Efficacy of diamide, neonicotinoid, pyrethroid, and phenyl pyrazole insecticide seed treatments for controlling the sugar beet wireworm, *Limonius californicus* (Coleoptera: Elateridae), in spring wheat

W.G. VAN HERK[1], T.J. LABUN[2], AND R.S. VERNON[3]

ABSTRACT

Four classes of insecticide applied on seed were evaluated for managing high populations of the sugar beet wireworm, *Limonius californicus* (Coleoptera: Elateridae), in spring wheat in southern Alberta, Canada. Three separate field trials were conducted, and assessments made for stand protection, yield, and wireworm survival. Imidacloprid and thiamethoxam applied at 10–30 g AI and cyantraniliprole applied at 10–40 g AI provided initial stand protection, but did not protect seedlings until harvest and did not decrease wireworm populations. λ-cyhalothrin applied at 30 g AI provided stand protection that persisted until harvest, but yields were considerably lower than observed in fipronil treatments and there was little (23%) decrease in populations relative to controls. Fipronil applied at 0.6, 1.0, and 5.0 g AI, either singly or in blend with thiamethoxam at 10 g AI, provided stand protection until harvest and significantly reduced numbers of wireworms larger than 10 mm (range: 74–96%). Very low numbers of small (<11 mm) wireworms were observed in all trials. These results are compared to data from laboratory and field studies for this and other wireworm species. The relation between crop stand protection and wireworm mortality, the potential of insecticide blends, and the importance of seed type, wireworm species, and activity periods for managing wireworms with seed treatments are discussed.

Keywords: *Limonius californicus*, wireworm, pest management, thiamethoxam, fipronil, insecticide blend

INTRODUCTION

Wireworms have long been important insect pests in cereal, sugar beet, and potato production in southern Alberta (AB) (Strickland 1927). Historically, the main pest species were the prairie grain wireworm, *Selatosomus destructor* (Brown) and *Hypnoidus bicolor* (Esch.) (Strickland 1927; Arnason 1931). Recent surveys indicate *S. destructor* and *H. bicolor* remain the most commonly occurring elaterid pests in AB and Saskatchewan (SK), while the sugar beet wireworm, *Limonius californicus* (Mann.), is of more regional importance (van Herk and Vernon 2014). Described as an occasional pest new to AB in the 1950s (MacNay 1954), and historically found only in low numbers alongside *S. destructor* and *H. bicolor* (Doane 1977), *L. californicus* is currently the third most prevalent wireworm species in arable land in the Prairie Provinces (van Herk and Vernon 2014). In southern AB, where it is often the predominant species in continuously cropped cereals, high *L. californicus* populations can cause complete stand destruction of spring wheat, even if treated with insecticides (T.J. Labun, personal observation). The relatively recent emergence of this species as a pest in this region may stem from changes in cultivation practices, including the implementation of minimal tillage practices in recent decades which have increased soil moisture retention. *Limonius californicus* is

[1] Corresponding author: Agassiz Research and Development Centre, Agriculture and Agri-Food Canada, P.O. Box 1000, Agassiz, British Columbia, Canada, V0M 1A0; wim.vanherk@canada.ca
[2] Syngenta Crop Protection (Canada) Inc., 6700 Macleod Trail Southeast, Calgary, Alberta, T2H 0M4
[3] Sentinel IPM Consulting, 4430 Estate Drive, Chilliwack, B.C., Canada V2R 3B5

known to prefer moist soils (e.g., irrigated land) and is typically not found on dry land (van Herk and Vernon 2014). Little else is known about the ecology and life history of this species, other than what was described by Stone (1941) for California, which suggests it is similar to the dusky wireworm, *Agriotes obscurus* L., and has a three- to five-year larval stage in the field.

In cereal production, wireworms have historically been managed by seed treatments, particularly chlorinated hydrocarbons (Toba *et al.* 1988; Grove *et al.* 2000). Treating seed with lindane decreased wireworm damage in cereal crops in the Canadian prairies by 90% and pest populations by 70% in the 1940s (Arnason and Fox 1948), and led to further decreases in damage between 1954 and 1961 (Burrage 1964). Similar results were obtained with other species, including *L. californicus*, in spring wheat in the Pacific Northwest (Toba *et al.* 1985, 1988). The effectiveness of lindane as a seed treatment stemmed from its ability to kill multiple pest species and all wireworm instars of these species, including neonates emerging from eggs laid after the seed is planted (Vernon *et al.* 2009). As a result of the latter property, wireworms would not repopulate fields to economic levels for several years after treatment, and farmers typically treated their cereal crops every 3–4 years (Arnason and Fox 1948). The reduction of wireworm populations achieved by planting lindane-treated seeds also protected high-value rotational crops such as sugarbeet, potato, and canola planted in subsequent years. Following lindane's de-registration (Canada in 2004; USA in 2006), there has been a gradual but continual increase in the incidence of wireworm damage in Canadian agricultural land. As a result, there is now a pressing need to identify and register new wireworm control measures for cereal crops. Such measures should be cost effective, pose negligible risk to humans and the environment, and offer similar efficacy to lindane by providing both stand and yield protection and reduction of wireworm populations (including controlling neonates) (Vernon *et al.* 2013a).

Initial results from laboratory and field evaluations of candidate insecticides to replace lindane as cereal seed treatments indicated neonicotinoid insecticides applied to wheat seed at 10–30 g AI/100 kg seed provide excellent stand and yield protection of spring wheat in the field in the presence of moderate to high populations of *A. obscurus*, but they did not decrease populations relative to control treatments (Vernon *et al.* 2009, 2013a). This disconnect between crop protection and lack of wireworm mortality was due to these insecticides inducing rapid and prolonged periods of morbidity, during which wireworms are unable to feed and after which they generally recover (Vernon *et al.* 2008, 2009). In contrast, the phenyl-pyrazole fipronil applied at 60 g AI/100 kg seed (a rate similar to that formerly registered for lindane) provided excellent stand and yield protection and eliminated *A. obscurus* populations (including neonate larvae) in the field (Vernon *et al.* 2009, 2013a). Laboratory studies indicated that dermal exposure of *A. obscurus* to fipronil causes rapid and irreversible morbidity, leading to complete mortality at higher rates. At low rates of fipronil exposure, wireworms showed no morbidity symptoms for several months, after which latent morbidity symptoms became apparent and mortality followed (Vernon *et al.* 2008). Exposing wireworms to wheat seed treated with low rates of fipronil permits them to feed normally until they succumb to latent mortality (Vernon *et al.* 2013a).

