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ORIGINAL PAPER

# Contact behaviour and mortality of wireworms exposed to six classes of insecticide applied to wheat seed

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**Abstract** Insecticide-treated seed is commonly used to manage wireworms, with insecticide toxicity generally being deduced from crop stand protection rather than from directly observed wireworm responses. We observed the behaviour of larvae of two economic elaterids exposed to wheat seeds treated with 11 insecticides at various rates or combinations in a soil environment. Wireworms were exposed for 3 or 24 h, and the post-contact health and mobility of 1030 larvae that contacted seeds were assessed (bi)weekly for 12–42 weeks. Considerable repellency was observed when wireworms were exposed to bifenthrin, tefluthrin, λ-cyhalothrin, and some repellency was also observed at high rates of various other insecticides. A high proportion of wireworms were moribund after 24 h when exposed to treatments containing thiamethoxam, fipronil, or high rates of ethiprole, cyazypyr, chlorpyrifos, and spinosad, but not after exposure to bifenthrin, tefluthrin, chlorantraniliprole, spirotetramat, or low rates of ethiprole, cyazypyr, and chlorpyrifos. High mortality was observed in all treatments containing fipronil, but none after exposure to bifenthrin, tefluthrin, λ-cyhalothrin, chlorantraniliprole, spirotetramat, spinosad, or low rates of cyazypyr. Combining thiamethoxam with fipronil or a high rate of chlorpyrifos decreased the toxicity of the second compound. These findings largely explain why we observe stand protection without wireworm population reduction in efficacy studies with wheat seed treated with various pyrethroid and neonicotinoid insecticides, and suggest a similar result for other insecticides that only induce temporary morbidity. This bioassay allows for rapid screening of insecticides proposed for wireworm management before these are evaluated in labour-intensive and costly field trials.

**Keywords** Wireworm · Insecticide repellency · Seed treatment · *Agriotes obscurus* · *Limonius canus* · Insect behaviour

## Key message

- Insecticide-treated seed is commonly used to control wireworms. Effectiveness is generally deduced indirectly from crop protection.
- Intoxication symptoms following contact with insecticides may be delayed and/or temporary.
- We observed contact behaviour and post-contact health of two economic species exposed to treated seed.
- Pyrethroid insecticides are repellent and nonlethal.
- Fipronil, but not ethiprole, is highly toxic and induces unique intoxication symptoms. Combining fipronil with thiamethoxam decreases fipronil's efficacy.
- Thiamethoxam, cyazypyr, and spinosad induce temporary morbidity from which most larvae recover.

## Introduction

Wireworms, the larvae of click beetles (Coleoptera: Elateridae), are important pests of potato, cereals, and many other agricultural crops (Vernon and van Herk 2012).

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Populations, and concomitant crop damages, appear to be increasing in many areas throughout the Holarctic as residues of deregistered effective insecticides such as heptachlor and other organochlorines are leaving agricultural land, and with the increasing implementation of sustainable farming practices beneficial to soil conservation (e.g. minimal tillage) (Vernon and van Herk 2012; Traugott et al. 2015). Management of this pest complex is complicated by the larvae's long life history (3–4 years for many pest species), subterranean ecology, seasonal periods of inactivity, and vertical movements in the soil profile (Vernon and van Herk 2012). These factors also contribute to the difficulty of obtaining consistent results between field efficacy studies for wireworm management conducted by different research programmes. Other factors complicating such studies are the polyphagous nature of many pest species, which causes organic material in the soil to distract them from contacting insecticides applied on seed or in furrow (Vernon and van Herk 2012), among species variability in insecticide susceptibility (Lange et al. 1949; van Herk et al. 2007), and the behavioural responses elicited by the insecticides applied. Regarding the latter, our laboratory studies have shown insecticides which when applied to wheat seed can elicit repellency (e.g. pyrethroids) (van Herk and Vernon 2007a) and/or temporary, reversible morbidity (e.g. neonicotinoids, pyrethroids) in wireworms (van Herk and Vernon 2007b), and that these responses at least partly explain why some insecticides provide stand protection without reducing wireworm populations in the field (Vernon et al. 2009, 2013a, b).

The considerations listed above have prompted us to develop several laboratory bioassays for determining the efficacy of insecticides on various wireworm pest species, enabling us to screen candidate insecticides before using them in labour-intensive field studies and to interpret results obtained in such studies. Among these bioassays, our soil window arenas are particularly useful, in that they permit the direct observation of wireworm behaviour in response to insecticides in a soil environment, and enable us to determine their response to these insecticides when applied in the field (van Herk and Vernon 2007a; van Herk et al. 2008a). In this paper, we report on the behaviour of the dusky wireworm, *Agriotes obscurus* L., an important pest species in Europe, Asia, and North America, and of the Pacific Coast wireworm, *Limonius canus* LeConte, an important pest in British Columbia and the Pacific Northwest, to a range of candidate insecticides and insecticide combinations proposed for their control. Discussed are contact behaviour, post-contact morbidity and mortality, and the implications of these responses for employing these insecticides for wireworm management.

## Methods

### Overview of studies

Three separate studies were conducted, and the methodology employed was largely consistent between them to allow for comparisons. Study 1 tested the response of *Agriotes obscurus* to seeds treated with combinations of thiamethoxam and chlorpyrifos; Study 2 tested the response of *Limonius canus* to seeds treated with bifenthrin and tefluthrin; and Study 3 evaluated the response of *A. obscurus* to ten different insecticides, alone or in combinations.

### Soil

Soil used in Study 1 and Study 2 was collected from the Pacific Agri-Food Research Centre (PARC) in Agassiz, British Columbia (BC) (49.2429°N, −121.7550°W), and consisted of a sandy clay loam containing approx. 5 % fine organic material. Soil used in Study 3 was collected from a fallow field that had previously been in long-term pasture, located west of Chilliwack, BC (49.1667°N, −122.0667°W), and consisted of a similar sandy clay loam. Prior to use in bioassay arenas or for wireworm storage, the soil used in each study was manually sifted through a 3 mm × 3 mm sieve to remove rocks and coarse organic material, and adjusted to 20 % moisture (by weight).

### Wireworms

Larvae of *A. obscurus* used in Study 1 were collected from an area not recently exposed to insecticides at PARC in the summer of 2007, identified using the keys developed by Eidt (1954) and Becker (1956), and stored in 40L Rubbermaid tubs with soil without food at 16 °C until needed. Wireworms were selected by placing potato pieces cut face down in the tubs, and those retrieved were considered to be in a feeding state (van Herk and Vernon 2013a). Only feeding wireworms were used in bioassays, as these are more likely to respond to germinating wheat seeds placed in the arena, or encounter them in the field (van Herk and Vernon 2013a). Wireworms do not feed for a large part of each instar, and a randomly selected sample will likely include a large proportion of non-feeding larvae, introducing additional variability in the results (Evans and Gough 1942; Falconer 1945; van Herk and Vernon 2013a; Sufyan et al. 2014). Wireworms were weighed with an analytic balance (Sartorius CP64; Sartorius AG, Goettingen, Germany) and their health and mobility assessed (see below) immediately before placement in and after removal from bioassay arenas to assess for weight change and whether this differed among treatments.

Larvae of *L. canus* used in Study 2 were collected from an organic vegetable farm in Kelowna, B.C. (49.8225°N, −119.4432°W) in June 2008, identified using the keys developed by Lanchester (1946) and Glen (1950), and stored in soil (as above) at 4 °C until needed. Tubs were moved to 16 °C to select feeding wireworms with small (150 ml) bait traps consisting of a moistened vermiculite–wheat mixture. Bait traps were left in the tubs for 4 days, and all wireworms located in the trap were considered feeding (van Herk and Vernon 2013a). In both studies, feeding wireworms were collected 2–3 days before being used in bioassays and were kept at room temperature (RT: 21 °C) during that time. Wireworms were weighed and their health assessed (as above) immediately before placement in and after removal from bioassay arenas.

Wireworms used in Study 3 were collected in the fall of 2011 and spring of 2012 from the same field as soil used in this study was removed from and stored (as above) at 15 °C. Adults that developed from larvae collected from this site were predominantly *A. obscurus* (prop. = 0.75), with the remainder being *A. lineatus* L.. These species are closely related and frequently found together, are nearly impossible to reliably distinguish from each other morphologically, and are therefore commonly studied as a single species complex (e.g. Subklew 1935; Lees 1943a; Falconer 1945). Feeding wireworms were selected with small bait traps placed in the tubs for 4 days (as above). Retrieved feeding wireworms were moved to an incubator set at 18 °C until used, within 7 days of retrieval, in window arenas. All wireworms used in the observation arenas were visually inspected for health and mobility immediately before placement in and after removal from the arenas, but were only weighed after removal.

In all three studies, only late instar larvae were selected for bioassays based on weight (>20.0 mg) and size (>15 mm) (Subklew 1935; Sufyan et al. 2014). All larvae were active and "Alive" (see below: "Health and mobility assessment") and showed no signs of entering a moulting phase.

## Soil window arenas

Soil window arenas were used to observe the behavioural response of individual wireworms exposed to insecticide-treated wheat seeds in soil. These arenas, described in van Herk and Vernon (2007a), consist of two 30 × 30 cm sections of transparent Plexiglas, into one of which a 26.0 cm dia × 4.0 mm deep circular chamber—the observation arena—has been machined. The second component, acting as a cover to the arena, is fastened to the bottom section with eight small carriage bolts positioned along the four edges, ensuring a tight seal between the two sections. This circular arena ensures that the wireworm stays within 13 cm of the insecticide-treated wheat seed at all times and cannot become trapped in corners. Transparent plastic grids overlaying both the top and bottom sections divide the arena into 113 equal-area cells arranged in eight concentric circles and four quadrants, with the innermost ring (ring 1) consisting of a single cell that touches all four quadrants. A 5 mm hole drilled into the arena cover aligns with the centre of the middle cell in the outer ring of one of the quadrants, (i.e. cell 89, 96, 103, or 110). Which cell the holes align with is determined by the orientation of the upper section on the section with the arena.

Arenas were carefully filled with an even layer of finely screened soil, then placed on a raised wooden frame to permit observation from both the top and the bottom, after which five wheat seeds (germinated 44–48 h at 21–24 °C between moist paper towels to ensure 1.0–1.5 cm-long roots) were placed in cell 1, and the upper section secured into place. These seeds were allowed to set up $CO_2$ gradients (which cause wireworms to orient towards the seeds; Doane et al. 1975) for 1 h (Studies 1, 2) or 30 min (study 3), after which a single wireworm was introduced 'head first' into the arena in cell 89, 96, 103, or 110, through the small hole in the top section. The placement of this hole, and therefore of the wireworm into the arena, was random between the four possible positions, and the hole was kept sealed with masking tape except for wireworm insertion. Wireworm position and behaviour monitoring commenced as soon as larvae were placed in the arenas, and were recorded every 5 min for 3 h. Up to 24 windows were monitored simultaneously, requiring 2–3 technicians familiar with wireworm behaviour and the bioassay procedure. To help simulate a subterranean environment, all observations were done under faint red light generated by 25 W incandescent bulbs.

## Wireworm observations

Of particular interest were the following: the time required to initially make contact with the seeds (contact being defined as a larva's head being in one of the centre five cells); duration of initial contact with seeds; incidence of repellent behaviour; predominant location of the wireworm in the arena subsequent to initial contact; and evidence of morbidity symptoms (discussed below: "Health and mobility assessment") during the exposure period. We define repellent behaviour as continuous contact with seeds for <16 min, and consider continuous contact for >45 min as indicative of normal feeding behaviour (van Herk et al. 2010). Contact lasting 16–45 min is typical of a non-repellent insecticide that induces morbidity upon ingestion and/or contact (van Herk et al. 2010). A repellency index (RI) was calculated based on the proportion of wireworms in contact for <16 min. Wireworms are not considered to be repelled (RI = 0) if this proportion is <0.30, and insecticide treatments are considered to elicit low, moderate, and high repellency (RI = 1, 2, 3, respectively) if this proportion is 0.30–0.49, 0.50–0.69, and 0.70+, respectively. The predominant location of wireworms after first making contact with the seeds was determined by calculating the mean proportion of time wireworms spent in rings 1–2 (in contact with seeds or rootlets), rings 3–4 (2.5–6.0 cm from the arena centre), and rings 5–8 (6–13 cm from the arena centre). Following the 3 h observation period, wireworms were either removed (some of those used in Study 1) or (all others) left in soil bioassays overnight to determine the result of a longer exposure, in which case their position and behaviour were recorded 24 h after introduction into bioassays. Recording the wireworm position immediately prior to their removal from the bioassay, and their health immediately after removal, permitted us to determine the position of moribund wireworms relative to the seeds. For wireworms remaining in arenas for 24 h, the date of removal was considered 1 day after initial exposure (i.e. 1 DAE).

