species should therefore include trials realistically simulating how non-target organisms would be exposed in the field.[20] The

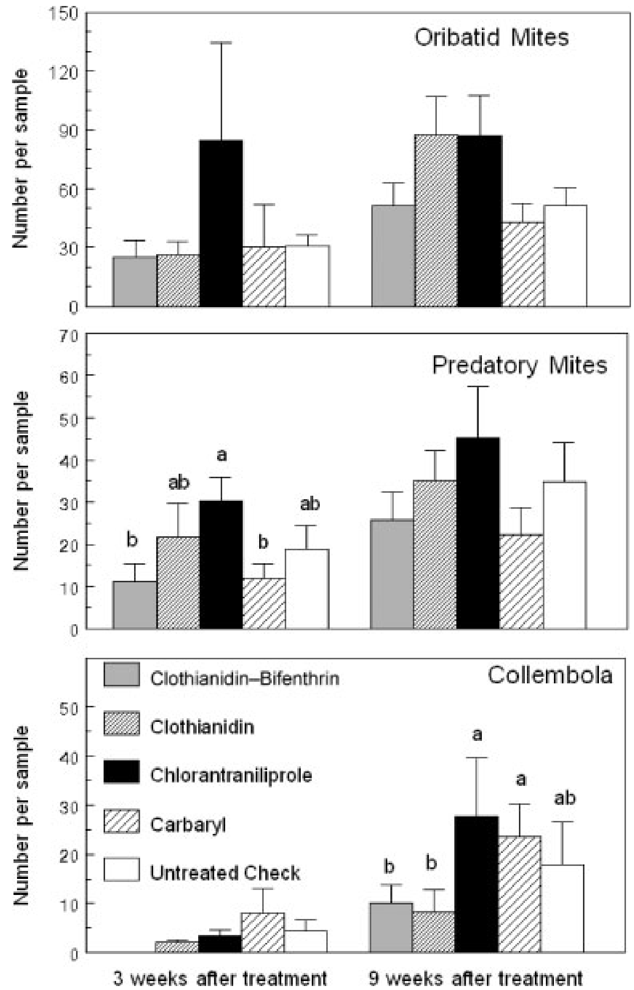

Figure 5. Abundance of soil microarthropods in samples from a golf course treated with representatives of different insecticide classes on 14 May 2010. Statistics are as in Fig. 1.

Table 3. Decomposition of tall fescue grass clippings in buried litter bags after plots on a golf course rough were treated with insecticides representing different chemical classes in May 2010

	Grass clippings (mg dry weight) remaining after[a]	
Treatment	2 months	4 months
Clothianidin–bifenthrin	517 ± 71 a	207 ± 34 a
Clothianidin	620 ± 119 a	291 ± 75 a
Bifenthrin	159 ± 71 b	81 ± 46 ab
Chlorantraniliprole	47 ± 42 b	114 ± 55 ab
Untreated check	155 ± 69 b	30 ± 16b

[a] Data are means ± SE. $F_{4,20}$ = 8.3, 3.7 and P < 0.01, 0.05 for amounts remaining after 2 and 4 months respectively. Within columns, means not followed by the same letter are significantly different (LSD, P < 0.05).

Table 4. Relative effectiveness of chlorantraniliprole versus bifenthrin application in reducing mounding activity by the ant *Lasius neoniger* on creeping bentgrass golf course tees

	Mounds (mean ± SE) per 6 m^2		
	Pretreatment	1 WAT	3 WAT
2010 trial			
Chlorantraniliprole	26.0 ± 2.8	26.7 ± 4.2 a	11.2 ± 2.4 b
Bifenthrin	27.8 ± 2.8	3.2 ± 1.3 b	7.3 ± 1.7 b
Untreated check	27.3 ± 3.5	33.3 ± 4.9 a	20.8 ± 3.5 a
2011 trial			
Chlorantraniliprole	21.0 ± 3.4	14.0 ± 1.8 a	36.0 ± 8.7 a
Bifenthrin	22.6 ± 4.5	0.8 ± 0.4 b	8.2 ± 3.3 b
Untreated check	21.6 ± 4.4	23.8 ± 6.6 a	47.4 ± 11.5 a

