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Response of the dusky wireworm, *Agriotes obscurus* (Coleoptera: Elateridae), to residual levels of bifenthrin in field soil

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Abstract To determine if bifenthrin residues elicit morbidity and surfacing behavior in wireworms, larvae of the dusky wireworm, Agriotes obscurus (Coleoptera: Elateridae) were placed in field soil treated with the pyrethroid insecticide bifenthrin ~ 1 year previous. Morbidity was immediate and lasted as long as wireworms remained in the soil, disappearing quickly after transfer to clean soil. In 2009, field soil treated 336 days previous with bifenthrin at 340 g AI/ha elicited morbidity symptoms similar to that elicited by soil freshly amended with bifenthrin at 100 g AI, and analysis of the field soil confirmed residual levels of bifenthrin exceeding 100 g AI/ha. In 2010, wireworms placed in field soil treated 343 days previous with bifenthrin at 100, 200, and 300 g AI/ha responded as in 2009, with the degree of morbidity increasing with the rate of insecticide, and with wireworms in a non-feeding state more affected than those in a feeding state at each rate. In both 2009 and 2010, moribund wireworms moved to the soil surface within 1 day of placement in the soil containing residual bifenthrin and remained there until reburied, after which they often resurfaced. To confirm that the bifenthrin residues elicited repellency, wireworms were placed in soil window bioassays containing field soil with residual bifenthrin. Wireworms behaved markedly different upon contacting soil containing the residues than when exposed to untreated soil, both in the presence and absence of an attractant, but were

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less likely to avoid soil containing residual bifenthrin when attracted by wheat seedlings placed inside it.

Keywords Wireworms · Insect behavior · Insecticide residue · Bifenthrin · Pyrethroid · Pest control

Introduction

Wireworms are important pests of many crops worldwide, and are becoming increasingly difficult to control as effective organophospate (OP), organochlorine (OC), and carbamate (C) insecticide treatments are being gradually phased out (Vernon et al. 2009; Kuhar and Alvarez 2008; Parker and Howard 2001). In Canada, the synthetic pyrethroid, bifenthrin, is a leading candidate for management of wireworms in potato production, and is currently registered for this purpose in the USA. Since bifenthrin is virtually insoluble in water (solubility = 0.1 mg/l) and has a high soil adsorption coefficient (Koc = $1.31-3.02 \times 10^5$), it is highly persistent in soil (Fecko 1999). Field dissipation studies indicate that, depending on soil type and condition, bifenthrin has a half-life of 122-345 days in soil, and the compound is virtually stable in soil under flooded conditions (Fecko 1999). The persistence of bifenthrin, as well as the high rate of application in the field (in-furrow atplanting spray rates in the USA exceed 200 g AI/ha), are variables favoring wireworm control, since protection of daughter tubers may be required for over 100 days post application. However, these variables also raise questions regarding post-harvest environmental impacts on non-target organisms in the soil.

In field studies conducted by our lab, the efficacy of an insecticide for wireworm control is determined both by crop stand and yield protection, and by bait trapping for surviving

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wireworms the following spring. Bait traps placed in the spring of 2009 in potato plots treated in 2008 with bifenthrin collected no wireworms, initially suggesting that all wireworms in the plot had been killed and/or repelled from the plots, particularly as bait traps placed in untreated plots collected a mean of 21 wireworms per trap. During bait trapping, however, several wireworms were observed on the surface of only the bifenthrin-treated plots, all of which showed characteristic symptoms of insecticide poisoning (i.e., "Writhing" according to Vernon et al. 2008). These observations suggested that at least some wireworms were still alive the spring following treatment, but were not entering the bait traps. It was hypothesized that wireworms were still being affected by bifenthrin residues in the soil 329 days after the insecticide had been applied, and that wireworms approaching bait traps placed in the bifenthrin treated soil became moribund and were repelled away from the bifenthrin-treated area, some of which came to the surface. Similar behavior has been reported for bifenthrin in soil bioassays in the laboratory (van Herk, unpublished data).

To confirm that sufficient residual bifenthrin remained to affect wireworm health (i.e., mobility) and behavior (i.e., could cause them to move to the soil surface in a moribund state), healthy wireworms were placed in soil collected in the spring of 2009 from the 2008 field plots treated with bifenthrin the previous year, and their mobility and behavior assessed over time. This study was conducted with feeding wireworms exposed to one rate of bifenthrin (applied in 2008 and soil collected in 2009) and was repeated the following year with both feeding and non-feeding wireworms exposed to three rates of bifenthrin (applied in 2009 and soil collected in 2010). In addition, observational studies were conducted in 2009 in which wireworms were placed in soil window arenas containing soils collected from both untreated and bifenthrin-treated plots to see if wireworms could recognize and avoid the presence of residual bifenthrin. These observational studies were conducted both in the presence and absence of wheat seedlings to determine if wireworm sensitivity to residual bifenthrin was affected by the presence of an attractant cue (i.e., CO₂) known to affect wireworm attractancy behavior (van Herk et al. 2010).

Materials and methods

Placement of wireworms in field soil with residual bifenthrin

Soil samples, 2009

Bifenthrin was evaluated in two insecticide efficacy field studies conducted at the Pacific Agri-Food Research Centre (PARC) in Agassiz, BC in 2008. These studies were located adjacent to each other and planted with potatoes on subsequent days (18 and 19 June, 2008). Both studies were designed as complete randomized block designs with four replications, and contained untreated control and bifenthrin treatments. Bifenthrin (Capture 2EC, containing 25.1% bifenthrin), was applied as an in-furrow spray at 340 g AI/ ha at planting in both studies. Treatment plots consisted of five 4.8 m long rows, of which the centre 3 (i.e., rows 2–4) received insecticide or no insecticide and the outer, "buffer", rows were untreated and shared with adjacent plots. Rows were spaced 1.0 m apart and 17 potatoes per row were hand-planted 15 cm deep and 30 cm apart.

Two soil samples were collected from each of rows 2 and 4 of each treatment plot in both studies on 13 May 2009 (i.e., 336 days after planting; DAP). These rows had not been harvested or otherwise disturbed since planting the previous year. Soil samples were taken 1.5 m into each row (i.e., between the 5th and 6th plant from either end). Since the furrows in which potatoes were planted were covered immediately after the sprays were applied with soil that had not been sprayed, the top 5 cm of soil were removed at the sampling location prior to taking a 7.5 cm deep \times 10 cm diameter soil sample with a golf cup cutter.