## Seed treatments

All insecticide rates are expressed as g AI/100 kg wheat seed, and hereafter abbreviated as g. Insecticides evaluated in Study 1 were thiamethoxam (Cruiser 5FS) applied at 10 g with and without fungicide, and combinations of thiamethoxam at 10 g + chlorpyrifos (Pyrinex 480EC) at 0.5, 5, or 50 g. Equal numbers of all wireworms were exposed for 3 and 24 h in all treatments except one, thiamethoxam at 10 g seed (no fungicide), in which all larvae were exposed for 24 h. Insecticides evaluated in Study 2 were bifenthrin (Capture 2EC) and tefluthrin (Tefluthrin 20SC) at 10 and 20 g, and all larvae were exposed for 24 h.

Insecticides evaluated in Study 3 were λ-cyhalothrin (Matador 120EC, Demand 100CS) at 10 g (both formulations); tefluthrin (Force 200SC) at 10 g; thiamethoxam (Cruiser 350FS) at 10 g; fipronil (Regent 500FS) at 1, 5, and 50 g; combinations of thiamethoxam at 10 g + fipronil at 1 or 5 g; ethiprole (Ethiprole FS350G) at 5 and 50 g; cyazypyr (DPX HGW86-599) at 10 and 30 g; a combination of cyazypyr at 10 g + thiamethoxam at 39 g + λ-cyhalothrin (Matador 120EC) at 10 g; a combination of cyazypyr at 30 g + thiamethoxam at 39 g; chlorantraniliprole (Coragen 200SC) at 30 g; spinosad (GF-976 Spinosad 480SC) at 30 g; spirotetramat (Movento 240SC) at 5 and 50 g; and chlorpyrifos (Pyrinex 480EC) at 5 and 50 g.

For all three studies, wheat seed (cv. AC Barrie) used in all treatments (except one treatment in Study 1, noted above) was also treated with the fungicide Dividend XLRTA (containing 3.21 % difenoconazole and 0.27 % mefenoxam) at 13 g AI/100 kg, and seeds treated with Dividend XLRTA alone served for a control treatment. Seed was treated with a Hege 11 liquid seed treater (Wintersteiger Inc., Salt Lake City, UT), either at PARC by the authors or by technicians at a Syngenta Crop Protection (Canada) seed treatment facility in Portage la Prairie, MB. Studies were conducted from August–November 2007 (Study 1), August 2008–March 2009 (Study 2), and February–November 2012 (Study 3). Generally several treatments, including the control treatment, were evaluated each day observations were conducted, and each treatment was evaluated over several days throughout the duration of the study.

## Health and mobility assessment

In all studies, wireworm mobility, a measure of their level of intoxication from insecticide exposure (Grove et al. 2000; Furlan and Campagna 2002), was assessed using the methodology and categories we developed previously (Vernon et al. 2008; van Herk and Vernon 2013b). Wireworms demonstrating spontaneous, directed movement and capable of moving to the outside of a 10 cm Petri dish lined with moist Whatman #1 filter paper (Whatman International Ltd., Maidstone, England), without falling over and within 2 min of being placed in the centre, were considered "Alive" (A). Wireworms were considered "Writhing" (W) if incapable of such directed movement or leaving the centre of the dish, but capable of spontaneous twisting movements involving the entire body; "Leg and mouthparts" (LM) if unable to make writhing movements, but capable of moving both legs and mouthparts; and "Mouthparts" (M) if capable of moving mouthparts only. Wireworms described in van Herk and Vernon (2013b) as "Writhing upon stimulus" (WR) are here included in the "Writhing" category, and those described as "Alive, clearly affected" (AC) and "Alive-slow" (AS) are here included in the "Alive" category. Wireworms exposed to the phenylpyrazole fipronil frequently display a combination of intoxication symptoms not observed when exposed to other treatments, including "Contraction" (T), in which the abdominal and thoracic segments have collapsed into each other and the wireworm is reduced to approximately 1/2–2/3 its original length; "Convulsions" (V), in which spasmic, shuddering tremors are seen along the length of the body; and "Continuous leg and mouthparts movement" (CLM), in which these move continuously. As these characteristic symptoms generally co-occur, we consider them together as CLMVT, and refer to their appearance as 'characteristic fipronil poisoning symptoms'. Wireworms categorised as either W, LM, M, or CLMVT are considered 'moribund'. Wireworms were not considered to be dead (D) until this was obvious from cuticle blackening or mycelial growth.

The health and mobility categories described above are generally determined within 30 s of wireworm placement in the Petri dish, and no more than 5 min was allowed to complete individual health assessments. Wireworms were stored in individual 150 ml containers with soil between assessments, at 20 °C in Studies 1 and 2, and at 15 °C in Study 3. Health checks were done weekly until 28 days after removal from the windows, weekly (Studies 1, 2), or biweekly (Study 3) thereafter, and continued until the health of all wireworms in a treatment had resolved, i.e. there were no changes in health categories of the remaining wireworms for at least 1 consecutive month. For some treatments, this required health assessments to continue for more than 200 days. In each study, health checks in the control treatments were continued as long as those in the treatment in which these assessments were required longest. Health observations were performed at 18 °C in Study 3 and at RT in the other studies. Great care was exercised and only soft tip tweezers (Fine Science Tools, Vancouver, British Columbia, Canada) were used to reduce stress from handling.

## Statistical analysis

For each study, the proportion of wireworms placed in arenas that contacted seeds during the 3-h observation period, and 24-h exposure period, was compared among treatments with $\chi^2$ analyses, and the proportion in each treatment was subsequently compared with that in the control treatment directly with Fisher's exact test. Of wireworms that contacted seeds during 3 h, mean larval weight, mean weight change during the bioassay period, and the time required to first establish contact, were compared among treatments with ANOVA followed by Tukey's separation of means. Initial analyses indicated that wireworm weight did not significantly affect the time required to make first contact ($P > 0.05$) in any study, and this covariate was consequently dropped from models. Further, the mean proportion of time spent in rings 1–2, 3–4, or 5–8 subsequent to making initial contact with seeds was compared among treatments with ANOVA followed by Tukey's separation of means. Prior to ANOVAs, normality of data was inspected (Proc Univariate, SAS 9.2), and data transformed using a power or arcsine function as appropriate.

Of wireworms that contacted seeds in 3 h, the proportions that contacted <16, 16–45, and >45 min were compared among treatments with $\chi^2$ analyses using the Yates correction to compensate for cells with low counts (Yates 1984), followed by comparison with each treatment separately to the control treatment (as above). Similar analyses were done to determine if there were differences among treatments in the proportion of wireworms that contacted in 24 h and that were moribund when removed from the arenas, and in the proportion of moribund larvae that were located near the seeds (i.e. in rings 1–4). These analyses were followed by Ryan's test (Ryan 1960) to separate proportions when $\chi^2$ analysis indicated significant differences among treatments. All analyses were done in SAS (SAS 9.2) and MS Excel.

The methods of Morales-Rodriguez and Wanner (2015) were followed to determine if treating seeds with two insecticides had a synergistic or antagonistic effect, e.g. on wireworm mortality in our previously published field studies (Vernon et al. 2013b). The percentage population decrease for both component insecticides ($P_1$, $P_2$) was calculated by comparing wireworm survivorship in these treatments to that observed in the control. An expected percentage of population decrease for the blended insecticide treatment ($P_{BE}$) was calculated using, $P_{BE} = P_1 + P_2 - (P_1 \times P_2)/100$. $P_{BE}$ was then compared to the percentage observed, $P_{BO}$, with $\chi^2$ analysis ($df = 1$). Statistical significance indicates either antagonism or synergy between the two component chemicals depending on the relative values of $P_{BE}$ and $P_{BO}$.

# Results

## Study 1: chlorpyrifos + thiamethoxam, *Agriotes obscurus*

### *Contact behaviour*

A high proportion of wireworms placed in bioassay arenas contacted seeds (Ring 1, cell 1) or roots (Ring 2, cells 2–5), herein referred to as 'seed' contact, within 3 h in all treatments (range 0.85–0.95; mean: 0.90; control treatment: 0.88; Table 1). This response to germinating seed was similar to that observed for *A. obscurus* in earlier soil bioassay studies (range 0.80–1.00; van Herk et al. 2008a), and indicated an absence of long-distance (i.e. pre-contact) repellence to the seed treatments tested. The time required to first contact seed (range 33.1–50.7 min) also did not differ significantly among treatments ($P > 0.05$) and was similar to that observed in previous studies with untreated seed (range 22.8–46.7 min, van Herk and Vernon 2007a). Of wireworms left in arenas for 24 h, there were no significant differences in the proportion that was in contact with seed at the end of the observation period (Table 1; Chi = 0.07, $df = 5$, $P = 1.00$).

**Table 1** Behaviour of *Agriotes obscurus* larvae exposed to seeds treated with chlorpyrifos and/or thiamethoxam in Study 1

| Insecticide | Rate[b] | N (C3)[c,d] | Mean (SE) initial weight (mg) of C3[e] | Mean (SE) weight change (mg) of C3[e] | Mean (SE) time to contact[e] | Proportion of wireworms in each contact duration category[d,f] | | | Mean (SE) proportion of time spent at different distances from seeds after first contact[d,h] | | | C24 (MB24)[e,f] | Prop. MB24 in rings 1–4[f] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | <16 min (RI)[g] | 16–45 min | >45 min | Rings 1–2 | Rings 3–4 | Rings 5–8 | | |
| Untreated control[a] | | 40 (35) | 26.7 (1.70) A | 1.93 (0.16) A | 50.7 (6.56) A | 0.14 (0) | 0.14 | 0.71 | 0.91 (0.03) | 0.03 (0.01) | 0.06 (0.02) | 20 (0) A | – |
| Thiamethoxam | 10 | 20 (17) | 25.7 (1.83) A | −0.89 (0.21) B | 45.6 (5.47) A | 0.24 (0) | 0.12 | 0.65 | 0.88 (0.05) | 0.07 (0.03) | 0.05 (0.03) | 20 (11) B | 0.73 |
| Thiamethoxam[a] | 10 | 40 (38) | 23.1 (1.36) A | −0.81 (0.21) B | 46.7 (5.90) A | 0.13 (0) | 0.08 | 0.79 | 0.83 (0.03) | 0.08 (0.02) | 0.09 (0.03) | 20 (18) B | 0.78 |
| Thiamethoxam + Chlorpyrifos[a] | 10 + 0.5 | 40 (37) | 23.4 (1.55) A | −0.59 (0.14) B | 45.8 (6.90) A | 0.24 (0) | 0.14 | 0.62 | 0.72 (0.04)* | 0.17 (0.03)** | 0.11 (0.03) | 19 (13) B | 0.77 |
| Thiamethoxam + Chlorpyrifos[a] | 10 + 5 | 40 (36) | 27.2 (1.46) A | −0.96 (0.20) B | 39.7 (5.58) A | 0.03 (0) | 0.22 | 0.75 | 0.75 (0.04)* | 0.13 (0.02)* | 0.12 (0.04) | 20 (11) B | 0.36 |
| Thiamethoxam + Chlorpyrifos[a] | 10 + 50 | 40 (35) | 24.6 (1.35) A | −0.65 (0.22) B | 33.1 (4.53) A | 0.29* (0) | 0.23 | 0.49 | 0.60 (0.04)** | 0.21 (0.03)** | 0.19 (0.04)* | 19 (13) B | 0.54 |
| Statistics | | Chi = 0.26<br>*df* = 5<br>*P* = 0.99 | F = 1.36<br>*df* = 5192<br>*P* = 0.24 | F = 43.11<br>*df* = 5191<br>*P* < 0.0001 | F = 1.25<br>*df* = 5191<br>*P* = 0.29 | Chi = 6.43<br>*df* = 5<br>*P* = 0.27 | Chi = 2.47<br>*df* = 5<br>*P* = 0.47 | Chi = 2.51<br>*df* = 5<br>*P* = 0.77 | F = 9.04<br>*df* = 5191<br>*P* < 0.0001 | F = 8.71<br>*df* = 5191<br>*P* < 0.0001 | F = 3.00<br>*df* = 5191<br>*P* = 0.012 | Chi = 14.40<br>*df* = 5<br>*P* = 0.013 | Chi = 1.55<br>*df* = 4<br>*P* = 0.82 |

*N* Number of wireworms (wws) exposed; C3, C24 = number of wws that contacted seeds at least once in 3 and 24 h (respectively). Contact time = time (min) required for wireworms to first contact seeds in the bioassay. RI = ww repellency index. MB24 = number of wws immobile (mobility categories W to M) after 24 h exposure

[a] Also treated with the fungicide Dividend XL RTA at 13 g AI/100 kg seed. Thiamethoxam = Cruiser 350FS; Chlorpyrifos = Pyrinex 480EC

[b] Rates are expressed as g AI/100 kg wheat seed

[c] In each treatment 20 wws left in arenas for 24 h, the others for 3 h

[d] Values in a column that differed significantly from the untreated control are marked with * if $P < 0.05$, ** if $P < 0.0001$

[e] Values followed by the same letter do not differ significantly, as per Tukey and Ryan's procedures for separating means and proportions, respectively ($\alpha < 0.05$)

[f] The Yates continuity correction was used for these $\chi^2$ comparisons

[g] RI = 0, 1, 2, 3, respectively, if the proportion contacting <16 min = 0–0.29, 0.30–0.49, 0.50–0.69, and 0.70+, respectively

[h] Rings 1–2: 0–2.5 cm from centre of arena; Rings 3–4: 2.5–6.0 cm; Rings 5–8: 6.0–13 cm

*Control, thiamethoxam* Most wireworms exposed to untreated control seeds remained in contact for >45 min (prop. = 0.71) and in rings 1–2 (prop. = 0.91) after making initial contact (Table 1). The contact behaviour of wireworms exposed to thiamethoxam with or without fungicide was similar to those exposed in the control treatment, most larvae remaining in contact for >45 min (prop. = 0.65–0.79) and within rings 1–2 (prop. = 0.83–0.88) after making initial contact (Table 1). There was no evidence of repellency (RI = 0).

