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ORIGINAL PAPER

Categorization and numerical assessment of wireworm mobility over time following exposure to bifenthrin

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Abstract We discuss the effect of the pyrethroid insecticide bifenthrin incorporated into soil at 100, 200, and 300 g AI/ha on late-instar larvae of the dusky wireworm, *Agriotes obscurus* (Coleoptera: Elateridae), and present a method of assessing wireworm health and mobility over time. Wireworms became moribund within 1 h of placement in soil amended with bifenthrin at all rates. After 2 weeks of morbidity in amended soils, wireworms recovered within 7 days of being placed in clean soil. A considerable proportion (0.13–0.93) of wireworms placed in amended soil moved to the soil surface and remained there for 2 weeks or more in a moribund state; wireworms transferred to clean soil no longer moved to the soil surface. Eight distinct mobility categories were observed and are described, and a new method for assessing wireworm health and mobility over time is discussed.

Keywords *Agriotes obscurus* · Insect behavior · Insecticide · Morbidity · Pyrethroid · Pest control

Introduction

The general increase in wireworm-related crop damage in North America (Vernon et al. 2001; Kuhar and Alvarez 2008) and Europe (Parker and Howard 2001), and the removal of effective organophosphate (OP), organochlorine (OC), and carbamate (C) insecticides from Canada and elsewhere, has prompted an increase in research activities to identify effective, lower-risk chemicals for wireworm management. In recent years, we have conducted both laboratory and field studies with pyrethroid, neonicotinoid, phenyl pyrazole, and other classes of insecticides, to assess their ability both to kill wireworms and/or protect key crops such as potato and wheat from damage. Our research has indicated that some insecticides (e.g., the pyrethroid tefluthrin) elicit repellency in wireworms (van Herk and Vernon 2007a; van Herk et al. 2008b), and that it is repellency, rather than mortality that protects tefluthrin-treated wheat seed from wireworm damage (Vernon et al. 2009). We have also shown that neonicotinoid insecticides (i.e., imidacloprid, clothianidin, and thiamethoxam) applied to wheat seed will provide stand and yield protection without reducing wireworm populations by inducing reversible, long-term (>150 day) morbidity/intoxication (van Herk et al. 2007, 2008a; Vernon et al. 2008). Wireworms exposed through contact with low doses of the phenyl pyrazole, fipronil, have been shown to remain symptomless for several weeks after exposure (during which time feeding damage can occur) before becoming moribund and dying (van Herk et al. 2008a; Vernon et al. 2008). These findings, collectively, indicate that protection of crops from wireworm damage with contemporary insecticides cannot automatically be equated with wireworm mortality, or vice versa. This is an important finding, in that formerly used OP, OC, and C insecticides have been shown to directly prevent wireworm damage through wireworm mortality (Lange et al. 1949; Lane 1954; Edwards and Thompson 1971; Vernon et al. 2009). Since wireworms often take several years to reach maturity in the soil (Furlan 1998, 2004; Parker and Howard 2001), the survival of populations in crops treated with non-lethal insecticides (i.e., pyrethroids and neonicotinoids) would present

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an ongoing threat to subsequent crops (Vernon et al. 2009), and require continuing treatments. To fully understand the overall effectiveness of contemporary insecticides on wireworms, therefore, it is essential to study their lethal, sublethal, and behavioral effects, and to do so over a prolonged period of time (Vernon et al. 2008, 2009). In previous studies, we have described a number of bioassays to study the repellency (van Herk and Vernon 2007a; van Herk et al. 2008c), and toxicity (van Herk and Vernon 2007b) of insecticides to wireworms, and have developed an approach for rating their short- and long-term effects on wireworm health and mobility (Vernon et al. 2008).

In this article, we present the results of a laboratory study in which wireworms were placed in soil amended with bifenthrin, a pyrethroid insecticide under consideration as an in-furrow spray for wireworm control in potato in Canada, and which is currently registered for this purpose in the USA. These results are used to expand the wireworm health and mobility criteria defined by Vernon et al. (2008), and numerical values have been assigned to the mobility categories to facilitate comparisons of wireworm mobility between various treatments over time. This study was conducted with bifenthrin to confirm recent field study observations (MS in prep.) that this chemical can cause wireworms to come to the soil surface and exhibit symptoms of morbidity up to 1 year after the chemical was applied.