Soil samples were placed immediately in clear plastic bags, coarse organic matter was removed, clods broken into 5 mm pieces, and the soil homogenized by hand. A 150.0 g sub-sample of soil (~25% moisture by weight) was taken from each bag and placed in 150 ml specimen cups (Fisher Scientific, Toronto, ON). All excess soil was placed in separate Rubbermaid tubs and homogenized for use in soil window bioassays (discussed below). Small samples (2 per treatment) of the homogenized soil were stored in a freezer at -18° C until they could be analyzed for bifenthrin residues. Residue analysis was conducted with a Perkin Elmer Turbomass Gold Gas Chromatography Mass Spectrometry system (CanTest Ltd., Burnaby, BC), capable of detecting 2 ng bifenthrin/g soil.

Soil samples, 2010

Three rates of bifenthrin were evaluated for wireworm control in an insecticide efficacy field study conducted at PARC in 2009. The study was laid out as in 2008 and potatoes were planted on 4 June 2009. The study contained an untreated control treatment and bifenthrin applied as an infurrow spray at 100, 200, and 300 g AI/ha. Plot size, row length and spacing, and potato planting were as in 2008. Unforeseen heavy flooding (~ 15 -30 cm) submerged replicates 1 and 2 continuously for ~ 3 months from November–February and due to slightly higher elevation, replicates 3 and 4 for only ~ 1 month. This flooding resulted in disruption of the unharvested potato plots in replicates 1 and 2 by geese excavating the remaining potatoes from rows. Plots

in replicates 3 and 4 from which soil samples were taken, however, were not disturbed by geese activity.

Four soil samples were collected from each of rows 2 and 4 in the control and bifenthrin-treated plots of replicates 3 and 4, on 13 May 2010 (343 DAP). Soil samples were taken as in 2009; sampling locations within a row were separated by at least 50 cm and were at least 50 cm from the ends of the rows. Samples were sealed in bags and stored at 10°C for ~40 days, at which time rocks and organic material were removed and the soil homogenized by hand (as in 2009). A 150.0 g subsample of soil was taken from each bag, after which the subsamples from the four samples taken from each row were homogenized and repartitioned into four 150 ml specimen cups to obtain cups containing similar soil.

Wireworms

For the study conducted in 2009, larvae of the dusky wireworm, Agriotes obscurus (L.), were collected at the Pacific Agri-Food Research Center (PARC) in Agassiz, BC in May 2009, where this is the predominant species (Vernon et al. 2001). Wireworms were stored without food in 40-1 Rubbermaid tubs with soil from the collection area at 10°C until needed. Feeding wireworms were retrieved from the storage tubs within 4 days of being used in the study using small bait traps composed of a vermiculite-wheat mixture soaked in water (van Herk et al. 2010). Wireworms were stored individually in small, 100 ml plastic containers with soil, and during the day of study were weighed with an analytical balance (Denver Instruments, model SI-403) and checked for mobility. Wireworms weighing 20.0-40.0 mg and scored as "Alive" (Vernon et al. 2008) were randomly selected and assigned to one of 64 cups of soil, and placed on the soil surface on 20 May 2009. Cups containing wireworms were placed in an environmental chamber (Model E7, Conviron, Winnipeg, MB) at $15^{\circ}C$ ($\pm 0.5^{\circ}C$) without light for the duration of the experiment, except when their mobility was assessed.

For the study conducted in 2010, wireworms were collected at PARC in May 2010 and stored as in 2009 until needed. Feeding wireworms were selected as in 2009. As this baiting procedure selects virtually all the wireworms in the feeding stage from a storage tub (WvH, personal observation), those wireworms that did not respond to two back-to-back baiting attempts within 2 weeks were considered non-feeding. For this study, 96 feeding and 96 nonfeeding wireworms were weighed and their mobility initially assessed as in 2009. Wireworms were placed in the cups (three per cup) on 1 July, two randomly selected cups from each row receiving feeding, and two cups receiving non-feeding wireworms. Weights of wireworms in a cup differed by at least 6.0 mg from each other to enable us to track the mobility scores of individual wireworms over time.

Placement of moribund wireworms in clean soil

To determine if wireworms affected by the soil from the bifenthrin-treated plots could make a full recovery from morbidity, larvae of 16 randomly selected cups of both the control and bifenthrin treated soil treatments in the 2009 study were placed in clean soil (i.e., were "resoiled"). These wireworms were placed in new 150 ml containers that contained 150.0 g of untreated soil 16 days from the start of the study, immediately after their mobility was assessed. The untreated soil had been amended to 25% moisture and prepared as the field soil above. Observations of wireworm mobility and surfacing were continued as before for both these "resoiled" wireworms and those left in their original soil. Resoiling was only done in the 2009 study.

Mobility and surface emergence monitoring

For the 2009 study, wireworm mobility was monitored at 1, 2, 8, 16, 23, 30, 37, and 65 days after (DA) first insertion into cups. Resoiled wireworms were also checked at 17, 18, and 21 DA (i.e., 1, 2, and 4 days after being resoiled). Wireworm mobility was assessed by placing wireworms in the centre of a 10 cm Petri dish arena lined with filter paper moistened with deionized water, using the descriptive and numerical criteria described by van Herk and Vernon (2011). Briefly, wireworms are considered "Alive" (A: 0) when capable of spontaneous, normal movement; "Aliveslow" (AS: 0.5) when they require more than 2 min to make it to outside of dish; "Alive-clearly affected" (AC: 1) when not capable of moving continuously for 2 min without falling over; "Writhing" (W: 2), when making spontaneous twisting movements of entire body, often bending into "C" or corkscrew shapes; "Writhing upon stimulus" (WR: 3), when only capable of "Writhing" motions in response to gentle prodding; "Leg and mouthpart movement" (LM: 4) when only capable of moving legs and mouthparts; "Mouthpart movement" (M: 4.5) when only capable of moving mouthparts; and "Dead" (D: 5) when this is obvious from decomposition and/or mycelial growth.

