Journal of Economic Entomology, 109(4), 2016, 1706–1712
doi: 10.1093/jee/tow140
Advance Access Publication Date: 21 June 2016
Research article

OXFORD

Field and Forage Crops

Efficacy of Entomopathogenic Nematodes and Sprayable Polymer Gel Against Crucifer Flea Beetle (Coleoptera: Chrysomelidae) on Canola

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Received 26 April 2016; Accepted 27 May 2016

Abstract

The crucifer flea beetle, *Phyllotreta cruciferae* (Goeze), is a key pest of canola (*Brassica napus* L.) in the northern Great Plains of North America. The efficacies of entomopathogenic nematodes (*Steinernema* spp. and *Heterorhabditis* spp.), a sprayable polymer gel, and a combination of both were assessed on canola for flea beetle management. Plots were treated soon after colonization by adult flea beetles, when canola was in the cotyledon to one-leaf stage. Ten plants along a 3.6-m section of row were selected and rated at pre-treatment and 7 and 14 d post treatment using the damage-rating scheme advanced by the European Plant Protection Organization, where 1 = 0%, 2 = 2%, 3 = 5%, 4 = 10%, and 5 = 25% leaf area injury. Under moderate flea beetle feeding pressure (1–3.3% leaf area damaged), seeds treated with Gaucho 600 (Bayer CropScience LP Raleigh, NC) (imidacloprid) produced the highest yield (843.2 kg/ha). Meanwhile, Barricade (Barricade International, Inc. Hobe Sound, FL) (polymer gel; 1%) + Scanmask (BioLogic Company Inc, Willow Hill, PA) (*Steinernema feltiae*) resulted in the highest yields: 1020.8 kg/ha under high (2.0–5.3% leaf area damaged), and 670.2 kg/ha at extremely high (4.3–8.6 % leaf area damaged) feeding pressure. Our results suggest that Barricade (1%) + Scanmask (*S. feltiae*) can serve as an alternative to the conventional chemical seed treatment. Moreover, Scanmask (*S. feltiae*) can be used to complement the effects of seed treatment after its protection has run out.

Key words: Canola, *Phyllotreta cruciferae*, entomopathogenic nematode, sprayable polymer gel, leaf area injury

In North America, canola (*Brassica napus* L.; Brassicales, Brassicaceae) is a major oilseed crop, grown especially in the Northern Great Plains of the United States and the prairies of Canada (Brown 1967, Burgess 1977, Lamb 1984, Thomas 2003). The crucifer flea beetle, *Phyllotreta cruciferae* (Goeze) (Coleoptera, Chrysomelidae), is an economically important pest of canola in this region (Brown 1967, Burgess 1977, Lamb 1984, Thomas, 2003). Adult flea beetles emerge from overwintering sites in spring as air temperatures reach 15–20°C (Lamb 1983). Overwintered adults initially feed on brassicaceous weeds, and as the crop emerges, beetles move into canola fields, where they immediately begin to feed on young cotyledons and true leaves, stems, and pods (Lamb 1988, Thomas 2003). The larval stages contribute to yield losses during summer months by feeding on plant roots, root hairs, and tap roots of seedlings, and root damage reduces yield of about 5% (Thomas 2003). Yield losses due to *P. cruciferae* feeding are estimated as tens of millions of U.S. dollars annually in the region (Burgess 1977, Lamb and Turnock 1982, Madder and Stermeroff 1988, Thomas 2003).

Phyllotreta cruciferae management is directed against adults in early spring when canola seedlings are vulnerable to flea beetle injury (Thomas 2003). Conventional control methods are chemical seed treatments or foliar sprays (Lamb and Turnock 1982, Antwi et al. 2007, Reddy et al. 2014). The majority of canola acreage in the Northern Great Plains is planted with insecticide-treated seed, as foliar chemical insecticides are only effective against *P. cruciferae* within a narrow window of opportunity before eggs have been laid in the soil (Turnock and Turnbull 1994, Glogoza et al. 2002, Tangtrakulwanich et al. 2014). This near uniform reliance on chemical insecticide-based pest management increases the risk of development of pesticide resistance. To help avoid this problem, entomopathogenic nematodes (EPNs), which are a group of nematodes that are lethal to many important insect pests, offer an alternative treatment regimen and may be used alone or in combination with reduced rates of conventional insecticides (Koppenhöffer and Kaya 1998). Concern over development of resistance and damage to pollinators and other beneficial insects makes the evaluation of alternative controls for *P. cruciferae* a current priority (Antwi et al. 2007, Reddy et al. 2014).