Materials and methods

Wireworms

Wireworms (*Agriotes obscurus)* were collected from a corn field at the Pacific Agri-Food Research Centre (PARC) in Agassiz, B.C., in April–May 2009 and stored in 60 l tubs of untreated soil at 10°C until needed. Feeding wireworms were isolated from these tubs with bait traps composed of a vermiculite–wheat mixture soaked in water 1–2 days before being used in the study, weighed individually with an analytical balance (Denver Instruments, model SI-403), and checked for health and mobility. Only healthy (mobility score = 0, see below) wireworms weighing 20–40 mg and >15 mm long (i.e., 3–4 years old; Subklew 1934) were used. Wireworms were randomly allocated to 64 150 ml plastic containers (Fisher Scientific, Ottawa, ON) filled with 150 g soil. The study contained 8 treatments, each replicated 8 times: the soil in the containers was amended with amounts of bifenthrin (Capture 2EC), equivalent proportionately to 100, 200, or 300 g bifenthrin AI/ha (for treatments 3 and 4, 5 and 6, 7 and 8, respectively), or was not amended (treatments 1 and 2). Four wireworms were placed in each container, and containers stored at 15°C for the duration of the experiment except during observations when they were transferred to room temperature (approx. 2–4 h). During initial placement and after each health and mobility assessment, wireworms were placed in a 3-cm deep hole made in the center of the cup.

Soil amendment and clean soil

Soil was collected from a fallowed, former field of untreated pasture at PARC, Agassiz, and consisted of a sandy clay loam (43% sand, 44% silt, and 13% clay) containing approx. 5% organic material. Collected soil was screened through a 2 × 2 mm mesh to remove coarse organic material and rocks, brought to 20% soil moisture by weight, and homogenized. As Capture 2EC (25.1% bifenthrin) tested in efficacy studies in the field is applied as a 15 cm banded in-furrow spray on potato rows planted 1.0 m apart, we estimated that in the field approx. 112,500 l soil/ha would contact the insecticide spray if we assume the product can penetrate up to 7.5 cm (Victoria R. Brookes, personal communication). This estimate was used to amend soil to 100, 200, and 300 g AI/ha rates by applying 0.10, 0.20, and 0.30 ml Capture 2EC, respectively, to 30 l batches of soil. These volumes of Capture 2EC were dissolved into 10.0 ml water and the resulting solution spread uniformly on soil spread 7.5 cm high. Immediately after spray application, the soil was mixed thoroughly.

Storage, mobility checks, and resoiling

Wireworm health (hereafter: mobility) was checked at 1, 2, 4, 7, 15, 22, and 29 days after (DA) placement in the cups with amended and non-amended soil. At 15 DA, wireworms in treatments 2, 4, 6, and 8 were placed in new 150 ml cups (as above) filled with non-amended soil (screened and prepared as above) immediately after mobility checks. Mobility checks of these "resoiled" wireworms continued as above and were also conducted at 16 and 18 DA (i.e., 1 and 3 days after placement in the clean soil). Wireworms in treatments 1, 3, 5, and 7 were kept in their original soil until 29 DA. As the wireworms were exposed to the same soil in treatments 1, 3, 5, and 7 as those in treatments 2, 4, 6, and 8, respectively, the study can be seen as containing 4 treatments (replicated 16 times) prior to the resoiling event (below) and 8 treatments (replicated 8 times) thereafter.

Mobility categories and numerical values

Wireworm mobility was recorded using eight categories based on their ability to move when placed on moist filter paper in a 10-cm Petri dish (described in Table 1). Generally, only 10–30 s was required to determine a wireworm's mobility category; observations did not exceed 5 min per larvae. Two of the 8 mobility categories were not

Table 1 Description of wireworm mobility categories and numerical values ascribed to categories for analyses

Category name	Abbreviations	Description	Numerical value
Alive	A	Wireworm is capable of spontaneous, normal movement and appears unaffected. Wireworm moves to the outside of a 10-cm Petri dish within 2 min of being placed in the center	0
Alive-slow	AS	Wireworm is capable of spontaneous, normal movement but moves slowly (requires 2–5 min to make it to outside of 10-cm Petri dish if placed in center)	0.5
Alive-clearly affected	AC	Wireworm is capable of spontaneous, normal movement but cannot do so continuously for 2 min without falling over. Wireworm may also move very quickly and move head hyperactively from side-to-side	1
Writhing	W	Wireworm not capable of normal movement and does not leave center of Petri dish but makes spontaneous twisting movements of entire body; often bends body into "C" or corkscrew shape	2
Writhing upon stimulus	WR	Similar to the "Writhing" category, but wireworm only makes full body movements in response to a stimulus (i.e., gentle prodding)	3
Leg and mouthpart movement	LM	Wireworm unable to make writhing movements but is capable of moving both legs and mouthparts; may require prodding to elicit movement	4
Mouthpart movement	M	Wireworm capable of moving mouthparts only; may require prodding to elicit movement	4.5
Dead	D	Wireworm is dead, as obvious from decomposition and/or mycelial growth	5