The number of wireworms found on the soil surface in each cup was recorded immediately after removal of the cups from the environmental chamber for mobility assessments. Wireworms were considered to be on the soil surface when any part was visible above the level of the soil. After mobility assessments, wireworms were all buried in the soil in a 3 cm deep hole in the middle of the container, so that any specimen found on the soil surface was known to have moved there since the previous assessment. Wireworms that were scored as Dead were not returned to the cups.

For the 2010 study, wireworm mobility was monitored as in 2009, at 1, 4, 7, 15, 22, 30, and 50 DA. Wireworms were not resoiled halfway through the study.

Observation of behavior of wireworms in soil containing bifenthrin residues

Bioassay setup

Two observational studies were conducted in the summer of 2009 with the field soil collected that year. Observations were conducted with Plexiglas soil window bioassays developed by van Herk and Vernon (2007). Bioassays consisted of a 4 mm deep circular arena (diameter = 26 cm) in which an even layer of soil was spread. Wireworm movement and behavior were observed under low light conditions through both the top and bottom of the arena. Soil used for these observations was taken from the control and bifenthrin-treated potato plots (discussed above), and wireworms were from the same collection used in the 2009 cup study. Only feeding wireworms that were observed to be Alive (A) immediately prior to use in observations were used.

Behavior in the presence of an attractant

In the first study, the outer ring of the arena was filled with soil from the control plots (Fig. 1a, unshaded area), and the centre area (diameter = 12 cm) filled with soil from either the control plots (i.e., "control" treatment) or from plots treated with bifenthrin (i.e., "bifenthrin" treatment). Care was taken to prevent cross-contamination between the soils in the two areas. Wireworms were released into the control soil along the outer edge of the arena and their movements observed continuously and their position (as determined by a transparent grid covering tops and bottoms of the arena) recorded every 5 min for 3 h. Wireworm position and behavior were also recorded at 24 h, immediately after which the arena was dismantled and wireworm mobility assessed (as above).

To see if wireworms were willing to enter soil treated with bifenthrin, five wheat seedlings (cv AC Barrie) that had germinated for 48 h were placed in the centre of the arenas 60 min before wireworms were introduced. The CO_2 evolved by wheat seedlings placed in this arrangement generally causes the majority of feeding wireworms to orient toward the centre of the arena within 2 h of their insertion (van Herk and Vernon 2007; van Herk et al. 2008). One wireworm was placed as above in each arena and 25 wireworms were observed per treatment. Wireworm movements were recorded as above for 3 h, and after 24 h wireworms were removed and stored individually in 50 ml specimen cups with screened soil and their mobility assessed. Mobility was also assessed 7 days later.

Behavior in the absence of an attractant

In the second observational study, one-half of the arena was filled with soil from the control plots (Fig 1b, unshaded area), and the other half filled with soil from either the control plots (i.e., "control" treatment) or from plots treated with bifenthrin (i.e., "bifenthrin" treatment). No wheat seedlings were placed in the arenas. One wireworm was placed in each arena along the outer edge (as above) and 25 wireworms were observed per treatment. Wireworm behavior and movements were observed and recorded as above and after 24 h wireworms were removed and their mobility assessed. Mobility was also assessed 7 days later.

Statistical analyses

Placement of wireworms in field soil with residual bifenthrin

For the 2009 study, wireworm mobility scores were averaged per cup, and mean wireworm mobility scores for the four treatments compared with repeated measures ANOVA using Proc MIXED (SAS 9.2) with an unstructured covariance matrix. Mobility scores of resoiled wireworms at 17, 18, and 21 DA were not included in this analysis as non-resoiled wireworms were not assessed on these dates. Comparisons (determined a priori) were made between the following treatments: control and control-resoiled, bifenthrin and bifenthrin-resoiled, control and bifenthrin, control-resoiled and bifenthrin-resoiled. Mean wireworm mobility scores in the two resoiled treatments at 17, 18, and 21 DA were compared with ANOVA (Proc GLM) for each day. Numerical scores of all wireworms were retained for the analyses. Proportions of wireworms Dead (D) were compared between treatments with Proc FREQ.

The proportions of wireworms found on the soil surface of cups were analyzed with ANOVA (Proc GLM) for each day separately, as the number on the surface was not correlated between observation days. Proportions were normalized with an arcsine transformation; normality of all variables was assessed using the UNIVARIATE procedure.

For the 2010 study, individual wireworm mobility scores were compared between treatments with repeated measures ANOVA using PROC MIXED, as with 2009 data. The model included additional variables to determine if there was a difference between feeding and non-feeding wireworms and to determine if the container in which wireworms were held had an effect; individual wireworm weight was included as a covariate in initial models but Fig. 1 Diagram of soil type distribution in window bioassays used to observe the behavior of wireworms in the presence of bifenthrin residues: the shaded area represents soil from either control plot (in the control treatment) or from plots treated with bifenthrin (Capture 2EC), the previous year. The unshaded area is filled with soil from control plots. Wireworms were released in the bottom corner, at right. Wheat seedlings were placed in the centre of a; no wheat was placed in b



was highly non-significant (P > 0.9) and dropped from subsequent analyses. As with 2009 data, the effect of the plot row was nested within the study replicate. In addition, the effect of individual cups was nested within the plot row. As wireworm mobility was tracked individually, only the ante-mortem mobility of those that died was included in the analyses.

Proportion of wireworms on the surface was compared per date with ANOVA with feeding status and treatment as variables. Replicate and row effect were included in initial models as variables, but were not significant (P > 0.05) and therefore removed from the final analyses.

Observational studies

In both observational studies, mobility of wireworms exposed to control and bifenthrin treated soil were compared at 24 h and at 7 days with PROC TTEST using numerical scores.

Results

Placement of wireworms in field soil with residual bifenthrin

Mobility scores over time, 2009

All wireworms were scored as "A" and moved normally immediately prior to insertion into cups. Wireworms placed in soil taken from control plots continued to move normally until the end of the study (Table 1). There was little incidence of mortality in any treatment until 16 DA (0.0 in control, 0.03–0.05 in bifenthrin). By 65 DA, the proportion dead had increased to 0.13 and 0.16 in the control resoiled and control not-resoiled (respectively), and to 0.23 and 0.25 in the bifenthrin-resoiled and bifenthrin not-resoiled treatments (respectively). Although the proportion dead differed significantly between the combined bifenthrin and control treatments ($\chi = 4.26$, df = 1, P = 0.039), it did not differ significantly between resoiled and non-resoiled wireworms placed in either control or bifenthrin treated soil (P > 0.6).