*Chlorpyrifos + thiamethoxam* Most wireworms exposed to seed treated with combinations of thiamethoxam at 10 g + chlorpyrifos at 0.5 and 5 g also contacted seed for >45 min (prop. = 0.62, 0.75, respectively) with no evident repellency (RI = 0) (Table 1). Exposure to thiamethoxam at 10 g + chlorpyrifos at 50 g, however, caused a considerable decrease in the proportion contacting seed for >45 min relative to the control (prop. = 0.49), and a significant increase in the proportion contacting seed for <16 min (prop. = 0.29). At all three rates of chlorpyrifos tested (0.5 g, 5 g, and 50 g), wireworms spent significantly less time in rings 1–2 and significantly more time in rings 3–4 after making initial contact relative to the control treatment (Table 1). This resulted from a number of wireworms (prop. = 0.22, 0.19, 0.43, respectively) becoming immobilized after moving out of rings 1–2. Despite this behaviour, no collective repellency was indicated (RI = 0).

### *Wireworm weight change*

Mean initial weights of wireworms contacting seeds did not differ significantly among treatments (range 23.1–27.2 mg; Table 1), and larval weight did not significantly affect the time required to first contact seeds ($P > 0.3$). Weight change over the observation period varied significantly between the control and other treatments, however, with larvae gaining mass in the untreated control (weight change: 7.2 %) and decreasing in all other treatments (range 2.5–3.5 %; Table 1). These estimates are based on adjusted means, as some wireworms were exposed for 3 h and some for 24 h. In general, exposure duration significantly affected the degree of weight change ($F = 46.45$, $df = 5191$, $P < 0.0001$), but this varied among treatments. Weight gain in larvae exposed to control seed for 3 h and 24 h was similar (3 h: 8.2 %, 24 h: 6.7 %), but in all other treatments weight loss increased with the duration of exposure. Weight change was +0.4 % and −5.8 % when exposed to thiamethoxam (with Dividend XLRTA) at 10 g for 3 and 24 h (respectively), and a decrease in weight was also observed when larvae were exposed to combinations of thiamethoxam at 10 g + chlorpyrifos at 0.5 g (weight change = −0.5 %, −3.8 %, respectively), 5 g (−1.1 %, −5.0 %, respectively), and 50 g (−0.3 %, −4.4 %, respectively).

### *Post-contact wireworm health*

No mortality due to *Metarhizium* infection was observed in Study 1. Wireworms that contacted control seeds showed no signs of morbidity when removed from arenas and (except one larva) did not die during the 84 days of post-contact health assessments (Fig. 1a). In all other treatments, a significant proportion (range 0.55–0.90, Table 1) was moribund at removal, and the majority of these (prop. >0.7) were found close to the seeds (rings 1–4; Table 1).

Wireworms moribund after exposure to seeds treated with thiamethoxam at 10 g with or without fungicide had recovered to normal health (i.e. “A”) by 4 DAE (thiamethoxam alone) or 7 DAE (thiamethoxam + fungicide) (Fig. 1b, c). Despite this recovery, a small proportion (0.29) of larvae exposed to thiamethoxam at 10 g alone had died by 42 DAE, which did not occur when exposed to thiamethoxam + fungicide (Fig. 1b, c). The recovery from morbidity, and subsequent low incidence of mortality after exposure to thiamethoxam at 10 g alone is similar to previous results reported for both *A. obscurus* and *L. canus* larvae after exposure to thiamethoxam + fungicide (van Herk et al. 2008a).

Most wireworms moribund after exposure to seeds treated with thiamethoxam at 10 g + chlorpyrifos at 0.5, 5, or 50 g seed had fully recovered by 7 DAE (Fig. 1d–f). A small proportion showed signs of “Writhing” after 14 DAE but this was generally due to them entering a moulting phase. The small proportion dead in the combined treatments by 84 DAE was not significantly different from either the control ($P > 0.5$) or thiamethoxam + fungicide treatments ($P > 0.9$).

## **Study 2: tefluthrin and bifenthrin, *Limonius canus***

### *Contact behaviour*

The proportion of *L. canus* wireworms placed in bioassay arenas that contacted seeds within 3 h (range 0.75–0.85; mean: 0.82; control treatment: 0.86; Table 2), and the time required to first contact seeds (range 38.4–59.3 min) did not differ significantly among treatments, and was similar to that observed in previous studies (van Herk et al. 2008a, 2010). There was no significant difference between treatments in the proportion of wireworms that had contacted seeds within 24 h (Table 2; Chi = 0.94, $df = 4$, $P = 0.92$). The high proportion of contacting wireworms suggests an absence of pre-contact repellence, but in all four pyrethroid treatments some (1–3) wireworms oriented

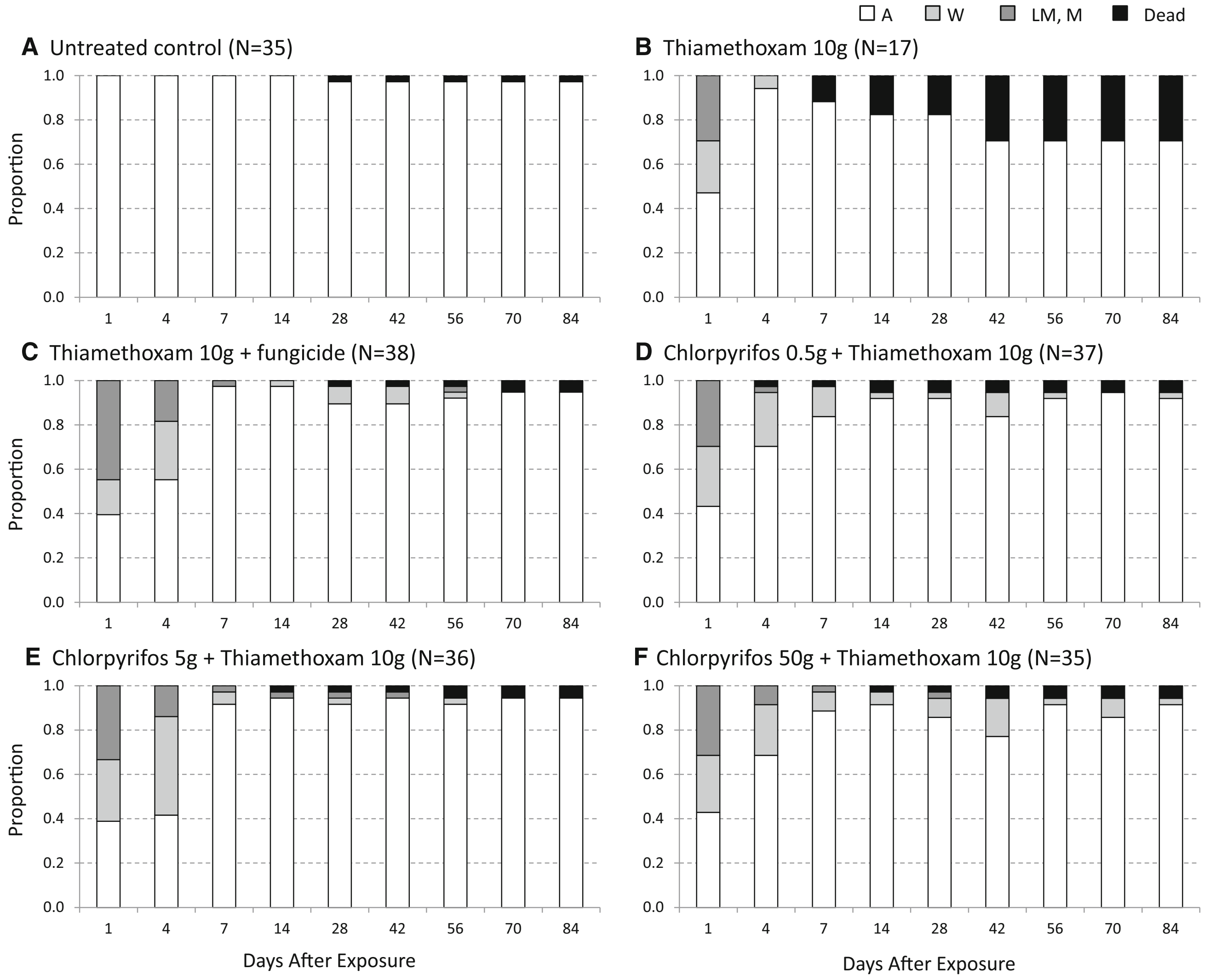


**Fig. 1** Proportion of *Agriotes obscurus* larvae in different health (mobility) categories after contacting seeds treated with thiamethoxam (Cruiser 350FS) with or without chlorpyrifos (Pyrinex 480EC) in Study 1. All rates in g AI/100 kg wheat seed. *N* Number of wireworms that contacted seeds. Mobility categories (see text for full explanation): *A* alive; *W* writhing; *LM* leg and mouthpart movement; *M* mouthpart movement only

towards the seeds and reached ring 3 before veering away. These individuals did not contact seed subsequently.

*Control* The proportion of wireworms contacting untreated control seeds for >45 min (prop. = 0.61, Table 2) was similar to that observed for *L. canus* in earlier studies (range 0.56–0.57, van Herk et al. 2010), but more larvae remained in contact for <16 (prop. = 0.28 vs. 0.06–0.09), and fewer in contact for 16–45 min (0.11 vs. 0.34–0.38) than previously observed (van Herk et al. 2010). Wireworms generally remained within rings 1–2 after initially contacting seeds (prop. time: 0.68, Table 2).

*Bifenthrin* Wireworm contact behaviour was affected by bifenthrin at both the 10 g and 20 g seed rates, with significantly fewer larvae contacting seed for >45 min (prop. = 0.00, 0.08, respectively), and a significantly lower proportion of time spent in rings 1–2 after initial contact (prop. = 0.46, 0.26, respectively; Table 2). Significantly more larvae contacted seed for 16–45 min (prop. = 0.80, 0.28, respectively) relative to the control treatment. Exposure to bifenthrin at 20 g elicited significant repellency (prop. contacting <16 min: 0.64; RI = 2) and significantly more time was spent in rings 3–4 and 5–8 after initial contact with seeds relative to the control treatment (Table 2). Repellent behaviour was not observed at the 10 g rate (RI = 0).

*Tefluthrin* Tefluthrin elicited moderate (RI = 2) and high (RI = 3) repellency at the 10 and 20 g rates, respectively

**Table 2** Behaviour of *Limonius canus* larvae exposed to seeds treated with bifenthrin and tefluthrin in Study 2

| Insecticide[a] | Rate[b] | N (C3)[c] | Mean (SE) initial weight (mg) of C3[d] | Mean (SE) weight change (mg) of C3[d] | Mean (SE) time to contact[d] | Proportion of wireworms in each contact duration category[c,e] | | | Mean (SE) proportion of time spent at different distances from seeds after first contact[c,g] | | | C24 (MB24)[e] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | <16 min (RI)[f] | 16–45 min | >45 min | Rings 1–2 | Rings 3–4 | Rings 5–8 | |
| Untreated control | | 42 (36) | 27.0 (1.03) A | 2.74 (0.34) A | 38.4 (6.52) A | 0.28 (0) | 0.11 | 0.61 | 0.68 (0.06) | 0.07 (0.02) | 0.25 (0.05) | 41 (0) |
| Bifenthrin | 10 | 20 (15) | 30.9 (1.71) A | −0.04 (0.12) B | 59.3 (13.79) A | 0.20 (0) | 0.80** | 0.00* | 0.46 (0.08)* | 0.15 (0.05) | 0.39 (0.08) | 16 (0) |
| Bifenthrin | 20 | 48 (39) | 27.5 (0.92) A | −0.02 (0.10) B | 49.1 (7.51) A | 0.64** (2) | 0.28* | 0.08** | 0.26 (0.03)** | 0.24 (0.04)* | 0.50 (0.04)* | 40 (6) |
| Tefluthrin | 10 | 20 (17) | 29.5 (1.14) A | 0.03 (0.09) B | 44.7 (10.94) A | 0.53 (2) | 0.29 | 0.18* | 0.38 (0.08)* | 0.22 (0.05)* | 0.40 (0.08) | 19 (2) |
| Tefluthrin | 20 | 48 (39) | 26.8 (0.82) A | −0.25 (0.09) B | 39.5 (6.54) A | 0.72** (3) | 0.26* | 0.03** | 0.26 (0.04)** | 0.22 (0.04)* | 0.52 (0.05)* | 40 (7) |
| Statistics | | Chi = 0.22 | F = 2.02 | F = 51.35 | F = 0.85 | Chi = 10.77 | Chi = 13.74 | Chi = 43.35 | F = 11.44 | F = 6.71 | F = 3.61 | Chi = 7.12 |
| | | *df* = 4 | *df* = 4141 | *df* = 4132 | *df* = 4140 | *df* = 4 | *df* = 4 | *df* = 4 | *df* = 4140 | *df* = 4140 | *df* = 4140 | *df* = 4 |
| | | $P = 0.99$ | $P = 0.095$ | $P < 0.0001$ | $P = 0.49$ | $P = 0.029$ | $P = 0.0082$ | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ | $P = 0.0078$ | $P = 0.13$ |