previously described (Alive-slow and Alive-clearly affected), and two others (Writhing and Writhing upon stimulus) were created from the "writhing" category described in Vernon et al. (2008). Wireworm mobility was graded according to new numerical values (0–5) ascribed to each of the 8 categories (Table 1). Wireworms that were incapable of any movement but did not appear decomposed were scored as "Probably dead," and a numerical value was subsequently ascribed to these wireworms based on their mobility at the following observation day—either 4.5 if the wireworm subsequently showed any sign of life, or 5 if it was dead. Wireworms that were scored as "Dead" were not returned to the cups after mobility checks. A numerical scale for wireworm movement has previously been described by Kring (1959) for larvae of *Limonius agonus* Say, but this scale is based on speed of movement and not on wireworm health, coordination, or level of intoxication or immobility.

Statistical methods

Statistical analyses were conducted using SAS 9.2 (SAS Institute). Normality of data was assessed with the UNIVARIATE procedure prior to analysis of (co)variance.

Mobility scores

To compare mobility scores among treatments, individual wireworm scores were first averaged per cup (4 wireworms per cup) and cup averages used for analyses, since individual wireworm mobility scores could not be compared between observations. Wireworm mobility scores over time were compared among treatments with repeated measures ANOVA using Proc MIXED with an unstructured covariance matrix. Comparisons (determined a priori) were set up with the estimate function among the following (groups of) treatments: 0 and 100, 200, 300 g AI/ha; 100 and 300 g; 0 g (resoiled) and 100 g (resoiled), 200 g (resoiled), 300 g (resoiled); 0 and 0 g (resoiled); 100 and 100 g (resoiled); 200 and 200 g (resoiled); and 300 and 300 g (resoiled). Separate analyses were conducted using mobility scores that included dead wireworms in the cup and mobility scores that did not. The proportion of dead wireworms at 29 DA was compared among treatments with chi-square analyses.

Wireworm weights

Mean wireworm weights were calculated per cup using weights of individual living wireworms and were compared among treatments with ANOVA. To determine whether wireworm weight affected mobility scores, mean wireworm weights per cup were regressed against mean mobility scores per cup using ANCOVA, including "treatment" as a variable and wireworm weight as a covariate. This analysis was conducted for mobility scores at 1, 2, 4, 7, and 15 DA (i.e., all mobility checks prior to resoiling) on combined data from similar treatments (i.e., treatments 1 with 2; 3 with 4, etc.). Mean wireworm weights were also included as a covariate in the mobility score analyses (above).

Surface wireworms

The proportion of wireworms on the soil surface was normalized with an arcsine transformation and compared among treatments with ANOVA, followed by mean separation with the REGWQ procedure, at 1, 2, 7, 15, 22, and 29 DA. As dead wireworms were removed from cups, the proportion per cup calculation changed over time in some cups.

Results

General observations

Mean wireworm weights per cup ranged from 29.5 (SEM = 0.99) to 32.7 mg (SEM = 1.21) and did not vary significantly among the eight treatments ($F = 0.85$, $df = 7.56$, $P = 0.55$). Including wireworm weight as a covariate in mobility score analyses indicated that it had no significant effect on wireworm mobility scores ($P > 0.8$), and so wireworm weight was not included in subsequent analyses.

Wireworm mortality

Mortality of wireworms generally began between 7 and 15 DA, and gradually increased until a small proportion of wireworms in all treatments had died (range: 0.13–0.31; Fig. 1) by the end of the experiment (29 DA). A high proportion of wireworms that died in each treatment (range: 0.67–1.00) displayed mold formation on the cadaver typical of *Metarhizium anisopliae* infection, suggesting this was the predominant, and likely the only, cause of death in all treatments. None of the wireworms appeared to have died from bifenthrin. Since wireworms that die from *M. anisopliae* infection often show no visible ill effects (i.e., mobility score = 0; WvH personal observation) until 3–4 days before dying, and as *M. anisopliae* infection was not evident and wireworm mortality did not occur until the latter half of the experiment (22–29 DA) in all treatments (Fig. 1), and as mortality occurred equally in control and amended soil treatments (Chi = 5.69, $df = 7$, $P = 0.58$), we did not consider *M. anisopliae* infection to have confounded the overall results. However, the presence of *Metarhizium* caused us to exclude mobility observations taken after 29 DA (i.e., at 38 and 50 DA), though inclusion of this data (not shown) did not alter the results of the statistical tests. The high levels of *M. anisopliae* infection in this study coincided with a rapid decline of wireworm stocks in the storage tubs due to *M. anisopliae,* both possibly been triggered by moving the tubs from storage to room temperature (approx. 24°C) without appropriate acclimatization. *Agriotes obscurus* collected at PARC appear to carry *M. anisopliae* spores but require an environmental stressor to trigger the infection (Kabaluk and Ericsson 2007). Based on the study temperature and low mortality until the end of the study, this infection was triggered prior to the wireworm selection (Kabaluk and Ericsson 2007).