Wireworms placed in bifenthrin soil showed symptoms of being affected within 1 DA and were on average in the Writhing (W) category as long as they remained in this soil (Table 1). Analysis of mean wireworm mobility scores over time indicated that treatment, time, and treatment \times time were all statistically significant (F = 51.48, df = 3,45, P < 0.0001; F = 13.42, df = 7,45, P < 0.0001;0.0001; F = 7.63, df = 21,45, P < 0.0001, respectively), but that neither the study replicate or plot row where soil was taken from were significant (F = 0.72, df = 7,45, P =0.65; F = 1.96, df = 8,45, P = 0.07, respectively). Between-treatment comparisons of Ismeans indicated that mobility scores did not differ significantly between control and control-resolled treatments (t = 0.51, df = 45, P =0.61), but did differ significantly between control and bifenthrin (t = 9.75, df = 45, P < 0.0001), control-resoiled and bifenthrin-resoiled (t = 6.85, df = 45, P < 0.0001), and between bifenthrin and bifenthrin-resoiled treatments (t = 3.30, df = 45, P = 0.0019).

Inspection of the effect of time indicated that although mean mobility scores were higher at 65 DA than at 37 DA in all four treatments (Table 1), this increase over time was only significant in the bifenthrin (not resoiled) treatment (F = 4.71, df = 7,45, P = 0.0005). Mobility scores did not increase significantly over time in either the control or control-resoiled treatments (F = 1.40, df = 7,45, P = 0.23; F = 1.01, df = 7,45, P = 0.44, respectively), and

Treatment	Ν	1 DA	2 DA	8 DA	16 DA	23 DA	30 DA	37 DA	65 DA
Control	16	0.02 (0.01)	0.02 (0.01)	0.01 (0.01)	0.08 (0.08)	0.23 (0.13)	0.34 (0.14)	0.48 (0.15) *	0.82 (0.22) *
Control- Resoiled	16	0.01 (0.01)	0.04 (0.02)	0.02 (0.01)	0.01 (0.01)	0.16 (0.11)	0.33 (0.14)	0.39 (0.18)	0.65 (0.32)
Bifenthrin	16	2.14 (0.23)**	2.36 (0.28)**	2.09 (0.23)**	2.32 (0.22)**	2.23 (0.23)**	2.17 (0.27)**	2.24 (0.26)**	3.10 (0.33)**
Bifenthrin- Resoiled	16	2.43 (0.13)**	2.48 (0.15)**	2.45 (0.18)**	2.20 (0.12)**	0.66 (0.23)*	0.63 (0.22)*	0.84 (0.29)*	1.30 (0.36)*

 Table 1 Mean (SE) mobility scores of A. obscurus placed in soil collected in 2009 from field plots treated 1 year previous with bifenthrin (Capture 2EC) or left untreated (Control)

At 16 days after insertion into cups (DA), half of all the samples were transferred to clean soil (Resoiled). N number of original cups. * Ismean is significantly different from 0 at P < 0.05, ** P < 0.0001, other values not significantly different P > 0.05

due to the resoiling decreased significantly in the bifenthrin-resoiled treatment (F = 29.18, df = 7,45, P < 0.0001).

Recovery was rapid when wireworms from the bifenthrin treatment were placed in clean soil (Table 1). Comparison of wireworm mobility between the resoiled treatments 1, 2, and 5 days after resoiling (i.e., at 17, 18, 21 DA) indicated that mobility scores decreased rapidly in the bifenthrin resoiled treatment [1.62 (SE = 0.25), 0.97 (0.20), 0.73 (0.20), respectively], compared to the control resoiled treatment [0.01 (0.01), 0.09 (0.08), 0.23 (0.10), respectively; F = 41.80 df = 1,30 P < 0.0001; F =13.01, df = 1,30, P = 0.0011; F = 5.04, df = 1,30, P = 0.032, respectively].

Mobility scores over time, 2010

All wireworms were scored as "A" and moved normally immediately prior to insertion into cups, and nearly all wireworms survived until the end of the study. Feeding wireworms placed in soil taken from untreated control plots continued to move normally until 30 DA (Table 2), at which time some (proportion = 0.34) were moving slowly and were scored as Alive-slow (AS). Some (proportion < 0.5) non-feeding wireworms placed in control soil were scored as AS on each observation day. Wireworms placed in soil taken from bifenthrin-treated plots showed symptoms of intoxication within 1 DA and were scored as Alive-clearly affected (AC) (proportion = 0.17, 0.54, 0.17for 100, 200, 300 g AI, respectively), "W" or Writhing upon stimulus (WR) (proportions = 0.75, 0.46, 0.83, respectively), and remained so over the course of the study. In general the level of intoxication increased with the rate of bifenthrin initially applied to the plots, although the wireworms exposed to residues from plots treated with 100 g AI appeared slightly more affected than those exposed to 200 g AI. On most observation days nonfeeding wireworms exposed to 200 or 300 g AI had higher mobility scores than feeding wireworms.

Analysis of mean mobility scores over time indicated that wireworm weight was not significantly affected by any of the treatments (discussed above), but that both treatment and feeding status were statistically significant (F =249.18, df = 3,127, P < 0.0001; F = 10.87, df = 1,127, P = 0.0013, respectively). There was no significant interaction between treatment and feeding status (F = 0.61, df = 3,127, P = 0.61), but between-treatment comparisons of Ismeans indicated a significant difference in mobility scores between feeding and non-feeding wireworms exposed to soil taken from plots treated with bifenthrin at 300 and 200 g AI (t = 2.44, df = 127, P =0.016 and t = 2.17, df = 127, P = 0.032, respectively), though not between those exposed to 100 g AI (t = 0.78, df = 127, P = 0.44) or the control treatment (t = 1.20, df = 127, P = 0.23).