Ecorational insecticides are products that are likely to do minimal harm to nontarget organisms or the environment (Ware 1989). Ecorational insecticides have been shown to be effective against many insect pests (Hajek et al. 1987, Miranpuri et al. 1992, Miranpuri and Khachatourians 1995, Sparks et al. 1999, Xu et al. 2010), and they may have potential value in *P. cruciferae* management. Because ecorational insecticides' modes of action are very different from those of chemical insecticides (Sparks et al. 2001, Thompson et al. 2000), they can be used to slow down or prevent the development of insecticide resistance (Liu and Stansly 1995, Copping and Menn 2000). EPNs are persistent, they recycle inside the host, and inundative application may provide short-term control of pests with few or no deleterious effects on nontarget organisms (Rosell et al. 2008). They are used where chemical insecticides fail (e.g., in soil, or in boring insect pest galleries) or insecticide resistance develops (Ehlers 2001, Rosell et al. 2008). Because EPNs actively search for hosts, they have special value in the management of soil-dwelling pests such as flea beetles in *Brassica* crops (Grewal et al. 2005, Trdan et al. 2008, Wei et al. 1992). According to Kakizaki (2004), treatment of radish roots with *Sternenema capocapsae* (Weiser) (at 250,000–500,000 infective juvenile nematodes/m^2) reduced *Phyllotreta striolata* (F.) (Coleoptera: Chrysomelidae) damage by 33 to 80%. EPNs in the genera *Steinernema and Heterorhabditis* can be used to manage many insect pests as biological control agents (Shapiro-llan et al. 2002, Shapiro-llan and Cottrell 2006, Grewal et al. 2005). However, ultraviolet radiation and desiccation effects can be a limiting factor for efficacy of EPNs at above-ground applications (Shappiro-Ilan et al. 2006, Glazer 1992). Shapiro-llan et al. (2010, 2016) concluded that EPNS treatments followed by a sprayable polymer gel application or as single spray can enhance the management of lesser peachtree borer, *Synanthedon pictipes* (Grote and Robinson) (Lepidoptera: Sesiidae) and other above-ground pests. Therefore, the use of sprayable gel in this study is meant to protect EPNs from harmful environmental conditions (ultraviolet radiation, desiccation). According to Koppenhöffer et al. (2000), various studies have shown that EPNs efficacy can be improved with other pathogens for white grubs (Coleoptera: Scarabaeidae) management. According to Thurston et al. (1993, 1994), EPNs and *Bacillus popilliae* Dutky (the Japanese beetle pathogen) (Bacillales: Paenibacillaceae) combination is only feasible in high economic threshold situations for long-term management. Moreover, EPNs and *Bacillus thuringiensis* Berliner Buibui strain (Bacillales: Bacillaceae) combination is only feasible for scarab species that are susceptible to this bacterium (Koppenhöfer and Kaya 1997, Koppenhöfer et al. 1999). However, due to limitations with these combinations, EPNs combined with chloronicotinyl insecticide (imidacloprid) would be more efficient with wider applicability (Koppenhöffer and Kaya 1998). The objective of our study was to evaluate the effect of low rates of several species of EPNs, combinations of EPN species, combinations of EPNs and a sprayable polymer gel, and a combination of EPN and imidacloprid on *P. cruciferae* feeding injury to seedling canola and resulting yield.