Rapid induction of morbidity and rapid recovery when resoiled

Symptoms of morbidity were evident within 1 h in most cups containing soil amended with bifenthrin at all rates (data not shown), and most wireworms in amended soil were in the "Writhing" categories at 1 DA (Fig. 1c–h). The majority of wireworms remained in the "Writhing" categories at all three rates, unless they were transferred into clean soil or ultimately died due to *M. anisopliae* infection (Fig. 1c–h). When wireworms were transferred to clean soil, there was a marked increase in the proportion of wireworms in the "Alive-slow" and "Alive-clearly affected" categories, and a rapid decrease in the proportion in the "Writhing", "Leg and Mouthpart," and "Mouthpart" categories by the following day (Fig. 1d, f, h), indicating wireworms were recovering from morbidity. By 18 DA, a considerable proportion of wireworms placed in clean soil had recovered from previous exposure to all three rates of bifenthrin, though the speed of recovery clearly decreased as the insecticide concentration increased (Fig. 1d, f, h). Unlike the non-resoiled treatments, there were almost no wireworms in the "Leg and Mouthpart" and "Mouthpart" categories after 18 DA, or in the "Writhing" categories after 22 DA (Fig. 1d, f, h). There was no evident change in mobility of wireworms exposed to 0 g when placed in clean soil, indicating that the resoiling process itself had no adverse effect on wireworm mobility or health (Fig. 1b).

Mobility scores over time

When only the living wireworms in each cup were included in the analyses, wireworm mobility scores remained below 0.2 over the course of the experiment in the two control