Comparison of mobility scores of feeding wireworms indicated significantly higher scores in those exposed to soil treated with 100, 200, and 300 g AI bifenthrin than those exposed to control soil (t = 14.24, df = 127, P <0.0001; t = 14.19, df = 127, P < 0.0001; t = 17.21, df =127, P < 0.0001, respectively), and significantly higher scores of those exposed to 300 g AI than those exposed to either 200 or 100 g AI (t = 2.97, df = 127, P = 0.0035; t = 2.79, df = 127, P = 0.0062; respectively). Similarly, non-feeding wireworms exposed to soil treated with 100, 200, and 300 g AI bifenthrin scored significantly higher than those exposed to control soil (t = 13.87, df = 127, P < 0.0001; t = 15.00, df = 127, P < 0.0001; t = 18.19, df = 127, P < 0.0001, respectively), and those exposed to 300 g AI scored significantly higher than those exposed to either 200 or 100 g AI (t = 3.22, df = 127, P = 0.0016; t = 4.48, df = 127, P < 0.0001, respectively). There was no significant difference between those exposed to soil treated with bifenthrin at 100 or 200 g AI in either feeding or non-feeding wireworms (t = 0.16, df = 127, P = 0.87; t = 1.24, df = 127, P = 0.22, respectively).

Mobility score analysis also indicated a significant effect of time (F = 11.12, df = 6,127, P < 0.0001) and a significant interaction between time, treatment, and feeding status (F = 2.42, df = 18,127, P = 0.0023). Further inspection indicated that there was no significant change in

Table 2 Mean (SE) mobility scores of *A. obscurus* placed in soil collected in 2010 from field plots treated 1 year previous with three rates of bifenthrin (Capture 2EC) or left untreated (Control)

Treatment	Ν	1 DA	4 DA	7 DA	15 DA	22 DA	30 DA	50 DA
Control								
F	24	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.02 (0.02)	0.00 (0.00)	0.17 (0.17)	0.13 (0.09)
NF	24	0.25 (0.17)	0.04 (0.03)	0.17 (0.07)	0.11 (0.09)	0.11 (0.09)	0.11 (0.09)	0.18 (0.10)
100 g AI Bit	fenthrin							
F	24	1.39 (0.16)*	0.87 (0.13)*	1.17 (0.23)*	1.48 (0.14)*	1.26 (0.09)*	1.22 (0.17)*	1.41 (0.11)*
NF	24	1.77 (0.14)*	1.21 (0.12)*	1.15 (0.13)*	1.08 (0.13)*	1.15 (0.12)*	1.17 (0.12)*	1.67 (0.16)*
200 g AI Bit	fenthrin							
F	24	1.21 (0.23)*	1.17 (0.13)*	1.46 (0.21)*	1.00 (0.15)*	1.13 (0.14)*	1.13 (0.22)*	1.62 (0.22)*
NF	24	1.71 (0.18)*	1.63 (0.13)*	1.75 (0.22)*	1.17 (0.15)*	1.22 (0.14)*	0.96 (0.10)*	1.59 (0.17)*
300 g AI Bit	fenthrin							
F	24	1.44 (0.17)*	1.83 (0.22)*	1.79 (0.23)*	1.29 (0.15)*	1.29 (0.19)*	1.30 (0.16)*	1.36 (0.25)*
NF	24	2.42 (0.17)*	1.42 (0.18)*	1.92 (0.19)*	1.48 (0.15)*	1.37 (0.15)*	1.27 (0.13)*	1.87 (0.13)*

N number of wireworms, F feeding wireworms, NF non-feeding wireworms, DA days after wireworm insertion into cups. * Ismean significantly different from 0 at P < 0.0001, other values not significantly different P > 0.05

mobility scores over time in the control treatments with feeding and non-feeding wireworms (F = 0.28, df = 6,127, P = 0.95; F = 0.33, df = 6,127, P = 0.92, respectively), but that there was a significant decrease in scores over time in all other treatments.

Mobility score analysis further indicated that on occasion there were significant differences in mobility scores between treatment rows 2 and 4 (F = 26.73, df = 3,127, P < 0.0001), and that this effect varied with treatment (F = 23.99, df = 21,127, P < 0.0001). Inspection of lsmeans revealed that these differences were due to two rows from which samples were taken, one row in the 300 g bifenthrin AI (replicate 4, row 4), the other in the 200 g AI treatment (replicate 3, row 4). In both cases, the Ismean mobility score calculated from wireworms exposed to soil from this row was significantly (P < 0.0001) lower than the scores calculated from wireworms exposed to soil from any of the other rows of this treatment. This was true for both feeding and non-feeding wireworms. The low and variable scores of wireworms exposed to soil taken from these rows also explained why the analysis suggested significant differences among cups within a row (F = 5.37, df = 4, 127, P = 0.0005), which differences varied with treatment (F = 3.20, df = 28,127, P < 0.0001).

Surface wireworms

In the 2009 study, wireworms were observed on the surface of soil taken from bifenthrin-treated plots within 1 DA of being placed in the containers, and continued to surface despite being reburied each observation date (Table 3). Wireworms on the surface were Writhing; those more affected did not appear capable of movement to the surface and those less affected could move down again. Wireworms placed in clean soil stopped coming to the surface within 7 days of resoiling in the bifenthrin-treated plots, and there was no significant difference in the proportion on the surface between the resoiled control and resoiled bifenthrin treatments as early as 2 days after resoiling (F = 1.00, df = 1,30, P = 0.33). In contrast, the proportion on the surface generally increased over time in the non-resoiled bifenthrin treatment (Table 3). Wireworms placed in control soil did not surface.

In the 2010 study, wireworms were observed on the surface of soil taken from bifenthrin-treated plots within 1 DA of being placed in the containers, and continued to surface despite being reburied each observation date (Table 4). For all three rates of bifenthrin, the proportion of wireworms on the surface of the soil was higher in the cups with non-feeders than with feeders (significant at the 300 g AI rate at 1, 4, 15, and 30 DA). The proportion of both feeders and non-feeders on the surface appeared to increase with rate of bifenthrin, numerically more wireworms surfacing when exposed to 300 g than when exposed to either 100 or 200 g AI on most dates. As in 2009, wireworms on the surface were generally Writhing and wireworms placed in soil from control plots did not surface.