*N* Number of wireworms (wws) exposed; C3, C24 = number of wws that contacted seeds at least once in 3 and 24 h (respectively). Contact time = time (min) required for wireworms to first contact seeds in the bioassay. RI = ww repellency index. MB24 = number of wws immobile (mobility categories W to M) after 24 h exposure

[a] Also treated with the fungicide Dividend XL RTA at 13 g AI/100 kg seed. Bifenthrin = Capture 2EC; Tefluthrin = Force 200SC

[b] Rates are expressed as g AI/100 kg wheat seed

[c] Values in a column that differed significantly from the untreated control are marked with * if $P < 0.05$, ** if $P < 0.0001$

[d] Values followed by the same letter do not differ significantly, as per Tukey's procedure for separating means ($\alpha < 0.05$)

[e] The Yates continuity correction was used for these $\chi^2$ comparisons

[f] RI = 0, 1, 2, 3, respectively, if the proportion contacting <16 min = 0–0.29, 0.30–0.49, 0.50–0.69, and 0.70+, respectively

[g] Rings 1–2: 0–2.5 cm from centre of arena; Rings 3–4: 2.5–6.0 cm; Rings 5–8: 6.0–3 cm

(Table 2), but the proportion contacting for <16 min was only significantly different from the control treatment at the 20 g rate (Table 2). As a result of this repellency, significantly fewer wireworms contacted normally at both the 10 and 20 g rates (prop. = 0.18, 0.03, respectively; Table 2), and significantly less time was spent in rings 1–2 after initial contact with seeds (prop. = 0.38, 0.26, respectively; Table 2). Larvae appeared to move further from the seeds subsequent to initial contact at the 20 g than 10 g rates, spending significantly more time in rings 5–8 in the 20 g tefluthrin than in the control treatment.

### *Wireworm weight change*

Mean initial weights of wireworms contacting seeds did not differ significantly among treatments (range 26.8–30.9 mg; Table 2), and larval weight did not significantly affect the time required to first contact seeds ($P > 0.06$). Weight change over the observation period varied significantly between the control and other treatments, however, with larvae gaining weight when exposed to the untreated control seeds (weight increase: 10.1 %), no weight change was observed when exposed to bifenthrin at 10 and 20 g or tefluthrin at 10 g, and decreases slightly when exposed to tefluthrin at 20 g (0.9 %; Table 2).

### *Post-contact wireworm health*

No mortality due to *Metarhizium* infection was observed. Wireworms that contacted control seeds showed no signs of morbidity at 1 DAE and did not die during the 84 days of post-contact health assessments. A small proportion (0.11–0.18) of larvae exposed to seeds treated with bifenthrin at 20 g and tefluthrin at both 10 and 20 g were moribund at 1 DAE, but these all had recovered by 7 DAE and there was no subsequent mortality. This rapid recovery from pyrethroid intoxication was previously observed for both *A. obscurus* and *L. canus* exposed to tefluthrin at 10 or 20 g (van Herk and Vernon 2007a; van Herk et al. 2008a).

## Study 3: *Agriotes obscurus/lineatus*

The proportion of wireworms in bioassay windows that contacted seeds within 3 h ranged from 0.43 to 0.88 (mean = 0.73; control treatment = 0.73; Table 3), with the proportion in only one treatment (the λ-cyhalothrin 10 g + cyazypyr 10 g + thiamethoxam 39 g combination) being significantly lower than the control ($P < 0.05$; Table 3), suggesting either pre-contact repellency or decreased attractiveness (e.g. reduced $CO_2$ production) of the seeds. The proportion of wireworms that contacted seeds within 24 h ranged from 0.77 to 1.00 (mean = 0.91; control = 1.00; Table 3), with no significant differences among treatments (Chi = 4.07, $df = 21$, $P = 1.00$), or between any treatment and the control ($P > 0.05$). The time required to first contact seeds ranged from 33.2 to 71.2 min, and while significant differences were observed among treatments, no significant differences were evident between any treatment and the control ($P > 0.05$; Table 3). Significant differences among treatments were also observed in post-assay wireworm weight (range of mean (SE) weights: 31.3 (1.89)–42.6 (1.34) mg; mean = 36.5 mg; control treatment = 41.6 mg), but individual wireworm weights did not significantly affect the time required to first contact seeds ($P > 0.1$).

### *Control, thiamethoxam*

The proportion of wireworms exposed to untreated seeds remaining in contact for <16, 16–45, >45 min (Table 3) was similar to that observed for *A. obscurus* exposed to the control treatment in Study 1 (above), with most larvae remaining in rings 1–2 (prop. = 0.83) and maintaining initial contact for >45 min (prop. = 0.63) (Table 3). The contact behaviour of wireworms exposed to thiamethoxam at 10 g was similar to that observed in the control treatment and to the thiamethoxam treatment in Study 1 (above), where larvae generally remained in contact for >45 min (prop. = 0.68; Table 3) and within rings 1–2 (prop. = 0.69; Table 3) after initial contact. There was no evidence of repellency (RI = 0).

### *Fipronil, thiamethoxam + fipronil, ethiprole*

Slight repellency (RI = 1) was observed when wireworms were exposed to fipronil at 50 g, the proportion contacting <16 min being significantly higher than in the control treatment (prop. = 0.35; Table 3). No repellency was evident at the 1 and 5 g rates (RI = 0, Table 3). The proportion of wireworms exposed to fipronil at 1, 5, and 50 g that contacted for >45 min (range 0.58–0.81; Table 3), and time spent in rings 1–2 after initially contact (range: 0.65–0.93, Table 3) did not differ significantly from the control treatment, but these values decreased as the rate of fipronil increased. Wireworms contacting seeds treated with combinations of thiamethoxam at 10 g + fipronil at 1 or 5 g were not repelled (RI = 0; Table 3), with contact behaviour being similar to those exposed to the control treatment or to seeds treated with either thiamethoxam at 10 g or fipronil at 1 or 5 g. In these combined treatments, most larvae contacted >45 min (prop.: 0.66, 0.71, respectively; Table 3) and remained within rings 1–2 after initial contact (0.72, 0.77, respectively, Table 3). Slight repellency was observed when wireworms were exposed to ethiprole at 5 g (prop. in contact <16 min = 0.30; RI = 1; Table 3), but not when

**Table 3** Behaviour of *Agriotes obscurus/lineatus* larvae exposed to seeds treated with various insecticides in 2012 (Study 3)

| Insecticide[a] | Rate[b] | N (C3)[c] | Mean (SE) time to contact[d] | Proportion of wireworms in each contact duration category[c,e] | | | Mean (SE) proportion of time spent at different distances from seeds after first contact[c,g] | | | C24 (MB24)[d,e] | Prop. MB24 in rings 1–4[e] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | <16 min (RI)[f] | 16–45 min | >45 min | Rings 1–2 | Rings 3–4 | Rings 5–8 | | |
| Untreated control | | 41 (30) | 59.7 (8.46) AB | 0.13 (0) | 0.23 | 0.63 | 0.83 (0.05) | 0.05 (0.02) | 0.12 (0.04) | 41 (0) A | – |
| λ-cyhalothrin (M) | 10 | 24 (21) | 55.7 (9.44) AB | 0.38 (1)* | 0.52* | 0.10* | 0.26 (0.05)** | 0.25 (0.06)* | 0.49 (0.07)* | 22 (1) A | 0.00 |
| λ-cyhalothrin (D) | 10 | 66 (45) | 67.2 (7.08) A | 0.51 (2)** | 0.36 | 0.13** | 0.33 (0.04)** | 0.36 (0.05)** | 0.31 (0.05) | 54 (22) A-C | 0.55 |
| Tefluthrin | 10 | 30 (20) | 65.8 (10.36) AB | 0.50 (2)** | 0.45 | 0.05* | 0.33 (0.05)** | 0.28 (0.04)** | 0.39 (0.07) | 28 (4) AB | 1.00 |
| Thiamethoxam | 10 | 31 (22) | 61.1 (9.93) AB | 0.18 (0) | 0.14 | 0.68 | 0.69 (0.07) | 0.13 (0.03) | 0.18 (0.06) | 26 (22) E–G | 0.64 |
| Thiamethoxam + Fipronil | 10 + 1 | 46 (28) | 33.2 (5.96) B | 0.11 (0) | 0.18 | 0.71 | 0.72 (0.06) | 0.13 (0.03) | 0.15 (0.04) | 36 (29) D-G | 0.69 |
| Thiamethoxam + Fipronil | 10 + 5 | 35 (29) | 42.9 (7.44) AB | 0.10 (0) | 0.24 | 0.66 | 0.77 (0.05) | 0.08 (0.02) | 0.14 (0.04) | 34 (32) G | 0.63 |
| Fipronil | 1 | 28 (21) | 49.5 (8.74) AB | 0.10 (0) | 0.10 | 0.81 | 0.93 (0.03) | 0.03 (0.02) | 0.04 (0.02) | 26 (15) C-E | 0.27 |
| Fipronil | 5 | 42 (30) | 71.2 (9.16) A | 0.17 (0) | 0.23 | 0.60 | 0.79 (0.06) | 0.08 (0.03) | 0.13 (0.04) | 37 (31) E–G | 0.65 |
| Fipronil | 50 | 37 (31) | 43.9 (6.04) AB | 0.35 (1)* | 0.06 | 0.58 | 0.65 (0.07) | 0.11 (0.03) | 0.24 (0.06) | 35 (35) G | 0.77 |
| Ethiprole | 5 | 36 (30) | 54.2 (8.22) AB | 0.30 (1)* | 0.17 | 0.53 | 0.72 (0.07) | 0.14 (0.04) | 0.14 (0.05) | 32 (4) AB | 0.75 |
| Ethiprole | 50 | 31 (21) | 56.2 (8.59) AB | 0.14 (0) | 0.29 | 0.57 | 0.65 (0.08) | 0.12 (0.03) | 0.23 (0.07) | 24 (14) C-E | 0.79 |
| Cyazypyr | 10 | 41 (31) | 41.3 (5.03) AB | 0.19 (0) | 0.19 | 0.61 | 0.71 (0.07) | 0.07 (0.02) | 0.21 (0.06) | 38 (7) AB | 1.00 |
| Cyazypyr | 30 | 24 (19) | 63.7 (11.00) AB | 0.21 (0) | 0.37 | 0.42 | 0.68 (0.07) | 0.15 (0.04) | 0.17 (0.06) | 23 (13) C-E | 0.85 |
| Thiamethoxam + Cyazypyr + λ-cyhalothrin (M) | 39 + 10 + 10 | 46 (20)* | 42.8 (7.49) AB | 0.45 (1)* | 0.30 | 0.25* | 0.38 (0.07)* | 0.34 (0.08)** | 0.27 (0.08) | 41 (29) D-F | 0.48 |
| Thiamethoxam + Cyazypyr | 39 + 30 | 33 (19) | 43.4 (7.54) AB | 0.37 (1)* | 0.11 | 0.53 | 0.57 (0.08) | 0.15 (0.03) | 0.28 (0.07) | 28 (26) FG | 0.81 |
| Chlorantraniliprole | 30 | 26 (19) | 54.0 (8.77) AB | 0.05 (0) | 0.47 | 0.47 | 0.61 (0.07) | 0.16 (0.05) | 0.23 (0.06) | 25 (1) A | 0.00 |
| Spinosad | 30 | 29 (17) | 55.3 (9.42) AB | 0.41 (1)* | 0.06 | 0.53 | 0.63 (0.08) | 0.12 (0.04) | 0.25 (0.07) | 24 (12) B-D | 0.50 |
| Spirotetramat | 5 | 24 (21) | 42.9 (9.40) AB | 0.14 (0) | 0.05 | 0.81 | 0.91 (0.06) | 0.02 (0.01) | 0.07 (0.05) | 24 (0) A | – |
| Spirotetramat | 50 | 22 (17) | 60.0 (10.62) AB | 0.35 (1)* | 0.00 | 0.65 | 0.79 (0.08) | 0.05 (0.02) | 0.17 (0.07) | 22 (0) A | – |
| Chlorpyrifos | 5 | 24 (20) | 59.3 (11.47) AB | 0.10 (0) | 0.10 | 0.80 | 0.93 (0.05) | 0 (0) | 0.07 (0.05) | 24 (0) A | – |
| Chlorpyrifos | 50 | 37 (26) | 49.6 (7.55) AB | 0.35 (1)* | 0.04 | 0.62 | 0.72 (0.08) | 0.09 (0.03) | 0.19 (0.06) | 32 (12) A-C | 0.42 |