Materials and Methods

Study Sites

Trials were conducted at three field locations: Western Triangle Agricultural Research Center (WTARC; 48° 18.627′ N, 111° 55.402′ W) in Conrad, Sweet Grass (48° 57.831′ N, 111° 40.801′ W), and Cutbank (48° 50.22′ N, 112° 17.746′ W), Montana, USA. Experimental plots were seeded on 13 April 2015 at WTARC, on 20 April 2015 at Sweet Grass, and on 30 May 2015 at Cutbank. Hy-Class canola seeds (WindField Solutions, LLC) were used for all three locations, at a rate of 12 seeds per 30 cm using a four-row plot drill with a row spacing of 30 cm. The herbicide RT3 (a.i. glyphosate) was applied at a rate of 2.5 L/ha before seeding. Fertilizers at an N, P, K, and S ratio of 134.5, 25.2, 61.6, and 22.4 kg/ha and N, P, K ratio of 12.3, 25.2, and 0 kg/ha were applied at the time of seeding. The trials were conducted under dryland (i.e., nonirrigated) conditions.

Treatments

Twelve treatments were used for the studies (Table 1): 1) Water, 2) Gaucho (imidacloprid), 3) Ecomask (*Steinernema carpocapsae*), 4) Hi (*Heterorhabditis indica*), 5) Scanmask (*Steinernema feltiae*), 6) Heteromask (*Heterorhabditis bacteriophora*), 7) Ecomask + Heteromask (*S. carpocapsae* + *H. bacteriophora*), 8) Ecomask + Scanmask (*S. carpocapsae* + *S. feltiae*), 9) Gaucho + Scanmask (imidacloprid + *S. feltiae*), 10) Barricade (Barricade polymer 4%), 11) Barricade 2% + Scanmask (Barricade polymer 2% + *S. feltiae*), and 12) Barricade + Scanmask (Barricade polymer 1% + *S. feltiae*).

Plot Design and Data Collection.

The plot design was a randomized complete block design. Plots sizes were 3.6 by 1.2 m, with a buffer zone of 1.2 m to avoid cross-contamination due to spray drift. Treatments were replicated four times at each location. Treatments were applied to plots with a SOLO backpack sprayer (SOLO, Newport News, VA) calibrated at 816.89 L/ha, after arrival of flea beetles in plots when air temperatures were 14–20°C, and canola was in the cotyledon or one- to two-leaf stage. Untreated plots sprayed with water served as the control. Before treatment applications (PT), each plot was rated for *P. cruciferae* feeding injury along one 3.6-m section of row, by sampling 10 plants at 0.3-m intervals. *Phyllotreta cruciferae* injury measurements were made by visual classification into the European Plant Protection Organization (EPPO) damage categories as 1 = no damage; 2 = up to 2% leaf area eaten; 3 = 3–10% leaf area eaten; 4 = 10–25% leaf area eaten; and 5 = >25% leaf area eaten (EPPO, 2004). The visual injury ratings were converted into percent leaf area injury (OEPP/EPPO, 2004), where 1 = 0%; 2 = 2%; 3 = 5%; 4 = 10%; and 5 = 25% leaf area injury. Duration of efficacy of treatments was determined by post-application ratings for *P. cruciferae* injury at 7 and 14 d after application of foliar insecticides (7 and 14 DPT). Feeding injury and yield from plots were evaluated to compare treatment effects. Plots were harvested on 5 August 2015 at WTARC, on 14 September 2015 at Sweet Grass, and on 3 October 2015 at Cutbank when 50% of the canola seeds were dark in color.

The canola crop was straight combined at 30% seed moisture, stored, and air dried for 7 d until the seeds were at 8-10% moisture. The seeds were then cleaned and weighed to determine the seed yield per plot (as kilograms per hectare) for each experimental unit between August and October 2015.

Data Analysis

Data were analyzed using multivariate analyses of covariance (SAS Institute 2015). Analyses of covariance were used to account for and eliminate effects of pre-foliar treatment ratings on change in *P. cruciferae* feeding injury across dates after treatments. Least square means (LSMEANs) was run following analysis of variance (SAS Institute 2015). Main and interaction effects of location by treatment on *P. cruciferae* feeding injury ratings and yields were determined using the PROC GLM procedure (PROC GLM, SAS Institute 2015).