Observational studies

Behavior in the presence of an attractant

Seventeen of 25 wireworms exposed in arenas in which the centre area (Fig. 1a) contained soil from control plots

Table 3 Mean (SE) proportion of *A. obscurus* that surfaced in cups filled with soil collected in 2009 from field plots treated one year previous with bifenthrin (Capture 2EC) or left untreated (Control)

Treatment	Ν	1 DA	2 DA	8 DA	16 DA	23 DA	30 DA	37 DA	65 DA
Control	16	0.00 (0.00) A	0.05 (0.03) AB	0.00 (0.00) A	0.00 (0.00) A	0.00 (0.00) A	0.00 (0.00) A	0.00 (0.00) A	0.00 (0.00) A
Control- Resoiled	16	0.00 (0.00) A	0.00 (0.00) A	0.00 (0.00) A	0.00 (0.00) A	0.00 (0.00) A	0.00 (0.00) A	0.00 (0.00) A	0.02 (0.02) A
Bifenthrin	16	0.17 (0.05) B	0.17 (0.06) BC	0.39 (0.09) B	0.34 (0.07) B	0.19 (0.05) B	0.46 (0.10) B	0.42 (0.09) B	0.41 (0.11) B
Bifenthrin- Resoiled	16	0.27 (0.07) B	0.30 (0.07) C	0.34 (0.09) B	0.27 (0.05) B	0.00 (0.00) A	0.00 (0.00) A	0.00 (0.00) A	0.00 (0.00) A
ANOVA statistics df = 3,60		F = 9.68 P < 0.0001	F = 8.58 P < 0.0001	F = 8.07 P < 0.0001	F = 17.16 P < 0.0001	F = 12.06 P < 0.0001	F = 15.47 P < 0.0001	F = 14.26 P < 0.0001	F = 11.45 P < 0.0001

DA days after wireworm insertion into cups. At 16 DA half of all the samples were transferred to clean soil (Resoiled). Values followed by the same letter in each column are not significantly different ($\alpha = 0.05$)

Table 4 Mean (SE) proportion of *A. obscurus* that surfaced in cups filled with soil collected in 2010 from field plots treated 1 year previous with three rates of bifenthrin (Capture 2EC) or left untreated (Control)

Treatment	Ν	1 DA	4 DA	7 DA	15 DA	22 DA	30 DA	50 DA
Control								
F	8	0 (0) A	0 (0) A	0 (0) A	0 (0) A	0 (0) A	0 (0) A	0 (0) A
NF	8	0 (0) A	0 (0) A	0 (0) A	0 (0) A	0 (0) A	0 (0) A	0 (0) A
100 g AI Bif	enthrin							
F	8	0.22 (0.09) AB	0.04 (0.04) A	0.16 (0.06) B	0.08 (0.08) A	0.04 (0.04) A	0 (0) A	0.12 (0.06) AB
NF	8	0.33 (0.08) ABC	0.16 (0.06) A	0.12 (0.06) AB	0 (0) A	0.12 (0.08) AB	0.08 (0.05) AB	0.12 (0.06) AB
200 g AI Bif	enthrin							
F	8	0.08 (0.05) AB	0.08 (0.05) A	0.04 (0.04) AB	0.04 (0.04) A	0 (0) A	0 (0) A	0.04 (0.04) A
NF	8	0.29 (0.13) BC	0.08 (0.05) A	0.12 (0.06) AB	0.08 (0.05) A	0.08 (0.05) AB	0.16 (0.08) BC	0.12 (0.06) AB
300 g AI Bif	enthrin							
F	8	0.20 (0.12) AB	0.12 (0.06) A	0.12 (0.08) AB	0.08 (0.05) A	0.16 (0.06) AB	0.08 (0.05) AB	0.29 (0.07) B
NF	8	0.54 (0.12) C	0.41 (0.12) B	0.12 (0.06) AB	0.25 (0.08) B	0.20 (0.10) B	0.25 (0.10) C	0.20 (0.10) B
ANOVA stat	istics							
Treatment, $df = 3,56$		F = 4.67, P = 0.0056	F = 5.97, P = 0.0013	F = 2.64, P = 0.058	F = 3.50, P = 0.021	F = 3.60, P = 0.019	F = 3.20, P = 0.03	F = 5.74, P = 0.0017
Feeding, $df = 1,56$		F = 4.65, P = 0.035	F = 5.29, P = 0.025	F = 0.04, P = 0.83	F = 0.65, P = 0.42	F = 1.70, P = 0.20	F = 6.93, P = 0.01	F = 0.00, P = 0.97
Treatment \times feeding, df = 3,56		F = 0.89, P = 0.45	F = 2.65, P = 0.058	F = 0.44, P = 0.73	F = 2.04, P = 0.12	F = 0.22, P = 0.88	F = 1.06, P = 0.37	F = 0.51, P = 0.68

N number of cups, *F* feeding wireworms, *NF* non-feeding wireworms, *DA* days after wireworm insertion into cups. Values followed by the same letter in each column are not significantly different ($\alpha = 0.05$)

approached and entered the centre area and proceeded to the seeds during the 3 h observation period. Of these, 14 remained for >30 min and 1 left within 5 min. Similarly, in arenas in which the centre area was filled with soil from bifenthrin-treated plots, 14 of 25 wireworms approached the centre area, of which 12 proceeded into the soil and 2 stayed at the interface of the two soils and then retreated. Wireworms that entered the treated soil remained in it for >30 min, but none proceeded to the seeds.

By 24 h, 21 out of 25 wireworms in the control treatment were in the centre area, all of which were in contact with the seeds. It was obvious from their burrows (Fig. 2a) that all but three wireworms had entered the centre area at some point in 24 h. In arenas with bifenthrin treated soil only in the centre area, two wireworms were in the centre area, four at the interface of the soil types (in contact with both soils), and none in contact with the seeds after 24 h. However, based on their burrows, most (17/25) wireworms had entered the centre area at some point in 24 h, and nearly all wireworms had made multiple approaches to the bifenthrin treated soil, often following the interface between the two soil types, indicating reluctance to enter (Fig. 2b).