Based on these observations, we hypothesized that applying low rates of both thiamethoxam and fipronil to wheat seed would both provide stand and yield protection equivalent to lindane, and significantly reduce wireworm (including neonate) populations in the field (Vernon *et al.* 2013a). Specifically, thiamethoxam would provide early-season crop protection, while fipronil, even at very low doses, would cause late-season wireworm mortality. This approach would require low amounts of chemical, thereby reducing the environmental and human risk posed by these insecticides. Subsequent studies with *A. obscurus* demonstrated that blends of thiamethoxam at 10 g AI/100 kg seed and fipronil at 1 g AI/100 kg seed provided plant protection and wireworm control equivalent to lindane (Vernon *et al.* 2013a). Similarly, Morales-Rodriguez and Wanner

(2015) found that blends of these insecticides provide plant protection and reduce numbers of *L. californicus* and *H. bicolor*, but their field studies evaluated a single rate of these chemicals and under low pest pressure.

Here, we present results from three trials conducted in southern AB in fields with very high populations of *L. californicus* to determine the efficacy of these blends and other candidate insecticides. As wireworm species differ in their susceptibility to insecticides, the results presented here constitute an important extension to the efficacy data previously reported for other species.

MATERIALS AND METHODS

Plot layout and preparation. All three trials were conducted in 2012 near Granum, AB, on a commercial field (approx. 240 ha) that had been planted to barley, peas, and wheat in 2009, 2010, and 2011, respectively, and that had a recent history of wireworm damage. No insecticides had been applied to crops planted in this field since ca. 2000.

Experimental design. All trials were randomized complete block designs with four replicates. Each trial contained seed not treated with insecticide as a control treatment, and included a combined thiamethoxam (Cruiser 5FS at 10 g AI/100 kg seed) and fipronil (Regent 500FS at 1 g AI/100 kg seed) as a common insecticide seed treatment (hereafter referred to as 'Standard T+F Blend') to permit between-trial comparisons. Individual treatment plots in all trials consisted of seven 6.0-metre-long rows of wheat oriented due West to East, with 0.20 m spacing between treatment rows, 1.6 m between adjacent treatment plots, and 2.0 m between replicates.

Seed treatments. Seeds (hard red spring wheat: Syngenta, WR859CL) were treated with a Hege 11 liquid seed treatment applicator (Wintersteiger Inc., Salt Lake City, UT) by technicians at a Syngenta Crop Protection (Canada) seed treatment facility in Portage la Prairie, Manitoba, with the following insecticides:

Trial 1: Cyantraniliprole and λ-cyhalothrin: Cyantraniliprole (Fortenza 600FS) at 10, 20, 30, and 40 g AI/100 kg seed, λ-cyhalothrin (Demand 100CS) at 30 g AI, thiamethoxam (Cruiser 5FS) at 30 g AI, fipronil (Regent 500FS) at 5 g AI, and the Standard T+F Blend. All treatments also contained the fungicide Dividend XLRTA at 13 g AI (containing 3.21% difenoconazole and 0.27% mefenoxam).

Trial 2: Fipronil, alone and blended with thiamethoxam: Thiamethoxam (Cruiser 5FS) at 10 g AI/100 kg seed, fipronil (Regent 500FS) at 0.6, 1, and 5 g AI, and blends of thiamethoxam at 10 g AI + fipronil at 0.6, 1, and 5 g AI. All treatments also contained the fungicides Proseed at 2.5 g AI (containing 40.3% fludioxonil) and Vibrance XL at 17.5 g AI (containing 1.2% sedaxane, 5.9% difenoconazofe, and 1.5% metalaxyl-M).

Trial 3: Imidacloprid and thiamethoxam: Imidacloprid (Stress Shield 480SC) at 10, 20, and 30 g AI, thiamethoxam (Cruiser 5FS) at 10, 20, and 30 g AI, and Standard T+F Blend. The imidacloprid treatments also contained the fungicide Raxil MD at 3.5 g AI; all other treatments also contained the fungicides Proseed 480FS at 2.5 g and Vibrance XL at 17.5 g AI.

Planting*:* All plots were planted on 8 May 2012 with a seven-row double disc drill, no till planter (Fabro Enterprises Ltd., Swift Current, SK) directly into the wheat stubble from the previous year's crop. No tillage was done in the previous fall nor immediately prior to planting. Seeds were planted approx. 2.5 cm deep, at 285 seeds/m^2. As rows were spaced 20 cm apart, this seeding rate was equivalent to approx. 57 seeds per 1 m of row, or 100 kg seed/ha.

Stand assessment. Plant survival (hereafter "stand") was determined by counting the number of wheat seedlings alive in the central two-metre sections of the middle three rows of each plot at 14 and 29 days after planting (DAP) (22 May and 6 June, respectively) in all three trials, and measuring the plant reflective index (NDVI; Crop Circle ACS-430, Holland Scientific, Lincoln NE) at 21, 29, and 37 DAP (29 May, 6 and 14 June, respectively).

Plot maintenance*:* Plots were kept weed free by treating with glyphosate on 4 May prior to seeding, and no further weed control was deemed necessary. After harvest, the remaining wheat stubble was left intact over winter to prevent disturbance of surviving wireworm populations, which were assessed by trapping the following spring.

Harvest. All trials were harvested on 28 August 2012 (112 DAP) using a small plot combine (Wintersteiger Inc., Salt Lake City, UT) that calculated both the moisture percentage in the seed and per hectare yield. Some plots (e.g., neonicotinoid treatments in Trial 3) were not harvested due to the lack of surviving plants.