For 2010, $F_{2,8}$ = 0.6, 24.1, 9.7 and P = 0.59, <0.01, <0.01; for 2011, $F_{2,8}$ = 0.5, 9.5, 13.2 and P = 0.61, <0.01, <0.01 for pretreatment and 1 and 3 weeks after treatment (WAT) respectively. Within years and columns, means not followed by the same letter are significantly different (LSD, P < 0.05).

present trials, done in golf course settings with rates, timings and application methods similar to those used by turf managers, support the hypothesis that chlorantraniliprole is less disruptive to epigeal predators, decomposers and some of their ecosystem services than are representatives of some other current classes of turf insecticides. The mode of entry of anthranilic diamides, including chlorantraniliprole, is mainly through ingestion; low contact activity contributes to their selectivity.[2] Chlorantraniliprole has shown low toxicity to parasitoids,[4] entomopathogenic nematodes[29] and pollinators[3,5] in laboratory studies. This study is the first to assess its effects on non-target invertebrates in turf.

The predominant families of epigeal predators in the present plots, which included ants, rove and ground beetles, Linyphiidae (sheet web and dwarf spiders) and Lycosidae (wolf spiders), are typical of those found in cool-season turf settings.[30–36] Chlorantraniliprole had little or no impact on any of these families, as determined by pitfall catches, which are composite measures of activity and abundance,[37] nor on predatory mites in the soil and thatch. In contrast, clothianidin, a representative nicotinoid, and the nicotinoid–pyrethroid premix had short-term impact on a number of predator groups. The temporary increase in pitfall trap captures of ants following application of the latter two products in late summer 2009 was likely due to sublethal neural excitation[38] and activity of foraging workers rather than increases in their populations. That view is consistent with the concurrent reduced predation on black cutworm eggs in plots treated with the nicotinoid–pyrethroid combination. Workers of *L. neoniger* and other common turfgrass ants would have been at peak abundance in late summer when treatments for that trial were applied.[24,31]

Chlorantraniliprole also had no measurable impact on populations of earthworms, Collembola or oribatid mites. These groups tend to be the predominant decomposers in cool-season turfgrass soils.[11,22,40,41] In contrast, both clothianidin and the clothianidin–bifenthrin combination significantly reduced populations of both earthworms and Collembola in 2009, and the latter also affected earthworms in 2010. Both clothianidin and the combination product also significantly retarded grass clipping decomposition.

Turf managers seeking to control surface and subsurface pests with the same application can apply a combination formulation with two active ingredients or use a single chemistry such as chlorantraniliprole that is active against both lepidopteran and coleopteran pests. Multiple targeting is attractive to golf superintendents, lawn care providers and grounds managers

Table 5. Mean ($\pm$ SE) earthworm casts per m^2 at indicated weeks after treating plots on a soil-based putting green, and relative earthworm (*Apporectodea* spp.) abundance at the end of the 5 week trial

Treatment	Casts pretreatment	Number of casts after[a] 3 weeks	5 weeks	Worms per sample[b]
Carbaryl	35.3 $\pm$ 5.8	13.0 $\pm$ 2.5 c	39.2 $\pm$ 8.0 ab	6.8 $\pm$ 2.0 a
Clothianidin–bifenthrin	36.2 $\pm$ 7.0	9.7 $\pm$ 1.7 c	11.3 $\pm$ 2.9 c	2.5 $\pm$ 0.7 b
Clothianidin	38.2 $\pm$ 7.9	19.8 $\pm$ 2.4 b	26.3 $\pm$ 6.5 b	8.0 $\pm$ 2.1 a
Chlorantriniliprole	32.7 $\pm$ 5.9	21.2 $\pm$ 2.1 ab	42.5 $\pm$ 3.7 ab	13.0 $\pm$ 2.9 a
Untreated check	37.7 $\pm$ 6.1	33.5 $\pm$ 5.5 a	59.0 $\pm$ 15.8 a	11.2 $\pm$ 4.7 a