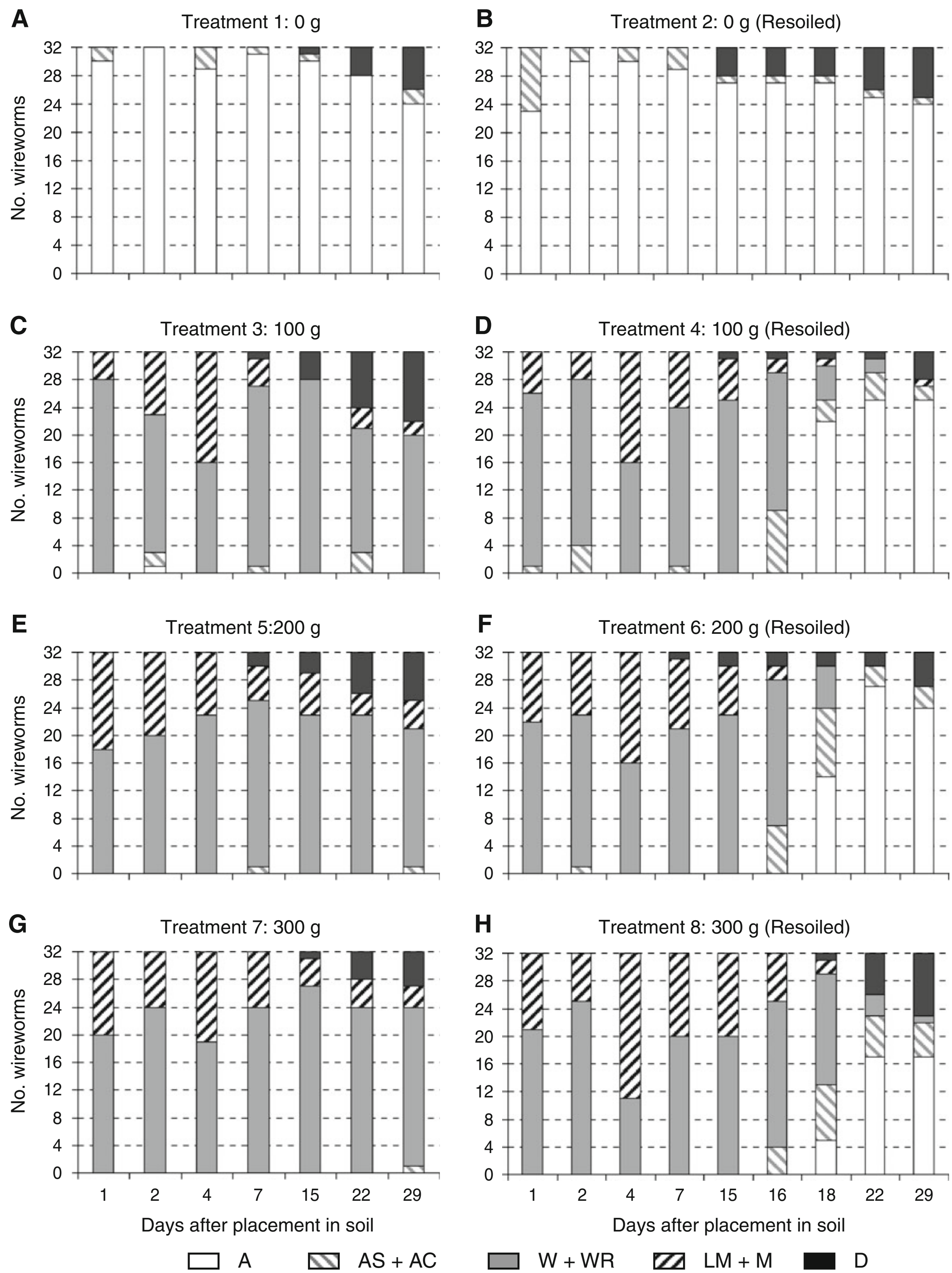

Fig. 1 Mobility of wireworms at various days after placement in soil amended with Capture 2EC, containing bifenthrin. Number following treatment numbers shows g bifenthrin AI/ha. Wireworms in treatments marked "Resoiled" were placed in clean soil after the 15 DA measurement. See Table 1 for explanation of wireworm mobility categories listed in legend

treatments (Table 2, t: 1, 2) and remained in the 2–3.5 range during the time wireworms were in contact with bifenthrin-amended soil (Table 2, t: 3–8). Analysis of variance indicated significant differences in wireworm mobility scores between some treatments ($F = 228.97$, $df = 7.56$, $P < 0.0001$). Mobility scores in control

Table 2 Mean (SEM) mobility scores of living wireworms obtained at various days after (DA) placement in soil amended with Capture 2EC, containing bifenthrin

Treatment: rate	1 DA	2 DA	4 DA	7 DA	15 DA	22 DA	29 DA
1: 0 g	0.03 (0.02)	0.00 (0.00)	0.05 (0.03)	0.02 (0.02)	0.02 (0.02) (31)	0.00 (0.00) (28)	0.04 (0.03) (26)
2: 0 g R	0.14 (0.04)	0.03 (0.02)	0.03 (0.02)	0.05 (0.03)	0.02 (0.02) (28)	0.04 (0.04) (26)	0.02 (0.02) (25)
3: 100 g	2.64 (0.13)**	2.44 (0.19)**	3.22 (0.15)**	2.32 (0.13) (31)**	2.21 (0.08) (28)**	2.35 (0.19) (24)**	2.27 (0.13) (22)**
4: 100 g R	2.63 (0.17)**	2.14 (0.15)**	3.06 (0.19)**	2.56 (0.16)**	2.65 (0.14) (31)**	0.24 (0.10) (31)	0.20 (0.16) (28)
5: 200 g	3.19 (0.19)**	2.80 (0.19)**	2.66 (0.17)**	2.62 (0.15) (30)**	2.76 (0.15) (29)**	2.67 (0.14) (26)**	2.46 (0.17) (25)**
6: 200 g R	2.83 (0.17)**	2.55 (0.17)**	3.23 (0.18)**	2.90 (0.16) (31)**	2.83 (0.14) (30)**	0.07 (0.04) (30)	0.06 (0.03) (27)
7: 300 g	2.86 (0.19)**	2.50 (0.16)**	3.01 (0.17)**	2.83 (0.15)**	2.55 (0.13) (31)**	2.57 (0.14) (28)**	2.37 (0.14) (27)**
8: 300 g R	2.91 (0.18)**	2.44 (0.15)**	3.42 (0.17)**	2.95 (0.17)**	3.08 (0.15)**	0.42 (0.15) (26)*	0.30 (0.12) (23)*