Synergistic, additive, or antagonistic interactions between Gaucho (imidacloprid) and EPNs in the combined treatments were determined with a χ^2 test (Finney 1964, Koppenhöffer and Kaya 1998, Koppenhöffer et al. 2002, McVay et al. 1977). Abbott

Table 1. Materials and rates of application in each treatment

Treatment	Active ingredient	Concentration	Amount/3.785 L water	Source
T1, Water spray (Control)	Water	Same volume as in mix	3.785 L	–
T2, Gaucho	Imidacloprid	190 ml/45 kg seed		Bayer Crop Science
T3, Ecomask	*Steinernema carpocapsae*	300,000/m^2	13.699 g	BioLogic Company Inc, Willow Hill, PA
T4, Hi	*H. indica*	300,000/m^2	208.5 g	Southeastern Insectaries, Perry, GA
T5, Scanmask	*Steinernema feltiae*	300,000/m^2	17.098 g	BioLogic Company Inc, Willow Hill, PA
T6, Heteromask	*Heterorhabditis bacteriophora*	300,000/m^2	16.995 g	BioLogic Company Inc, Willow Hill, PA
T7, Ecomask + Heteromask	*S. carpocapsae* and *H. bacteriophora*	300,000/m^2 (150,000 each nematode	6.85 g + 8.496 g	BioLogic Company Inc, Willow Hill, PA
T8, Ecomask + Scanmask	*S. carpocapsae* and *S. feltiae*	300,000/m^2 (150,000 each nematode)	6.85 g + 8.549 g	BioLogic Company Inc, Willow Hill, PA
T9, Gaucho + Scanmask	Imidacloprid and *S. feltiae*	190 ml/ 45 kg seed and 300,000/m^2 nematode	190 ml/45 kg seed (seed treatment) + 17.098 g product	See above
T10, Barricade	Barricade polymer	Barricade 4%	151.4 ml (g)	Barricade Internationalhttp./firegel.com/
T11, Barricade + Scanmask	Barricade and *S. feltiae*	Barricade 2% and 300,000/m^2 nematode	75.7 ml (g) + 17.098 g	See above
T12, Barricade (1%) + Scanmask	Barricade and *S. feltiae*	Barricade 1% and 300,000/m^2 nematode	37.5 ml (g) + 17.098 g	See above

method (Abbott 1925) was used to correct for control leaf area damage. The expected additive proportional percentage leaf area damage Me for the nematode–Gaucho combinations was determined as $Me = Mn + Mi\ (1 - Mn)$, where Mn and Mi are the observed proportional percentage leaf area damage by EPNs and Gaucho (imidacloprid) alone, respectively. A χ^2 test determined as ($\chi^2 = (Mni - Me)^2/Me$) whereby Mni = the observed percentage leaf area damage for the EPN–Gaucho combinations was compared with the χ^2 table value for 1 df. A nonadditive effect between two actives was suspected when the calculated χ^2 value exceeded the table value (Finney 1964). A significant interaction was considered as synergistic when the difference Mni − Me had a positive value. When the difference Mni − Me had a negative value, a significant interaction is considered antagonistic.

Results

Due to an especially cool spring, pest pressure at the research sites varied greatly among sites depending on sowing date. The average monthly weather parameters were precipitation 75.7 mm, temperature 6.2°C, and relative humidity 63.33% in April; precipitation 96 mm, temperature 9.2°C, and relative humidity 69.5% in May; and precipitation 136.5 mm, temperature 17.5°C, and relative humidity 68.4% in June (USDA-NRCS 2016). These conditions together with other biotic and abiotic factors caused *P. cruciferae* feeding pressure as determined by pre-treatment ratings to be moderate at WTARC (1–3.3% leaf area injury), high at Sweet Grass (2–5.3% leaf area injury), and extremely high at Cutbank (4.3–8.6% leaf area injury). Cotyledon and leaf injury did exceed the economic threshold of 15–20% leaf area defoliation across locations, especially at 14 DPT (Table 2; Tangtrakulwanich et al. 2014). In the water treatments, there were high levels of damage (WTARC: 5.9–15.6%, Sweet Grass: 8.9–21.9%, and Cutbank: 16.8–22.3%) at 7 to 14 DPT. Under these conditions of pest pressure, it was not favorable to discern the value of the biorational treatments. Across the locations, the seed treatment Gaucho 600 resulted in the lowest leaf area injury at 7 to 14 DPT (Table 2).