Wireworm trapping. To determine the longer-term effects of the various treatments on wireworm mortality, wireworms were sampled in the spring of the following year using a bait-trapping procedure similar to that described in Vernon *et al.* (2009). Bait traps were installed in the plots (three per plot) on 1–2 May, 2013, and removed on 13 May. Bait trap locations were spaced 1 m apart along the middle of each plot, so that the traps were 2, 3, and 4 m from the front and 75 cm from the outer rows of each plot. Each bait trap consisted of a 450-ml plastic flower pot filled with coarse-grade vermiculite and 100 ml untreated hard red spring wheat placed in a layer in the middle of the pot. Traps were soaked to run-off with lukewarm water twice several hours before placement in circular holes (10 cm diameter, 15 cm deep) cored into the ground. Soil was carefully and consistently packed around and on top of the bait traps, and a 20-centimetre-diameter inverted tray placed 5 cm above the trap and level with the ground. To reduce variability in data, considerable effort was taken to ensure each trap was prepared and installed identically. After removal, bait traps were immediately transported to the Agassiz Research and Development Centre (AAFC, Agassiz, BC), where they were placed in Tullgren funnels on 15 May for 2 weeks to extract wireworms. Extracted wireworms were counted, measured to the nearest millimetre, and identified to species. As wireworms shrink when they desiccate after extraction, 200 living *L. californicus* larvae were individually placed directly under the funnel heat source (25W incandescent light bulbs) for 48 h, and measured and weighed to 0.1 mg (Sartorius CP64 analytic balance; Sartorius AG, Goettingen, Germany) both before and after desiccation. Simple linear regression of desiccated to living wireworm length yielded the relation, living length = (desiccated length + 0.5391) / 0.6655; $R^2 = 0.81$, which was subsequently used to convert the lengths of desiccated wireworms to the corresponding size of living ones. For analyses, larval lengths were combined in three millimetre categories, since binning into two millimetre categories or showing each size separately would produce artifacts due to sizes calculated from desiccated lengths being rounded to the nearest 1 mm, which causes underestimations of the number that were 6, 9, 12 mm, etc. long. Wireworms were considered small, or neonate, if equal or less than 10 mm long, and large (or resident) if greater than 10 mm.

Statistical Analysis. All data analyses were conducted using SAS (SAS 9.2, SAS Institute, Cary, NC). Treatment means were compared by ANOVA (Proc GLM), followed by mean separation with Tukey's standardized range honestly significant difference (HSD) test at $\alpha = 0.05$. Where data could not be easily normalized using a power transformation (Trial 3, reflective index and yield only), the Kruskal–Wallis test (Proc NPAR1WAY) was used, after which normalized rank values were assigned to treatments (Proc RANK) and the standard ANOVA and the Tukey procedures performed on the rank values. The relationship between the amount of stand reflectivity and plant stand counts recorded on the same day was analyzed with linear regression (Proc GLM). Count data were analyzed with chi-square tests (Proc FREQ).

RESULTS

Wireworm sampling

Post-treatment bait-trapping results confirmed the trial areas had very high wireworm populations, with 403 larvae collected from the combined control treatments in the three trials (12 plots, 36 traps), and 2,234 from the combined treated plots (88 plots). Of the latter, only 190 wireworms were in plots with seeds treated with fipronil alone or in blend with another insecticide (36 plots). Similar numbers were found in the control plots of all three trials (range of means: 8.8–13.4/trap, Tables 1–3), suggesting a fairly homogeneous population in the study area. Wireworm populations were predominantly *L. californicus* (97.3%), with very low numbers of *H. bicolor* (2.0%), *S. destructor* (0.7%), and *Aeolus mellillus* (Say) (<0.1%) — the latter species are included in the totals presented in Tables 1–3. To compare the age structures of wireworm populations retrieved from the various treatments, the distribution of larval lengths (range: 3–28 mm) were compared for wireworms retrieved from control plots, plots seeded to treatments containing fipronil, and plots seeded to treatments containing other insecticides (Fig. 1). Chi-square analyses indicated significant differences in population structures (i.e., in the relative number in each of the size classes), both between control and fipronil treatments (Chi=1089.3, df=7, P<0.0001) and between control and other treatments (Chi=144.9, df=7, P<0.0001). Comparison of the age structures (Fig. 1A–C) indicates control treatments had significantly lower numbers of small (3–10 mm) wireworms per plot (1.08) than fipronil (1.94) and non-fipronil (2.92) insecticide treatments (Chi=104.3, df=1, P<0.0001; Chi=8.85, df=1, P=0.0016; respectively). In contrast, the control and non-fipronil treatments had a similar number of large (>10 mm) wireworms per trap (32.5 and 36.4, respectively per plot), while very low numbers (3.3 per plot) were retrieved from treatments containing fipronil (Fig. 1A–C).

Relation between plant reflectivity and plant stand

A direct, highly significant relationship was observed between plant reflectivity index (RI) and plant stand when the two were measured on the same day (29 DAP). This was true for trials with cyantraniliprole (t=7.95, df=1,34, P<0.0001, R^2=0.64), fipronil (t=11.17, df=1,30, P<0.0001, R^2 = 0.80), and imidacloprid and thiamethoxam (t =7.06, df=1,30, P<0.0001, R^2=0.61), and indicates that plant RI is an acceptable metric for assessing plant stand (e.g., at 37 DAP, when individual plant counts were not conducted).

Trial 1: Cyantraniliprole and λ-cyhalothrin

Stand protection and yield

Greatest stand protection was provided by fipronil at 5 g AI and Standard T+F Blend treatments. These treatments had higher stand counts than the control at 14 DAP (1.55x) and 29 DAP (6.03x, 5.61x, respectively). However, RI readings at 37 DAP indicate better stand protection in fipronil (5 g AI) than the Standard T+F Blend (2.08x vs 1.77x control, respectively), which resulted in higher yields at harvest (respectively, 18.3 vs 11.5x the control; Table 1). Thiamethoxam applied at 30 g AI provided good initial plant protection (respectively, 1.54x and 3.14x control at 14 and 29 DAP), but the RI at 37 DAP and yield at harvest were similar to control and significantly less than fipronil (5 g AI) and Standard T+F Blend treatments. Similarly, λ-cyhalothrin at 30 g AI provided stand protection (respectively, 1.80x and 4.60x control at 14 and 29 DAP) that resulted in similar yield to the Standard T+F Blend, but yield was significantly lower than observed for fipronil at 5 g AI (Table 1).

Cyantraniliprole applied at 10–40 g AI provided stand protection equivalent to or greater than thiamethoxam at 30 g AI (i.e., 1.60-1.80x control at 14 DAP, 3.02-3.85x control at 29 DAP), which resulted in numerically higher yields (1.73-2.56x thiamethoxam). Stand protection with cyantraniliprole was equivalent to that provided by λ-cyhalothrin and fipronil (5 g AI) at 14 DAP, but this had diminished by 29 DAP (0.50-0.64x fipronil), and the RI at 37 DAP and yields at harvest were significantly lower than fipronil (5 g AI) (Table 1). There were no significant differences in stand protection or yield between rates of cyantraniliprole (Table 1).