The two wireworms found in the bifenthrin treated soil and 2 (of 4) found on the interface of the two soils were scored as "W" or "WR"; the other 2 were scored as "A". Most wireworms exposed to arenas with bifenthrin treated soil exhibited morbidity effects at 24 h [scores: 7A, 4AC, 11 W, 2WR, 1 M]. In contrast, nearly all wireworms exposed to the control soil alone were unaffected [scores: 23A, 1AS, 1D], causing a significant difference in mobility scores from those exposed to bifenthrin treated soil (mean = 1.46 (SD = 1.17), 0.22 (1.00), respectively;t = 4.02, df = 48, P = 0.0002). At 7 DA most of the wireworms exposed to arenas with bifenthrin treated soil had fully recovered and their mobility was similar to those exposed to control soil [19A, 2AS, 3AC, 1 W; 16A, 6AS, 3D, respectively], eliminating significant differences in numerical scores [0.24 (0.50), 0.72 (1.63), respectively; t = 1.39, df = 48, P = 0.17]. The three dead wireworms in the control treatment died from Metarhizium infection and were the ones that had not responded to the wheat.

Behavior in the absence of an attractant

Twenty-one of 25 wireworms exposed to soil from control plots alone entered the "treated" side (side in arena opposite release point) at least once during the 3 h observation, and 9 of 25 larvae were found there at 24 h (Fig. 1b). While nearly all (24/25) wireworms had entered the side with bifenthrin treated soil at least once during the 3 h observation period, their behavior was considerably

different from control wireworms and appeared to follow four patterns. Some (9) wireworms showed no recognition of the bifenthrin residues and entered normally (i.e., just as the control wireworms entered their "treated" soil). Of these, five retreated to the control soil after 30-60 min and became immobile, and four continued to move apparently unaffected. Some (7) appeared to recognize the treated soil too late, entering 1-2 cm before stopping. Of these, three stopped entirely and became immobile in the treated soil and the other four moved around slightly, before retreating and leaving. Some (7) appeared to recognize the treated soil prior to entering it, approaching it normally but turning abruptly before entering. Of these, five turned at the interface of the two soil types and moved along it for approx. 60 min before either leaving (4), entering and becoming immobile (1), or turning away completely at first approach but entering the treated soil upon their second approach (2). Finally, one wireworm appeared to show learned recognition, entering the bifenthrin treated soil briefly (20 min) upon first approach, but then leaving and moving along the interface without entering it upon its second approach. At 24 h, 10 of 25 were positioned in the bifenthrin treated soil, all of which were scored as "W" and were immobile.

All larvae exposed to control soil were scored as "A" at 24 h [score = 0.00 (0.00)], and 23 were "A" and 2 "AC" after 1 week [0.04 (0.14)]. In contrast, larvae exposed to bifenthrin treated soil were either "W" (21) or "AC" (4) at 24 h [1.84 (0.37); t > 100, df = 48, P = 0.00]. After 1 wk these wireworms had recovered after placement in cups with untreated soil: 22 were "A", 1 "AS", and 2 "AC" [0.10 (0.29); t = 0.93, df = 48, P = 0.36].

Soil analyses

Treated soil samples (2008) collected for residue analysis in 2009 (329 DAP) contained a mean of 1057.5 (SD = 68.23) ng bifenthrin/g soil (wet weight) [872.25 (62.98) ng/g soil (dry weight)]. As bifenthrin tested in efficacy studies in the field was applied as a 15 cm-band in-furrow spray

Fig. 2 Pictures of wireworm burrows in observation arenas (top cover removed) containing in the centre area soil from the control plots (a) or soil from plots treated with bifenthrin (Capture 2EC) the previous year (b). See Fig 1a for soil distribution



(Capture 2EC) in potato rows planted 1.0 m apart, we can estimate that in the field ~112,500 l soil/ha would have contacted the insecticide spray if we assume that the product can penetrate up to 7.5 cm (Victoria R. Brookes, personal communication). As 1L PARC soil weighs ~1 kg, this means that applying 340 g AI/ha is equivalent to applying 3.0222×10^{-6} g AI bifenthrin/1 g soil. Since the amount of bifenthrin found was 1.0575×10^{-6} g AI/g soil, this means 35.0% of what was applied was still in the soil, equivalent to 119 g AI/ha. No detectable amounts of bifenthrin were found in the control samples.

Discussion

Placement of wireworms in field soil with residual bifenthrin

The level of immobility induced in healthy wireworms placed in cups with soil collected in 2009 from plots treated with 340 g AI/ha bifenthrin in 2008 was similar to that of wireworms placed in soil freshly amended with bifenthrin at 100 g AI/ha in a laboratory study (van Herk and Vernon 2011), indicating that ~ 100 g AI/ha bifenthrin remained in the field soil after one year of degradation. This predicted value of residual bifenthrin was remarkably similar to the value subsequently obtained from the soil analyses, suggesting that in some circumstances the numerical values of wireworm mobility can be used to quantify the amount of chemical left in the soil.

The rapid recovery of moribund wireworms when placed in clean soil was similar to that of wireworms moved to clean soil after morbidity induced by soil freshly amended with bifenthrin (van Herk and Vernon, 2011), and suggests that while the bifenthrin residues kept the wireworms in a moribund state and therefore susceptible to predation or mortality induced by secondary pathogens, they were not sufficient to kill wireworms directly. For example, most wireworms that died after exposure to bifenthrin field soil showed clear signs of Metarhizium. As Metarhizium equally killed wireworms in the control treatments (proportion = 0.05-0.06 of wireworms exposed), contact with bifenthrin residues itself likely did not affect wireworm mortality from Metarhizium. This conclusion is supported by results from the 2010 study, in which a total of two wireworms died from Metarhizium. All other wireworms that died in the 2010 study were killed by Mermithid nematodes, a common endoparasite of A. obscurus found in long-term pasture in Agassiz (WvH, personal observation).

Wireworms exposed to soil collected from bifenthrin treatments applied at three rates in 2009 also exhibited a rapid decrease in mobility and also remained moribund as long as they were kept in the soil. This confirms the results of the 2009 study and indicates that there was enough residual bifenthrin in the plots treated even with the lowest rate, 100 g AI, to induce illness in wireworms nearly one year later, despite periods of flooding in the plots.

While wireworm weight did not appear to affect the degree of intoxication over time, non-feeding wireworms were significantly more affected by bifenthrin residues than feeding wireworms, a difference that became more pronounced as the insecticide rate increased. This was possibly because non-feeding wireworms had either just completed a molt, or were preparing to molt, at which times wireworms do not feed (Furlan 1998). Wireworms that have recently molted appear to have softer cuticles (WvH, personal observation), and larvae of A. obscurus that are preparing to molt have increased spaces between the mediotergite and mediosternites on their abdominal segments, exposing the pleural membranes (WvH, personal observation). In both cases, dermal absorption of bifenthrin residues by the wireworm is likely to increase. Non-feeding wireworms may also be less active than feeding wireworms and thus move less through the soil. Wireworm handling was probably not responsible for the increased loss of mobility, as the apparent differences in the mobility of feeding and non-feeding wireworms exposed to soil from the untreated control plots were not significant.