**Table 3** continued

| Insecticide[a] | Rate[b] | N (C3)[c] | Mean (SE) time to contact[d] | Proportion of wireworms in each contact duration category[c,e] | | | Mean (SE) proportion of time spent at different distances from seeds after first contact[c,g] | | | C24 (MB24)[d,e] | Prop. MB24 in rings 1–4[e] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | <16 min (RI)[f] | 16–45 min | >45 min | Rings 1–2 | Rings 3–4 | Rings 5–8 | | |
| Statistics | | Chi = 12.37<br>*df* = 21<br>$P = 0.93$ | F = 1.64<br>*df* = 21,515<br>$P = 0.036$ | Chi = 33.72<br>*df* = 21<br>$P = 0.39$ | Chi = 37.36<br>*df* = 21<br>$P = 0.015$ | Chi = 41.32<br>*df* = 21<br>$P = 0.0051$ | F = 8.83<br>*df* = 21,515<br>$P < 0.0001$ | F = 8.86<br>*df* = 21,515<br>$P < 0.0001$ | F = 3.22<br>*df* = 21,515<br>$P < 0.0001$ | Chi = 174.06<br>*df* = 21<br>$P < 0.0001$ | Chi = 9.89<br>*df* = 17<br>$P = 0.91$ |

*N* Number of wireworms (wws) exposed; C3, C24 = number of wws that contacted seeds at least once in 3 and 24 h (respectively). Contact time = time (min) required for wireworms to first contact seeds in the bioassay. RI = ww repellency index. MB24 = number of wws immobile (mobility categories W to M) after 24 h exposure

[a] All treatments also treated with the fungicide Dividend XL RTA at 13 g AI/100 kg seed. λ-cyhalothrin (M) = Matador 120EC; λ-cyhalothrin (D) = Demand 100CS; tefluthrin = Force 200SC; thiamethoxam = Cruiser 350FS; fipronil = Regent 500FS; ethiprole = Ethiprole FS350G; cyazypyr = DPX HGW86-599; chlorantraniliprole = Coragen 200SC; spinosad = GF-976 Spinosad 480SC; spirotetramat = Movento 240SC; chlorpyrifos = Pyrinex 480EC

[b] Rates are expressed as g AI/100 kg wheat seed

[c] Values in a column that differed significantly from the untreated control are marked with * if $P < 0.05$, ** if $P < 0.0001$

[d] Values followed by the same letter do not differ significantly, as per Tukey and Ryan's procedures for separating means and proportions, respectively ($\alpha < 0.05$)

[e] The Yates continuity correction was used for these $\chi^2$ comparisons

[f] RI = 0, 1, 2, 3, respectively, if the proportion contacting <16 min = 0–0.29, 0.30–0.49, 0.50–0.69, and 0.70+, respectively

[g] Rings 1–2: 0–2.5 cm from centre of arena; Rings 3–4: 2.5–6.0 cm; Rings 5–8: 6.0–13 cm

exposed at 50 g (RI = 0; Table 3). The proportion contacting >45 min at both rates (0.53, 0.57, respectively; Table 3), and amount of time spent in rings 1–2 after initial contact (prop. = 0.72, 0.65, respectively; Table 3) did not differ significantly from the control treatment.

### *Pyrethroids*

Repellent behaviour was elicited upon exposure to the 10 g rate of tefluthrin (RI = 2) and the two formulations of λ-cyhalothrin (Demand 100CS: RI = 2; Matador 120EC: RI = 1; Table 3). Significantly ($P < 0.05$) fewer wireworms remained in contact for >45 min in all three treatments relative to the control (prop. range = 0.05–0.13, Table 3), significantly less time was spent in rings 1–2 (range 0.26–0.33), and more time was spent in rings 3–4 (range 0.25–0.36; Table 3) after contact. When λ-cyhalothrin (Matador 120EC formulation) at 10 g was combined with cyazypyr at 10 g + thiamethoxam at 39 g, a similar proportion of wireworms were repelled (prop. = 0.45; RI = 1) as when larvae were exposed to seeds treated with λ-cyhalothrin (Matador 120EC formulation) at 10 g alone (Table 3), but more larvae remained in contact for >45 min (prop. = 0.25 vs. 0.10; $P > 0.2$; Table 3) and within rings 1–2 after initial contact (prop. = 0.38 vs. 0.26; $P > 0.5$; Table 3). While the proportion of wireworms that contacted seeds treated with this combination in 3 h was significantly lower than in the control treatment (prop. = 0.43 vs. 0.73; Table 3), a similar proportion had contacted by 24 h (prop. = 0.89; Table 3). Some initial pre-contact repellency was suggested by 6 wireworms that oriented towards the seeds and turned abruptly upon reaching ring 3, but 5 of these had contacted by 24 h. If initially repelled prior to contact, these wireworms were either desensitised to the repellent stimulus, or their repellency was overcome by the attraction of the seeds.

### *Diamides*

Wireworms were not repelled by seeds treated with cyazypyr at 10 or 30 g, or chlorantraniliprole at 30 g (RI = 0, Table 3), but the proportion remaining in contact for >45 min at the 30 g rates of cyazypyr and chlorantraniliprole (prop. = 0.42, 0.47, respectively, Table 3) was considerably lower than observed in the control treatment. Some wireworms (prop. = 0.26) exposed to chlorantraniliprole at 30 g became moribund after leaving rings 1–2, partially explaining why the time spent there after contact was numerically lower (prop. = 0.61) than in the control treatment. No morbidity was observed during the bioassay period for the 10 and 30 g rates of cyazypyr. Slight repellency was observed when wireworms were exposed to seeds treated with a combination of cyazypyr at 30 g + thiamethoxam at 39 g (RI = 1, Table 3), with a significantly higher proportion remaining in contact for <16 min (0.37, Table 3). The proportion contacting for >45 min (0.53) and the amount of time spent within rings 1–2 after initial contact (0.57) were numerically but not significantly lower than that observed in the control treatment (Table 3).

### *Chlorpyrifos*

Chlorpyrifos at the 50 g rate elicited slight repellency (RI = 1), with significantly more wireworms contacting seeds for <16 min (prop. = 0.35; Table 3) than in the control treatment. No repellency (RI = 0) was evident at the 5 g rate. The proportion of larvae contacting for >45 min at both 5 and 50 g (0.80, 0.62, respectively, Table 3), and the time spent within rings 1–2 after initial contact (0.93, 0.72, respectively, Table 3) were similar to that observed in the control treatment. At the 50 g rate, some (prop. = 0.12) larvae were immobilized, while in contact during the 3 h of observation. When wireworms were removed from bioassay arenas at 24 h, approximately half (prop. = 0.52) of those that contacted seeds treated with the 50 g rate were moribund (and therefore immobilized), of which the majority (prop. = 0.80) were in rings 3–8.

### *Spirotetramat*

Slight repellency was observed when wireworms were exposed to seeds treated with spirotetramat at 50 g (RI = 1), and significantly more wireworms contacted seed for <16 min (prop. = 0.35; Table 3) than in the control treatment. No repellency (RI = 0) was evident when wireworms were exposed to the 5 g rate, and the proportion contacting for >45 min at both 5 and 50 g (0.81, 0.65, respectively, Table 3), and the time spent within rings 1–2 after initial contact (0.91, 0.79, respectively, Table 3), were similar to that observed in the control treatment.

### *Spinosad*

Slight repellency was observed when wireworms were exposed to spinosad at 30 g (RI = 1), with significantly more wireworms contacting for <16 min (prop. = 0.41; Table 3) than in the control treatment. In addition, the proportion contacting for >45 min (0.53, Table 3), and time spent within rings 1–2 after making initial contact (0.63, Table 3) were numerically lower than observed in the control treatment.

## Post-contact wireworm health

### *Metarhizium infection*

A considerable proportion of wireworms died from *Metarhizium* infection in all treatments (range prop. 0–0.35 by 84 DAE) except λ-cyhalothrin at 10 g (Matador 120EC formulation), where there was no mortality (Figs. 2, 3, 4, 5). Mortality from this entomopathogen is not unusual in laboratory studies with wireworms (Zacharuk and Tinline 1968; Kabaluk et al. 2013) and has affected our previous studies with *A. obscurus* (van Herk and Vernon 2011, 2013a). Here *Metarhizium* infection was first observed at 7 DAE, and its incidence increased gradually until approx. 126 DAE, after which no further mortality was observed (Fig. 2a). Due to the high incidence of *Metarhizium*, Figs. 3, 4, 5 show the mortality of wireworms with and without infection symptoms, as well as the proportion of larvae that had died from *Metarhizium* by the last date of health checks. Hence, in most treatments, the number of wireworms on which these health profiles (Figs. 3, 4, 5) are based decreased over time as larvae that died from *Metarhizium* were removed. It should be noted that wireworms that die from *Metarhizium* infection do not show morbidity symptoms or changes in feeding and contact behaviour until $<7$ days of dying from the infection and the appearance of fungal hyphae on the cuticle) (Zacharuk and Tinline 1968; van Herk and Vernon 2013a). As health checks in the various treatments continued for 84, 126, 154, 182, and 210 DAE, depending on the time required for morbidity symptoms to resolve, we compared the incidence of *Metarhizium* in individual treatments by the last health check with that in the control treatment after the same DAE. This analysis indicated that the incidence of *Metarhizium* by the last health check differed significantly ($P < 0.05$) from the control in two treatments: λ-cyhalothrin (Matador 120EC) at 10 g (no *Metarhizium*: Chi $= 6.92$, $df = 1$, $P = 0.0085$, at 182 DAE), and thiamethoxam at 10 g + fipronil at 1 g (more *Metarhizium*; Chi $= 4.21$, $df = 1$, $P = 0.04$, at 210 DAE). The degradation of the larvae due to *Metarhizium* infection prevented post-assay molecular identification, which would have been able to separate the two cryptic *Agriotes* species used in this study and confirm identifications based on larval characters (Staudacher et al. 2011; Benefer et al. 2013).

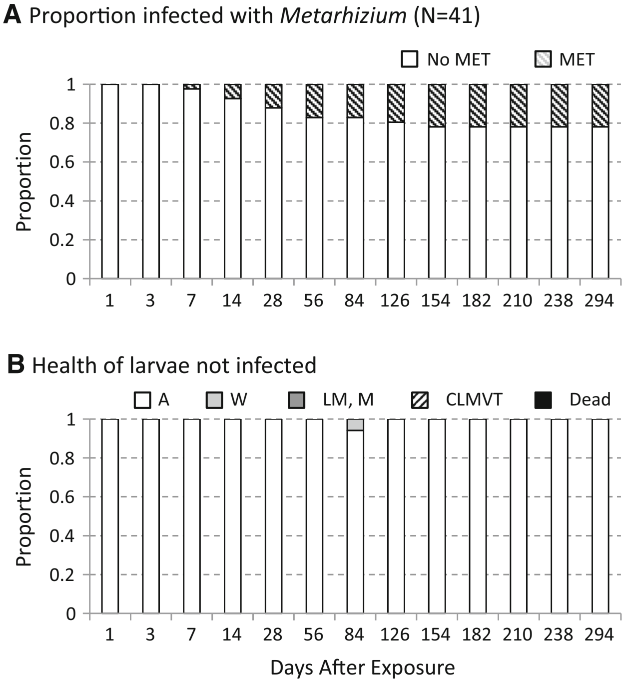


**Fig. 2** Proportion of *Agriotes obscurus/lineatus* larvae in different health (mobility) categories after contacting seeds treated with Dividend XLRTA in Study 3. "MET" = wireworms that died from *Metarhizium anisopliae* infection, "No MET" = wireworms that were not infected. Mobility categories (see text for full explanation): *A* alive; *W* writhing; *LM* leg and mouthpart movement; *M* mouthpart movement only; *CLMVT* characteristic fipronil poisoning symptoms

### *Thiamethoxam*

Wireworm health after contact with thiamethoxam at 10 g was similar to that observed in Study 1 (reported above). A high proportion (0.85) was moribund at 1 DAE, and these had all fully recovered by 14 DAE (Fig. 3a). There was very little mortality, and no relapse into morbidity after recovery. As in Study 1, most (prop. = 0.64, Table 3) wireworms moribund at 1 DAE were located near the seeds (rings 1–4).

### *Fipronil*

With the exception of one wireworm (discussed below), the characteristic fipronil poisoning symptoms (i.e. CLMVT) were only observed in wireworms that contacted treatments containing this insecticide. At all three rates tested, CLMVT symptoms generally did not appear immediately (i.e. 7 DAE for 1 g rate; 3 DAE for 5 g, 50 g), with typical morbidity symptoms (e.g. W, LM) appearing first (Fig. 3b, d, f). All wireworms showing CLMVT symptoms subsequently died. Both the proportion moribund at 1 DAE (range 0.58–1.00) and proportion of wireworms moribund, while in close proximity to the seeds (rings 1–4; range 0.27–0.77; Table 3), increased with the rate of fipronil applied.