Table 1

Plant stand, crop yield, and wireworm survival in plots treated with cyantraniliprole and λ-cyhalothrin. Shown are mean (SE) values, based on four replicates (Rep). Wireworm numbers are calculated per plot (i.e., three bait traps combined). Plant stand (number of plants per 6.0-m row) and plant reflective index were measured at 14, 29, and 37 days after planting (DAP). Wireworms (wws) were considered 'large' if >10mm and 'small' if ≤10mm long (see text for explanation). Numbers followed by the same letter in a column are not significantly different at $\alpha = 0.05$.

Treatment *	Rate (g AI/ 100kg seed)	Plant stand: 14 DAP	Plant stand: 29 DAP	Reflective Index: 37 DAP	Yield (kg/ha) at harvest	Small wws	Large wws	All wws
Control	13	79.8 (5.7) B	25.3 (4.6) C	0.13 (0.008) D	206.8 (206.8) C	1.3 (0.5) AB	39.0 (5.5) A	40.3 (5.8) A
Cruiser 5FS	30	122.5 (12.7) AB	86.3 (28.2) ABC	0.15 (0.013) CD	373.2 (171.3) C	0.5 (0.5) B	32.8 (2.8) A	33.3 (3.4) A
Regent 500FS	5	124.0 (10.4) AB	152.5 (10.9) A	0.27 (0.021) A	3675.3 (295.8) A	0.8 (0.5) AB	2.2 (1.1) B	3.0 (1.5) B
Cruiser 5FS + Regent 500FS	10+1	123.5 (16.0) AB	142.0 (13.0) AB	0.23 (0.023) AB	2305.0 (177.9) B	1.3 (0.5) AB	8.2 (2.1) B	9.5 (1.9) B
Demand 10CS	30	143.3 (16.2) A	116.3 (13.3) AB	0.21 (0.007) BC	2342.6 (271.9) AB	1.0 (1.0) AB	30.0 (4.9) A	31.0 (5.7) A
Fortenza 600FS	10	143.8 (15.1) A	87.5 (19.0) ABC	0.16 (0.017) CD	955.0 (249.1) BC	1.5 (0.9) AB	46.5 (4.9) A	48.0 (5.5) A
Fortenza 600FS	20	135.8 (10.7) A	76.3 (14.0) BC	0.16 (0.009) CD	643.9 (226.7) C	2.3 (0.9) AB	48.5 (7.0) A	50.8 (6.9) A
Fortenza 600FS	30	127.8 (13.6) AB	97.3 (19.6) AB	0.17 (0.027) BCD	912.9 (445.9) BC	3.5 (0.9) A	44.5 (6.5) A	48.0 (7.3) A
Fortenza 600FS	40	130.8 (7.3) A	90.8 (14.3) ABC	0.15 (0.005) CD	955.0 (210.2) BC	3.0 (0.7) AB	39.3 (4.8) A	42.3 (5.4) A
Trt df=8,23		F=3.14, P=0.015	F=6.90, P=0.0001	F=12.11, P<0.0001	F=17.91, P<0.0001	F=2.87, P=0.02	F=16.46, P<0.0001	F=15.78, P<0.0001
Rep df=3,23		F=4.31, P=0.015	F=4.05, P=0.019	F=6.35, P=0.0027	F=0.51, P=0.68	F=4.60, P=0.011	F=4.53, P=0.012	F=5.11, P=0.007

All treatments contained the fungicide Dividend XLRTA at 13 g AI

Table 2

Plant stand, crop yield, and wireworm survival in plots treated with fipronil alone or in blend with thiamethoxam. Shown are mean (SE) values, based on four replicates (Rep). Wireworm numbers are calculated per plot (i.e., three bait traps combined). Plant stand (number of plants per 6.0-m row) and plant reflective index were measured at 14, 29, and 37 days after planting (DAP). Wireworms (wws) were considered 'large' if >10mm and 'small' if ≤10mm long (see text for explanation). Numbers followed by the same letter in a column are not significantly different at $\alpha = 0.05$

Treatment *	**Rate (g AI/ 100kg seed)**	**Plant stand: 14 DAP**	**Plant stand: 29 DAP**	**Reflective Index: 37 DAP**	**Yield (kg/ha) at harvest**	**Small wws**	**Large wws**	**All wws**
Control		83.5 (10.1) B	43.5 (15.6) C	0.13 (0.009) C	210.2 (205.7) D	1.0 (0.7) A	25.3 (3.8) A	26.3 (3.5) A
Cruiser 5FS	10	97.0 (9.3) AB	71.8 (6.1) BC	0.13 (0.006) C	432.1 (143.9) D	2.3 (0.3) A	30.0 (2.3) A	32.3 (2.4) A
Regent 500FS	0.6	104.3 (3.7) AB	116.3 (5.1) AB	0.19 (0.019) ABC	3024.6 (152.1) BC	1.3 (0.5) A	4.0 (1.6) B	5.3 (1.9) B
Regent 500FS	1	132.8 (8.9) AB	145.3 (10.0) A	0.20 (0.009) ABC	2891.8 (333.6) BC	3.3 (1.3) A	5.8 (1.9) B	9.0 (2.9) B
Regent 500FS	5	135.0 (18.0) AB	160.5 (10.9) A	0.26 (0.010) A	3767.7 (183.9) AB	0.5 (0.5) A	0.8 (0.5) B	1.3 (0.5) B
Cruiser 5FS + Regent 500FS	10+0.6	118.5 (16.3) AB	115.5 (13.7) AB	0.17 (0.012) BC	2797.6 (114.4) C	3.0 (1.1) A	6.5 (1.4) B	9.5 (2.2) B
Cruiser 5FS + Regent 500FS	10+1	117.5 (15.0) AB	135.0 (19.4) A	0.21 (0.026) ABC	3542.4 (249.0) ABC	0.3 (0.3) A	2.0 (1.2) B	2.3 (1.4) B
Cruiser 5FS + Regent 500FS	10+5	142.8 (16.1) A	164.0 (21.8) A	0.24 (0.034) AB	4016.6 (216.7) A	1.5 (0.6) A	0.8 (0.8) B	2.3 (1.1) B
Trt df=7,21		F=2.95, P=0.026	F=10.32, P<0.0001	F=7.17, P=0.0002	F=60.34, P<0.0001	F=2.51, P=0.048	F=34.19, P<0.0001	F=27.32 P<0.0001
Rep df=3,21		F=2.58, P=0.08	F=1.94, P=0.15	F=2.38, P=0.10	F=2.97, P=0.06	F=1.99, P=0.15	F=0.63, P=0.61	F=0.50, P=0.68