While for both feeding and non-feeding wireworms mobility scores were consistently higher in the 300 than the 100 and 200 g AI rates in the 2010 study, the overall higher scores of wireworms exposed to 100 than 200 g AI resulted from the consistently lower scores for both feeding and non-feeding wireworms exposed to soil taken from one of the rows in the 200 g bifenthrin AI than the other three rows. To a lesser extent this also occurred in the 300 g treatment, explaining why there was a significant effect of row and row \times treatment, and perhaps reflecting a variable rate of bifenthrin degradation in the field (Fecko 1999).

A considerable number of wireworms moved to the surface of the soil in both the 2009 and 2010 studies, and remained there in a moribund state. These wireworms were reburied into the soil after every observation and often reappeared at the soil surface later, confirming that moribund wireworms are capable of vertical movement in the soil. The writhing motions may themselves be a mechanism for moribund wireworms to remove themselves from a substance that induced morbidity. Previous work has indicated that wireworms in the Writhing stage are unable to burrow back into the soil, and that less motile wireworms (e.g., those exposed to higher concentrations of bifenthrin) are unable to come to the soil surface (van Herk and Vernon, 2011). This suggests that wireworms may be unable to move to the soil surface directly after application of a high rate of bifenthrin (e.g., 300 g AI), but will begin

to surface after the product has partly broken down and the wireworms have become more motile.

The presence of "Writhing" wireworms on the soil surface exposes them to the risk of predation by birds. Wireworms found on the surface of the soil in the field plots in 2009 soon disappeared, and thereafter reappeared as partial carcases in bird feces in the same plots (WvH, personal observation). Considering bifenthrin's high bioconcentration factor (Fecko 1999), it is likely that the chemical is readily absorbed by wireworms as they move through soil containing residues, in which case the presence of wireworms that have absorbed bifenthrin residues on the soil surface may provide some risk for the compound's bioaccumulation in birds. This concern merits further examination, particularly as little is known about the effect of residual bifenthrin on other insect species that might also be scavenged in the field.

Observational studies

Wireworms approached wheat seeds placed in the centre of soil arenas equally, whether or not soil surrounding the wheat seeds came from untreated or bifenthrin-treated field plots, indicating that bifenthrin residues did not elicit longrange repellency. This behavior is similar to that observed for wireworms exposed to wheat seeds treated with tefluthrin, another pyrethroid insecticide (van Herk and Vernon 2007; van Herk et al. 2008). Wireworms that entered control soil proceeded to the seeds and exhibited normal feeding behavior, most still being at the seeds at 24 h, but those that entered the soil containing bifenthrin residues did not continue to the seeds. While most of the wireworms had entered the soil in the centre area at some point in 24 h, only those immobilized remained there. It appeared from the burrows that wireworms made multiple approaches to the treated soil, often entering slightly before retreating. These findings suggest that the onset of morbidity induced by the residues is a cue for the wireworms to retreat from the area, a behavior previously documented for wireworms exposed to tefluthrin and thiamethoxam (van Herk and Vernon 2007; van Herk et al. 2008). For effective wireworm management, wireworms must be able to accumulate sufficient product to kill them before the onset of morbidity causes them to retreat. Previous work has shown that this sometimes causes a lower rate of insecticide to be more effective than a higher rate, though the most effective rate may vary with wireworm species (van Herk et al. 2008). The rapid recovery of wireworms after removal from the treated soil indicates that the level of bifenthrin contacted in the observation arenas after 24 h was not sufficient to induce mortality.

Wireworms exposed to soil containing bifenthrin residues in the absence of an attracting cue (i.e., CO_2) were

expected to show more repellency than those exposed in the presence of wheat seedlings. However, most wireworms entered the treated soil in the absence of wheat seedlings and did not retreat until shortly before morbidity signs became evident. All wireworms that moved away from the treated soil after approaching it during the 3 h observation made direct contact with the soil before moving away, again indicating the absence of long range repellency. While wireworm behavior was variable, those that turned upon reaching the treated soil and/or that followed the interface between the two soil types are of interest, as they demonstrate wireworms may be able to detect deleterious compounds in the soil in time to avoid them. The higher repellency observed in the absence of wheat also confirms that the presence of an attractive cue may over-ride a deterrent response (van Herk et al. 2010). Finally, while all wireworms exposed to soil containing bifenthrin residues were moribund by 24 h, this was likely due to the limited space for the wireworms to move in 24 h, which ensured repeated contact with the residues.

Implications for control of wireworms in the field

The low levels of wireworm damage to potatoes, and the virtual absence of wireworms in bait traps placed in bifenthrin-treated plots (but not in untreated plots) the spring following the year of application, might initially suggest that high mortality of wireworms had occurred. The results from the soil container and observational studies, however, suggest that other explanations than mortality alone should be considered. Bait traps were placed directly into the areas of former potato rows that were initially treated with bifenthrin, the soil of which was proven to have residues sufficient to illicit morbidity and repulsive behaviors in the lab. The low number of wireworms taken in bait traps, therefore, may simply be due to the inability of surviving wireworms to move through the surrounding bifenthrin treated soil and enter traps without becoming moribund. Moribund wireworms discovered on the soil surface during the time of bait trapping would have been survivors from the year previous that exhibited the surfacing behavior described in the current studies. During the growing season when bifenthrin was applied, wireworms were likely either repelled from the treated furrows or rendered moribund periodically after exposure to the treated area, which would account for the low damage occurring to mother and daughter tubers observed in the 2008 and 2009 field studies. Since significant mortality of wireworms exposed continuously (for 50 or 65 days) to bifenthrin residues in soil containers did not occur in these studies, levels of mortality in field soil should also not be high, since wireworms could escape to untreated surrounding soil and recover. In harvested potato fields that would typically be re-planted with other crops in the following spring, it is possible that surviving wireworms could feed on these crops in planted areas where bifenthrin residues were low or absent.

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