Means are taken of 32 wireworms unless indicated otherwise in parentheses following SEM. Values followed by * and ** are significantly different from 0 at $P < 0.05$ and $P < 0.0001$, respectively. *Rate* equivalent rate of bifenthrin in g AI/ha. Wireworms in treatments marked R (for resoiling) were placed in clean soil after the 15 DA measurement

treatments did not differ significantly ($P < 0.05$) from 0 on any day (Table 2), but scores in all insecticide treatments differed significantly ($P < 0.0001$) from 0 at 1, 2, 4, 7, and 15 DA. Inspection of mean mobility scores suggested that there was no significant differences in mobility scores between similar treatments prior to resoiling, except between treatments 7 and 8 at 15 DA which resulted from the removal of the dead wireworm in treatment 7 (Fig. 1g). Specific comparisons between treatments indicated that there were no differences in mobility between the two control treatments (0 and 0 g AI resoiled; Table 3), but that there was a significant difference if wireworms were exposed to 0 versus 100, 200, and 300 g AI (Table 3). Similarly, there were significant differences in wireworm mobility if wireworms were exposed to 0 resoiled versus 100 resoiled, 200 resoiled, and 300 g AI resoiled; 100 versus 100 g AI resoiled; 200 versus 200 g AI resoiled; and 300 versus 300 g AI resoiled (Table 3). However, the difference in wireworm mobility if wireworms were exposed to 100 versus 300 g AI was not significant (Table 3). These trends were similar if dead wireworms were included in the analysis, except that the difference in mobility of larvae exposed to 300 versus 300 g AI resoiled was not significant due to the slower recovery from morbidity at 300 g AI than lower rates of bifenthrin (Table 3).

When wireworms exposed to bifenthrin were placed in clean soil, mobility scores rapidly decreased, resulting in scores that were not significantly different from 0 by 29 DA except in 300 g AI (Table 2), and reflected in the significant differences in mobility scores between the 100 versus 100 g AI resoiled; 200 versus 200 g AI resoiled; and 300 versus 300 g AI resoiled treatments. Inspection of mean mobility scores suggested that there were no significant differences between resoiled and control treatments by 29 DA ($P > 0.05$).

Analysis of mobility scores over time with repeated measures ANOVA indicated a significant decrease in mobility scores over time ($F = 102.22$, $df = 6.56$, $P < 0.0001$), resulting from both the recovery of the resoiled wireworms and the removal of dead wireworms in the non-resoiled treatments (Table 2). However, this decrease over time differed significantly between treatments ($F = 17.96$, $df = 42.56, P < 0.0001$). There was no significant change in mobility scores over time in either the control or the control-resoiled treatment ($F = 0.02$, $df = 6.56$, $P = 1.00$; $F = 0.11, df = 6.56, P = 1.00$, respectively). As expected, the decrease in scores over time was more highly significant in the resoiled than non-resoiled treatments for each concentration of insecticide: 100 g ($F = 61.11$, $P < 0.0001$; $F = 5.58$, $P < 0.0001$, respectively), 200 g ($F = 81.57$, $P < 0.0001$; $F = 3.38$, $P = 0.0064$, respectively), and 300 g ($F = 72.47$, $P < 0.0001$; $F = 2.69$, $P = 0.0231$, respectively).

Table 3 Statistical comparisons between (groups of) treatments

Comparison	Without dead wireworms		Including dead wireworms	
Treatments (rate)	Estimate of difference (SE)		Estimate of difference (SE)	
1 versus 3, 5, 7 (0 g vs. 100 g, 200 g, 300 g)	7.83 (0.25)	$t = 30.86$, $P < 0.0001$	7.70 (0.39)	$t = 19.73$, $P < 0.0001$
3 versus 7 (100 g vs. 300 g)	0.18 (0.10)	$t = 1.70$, $P = 0.0944$	0.01 (0.16)	$t = 0.08$, $P = 0.93$
2 versus 4, 6, 8 (0 g R vs. 100 g R, 200 g R, 300 g R)	6.07 (0.25)	$t = 23.93$, $P < 0.0001$	5.55 (0.39)	$t = 14.22$, $P < 0.0001$
1 versus 2 (0 g vs. 0 g R)	0.02 (0.10)	$t = 0.24$, $P = 0.81$	0.16 (0.16)	$t = 0.99$, $P = 0.32$
3 versus 4 (100 g vs. 100 g R)	0.57 (0.10)	$t = 5.53$, $P < 0.0001$	0.73 (0.16)	$t = 4.58$, $P < 0.0001$
5 versus 6 (200 g vs. 200 g R)	0.66 (0.10)	$t = 6.35$, $P < 0.0001$	0.69 (0.16)	$t = 4.33$, $P < 0.0001$
7 versus 8 (300 g vs. 300 g R)	0.46 (0.10)	$t = 4.40$, $P < 0.0001$	0.26 (0.16)	$t = 1.61$, $P = 0.1127$