* All treatments contained the fungicides Proseed at 2.5 g AI and Vibrance XL at 17.5 g AI

Table 3

Plant stand, crop yield, and wireworm survival in plots treated with imidacloprid and thiamethoxam. Shown are mean (SE) values, based on four replicates (Rep). Wireworm numbers are calculated per plot (i.e., three bait traps combined). Plant stand (number of plants per 6.0-m row) and plant reflective index were measured at 14, 29, and 37 days after planting (DAP). Wireworms (wws) were considered 'large' if >10mm and 'small' if ≤10mm long (see text for explanation). Numbers followed by the same letter in a column are not significantly different at $\alpha = 0.05$.

Treatment *	**Rate (g AI/ 100kg seed)**	**Plant stand: 14 DAP**	**Plant stand: 29 DAP**	**Reflective Index: 37 DAP ****	**Yield (kg/ha) at harvest ****	**Small wws**	**Large wws**	**All wws**
Control		88.8 (8.7) B	29.5 (5.6) C	0.12 (0.003) A	0 (0) A	0.8 (0.8) A	33.5 (6.5) A	34.3 (6.9) A
Stress Shield 480SC	10	118.0 (9.1) AB	72.0 (12.2) BC	0.13 (0.009) ABC	0 (0) A	1.8 (1.4) A	38.5 (6.8) A	40.3 (8.2) A
Stress Shield 480SC	20	141.5 (9.6) A	91.8 (13.0) ABC	0.13 (0.009) ABC	0 (0) A	3.3 (1.7) A	25.0 (3.7) AB	28.3 (5.2) AB
Stress Shield 480SC	30	151.5 (17.9) A	101.8 (18.8) AB	0.14 (0.009) BC	0 (0) A	3.3 (1.4) A	38.5 (2.6) A	41.8 (1.9) A
Cruiser 5FS	10	109.5 (11.0) AB	38.5 (7.5) C	0.12 (0.002) AB	0 (0) A	2.0 (0.8) A	26.5 (8.7) AB	28.5 (9.0) AB
Cruiser 5FS	20	133.5 (14.4) AB	66.3 (4.9) BC	0.13 (0.003) ABC	0 (0) A	1.5 (0.3) A	39.0 (5.6) A	40.5 (5.4) A
Cruiser 5FS	30	137.5 (14.5) A	76.0 (13.4) BC	0.13 (0.005) ABC	0 (0) A	2.3 (0.9) A	44.0 (5.5) A	46.3 (5.3) A
Cruiser 5FS + Regent 500FS	10+1	140.5 (10.2) A	144.0 (18.8) A	0.18 (0.019) C	2824.5 (326.4) B	4.0 (2.5) A	1.5 (0.9) B	5.5 (2.4) B
Trt df=7,21		F=4.41, P=0.004	F=7.57, P=0.0001	F=4.89, P=0.0021	F=95.67, P<0.0001	F=0.60, P=0.75	F=5.86, P=0.0007	F=4.60, P=0.003
Rep df=3,21		F=5.52, P=0.006	F=0.59, P=0.63	F=2.65, P=0.08	F=1.00, P=0.41	F=0.57, P=0.64	F=0.98, P=0.42	F=1.00, P=0.41

* Stress Shield 480SC treatments contained the fungicide Raxil MD at 3.5 g AI; all other treatments contained the fungicides Proseed at 2.5 g AI and Vibrance XL at 17.5 g AI
** ANOVA and mean separation conducted on normalized ranks. The non-parametric Kruskal–Wallis test was used to conduct initial analyses — Reflective Index: 37 DAP: H=15.87, df=1, P=0.0266; Yield (kg/ha) at harvest: H=30.83, df=1, P<0.0001.

Wireworm survivorship

Significantly fewer large (>10 mm) wireworms were collected in bait traps in both the fipronil (0.06x control) and Standard T+F Blend (0.21x control) treatments (Table 1), indicating high mortality in these treatments. In contrast, there were no significant reductions in large wireworms caught in the thiamethoxam (30 g AI), λ-cyhalothrin (30 g AI), and cyantraniliprole treatments relative to the control treatment (Table 1). Relatively few small (neonate) wireworms were collected in all insecticide treatments, and this was similar to numbers taken in the control treatment (Table 1).

Trial 2: Fipronil, alone and blended with thiamethoxam

Stand protection and yield

Higher stand protection was observed in fipronil (0.6, 1.0, and 5.0 g AI) treatments relative to the untreated control (range: 1.25–1.62x stand at 14 DAP, 2.67–3.69x stand at 29 DAP) and the thiamethoxam (10 g AI) treatments (1.08–1.39x stand at 14 DAP, 1.62–2.24x stand at 29 DAP). Stand protection increased with the rate of fipronil applied. As in the other trials, thiamethoxam failed to provide lasting plant protection, leading to very low yields at harvest (Table 2). In contrast, all rates of fipronil provided significantly higher yields than either the thiamethoxam or untreated control treatments (13.76–17.92x control; Table 2). No significant differences in yield were observed between the fipronil rates. Combining thiamethoxam at 10 g AI with fipronil at 0.6, 1.0, or 5 g AI provided similar stand protection than the fipronil treatments alone at the same rates, and did not significantly increase yields (13.31–19.11x control; Table 2).

Wireworm survivorship

Populations of large wireworms were significantly reduced in the fipronil (0.6, 1.0, and 5.0 g AI) (range: 0.03–0.23x control) and combined thiamethoxam (10 g AI) and fipronil (0.6, 1.0, and 5.0 g AI) treatments (0.03–0.26x control) (Table 2). Mortality was highest in treatments with the 5 g AI rate of fipronil. Although not statistically significant, there was notably higher mortality in the Standard T+F Blend than in the treatment with fipronil at 1 g AI alone (Table 2). In contrast, more (1.19x control) large wireworms were collected from the thiamethoxam than the control treatment (Table 2). Low and similar numbers of neonate wireworms were collected from all treatments.