Under moderate pest pressure (WTARC), Gaucho, the chemical seed treatment, was the most efficacious among the treatments. Among the treatment combinations or mixtures, Gaucho + Scanmask was the only treatment that resulted in a lower leaf area injury at 7 DPT. At 14 DPT, Gaucho 600 resulted in lower leaf area injury of 8.0%. *Phyllotreta cruciferae* fed more on plots with Barricade + Scanmask than on those with Gaucho + Scanmask. The seed treatment Gaucho 600, with imidacloprid (a neonicotinoid) as the active ingredient, was fed on the least, likely because it is a broad-spectrum systemic insecticide acting as a contact and stomach poison on sucking and some biting insects (Sur and Stork 2003). It antagonizes the nicotinic acetylcholine receptor, resulting in paralysis and death of pest organisms (Bai et al. 1991, Nauen et al. 1998, Schmuck et al. 2003).

At high feeding pressure (Sweet Grass), Gaucho 600 and Gaucho + Scanmask resulted in the lowest *P. cruciferae* feeding pressure with leaf area defoliation of 4.7–12.4 and 6.0–14.3%, respectively, at 7 to 14 DPT.

Under extremely high *P. cruciferae* pressure (Cutbank), and at 7 DPT, none of the treatments performs better compared with the water control. However, at 14 DPT, only Gaucho 600 and Gaucho + Scanmask treatments perform better when compared with the water control.

Table 2. Crucifer flea beetle *Phyllotreta cruciferae* feeding leaf area injury (as % area damaged) to seedling canola plants treated with entomopathogenic nematodes and sprayable polymer gel in Montana at three time points, based on visual estimates

Treatment	WTARC[a]			Sweet Grass			Cutbank		
	PT[b]	7 DPT[c]	14 DPT[d]	PT	7 DPT	14 DPT	PT	7 DPT	14 DPT
	Leaf area injury (%)								
Water	2.5	5.9abc	15.6b	5.3	8.9bcd	21.9d	8.6	16.8ab	22.3c
Gaucho 600	2.0	3.1a	8.0a	2.0	4.7a	12.4a	4.5	14.3a	13.2a
Ecomask	3.3	7.6bcd	15.9bc	2.7	7.4abc	21.3cd	8.3	18.9ab	19.6bc
Hi	2.1	11.3def	15.1b	2.5	8.9bcd	19.2bc	5.2	15.6ab	19.9c
Scanmask	1.9	14.7f	19.2c	3.3	9.7cd	20.9cd	7.1	15.7ab	19.2bc
Heteromask	1.4	14.7f	19.2c	4.1	9.8cd	18.9bc	6.5	19.0ab	21.3c
Ecomask + Heteromask	1.3	12.2ef	19.2c	3.5	8.2bcd	20.2bcd	7.9	20.2b	21.3c
Ecomask + Scanmask	1.7	9.8de	15.3b	4.2	8.1bcd	20.6bcd	8.2	16.0ab	19.6bc
Gaucho + Scanmask	1	5.8ab	9.8a	2.2	6.0ab	14.3a	4.3	14.2a	16.5b
Barricade (4%)	1.4	9.8cde	14.4b	3.8	9.7cd	18.2b	6.6	15.5ab	20.6c
Barricade (2%) + Scanmask	1.7	10.0de	15.9bc	3.0	7.4abc	18.2b	8.2	15.7ab	20.6c
Barricade (1%) + Scanmask	1.9	10.1de	16.4bc	3.1	10.5d	19.2bc	6.6	17.0ab	19.9c

Means within a column followed by the same letter are not significantly different at $P < 0.05$.
[a] WTARC, Western Triangle Agricultural Research Center.
[b] PT, pre-foliar application.
[c] 7 DPT, days after foliar and granular application.
[d] 14 DPT, days after foliar and granular application.