Wireworms were exposed to soil treated with Capture 2EC, containing bifenthrin, for 29 days. *Rate* rate of bifenthrin in g AI/ha. Wireworms placed in treatments marked R were “resoiled”; i.e., placed in clean soil after the 15 DA measurement; wireworms in other treatments remained in the amended soil. As some wireworms died from *Metarhizium* infection, the analysis was conducted both without and with wireworms that died during the study

Surface wireworms

Wireworms did not come to the soil surface when placed in cups with untreated soil, except for 1 wireworm found actively crawling along the edge in one cup at 2 DA (Table 4). This is not unusual, as wireworms occasionally come to the soil surface briefly and then re-enter the soil (Vernon et al. 2008). In contrast, a high proportion (mean > 0.9 on some days) of wireworms came to the soil surface in all cups with treated soil (Table 4). While there was no significant difference in the proportion that came to the surface between treatments with the same concentrations prior to resoiling, the proportion on the surface appeared to decrease as the concentration increased. This trend did not lead to significant differences between treatments except at 7 DA, where the proportion on the soil surface in treatment 4 (100 g AI) was significantly higher than in treatments 5–8 (200–300 g AI), and at 50 DA, where the proportion on the surface in treatment 7 (300 g AI, not resoiled) was significantly lower than in treatment 5 (200 g AI, not resoiled) and treatment 3 (100 g AI, not resoiled) (Table 4). These differences correspond to the numerical mobility scores of the wireworms, with sicker wireworms in higher treatment rates being less able to move (i.e., to the surface) than healthy ones. The surfacing behavior disappeared quickly after wireworms were placed in clean soil, the mean proportion that came to the surface dropping to <0.15 by 22 DA and <0.1 thereafter. By 22

Table 4 Mean (SEM) proportion of wireworms on surface of soil at various days after (DA) placement in soil amended with Capture 2EC, containing bifenthrin

Treatment: rate	1 DA	2 DA	7 DA	15 DA	22 DA	29 DA
1: 0 g	0.00 (0.00)A	0.03 (0.03)A	0.00 (0.00)A	0.00 (0.00)A	0.00 (0.00)A	0.00 (0.00)A
2: 0 g R	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
3: 100 g	0.41 (0.09)AB	0.34 (0.12)AB	0.56 (0.11)BC	0.66 (0.12)B	0.70 (0.08)B	0.86 (0.07)B
4: 100 g R	0.53 (0.12)B	0.38 (0.12)B	0.75 (0.12)C	0.75 (0.07)B	0.06 (0.04)A	0.00 (0.00)A
5: 200 g	0.28 (0.12)AB	0.13 (0.07)AB	0.34 (0.08)AB	0.68 (0.06)B	0.50 (0.09)B	0.79 (0.08)B
6: 200 g R	0.56 (0.09)B	0.19 (0.06)AB	0.44 (0.06)AB	0.56 (0.11)B	0.00 (0.00)A	0.00 (0.00)A
7: 300 g	0.53 (0.10)B	0.16 (0.08)AB	0.22 (0.13)AB	0.63 (0.08)B	0.50 (0.09)B	0.71 (0.10)B
8: 300 g R	0.44 (0.10)AB	0.13 (0.05)AB	0.31 (0.08)AB	0.53 (0.15)B	0.13 (0.07)A	0.04 (0.04)A
Statistical comparison F, P, $df = 7.56$	4.92, 0.0002	2.96, 0.0101	7.60, <0.0001	6.13, <0.0001	12.07, <0.0001	26.56, <0.0001

Means are taken of 8 cups, but number of wireworms per cup varies over time as dead wireworms were removed. Values in a column followed by the same letter are not significantly different ($\alpha = 0.05$). *Rate* Rate of bifenthrin in g AI/ha. Wireworms in treatments marked R (for resoiling) were placed in clean soil after the 15 DA measurement

DA, there was no significant difference in the proportion that came to the surface in the control treatments or the resoiled treatments (Table 4), and for each of the three insecticide concentrations, the proportion that came to the surface in the resoiled treatments was significantly less than in the corresponding non-resoiled treatments.