Trial 3: Imidacloprid and thiamethoxam

Stand protection and yield

Both imidacloprid (10, 20, and 30 g AI) and thiamethoxam (10, 20, and 30 g AI) provided initial stand protection (1.33–1.71x, 1.23–1.55x control at 14 DAP, respectively; 2.44–3.45x, 1.31–2.58x control at 29 DAP). For each rate tested, imidacloprid provided numerically greater protection than thiamethoxam, with protection increasing with rate for both chemicals (Table 3). Stand protection disappeared after 37 DAP, leading to complete destruction of the plots and no harvestable plants.

Good initial plant protection was observed in the Standard T+F Blend (1.58x and 4.88x control at 14 and 29 DAP, respectively). The effect of fipronil in the Standard T+F Blend was evident when compared to thiamethoxam applied alone at 10 g AI (1.28x and 3.74x thiamethoxam at 14 and 29 DAP, respectively). Plant stand protection in the Standard T+F Blend persisted throughout the season, and this was the only treatment with harvestable plants. Yields at harvest were similar to that observed for the same treatment evaluated in the other two trials (respectively, 2305, 3542, and 2824 kg/ha, Tables 1–3).

Wireworm survivorship

Populations of large wireworms were not reduced in any of the imidacloprid or thiamethoxam treatments (range: 0.75–1.15x, 0.79–1.31x control, respectively), and highest numbers were collected from plots seeded to the highest rates of these chemicals (Table 3). In contrast, very low numbers of large wireworms (0.04x control) were collected from the Standard T+F Blend treatment, indicating high mortality. Low and similar numbers of neonate larvae were collected from all treatments (Table 3).

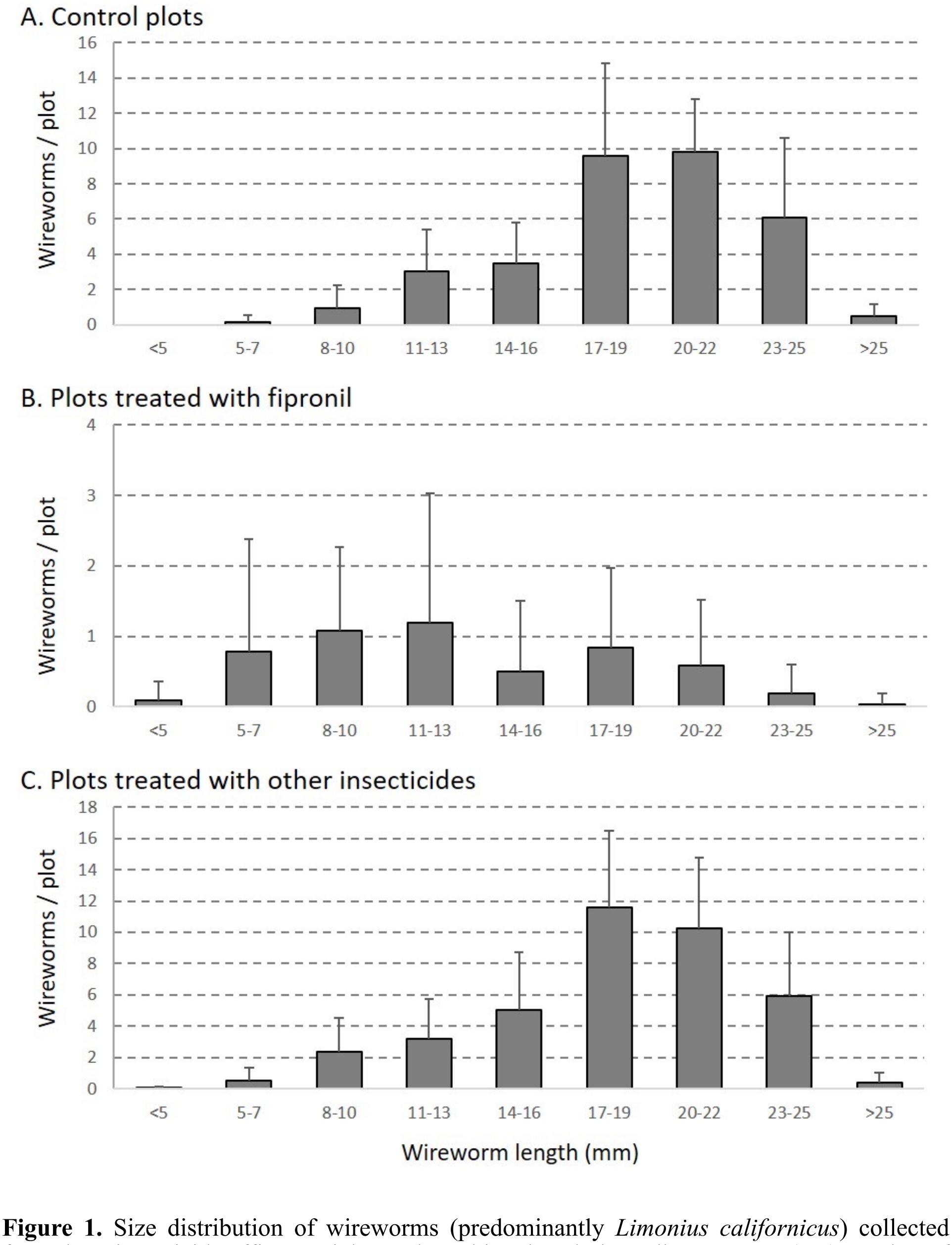

Figure 1. Size distribution of wireworms (predominantly *Limonius californicus*) collected from three insecticide efficacy trials conducted in Claresholm, Alberta. Mean (SD) number of wireworms retrieved from bait traps placed in control plots (**A.**, N = 12 plots), in plots treated with fipronil alone or in blend with another insecticide (**B.**, N = 36), and in plots treated with an insecticide other than fipronil (**C.**, N = 52). Note the differences in vertical axes between **B** and **A, C.**

DISCUSSION

Neonate versus resident wireworm mortality

The number of small (neonate) wireworms that would have been produced during this study was low (approx. 10%) in all treatments relative to the number of large (resident) wireworms that would have been present at the time of planting. This is in contrast to field studies with *A. obscurus* in which higher numbers of neonates were trapped in control plots and plots treated with neonicotinoids relative to fipronil-containing plots (cf. Vernon *et al.* 2009). There are a number of possible reasons for the differences in neonate catches between the previous and current studies. In the current study, plant stand in some (e.g., control, neonicotinoids; Tables 1–3) treatments was poor to non-existent, which would have reduced oviposition and food availability relative to treatments with higher stands (e.g., fipronil-containing treatments). This is partially substantiated by the cyantraniliprole treatments in Trial 1, where stand and yield were higher than in the control treatment, and neonate numbers were numerically higher (4.0–4.8 per plot) than in the other treatments (1.5–2.7 per plot) (Table 1). This also suggests cyantraniliprole may not be lethal to neonate wireworms.