Percentage Leaf Area Injury

Generally at WTARC and Sweet Grass, Gaucho and Gaucho + Scanmask treatments resulted in a lower percent leaf area injury at 7 to 14 DPT (Table 2).

At WTARC, the leaf area injury ranged from 1 to 3.3% at PT (Table 2). Except water and Gaucho + Scanmask treatments at 7 DPT, Gaucho had a significant leaf area injury of 3.1% compared with the other treatments (Table 2). Among the treatment combinations, Gaucho + Scanmask was the only one that resulted in a lower leaf area injury of 5.8% and this was not significant when compared with Gaucho and water (Table 2). At 14 DPT, Gaucho and Gaucho + Scanmask resulted in a significantly lower leaf area injury of 8.0 and 9.8%, respectively (Table 2). The treatments Scanmask, Heteromask, and Ecomask + Heteromask had leaf area injuries that were significantly higher compared with the control.

At Sweet Grass, the leaf area injury varied from 2.0 to 5.3% at PT (Table 2). Treatment of canola with Gaucho 600 resulted in the lowest leaf area injury of 4.7% at 7 DPT compared with the water control (Table 2). At 14 DPT, Gaucho 600 (12.4%) and Gaucho + Scanmask (14.3%) had leaf area injury levels significantly lower than the rest of the treatments (Table 2). Except for Ecomask, Scanmask, Ecomask + Heteromask, and Ecomask + Scanmask, all treatments had leaf area injuries significantly lower than the water control (Table 2).

The leaf area injury at Cutbank ranged from 4.3 to 8.6% at PT (Table 2). At 7 DPT, Gaucho + Scanmask and Gaucho 600 treatments had leaf area injuries of 14.2 and 14.3%, respectively (Table 2). At 14 DPT, Gaucho 600 (13.2%) and Gaucho + Scanmask (16.5%) were the only treatments that had a leaf area injury significantly lower than the water control (22.3%; Table 2).

Effect of Treatments on Seed Yield

Overall, yield ($F = 2.69$; df = 35, 143; $P < 0.0001$) and location ($F = 28.97$; df = 2, 22; $P < 0.0001$) effects were significantly different among treatments. However, overall treatment ($F = 1.63$; df = 11, 22; $P = 0.1014$) and location × treatment ($F = 0.84$; df = 2, 22; $P = 0.6704$) effects were not significant.

Table 3. Canola seed yield after treatment of seedlings with entomopathogenic nematodes and sprayable gel in Montana

Treatment	Location		
	WTARC[a]	Sweet Grass	Cutbank
	Yield (kg/ha)		
Water	354.8d	588.2b	427.1ab
Gaucho 600	843.2a	810.6ab	305.1b
Ecomask	620.0c	778.1ab	222.7b
Hi	804.0ab	645.1ab	405.8b
Scanmask	641.7bc	699.4ab	351.5b
Heteromask	669.1abc	560.3b	403.9b
Ecomask + Heteromask	665.3abc	701.6ab	388.6b
Ecomask + Scanmask	801.8ab	721.8ab	358.0b
Gaucho + Scanmask	650.6bc	761.6ab	413.0b
Barricade (4%)	758.3abc	720.3ab	357.9b
Barricade (2%) + Scanmask	699.5abc	604.5b	469.5ab
Barricade (1%) + Scanmask	739.5abc	1020.8a	670.2a

Means within a column followed by the same letter are not significantly different at $P < 0.05$.
[a] WTARC, Western Triangle Agricultural Research Center.

At WTARC, all treatments differ significantly from the water control (Table 3). Gaucho 600 resulted in the highest yield of 843.2 kg/ha compared with Ecomask (620.0 kg/ha), Scanmask (641.7 kg/ha), and Gaucho + Scanmask (650.6 kg/ha; Table 3). Although Hi and Ecomask + Scanmask had yields of 804.0 kg/ha, and 801.8 kg/ha, respectively, none of the treatments had yields that were as good as Gaucho the chemical standard (Table 3).