Discussion

Mobility categories and numerical scores

Wireworms recovering from exposure to bifenthrin moved upwards through each of the mobility stages shown in Table 1. This is similar to what was observed for *L. canus* exposed to tefluthrin in earlier studies (van Herk and Vernon 2007b, 2008). When exposed for brief periods of time, or to low concentrations of tefluthrin, *L. canus* also showed the "Alive-slow" and "Alive-clearly affected" stages (categories not previously described in Vernon et al. 2008) before entering the "Writhing" state and showed the same stages again when coming out of the "Writhing" state; and occasionally did not go below "Alive-clearly affected". When exposed to high concentrations of tefluthrin or for longer periods of time, *L. canus* went directly from "Alive" to "Writhing" or "Writhing upon stimulus" (van Herk and Vernon 2007a, b), as did *A. obscurus*, *A. sputator*, *L. canus*, *Ctenicera destructor*, and *C. pruinina* larvae exposed to high concentrations of neonicotinoid, OP, and other insecticides in a Potter Spray Tower (Vernon et al. 2008; van Herk et al. 2007, 2008a). It appears that *A. obscurus* exposed to bifenthrin goes through the same transitions as *L. canus*, so this may be typical of wireworms exposed to pyrethroid insecticides. At the concentrations tested herein, *A. obscurus* may have initially gone through both the "Alive-slow" and the "Alive-clearly affected" stages but this could not be observed, as wireworms were first observed 1 day after introduction.

Assigning a numerical value to each of the mobility categories described herein provided an index whereby statistical comparisons between chemicals or concentrations could be made and allowed assessment of mobility scores over time. Certain considerations are necessary when performing these comparisons, however. In the data presented here, *A. obscurus* exposed to soil treated with bifenthrin showed a statistically significant decrease in numerical scores over time that resulted from removal of dead wireworms from the average cup scores, not because wireworms were gradually improving over time. When the analysis was repeated with average cup scores that included dead wireworms, the change over time remained statistically significant, but now from a general increase in scores in all treatments due to mortality caused by *Metarhizium anisopliae*. Although mortality of wireworms due to *M. anisopliae* is atypical, studies in which there is mortality due to both the fungus and the insecticide itself will require data correction to separate the two effects (e.g., with Abbott's formula).

Implications for control of wireworms by bifenthrin

The results reported herein indicate that bifenthrin at the rates tested in soil may not be effective in killing wireworms directly, which may have implications for wireworm control in the field. In the current study, wireworms were confined to cups with all of the soil therein treated with bifenthrin. The only avenue of escape by wireworms that were not in advanced states of immobility (stages 3–5) was by moving to the soil surface, which in some cases was >90% (Table 3). When used as an in-furrow spray at time of potato planting, just the zone of soil surrounding the opened furrow with potato seed pieces would have been treated, and so wireworms moving into this treated zone could escape by moving to the soil surface, or to the untreated soil areas surrounding these cores. Those that moved to untreated areas of soil would not likely die, but might have made repeated attempts to approach the treated zone where potato seed pieces and developing daughter tubers were located. Those that moved to the soil surface and remained there immobilized (mobility categories: W–WR) would be more susceptible to mortality through predation. This has been observed in the field, where wireworms in a moribund state were at the soil surface in bifenthrin-treated plots approximately 11 months after the insecticide was applied (MS in prep.). Routine inspection of these wireworms indicated that some had desiccated, died, and later disappeared, indicating they may have been eaten. These observations coincided with observations of wireworm remnants found in bird feces nearby.

While rapid wireworm mortality may not occur in new plantings of potato treated with bifenthrin, this does not preclude the potential effectiveness of bifenthrin in protecting mother and late season daughter tubers from wireworm feeding damage. Since wireworms encountering the zone of soil treated with bifenthrin would be either repelled or rendered moribund for a long period of time, they would not be available to feed on tubers, provided bifenthrin was still present by harvest and tubers were safely within the treatment zone. Bifenthrin is known to last for considerable time in soil (Fecko 1999), and we have shown that bifenthrin-treated soil will render wireworms moribund 11 months after application (MS in prep.). The effectiveness of bifenthrin in reducing wireworm blemishes in potatoes (Kuhar and Alvarez 2008; Vernon and van Herk, unpublished data), therefore, may be due to the sublethal effects of bifenthrin reported in this study. Similarly, as contact with wheat seed treated with bifenthrin elicits both repellency and morbidity in *A. obscurus* and *L. canus* similar to that observed with tefluthrin (van Herk and Vernon, unpublished data; van Herk and Vernon 2007a, b), bifenthrin may provide stand protection to cereal crops even if it does not effectively reduce wireworm populations (Vernon et al. 2009).

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