In plots containing fipronil, which had excellent stand protection, low neonate numbers were likely due to the residual and toxic effect of this chemical. Numbers of resident wireworms were also very low in these treatments, and fipronil has previously been shown to be highly toxic to both resident and neonate *A. obscurus* (Vernon *et al.* 2009, 2013a, 2016). The effect of the pyrethroid, λ-cyhalothrin, in reducing neonate populations in the current study is more difficult to ascertain. Because stand protection and yield were similar to the Standard T+F Blend, the reduction in resident populations was not significantly different from thiamethoxam or the control (Table 1), and the neonate numbers were low, it appears that λ-cyhalothrin is persistent and toxic to this stage and/or that the presence of this insecticide in plots reduced egg laying due to repulsion of female beetles. We have previously shown that residues of another pyrethroid, bifenthrin, are repulsive to *A. obscurus* larvae >200 d after an in-furrow application to soil in potatoes (van Herk *et al.* 2013). While the overall low number of neonates in this study might be attributed to low click beetle emergence and egg-laying, this typically occurs in fields treated with an insecticide (e.g., that induces prolonged morbidity and prevents late-instar larvae from feeding sufficiently to pupate in the fall), whereas no insecticides had been applied to the study field since approx. 2000 (T.J. Labun, unpublished data).

It is interesting that the lack of food in certain plots did not appear to affect the survival and retention of resident wireworms, with high numbers of larvae trapped from plots with little or no plant survival (e.g., neonicotinoid treatments, Table 3). This supports the concern that later instars of some pest species can survive with minimal food for prolonged periods of time (Vernon and van Herk 2013). Also worth noting is that none of the fungicide treatments used in these trials appeared to negatively affect wireworm populations. This is consistent with results from lab and field studies with both *A. obscurus* and *L. canus* LeC. (Vernon *et al.* 2009, 2013a; van Herk *et al.* 2008, 2015).

Crop protection vs. wireworm mortality, and benefits of blended treatments

The above results underscore the importance of evaluating wireworm mortality (inferred here from the difference in wireworm numbers collected from treatment vs control plots) in field efficacy studies. While wireworm mortality could be deduced from crop protection in earlier insecticide efficacy studies with OP and OC insecticides, this is usually not possible with newer chemistries (Vernon *et al.* 2009), as exposure to neonicotinoid insecticides generally induces prolonged, reversible morbidity during which time wireworms are unable to feed (Vernon *et al.* 2008). Hence, these insecticides may protect plants from feeding damage without decreasing wireworm populations (Vernon *et al.* 2009, 2013a). A similar result was seen in efficacy studies with potatoes, where neonicotinoid treatments applied at planting reduced feeding damage to daughter tubers without decreasing wireworm numbers (Vernon *et al.* 2013b). Pyrethroid insecticides also protect wheat and potatoes from wireworm feeding damage without reducing populations, but here the mechanism is mainly repellency (van Herk *et al.* 2008, 2015). Conversely, exposure to an insecticide that induces morbidity and mortality latently can result in wireworm population reductions without providing adequate stand protection (Vernon *et al.* 2013a).

Contrary to results with *A. obscurus* in BC, high rates of imidacloprid and thiamethoxam failed to protect wheat seedlings from *L. californicus* past 29 DAP in these trials. This could result from differences in insecticide susceptibility between species or from the very high wireworm populations in the field. In southern Alberta, high populations of *L. californicus* can cause complete crop destruction in fields of spring wheat treated with a high (39 g AI) rate of thiamethoxam (T.J. Labun, personal observation). The observed failure of high rates of these commonly used insecticides to reduce populations of *L. californicus* is similar to findings by Esser *et al.* (2015) with *L. californicus* and *L. infuscatus* Mots., and likely explains why damage in wheat from these species is increasing in severity and frequency across the region.

Both cyantraniliprole and λ-cyhalothrin provided greater protection at the rates tested than either imidacloprid or thiamethoxam, although this was likely through different mechanisms. While λ-cyhalothrin and other pyrethroids (e.g., tefluthrin, bifenthrin) induce repellency and thereby reduce feeding (van Herk *et al.* 2008, 2015), cyantraniliprole is not repulsive and likely induces morbidity after feeding (van Herk *et al.* 2015). Considering the high wireworm populations in these trials, the partial plant protection observed is encouraging, and cyantraniliprole may be a potential candidate for blending with low rates of a lethal insecticide. It should be noted that at the rates tested, cyantraniliprole and λ-cyhalothrin by themselves did not cause significant wireworm mortality in either this study or in previous work with *A. obscurus* (Vernon *et al.* 2013b; van Herk *et al.* 2015).

Combining a non-lethal insecticide that rapidly induces morbidity with a low rate of a chemical that causes mortality latently can provide both stand protection and long-term population reductions in the field (Vernon *et al.* 2013a). Since wireworms live for up to 4–5 years in the soil, one application with an insecticide lethal to all wireworm stages can remove the economic threat of wireworms for three or more years. This blended treatment concept was evaluated in numerous lab and field studies with *A. obscurus, A. sputator,* and *L. canus,* which demonstrated that combinations of thiamethoxam at 5 or 10 g AI with fipronil at rates as low as 1 g AI will provide both acceptable crop protection and high neonate and resident wireworm mortality for these species (Vernon *et al.* 2009, 2013a). These results provided the basis for the current study with *L. californicus* and allowed the concept to be extended to using insecticide-blended wheat seed as an in-furrow treatment that both protects potato tubers from damage and reduces wireworm populations (Vernon *et al.* 2016).