### *Thiamethoxam + fipronil*

The health of wireworms that contacted seeds treated with the thiamethoxam at 10 g + fipronil at 1 g combination

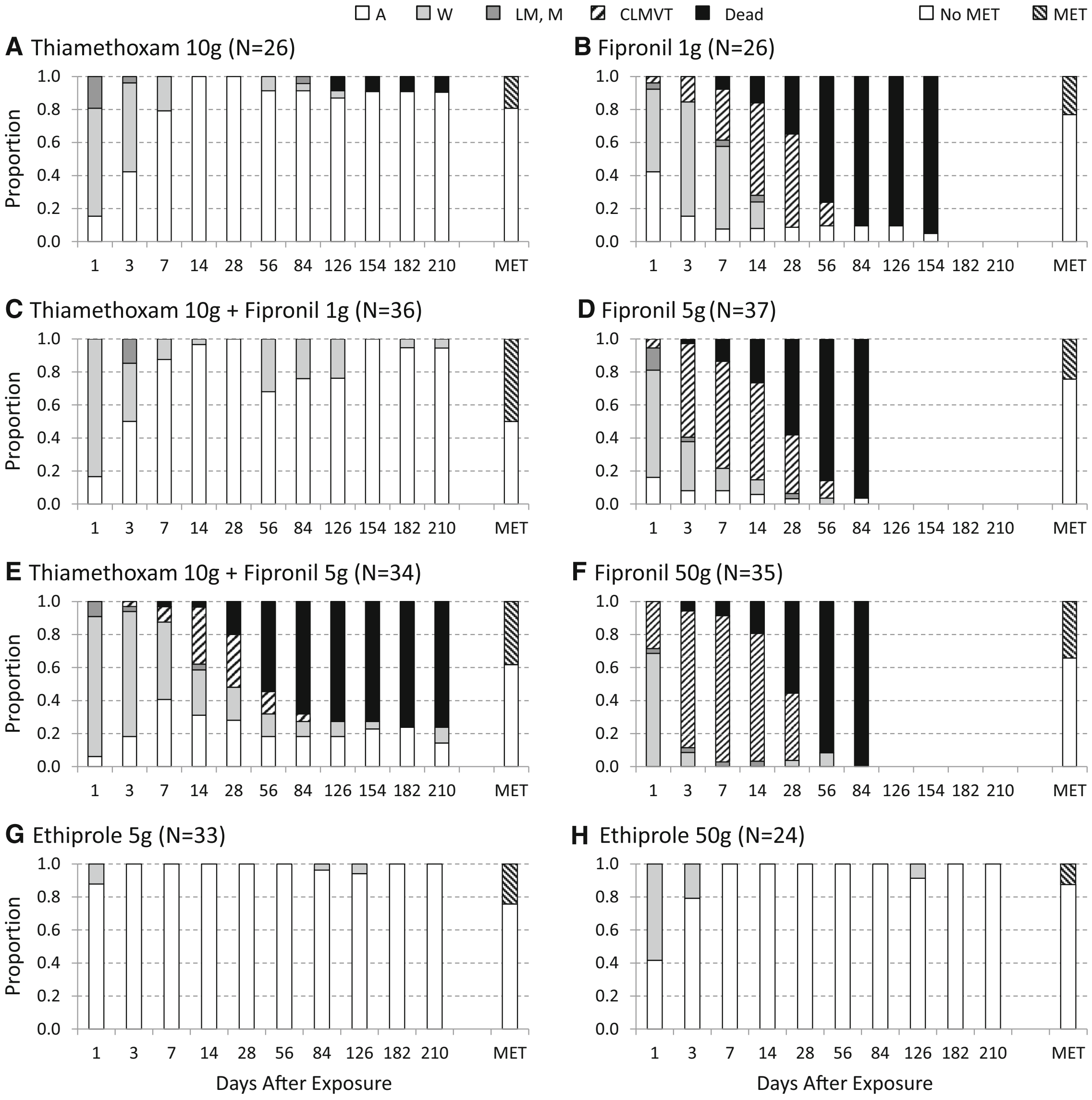


**Fig. 3** Proportion of *Agriotes obscurus/lineatus* larvae in different health (mobility) categories after contacted seeds treated with thiamethoxam (Cruiser 350FS), fipronil (Regent 500FS), and ethiprole (Ethiprole FS350G) in Study 3. Mobility categories (see text for full explanation): *A* alive; *W* writhing; *LM* leg and mouthpart movement; *M* mouthpart movement only; *CLMVT* characteristic fipronil poisoning symptoms. *N* Number of wireworms at 1DAE

was similar to those exposed to thiamethoxam at 10 g alone (Fig. 3c). Most wireworms (prop. = 0.81) showed normal morbidity symptoms (e.g. W, LM) at 1 DAE, mostly while in rings 1–4 (prop. = 0.69), from which they had recovered fully by 28 DAE. Of note is that the characteristic symptoms resulting from fipronil uptake (i.e. CLMVT) were absent, with no mortality occurring other than from *Metarhizium* (Fig. 3c). Since CLMVT symptoms and considerable mortality were seen when larvae were exposed to seeds treated with fipronil at 1 g alone, this suggests that the onset of morbidity caused by contacting thiamethoxam at 10 g prevented ingestion of sufficient fipronil to cause mortality. Larvae exposed to seeds treated with the combination of thiamethoxam at 10 g + fipronil at 5 g similarly appeared to have taken up less fipronil than those exposed to fipronil at either 1 g or 5 g rates alone, as appears from the lower, and later, incidence of CLMVT symptoms and lower mortality ultimately (Fig. 3e cf. Fig. 3b, d).

### *Ethiprole*

Wireworms exposed to ethiprole, the other phenylpyrazole insecticide evaluated in this study, did not show CLMVT symptoms, and there was no mortality other than from *Metarhizium* (Fig. 3g, h). At the high rate (50 g) tested,

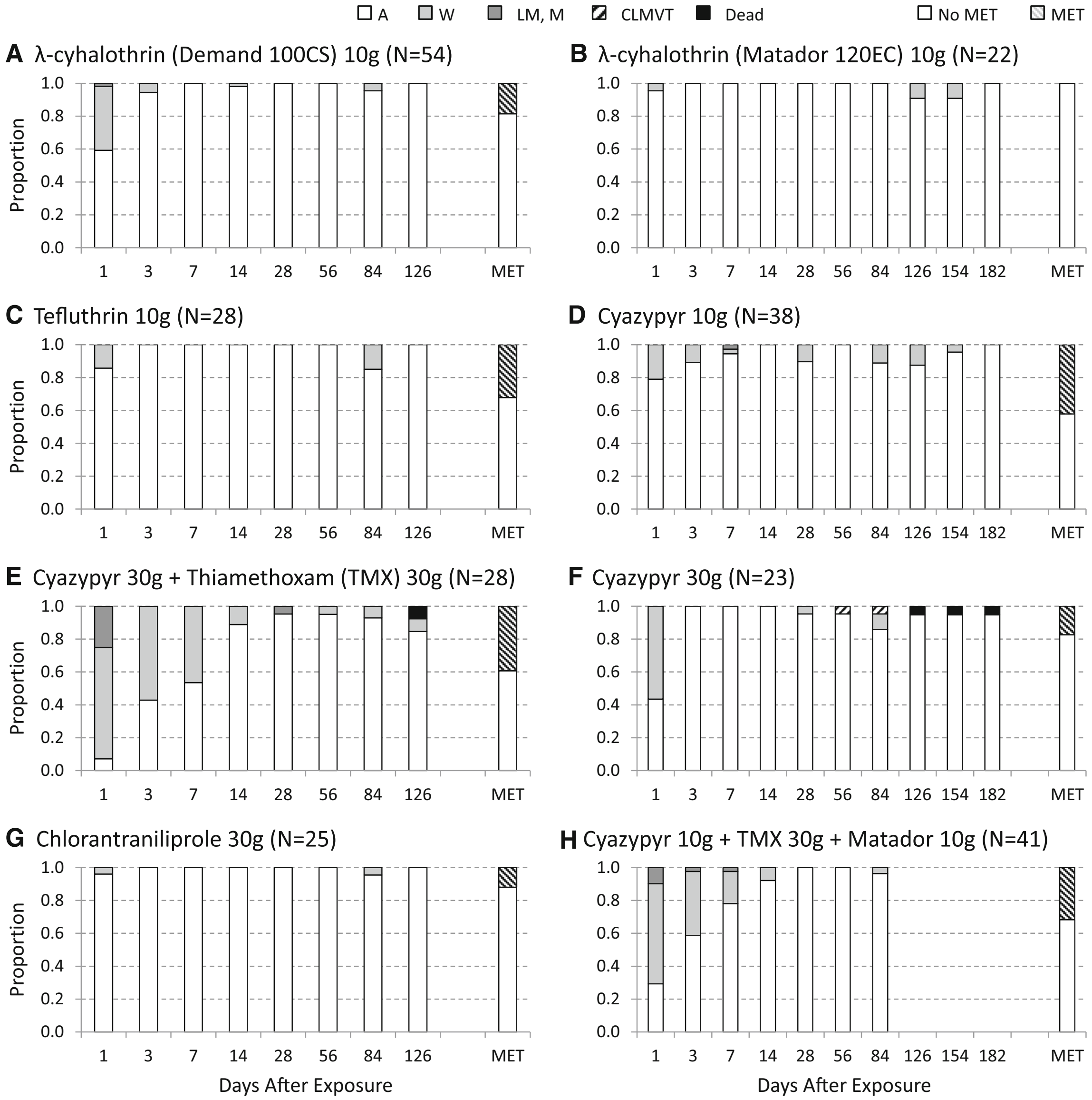
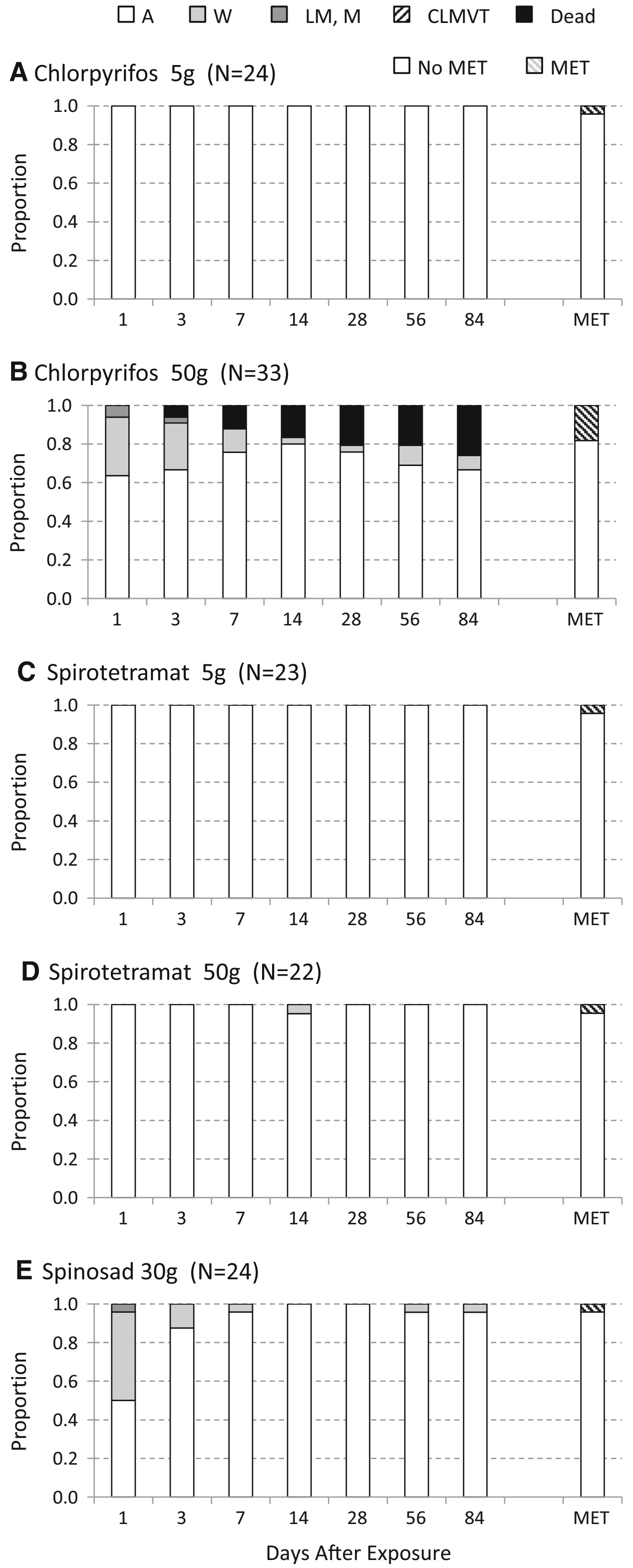


**Fig. 4** Proportion of *Agriotes obscurus/lineatus* larvae in different health (mobility) categories after contacted seeds treated with λ-cyhalothrin (Matador 120EC, Demand 100CS), tefluthrin (Force 200SC), cyazypyr (DPX HGW86-599), chlorantraniliprole (Coragen 200SC), and thiamethoxam (Cruiser 350FS) + cyazypyr blends in Study 3. Mobility categories (see text for full explanation): *A* alive; *W* writhing; *LM* leg and mouthpart movement; *M* mouthpart movement only; *CLMVT* characteristic fipronil poisoning symptoms. *N* Number of wireworms at 1DAE

approximately half (prop. 0.58) of larvae were moribund at 1 DAE, most occurring in rings 1–4 (prop. = 0.79), but all had fully recovered by 7 DAE (Fig. 3h). At the lower rate (5 g), only a low proportion (0.13) of larvae were moribund at 1 DAE, and these had recovered by 3 DAE (Fig. 3g). Health checks in both ethiprole treatments were continued until 210 DAE, as our previous toxicology studies with fipronil showed that morbidity symptoms and subsequent mortality may first appear long after exposure. When *A. obscurus* larvae were topically exposed to fipronil at the LC50 concentration in a Potter Tower, virtually no morbidity symptoms were observed until 84 DAE (van Herk et al. 2008c).