[a] $F_{4,20} = 1.7, 9.7, 6.1, 3.9$ and $P = 0.2, <0.001, <0.01, <0.02$ for pretreatment, 3 and 5 week casts and worms per sample respectively. Within columns, means not followed by the same letter are significantly different (LSD, $P < 0.05$).

because it simplifies treatment decisions and may also save time, labor and fuel costs.[18] Another purported benefit of combination products is potential synergistic or additive effects between the different chemistries.[19] Such effects, however, would also seemingly occur against beneficial invertebrates, and, indeed, more significant effects were seen on non-target arthropods and earthworms from the combination product than from either of its components alone. The present results indicate that chlorantraniliprole can provide the benefits of controlling multiple key pests without disrupting beneficial invertebrates and their activities.

Most insecticide-related reductions in activity or abundance of non-target invertebrates seen in this study lasted no more than a few weeks. Relatively rapid recovery of predatory invertebrates following insecticide perturbations is consistent with the pattern seen in earlier studies with older chemistries.[11,32,33,40,41] Plot size in studies of this type is invariably constrained by trade-offs between need for replication, practical issues such as amount of turf available and starting with reasonable consistency in site characteristics. Invertebrate repopulation of treated turf occurs both by immigration from surrounding areas and by reproduction of survivors or individuals originating from life stages not killed by the treatment. Plot sizes in the present main trials were large relative to the probable dispersal capabilities of earthworms and other decomposers, as well as smaller epigeal predators, but probably not for more mobile groups such as ground beetles that could recolonize treated areas when contact activity of residues declined. Although turf is a relatively resilient system, beneficial invertebrates would doubtless be slower to repopulate treated golf fairways, sports fields or home lawns than occurred in the present plots. Furthermore, repeated applications, within a growing season or over successive years, may have cumulative impacts on beneficial invertebrates.[41]

Ants and earthworms, beneficial in most turf settings, can become pests on golf courses and sports fields when their mounds or casts disrupt the smoothness and uniformity of playing surfaces, dull mower blades or smother small patches of grass. The present trials with clothianidin and previous ones with imidacloprid[11,13] indicate that application of nicotinoids for control of soil insects tends partially to suppress earthworms and casts. The clothianidin–bifenthrin premix had even greater impact on earthworms, greater than the impact of carbaryl. The trials on tees showed that applying a pyrethroid alone, done by many golf superintendents every 3–4 weeks during the growing season for cutworm control, secondarily suppresses ant mounds, albeit for only a few weeks after application. Because chlorantraniliprole does not suppress ants or earthworms, turf managers switching to the anthranilic diamide for its effectiveness against primary pests and favorable toxicology may need to spot treat more often for those secondary problems that had been suppressed by formerly used less selective insecticides.

Worldwide industry initiatives towards more sustainable turf management will require effective pesticides that are both non-toxic to vertebrates and compatible with non-target invertebrates that contribute to pest suppression, decomposition of plant matter, nutrient recycling and good soil tilth.[6–9,14–17,42] This study indicates that, when compared with representatives of other classes of chemical insecticides, chlorantraniliprole has particularly low impact on beneficial invertebrates and their services.

ACKNOWLEDGEMENTS

The authors thank B Barnes (Idle Hour CC), J Ducker (Lexington CC), M Johnson and C Gray (Champion Trace Golf Course) and DW Williams for access to study sites, C Elder and EK Dobbs for research assistance and two anonymous reviewers for constructive criticism. This study was supported in part by the University of Kentucky's BC Pass Research Professorship and Graduate Assistantship to DAP and JLL respectively. This is paper No. 11-08-045 of the Kentucky Agricultural Experiment Station.

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