In the work reported here, both the fipronil and various thiamethoxam + fipronil blend treatments provided significant stand protection and reduction in populations of resident wireworms, relative to the untreated control and all other treatments tested. Of note is that, in Trial 2, combining thiamethoxam at 10 g AI with fipronil at 0.6, 1.0, and 5.0 g AI did not improve stand protection and yield, nor increase resident wireworm mortality relative to the corresponding fipronil treatments. This suggests that *L. californicus* may respond differently to neonicotinoid and fipronil insecticide blends than *A. obscurus*, where the presence of thiamethoxam considerably improved stand and yield (Vernon *et al.* 2013a). Also of note is that stand, yield, and mortality were notably higher at the 5.0 g than 1.0 g and 0.6 g AI rates of fipronil. Similarly, in Trial 1, fipronil at 5 g AI provided 1.6x greater yield and 3.6x higher mortality than the Standard T+F Blend. This suggests that where fipronil is used alone as a seed treatment to control high populations of *L. californicus*, it should be applied at a rate higher than 1 g AI, and that (unlike for *A.*

obscurus) there is no additional benefit from combining fipronil with a neonicotinoid such as thiamethoxam.

Neonicotinoid and fipronil insecticide blends on wheat seed have been evaluated for wireworm management elsewhere. Morales-Rodriguez and Wanner (2015) observed high (>70%) mortality in *L. californicus* and *H. bicolor* exposed in laboratory assays to wheat seed treated with fipronil at 1 and 5 g AI/100 kg seed but low mortality (<30%) if exposed to thiamethoxam at 39 g AI. In field trials, seed treated with thiamethoxam at 39 g AI provided plant protection but resulted in higher wireworm populations than control plots, while seed treated with both thiamethoxam at 39 g AI and fipronil at 5 g AI significantly reduced populations. Combining thiamethoxam at 39 g AI with fipronil at 1 g AI/100 kg seed caused less mortality in lab studies than either insecticide alone, and we suggest that the high rate of thiamethoxam in this blend may have induced morbidity before sufficient fipronil was ingested. Higher rates of thiamethoxam decrease the duration of feeding in *L. canus* (van Herk *et al.* 2008), and in lab studies mortality is greater when wireworms are exposed to fipronil at 1 g AI alone than in combination with thiamethoxam at 10 g AI (van Herk *et al.* 2015). However, when larvae were exposed to a blend of thiamethoxam at 10 g AI and a higher rate of fipronil (e.g., 5 g AI), enough of the latter chemical was ingested to cause high mortality (van Herk *et al.* 2015). Under field conditions, high mortality of *A. obscurus* was observed with blends of thiamethoxam at 5 or 10 g AI and fipronil at both 1 and 5 g AI (Vernon *et al.* 2013b), likely because of longer exposure to the seeds than in laboratory studies and because other factors (i.e., desiccation, predation on moribund wireworms) contribute to mortality in the field (Vernon *et al.* 2009).

Potential of seed treatments for controlling wireworms in cereals

In a recent review of insecticides for controlling wireworms in cereals, it was observed that, in general, the most effective chemistries appear to be those that target GABA-gated chloride channels (e.g., fipronil, lindane) (van Herk *et al.* 2015). As noted by Lange *et al.* (1949), the efficacy of seed treatments also depends on "the species of wireworms involved, wireworm activity at the time the seed is planted, the proportion of the population attracted to the seed, the type of seed, and the time of planting." Some of these observations are briefly considered here.

Time of planting and wireworm activity

Seed treatments are most likely to be effective when seed is planted shortly before larvae become active (Vernon and van Herk 2013). Many pest wireworm species have two main periods of feeding activity (spring and fall), between which they burrow downwards to avoid desiccation (Traugott *et al.* 2015). Planting seed treated with a non-residual insecticide after wireworms have fed would therefore reduce exposure and resultant mortality. This would be a concern where cropping practices (e.g., continuous cropping, minimal tillage) provide alternative food sources before or after the seeds are planted (e.g., roots and decaying plant matter from the previous year's crop). Under these conditions, wireworms would presumably feed less on the treated seeds, if at all, and therefore ingest less insecticide (Vernon *et al.* 2013b). Early season planting, before wireworms become active in the spring, may not be feasible, as wireworms can cause considerable feeding damage even at low soil temperatures (van Herk and Vernon 2013).

Determining when wireworms become active in the spring has been the focus of considerable research (reviewed in Traugott *et al.* 2015 and Vernon and van Herk 2013), and the high mortality observed in the fipronil treatments reported here suggests the spring activity period of *L. californicus* coincides with spring wheat planting in southern Alberta.

Differences between species

Insecticide seed treatment efficacy may vary between wireworm species due to differences in species phenology (e.g., when they begin to feed) and different susceptibilities to insecticides (Vernon *et al.* 2008). Lange *et al.* (1949) noted that *L. canus* is more susceptible to lindane than *L. californicus,* possibly because of differences in the activity levels of these species. In eastern Washington State, repeated exposure to thiamethoxam-treated spring wheat resulted in no observed changes in populations of *L. californicus*, whereas at a nearby site it appeared to reduce *L. infuscatus* populations (Esser *et al.* 2015; Milosavljevic *et al.* 2016). Hence, it is critically important to know what species are present in the field before applying a management approach, particularly as pest species frequently co-occur.

Differences between cereals

In laboratory studies, Edwards and Evans (1950) observed no difference in wheat and oat (*Avena sativa* L.) seedling survival when exposed to *Corymbites cupreus* Fabr., *Agriotes* spp., or *Athous (=Hemicrepidius) niger* L. larvae, but slightly higher survival of barley (*Hordeum vulgare* L.) than wheat and oat seedlings exposed to *Agriotes* spp. and *C. cupreus*. In contrast, recent work suggests both oat and barley seedlings may be less susceptible to *L. infuscatus* and *L. californicus* feeding (respectively) than wheat (Higginbotham *et al.* 2014, Rashed *et al.* 2017). Recent field studies in Alberta suggest insecticides (e.g., fipronil) applied on barley cause lower mortality in *L. californicus* than when applied to spring wheat seed (van Herk *et al.*, unpublished data). This may be due to the barley seed hull absorbing some of the seed dressing, or to the susceptibility of the seed itself to wireworm feeding (cf. Higginbotham *et al.* 2014). While more data is required to determine if these results are real or result from the usual sources of variability that plague wireworm field studies (e.g., patchy distributions in the field), insecticides used as seed treatments may need to be applied at higher rates on barley than wheat to achieve the same level of population reduction, but at lower rates to achieve the same level of stand protection.

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