*Pyrethroids*

Only a small proportion of wireworms that contacted seeds treated with the 10 g rate of the two formulations of λ-cyhalothrin (Demand 100CS, Matador 120EC), and the 10 g rate of tefluthrin showed intoxication symptoms at 1 DAE (prop. = 0.41, 0.05, 0.14, respectively), and virtually all symptoms had disappeared by 3 DAE (Fig. 4a–c). In all



◀**Fig. 5** Proportion of *Agriotes obscurus/lineatus* larvae in different health (mobility) categories after contacted seeds treated with spinosad (GF-976 Spinosad 480SC), spirotetramat (Movento 240SC), and chlorpyrifos (Pyrinex 480EC) in Study 3. Mobility categories (see text for full explanation): *A* alive; *W* writhing; *LM* leg and mouthpart movement; *M* mouthpart movement only; *CLMVT* characteristic fipronil poisoning symptoms. *N* Number of wireworms at 1DAE

three treatments, there was no post-recovery relapse into morbidity and no mortality other than from *Metarhizium*. In the Matador 120EC at 10 g formulation, no wireworms died from *Metarhizium* infection (discussed above). This post-contact intoxication profile—a low incidence of morbidity at 1 DAE, rapid recovery from morbidity, and absence of mortality—is similar to that observed in Study 2 for *L. canus* exposed to the pyrethroids bifenthrin and tefluthrin at 10 and 20 g, and for *A. obscurus* and *L. canus* after exposure to tefluthrin at 10 or 20 g in previous studies (van Herk and Vernon 2007a; van Herk et al. 2008a).

### *Diamides*

Wireworms moribund after exposure to seeds treated with cyazypyr at 10 g or 30 g (prop. = 0.21, 0.52, respectively) at 1 DAE (Fig. 4d, f) were mostly located within rings 1–4 (prop. = 1.00, 0.85, respectively; Table 3), and had fully recovered by 14 DAE. Very low mortality (prop. = 0.06) was observed only at the 30 g rate at 126 DAE, due to a single larvae previously showing CLMVT symptoms (Fig. 4f). Wireworms exposed to the combined cyazypyr at 30 g + thiamethoxam at 39 g, and cyazypyr at 10 g + thiamethoxam at 39 g + λ-cyhalothrin at 10 g treatments showed a post-contact intoxication profile similar to larvae that contacted seeds treated with thiamethoxam at 10 g alone (Fig. 4e, h cf. Fig. 3a, c), with a high proportion (0.93, 0.71, respectively) moribund at 1 DAE from which nearly all (prop. = 0.89, 0.92, respectively) had recovered by 14 DAE, with very little subsequent mortality. Wireworms that contacted seeds treated with chlorantraniliprole at 30 g appeared unaffected by the chemical, with virtually no morbidity at 1 DAE (prop. = 0.04), and no subsequent mortality (Fig. 4g).

### *Chlorpyrifos*

The absence of morbidity symptoms in wireworms exposed to seeds treated with chorpyrifos at 5 g suggests this treatment did not affect wireworm health (Fig. 5a), and that post-contact morbidity in *A. obscurus* exposed to thiamethoxam at 10 g + chorpyrifos at 5 g in Study 1 was likely due to the thiamethoxam. Some wireworms (prop = 0.38) exposed to chorpyrifos at 50 g were moribund at 1 DAE, and a considerable prop. (0.27) had died by 84 DAE (Fig. 5b). Since virtually no mortality was observed when *A. obscurus* were exposed to thiamethoxam at 10 g + chorpyrifos at 50 g in Study 1 (prop = 0.06 at

84DAE, Fig. 1f), it appears that the morbidity induced by thiamethoxam in the combination treatment reduced the uptake of chlorpyrifos, and hence wireworm mortality.

### *Spirotetramat, spinosad*

Wireworms exposed to seeds treated with spirotetramat at 5 and 50 g appeared unaffected, with no morbidity at 1 DAE or subsequently (Fig. 5c, d). Of larvae exposed to seeds treated with spinosad at 30 g, one half (prop. = 0.50) were moribund by 1 DAE, while all of them were in rings 1–4 (Table 3). These had fully recovered by 14 DAE, and there was no subsequent mortality or relapse into morbidity (Fig. 5e).

## Discussion

### Importance of direct observations of behaviour

The importance of determining the behavioural response(s) of wireworms to insecticides was recognised by Long and Lilly (1958, 1959), who suggested larvae of *Melanotus communis* (Gyllenhal) were repelled by seeds treated with lindane and other organochlorine insecticides, or experienced other sublethal effects (e.g. cessation of feeding) after contact. We have shown that lindane does not repel larvae of *A. obscurus* and *L. canus* when placed on germinating wheat seeds in soil (van Herk et al. 2008a), but is repellent to *A. obscurus* in a soil-less environment and in the absence of food (van Herk et al. 2008b). Our observations have also shown that the cessation of feeding reported by Long and Lilly (1958, 1959) was likely due to wireworms entering intermediate morbidity stages during which the larvae lose varying degrees of mobility (van Herk and Vernon 2007a). Several others have reported similar decreases in wireworm mobility after contact with insecticides, notably Grove et al. (2000) and Furlan and Toffanin (1998).

Our bioassay methods, including pre-selection of feeding larvae, direct observation of their behaviour in soil, and long-term post-bioassay health assessments are a unique and efficient approach to helping us understand how insecticides affect wireworms in the field. The methods described herein ensure a high proportion of wireworms contact the treated seeds and enable us to determine if an insecticide is repellent, and if contact induces morbidity and mortality. Determining where wireworms become moribund relative to the treated seeds is also important, as some insecticides set up a small toxic halo around treated seeds (Raveton et al. 2007). This presumably exposes wireworms immobilized near the seeds to prolonged contact with the insecticide or its metabolites, which in some cases (e.g. fipronil) are as toxic as the parent compound (Scharf et al. 2000). The importance of continuing wireworm health observations long after exposure to insecticides was recognised long ago: Lehman (1933b) continued health checks on *L. californicus (Mannerheim)* larvae exposed to carbon disulfide for 60 days, during which some wireworms that initially appeared dead made a complete recovery; Lange et al. (1949) and Long and Lilly (1958) conducted regular observations on wireworms after laboratory studies for 16 and 20 weeks, respectively. We have stressed the importance of continuing health checks for up to a year, depending on the chemical class under evaluation, in previous work. Wireworms may be moribund after topical exposure to an insecticide at a rate 10x below the LC50 and recover thereafter (e.g. in neonicotinoids). In other cases, toxicity symptoms may be delayed: *A. obscurus* larvae exposed to fipronil at the LC50 rate did not show intoxication symptoms for 84 days after exposure, during which time they were able to feed and behave normally (van Herk et al. 2008c).

### Repellency and feeding deterrence

A review of wireworm literature indicates that the term ‘repellency’ is commonly used to describe observations (e.g. the absence of wireworms at insecticide-treated seedlings) better explained by other sublethal effects of insecticides (e.g. Lehman 1933a; Long and Lilly 1958, 1959). Arguably a chemical that elicits repellency causes a wireworm to be repelled immediately upon contact, often after showing a characteristic “shock reaction” (van Herk et al. 2008b). In this response, described by Lees (1943b) and Falconer (1945), wireworms immediately recoil and rapidly back away upon encountering an unfavourable situation, e.g. a pronounced difference in soil temperature or humidity (Lees 1943b; Falconer 1945), or an insecticide droplet on filter paper placed in its path (van Herk et al. 2008b). We have observed such repellent behaviour in response to tefluthrin, lindane, and chlorpyrifos under soil-less conditions and in the absence of food (van Herk et al. 2008b), but herein, wireworms remained in contact for some time when exposed to seeds treated with these chemicals in soil, and did not show a “shock reaction”. This discrepancy may indicate that the attractiveness of the seeds (e.g. because of $CO_2$ production) may interfere with the repellent cue, just as the presence of food causes some wireworms (e.g. *Selatosomus destructor* (Brown)) to endure suboptimum soil temperatures they would otherwise avoid (Zacharuk 1962). Our functional definition of repellency as <16 min contact used here remains useful when considering insecticides placed on germinating seeds, and the insecticides reported as repellent using this criterion also elicited the shock response when wireworms were assayed under soil-less conditions and in the absence of food (van Herk et al. 2008b).

We suggest the wireworm movement away from the seeds herein observed may correspond with the onset of morbidity. Contact with a single pyrethroid-treated seed can cause a rapid onset of morbidity (e.g. within <20 min for tefluthrin; van Herk and Vernon 2007b), which may explain why most larvae exposed to these insecticides (i.e. bifenthrin and tefluthrin at 20 g) contacted for <16 min (Table 2). The onset of morbidity depends on both the type and rate of insecticide larvae are exposed to. Exposure to a high rate of a chemical may cause sufficient ingestion of a toxin to effect rapid immobility, but insufficient ingestion to cause mortality. In such cases, a lower rate that allows for a longer period of ingestion prior to the onset of morbidity may cause more mortality. However, such responses will likely vary among species and between individuals. Combining a nonlethal insecticide that causes the rapid induction of temporary morbidity (e.g. a neonicotinoid or pyrethroid) with a lethal compound in which intoxication symptoms are delayed (e.g. fipronil) may decrease the effectiveness of the latter by preventing sufficient intake to cause mortality. Evidence of this is presented below.

## Wireworm energetics and LD estimation from feeding

Little has been reported on wireworm food uptake and its relation to larval growth and rate of insecticide ingestion. From the scant literature, it appears that food ingestion can be considerable and digestion rapid, though species, instar, and temperature dependent. Stone (1941) calculated *L. californicus* larvae required approximately 106 wheat seeds to complete development in 2 years, with fastest larval growth and highest food consumption occurring during the first year. Evans and Gough (1942) report a 14 % weight gain in 10 weeks when medium size *Agriotes* spp. larvae were raised on wheat, and Davis (1959) reports *S. destructor* larvae doubled in weight when exposed to germinated wheat seeds for 28 days.

To our knowledge, food energetics has only been studied for *Melanotus rufipes* (Herbst). Dutton (1968) measured larval feeding of this predatory elaterid on blowfly larvae, *Calliphora erythrocephala* (Meigen) to be 0.05 mg prey/mg wireworm in 24 h, and by measuring faecal production calculated the percentage of food assimilated to be 86.5 % by weight (90.3 % by calories). This high assimilation rate was attributed to the wireworms' extra-oral digestion and liquid feeding habit (Dutton 1968). If herbivorous wireworms have a similar food assimilation rate, the 2.74 mg increase in weight reported here for *L. canus* after feeding for 24 h would require consumption of less than 10 % of a single wheat seed (seed weight: approx. 0.04 g). This estimate can be used to approximate the amount of insecticide required to cause the (sub)lethal effects observed after contact. For example, we observed 95 % mortality in *A. obscurus* larvae that contacted seeds treated with fipronil at 1 g during the 24-h bioassay period. Since individual wheat seeds weighed no more than 0.04 g, these wireworms would have ingested approx. 0.9 nmol fipronil (molar mass = 437.15 g/mol) if they consumed one entire seed, and much less if they consumed as little as 10 % of a single seed. In comparison, Chaton et al. (2008) report the LD100 of fipronil on *Agriotes* larvae to be a minimum of 0.1 nmol, but do not report how this value was derived.

## Neonicotinoid insecticides

The contact behaviour and low post-contact mortality of *A. obscurus* exposed to seeds treated with thiamethoxam at 10 g observed in Studies 1 and 3 are similar to that observed in our previous laboratory studies (van Herk et al. 2008a), and explain the effectiveness of this insecticide when used as a seed treatment in the field. In field efficacy studies, thiamethoxam provided early protection and stand establishment of wheat without significantly decreasing populations of *A. obscurus* (at 10–30 g; Vernon et al. 2009, 2013a, b) and *L. californicus* and *Hypnoidus bicolor* (Eschscholtz) (at 39 g; Morales-Rodriguez and Wanner 2015). Similar results were obtained with other neonicotinoids used as seed treatments, e.g. clothianidin, imidacloprid (Vernon et al. 2009). In other laboratory studies, plant protection but low (or no) wireworm mortality was observed when thiamethoxam was applied on wheat seed (*L. californicus, H. bicolor*: Morales-Rodriguez and Wanner 2015) and sugarcane billets (*Melanotus communis*: Hall 2003), and when imidacloprid was applied to corn and sugarbeet seeds (various European *Agriotes* spp.: Furlan and Toffanin 1998; Furlan and Campagna 2002).

Our behavioural studies indicate the mechanism responsible for thiamethoxam providing plant protection is not repellency, as in pyrethroid insecticides, but temporary morbidity. The rapid induction of morbidity after contact with thiamethoxam (and other neonicotinoids; Vernon et al. 2008) prevents wireworm feeding on seedlings long enough for them to reach a size at which they can compensate for root herbivory. This rapid induction of morbidity may prevent ingestion of a lethal dose of thiamethoxam, or other insecticides (i.e. fipronil or chlorpyrifos) used in combination (discussed below).

## Phenylpyrazole insecticides

Our results confirm that fipronil is not repellent to *A. obscurus* larvae when applied to seeds at 1 or 5 g. Morbidity induction was rapid, with most of the wireworms that died after contact already moribund at the end of the 24h observation period. In contrast, when fipronil is applied topically, morbidity symptoms generally do not appear immediately (discussed above), and this discrepancy may simply result from the method of exposure. Fipronil's high toxicity to wireworms has been shown in topical application studies (e.g. LC50 = 0.0001, 0.06 for fipronil and lindane, respectively; van Herk et al. 2008c), field studies conducted for *A. obscurus* (Vernon et al. 2009, 2013a, b), *L. californicus* and *H. bicolor* (Morales-Rodriguez and Wanner 2015), and in laboratory studies with various *Agriotes* spp. (Furlan and Toffanin 1998; Furlan and Campagna 2002; van Herk et al. 2008c, Vernon et al. 2008).

To protect wheat seedlings from wireworm damage and reduce wireworm populations, a minimal amount of fipronil can be combined with a second compound that offers good stand protection (e.g. a neonicotinoid such as thiamethoxam). Such a compound should not preclude wireworms from contacting seeds (i.e. be repellent), but need not cause wireworm mortality itself as this would be done by fipronil. Field studies have shown that combinations of thiamethoxam and fipronil achieve both crop establishment and pest population reduction (Vernon et al. 2013a, b; Morales-Rodriguez and Wanner 2015). In laboratory studies, combining fipronil with imidacloprid also achieved both objectives when used on corn and sugarbeet as seed treatments against various *Agriotes* spp. (Furlan and Toffanin 1998; Furlan and Campagna 2002). Our laboratory studies suggest, however, that combining a neonicotinoid insecticide with a low rate of fipronil can decrease the effectiveness of the latter (discussed below).

The absence of mortality after exposure to ethiprole is notable, and was not due to their failure to contact the chemical. Hall (2003) reports dipping sugarcane billets into a liquid formulation of ethiprole provided some plant protection from *M. communis*, but did not cause significant wireworm mortality in 3 of 4 laboratory studies. Preliminary field studies with ethiprole wheat seed treatments indicated both poor stand protection and poor wireworm control (RS Vernon, unpublished data).

Factors that may account for the difference in toxicity between these two phenylpyrazoles are that fipronil is more lipophilic than ethiprole (presumably leading to higher transintegumental penetration), and that fipronils' strongly electron-withdrawing trifluoromethylsulfinyl substituent is replaced by an electron-donating ethylsulfinyl group in ethiprole (Caboni et al. 2003). Interestingly, older insecticides that effectively controlled wireworms (e.g. lindane, cyclodienes) also function as non-competitive antagonists (blockers) of insect γ-aminobutyric acid (GABA)-gated chloride channels and have strong electron-withdrawing groups (Chen et al. 2006). For developing future chemistries to control wireworms, it would be useful to determine the subunit structures of their GABA receptors, as different receptor subunit combinations confer differential susceptibility to fipronil, and if fipronil's high toxicity to wireworms is related to its unique ability to also block glutamate-activated chloride channels (Zhao et al. 2003).

## Pyrethroid insecticides

Repellent behaviour was observed for *L. canus* exposed to tefluthrin at 10 and 20 g and bifenthrin at 20 g and *A. obscurus* exposed to tefluthrin at 10 g and λ-cyhalothrin at 10 g, and resulted in an absence of mortality in both species. Repellency to tefluthrin was expected in light of observations from other studies (van Herk and Vernon 2007a; van Herk et al. 2008a). In earlier laboratory studies, tefluthrin droplets placed on filter paper elicited repellency in *A. obscurus* at amounts as low as 0.48 nmol (calculated from 20 µl droplet of 0.01 % AI tefluthrin, and a molar mass of 418.73 g/mol; van Herk et al. 2008b). In comparison, a single seed treated at 5 g AI/100 kg seed would have 4.8 nmol tefluthrin (assuming an individual seed weight of 0.04 g). Pyrethroid repellency is suspected for providing plant protection without reducing wireworm populations for tefluthrin placed on wheat (*A. obscurus*; Vernon et al. 2009) and for tefluthrin, bifenthrin, and zeta-cypermethrin applied on sugarcane billets (*M. communis*: Hall 2003). Of note is that the post-contact health of *A. obscurus* exposed to λ-cyhalothrin at 10 g differed between the Matador 120EC and Demand 100CS formulations, with a much higher percentage moribund at the end of the bioassay period in the latter (5 vs. 41 %). This did not affect the overall efficacy of the insecticide, however, and subsequent wireworm health was similar in both formulations.

## Chlorpyrifos

In both Study 1 and Study 3, wireworm contact behaviour was affected at the 50 g (but not 5 g) rate of chlorpyrifos, significantly more larvae contacting for <16 min than in the respective control treatments. Wireworm repellency to chlorpyrifos has previously been suspected (but not confirmed) for *Hapatesus hirtus* Candeze (Horne and Horne 1991) and *Agriotes* spp. (Missonnier and Brunel 1979), and in soil-less bioassays, we have observed slight and moderate repellency of *A. obscurus* to (respectively) 57 and 570 nmol chlorpyrifos (calculated from 20 µl droplet of 1 and 10 % AI chlorpyrifos, respectively, and a molar mass of 350.59 g/mol; van Herk et al. 2008b). In comparison, at the 50 g rate, the five seeds used in the current bioassay would contain 285 nmol (seed weight: 0.04 g).

The intoxication and recovery profiles of wireworms exposed to the blends of thiamethoxam at 10 g + chlorpyrifos at 0.5, 5, and 50 g were very similar to that observed for larvae exposed to thiamethoxam at 10 g alone, and wireworms exposed to chlorpyrifos at 5 g alone did not become moribund. This suggests the temporary morbidity observed in the blended treatments resulted mainly (or entirely) from thiamethoxam uptake. Since wireworm mortality was lower when exposed to both thiamethoxam and chlorpyrifos at 50 g than when exposed to chlorpyrifos at this rate alone (6 % vs. 27 %), the morbidity induced by thiamethoxam may have reduced chlorpyrifos uptake and thereby decreased the effectiveness of the latter.

Chlorpyrifos (Pyrinex 480EC) applied at 5 and 50 g, both with and without thiamethoxam (Cruiser 350FS) at 10 g provided good wheat stand protection relative to untreated plots in a field study conducted at PARC in 2008 (RS Vernon, unpublished data). When these plots were baited for wireworms the following spring to assess mortality, a 70.7 and 31.5 %, decrease in wireworm numbers was observed relative to untreated control plots, when seeds were treated with chlorpyrifos at 50 g with and without thiamethoxam (respectively). In comparison, when seeds were treated with thiamethoxam at 10 g alone, a 62.5 % reduction in populations was observed, suggesting that adding the chlorpyrifos at 50 g did not significantly increase mortality. When seeds were treated with chlorpyrifos at 5 g with and without thiamethoxam, wireworm populations decreased by 21.7 % and increased by 3.3 % relative to control plots, respectively, suggesting chlorpyrifos at this rate did not cause wireworm mortality and had an antagonistic effect when used in a blended treatment with thiamethoxam (Chi $= 28.0$, $df = 1$, $P < 0.0001$). The different interactions between the two chemicals in these field and lab studies underscore that insecticide efficacy data collected from laboratory studies must be confirmed by field studies.

## Chlorantraniliprole, cyazypyr, spirotetramat, and spinosad

The results reported herein suggest chlorantraniliprole and cyazypyr are not effective for controlling wireworms at the 10–30 g rates. Wireworms were not repelled by either chemical, post-contact morbidity symptoms, if present, disappeared quickly, and there was little post-contact mortality. While combining cyazypyr at 30 g with thiamethoxam at 30 g increased the proportion moribund at 1–7 DAE, this was probably due to the thiamethoxam, and did not appreciably increase wireworm mortality. Combining cyazypyr with thiamethoxam and λ-cyhalothrin caused a more rapid recovery from morbidity than observed in the blend of the first two alone, possibly due to the lower rate of cyazypyr used in this blend, but more likely due to repellency elicited by λ-cyhalothrin decreasing the duration of contact with the seeds. This repellency also appeared to delay the time needed to first contact seeds in the three insecticide blend, but since a similar proportion of wireworms did eventually contact seeds in this as in other treatments, these repelled wireworms either became habituated to the repellent cue and/or were overcome by the attraction of the seeds and their own physiological need to feed.

Wireworms exposed to spirotetramat showed no morbidity or subsequent mortality, possibly due to the chemical's mode of action (inhibition of lipogenesis, reproduction, and growth) being less effective for long-lived insects, and not from failure to contact seeds.

While some repellency and brief morbidity were observed when wireworms were exposed to spinosad at 30 g, there was no mortality due to the insecticide observed. Previous topical application studies indicated spinosad was 8.5× and 5100× less toxic to *A. obscurus* than lindane and fipronil, respectively, (based on LC50 values; van Herk et al. 2008c).

## Combining insecticides on seeds

Combining two or more insecticides into a blended seed treatment is a common practice, the reasoning being that each component targets different neural targets and/or requires different detoxification pathways and that combining them together has additive or synergistic effects. However, applying more than one insecticide to a seed can also have antagonistic effects. We have previously shown that adding tefluthrin at 10 g to thiamethoxam at 10 g reduced contact time from 141.2 to 23.5 min in *L. canus* and from 104.5 to 13.8 min in *A. obscurus*, which was similar to the contact duration when these two species were exposed to tefluthrin at 10 g alone (22.5, 15.5 min, respectively; van Herk et al. 2008a). Here we show that adding λ-cyhalothrin to a combination of thiamethoxam + cyazypyr decreased the proportion contacting >45 min, the proportion moribund in rings 1–4, and the time spent in rings 1–2 after contact.

Adding thiamethoxam at 10 g to fipronil at 1 or 5 g decreases the efficacy of the latter, likely due to a reduction in fipronil uptake. Antagonism between these two chemicals was recently suggested by Morales-Rodriguez and Wanner (2015) for *L. californicus* and *H. bicolor*, but was observed only in their laboratory studies and not in their concurrent field studies. Similarly, we have not observed antagonism between the two insecticides at the above rates on wheat in five insecticide efficacy field studies conducted on *A. obscurus* at PARC (Vernon et al. 2013b).

Combining thiamethoxam with chlorpyrifos decreased the percentage mortality of the latter in these laboratory studies, and of the former in concurrent field studies. The field data for the chlorpyrifos and fipronil blends with thiamethoxam should be interpreted with some caution; however, as we often observe higher wireworm populations in plots treated with thiamethoxam and other neonicotinoid insecticides than in respective control plots (Vernon et al. 2009), possibly because late instar larvae temporarily rendered moribund from neonicotinoid exposure failed to pupate during the summer and remained larvae for an additional year. Such an artificial increase in wireworm populations may mask the actual mortality (if any) caused by the neonicotinoid insecticide and confound standard synergy/antagonism calculations.

Antagonistic and synergistic effects between two or more insecticides used as a seed treatment blend are likely to vary with the rates of insecticide used and wireworm species involved. Such blending should take into account the possible antifeedant, repellent, and other sublethal responses induced by the component chemistries. These behavioural responses can be elucidated, and suspected insecticide synergy or antagonism confirmed, by using both contact application and observational studies with the component chemicals singly and as a blend. Observational studies as herein described will indicate if morbidity induced by a nonlethal insecticide prevents sufficient ingestion of a lethal compound to cause mortality.

## Author contribution statement

WVH and RSV conceived the research. WVH, BV, SS, JF, and CF conducted studies. WVH conducted statistical analyses and wrote the manuscript. RSV secured funding.

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