At Sweet Grass, Barricade (1%) + Scanmask resulted in the highest yield of 1020.8 kg/ha, and this was the only treatment that differed significantly from the water control (Table 3). None of the other treatments had yields that were significantly as better than Gaucho the chemical seed treatment (Table 3).

At Cutbank, Barricade (1%) + Scanmask resulted in the highest yield of 670.2 kg/ha, and this did not differ significantly from the water control (Table 3). However, none of the other treatments had

Table 4. Interaction between Gaucho and entomopathogenic nematodes (Ecomask, Hi, Scanmask, and Heteromask) against crucifer flea beetle *Phyllotreta cruciferae* at two time points in Montana

Treatment	WTARC											
	7 DPT						14 DPT					
	Mn[a]	Mi[b]	Me[c]	Mni[d]	Mni – Me[e]	$(\text{Mni-Me})^2$ /Me	Mn	Mi	Me	Mni	Mni - Me	$(\text{Mni – Me})^2$ /Me
Gaucho 600	−2.98	−2.98	−14.81	−0.11	14.70	−14.70	−9.00	−9.00	−99.10	−6.87	92.22	−85.83
Ecomask	1.81	−2.98	4.21	−0.11	−4.31	4.31	0.36	−9.00	−5.45	−6.87	−1.42	−0.37
Hi	5.74	−2.98	19.84	−0.11	−19.95	19.95	−0.59	−9.00	−14.93	−6.87	8.06	−4.35
Scanmask	9.35	−2.98	34.20	−0.11	−34.31	34.31	4.27	−9.00	33.67	−6.87	−40.54	48.82
Heteromask	9.35	−2.98	34.20	−0.11	−34.31	34.31	4.27	−9.00	33.67	−6.87	−40.54	48.82
Gaucho + Scanmask	−0.11	−2.98	−3.40	−0.11	3.29	−3.29	−6.87	−9.00	−77.76	−6.87	70.89	−64.62
	Sweet Grass											
Gaucho 600	−4.61	−4.61	−30.48	−3.18	27.29	−11.46	−12.16	−12.16	−172.29	−9.73	162.56	−153.38
Ecomask	−1.65	−4.61	−13.85	−3.18	10.67	−59.40	−0.77	−12.16	−22.28	−9.73	12.55	−7.07
Hi	0	−4.61	−4.61	−3.18	1.43	−254.15	−3.46	−12.16	−57.67	−9.73	47.94	−39.85
Scanmask	0.88	−4.61	0.32	−3.18	−3.50	4371.14	−1.28	−12.16	−29.02	−9.73	19.29	−12.82
Heteromask	0.99	−4.61	0.93	−3.18	−4.12	1513.27	−3.84	−12.16	−62.73	−9.73	53.00	−44.78
Gaucho + Scanmask	−3.18	−4.61	−22.47	−3.18	19.29	−24.58	−9.73	−12.16	−140.26	−9.73	130.53	−121.48
	Cutbank											
Gaucho 600	−3.01	−3	−15.04	−3.13	11.91	−237.40	−11.71	−11.71	−160.59	−7.47	153.12	−146.01
Ecomask	2.52	−3	7.10	−3.13	−10.23	809.92	−3.48	−11.71	−55.88	−7.47	48.42	−41.95
Hi	−1.44	−3	−8.78	−3.13	5.66	−470.85	−3.09	−11.71	−50.98	−7.47	43.51	−37.14
Scanmask	−1.32	−3	−8.30	−3.13	5.18	−503.59	−3.99	−11.71	−62.43	−7.47	54.96	−48.39
Heteromask	2.64	−3	7.59	−3.13	−10.71	765.53	−1.29	−11.71	−28.07	−7.47	20.61	−15.13
Gaucho + Scanmask	−3.13	−3	−15.52	−3.13	12.40	−227.35	−7.47	−11.71	−106.60	−7.47	99.14	−92.19

[a] Observed proportional percentage leaf area damage caused by nematodes alone.

[b] Observed proportional percentage leaf area damage caused by Gaucho (imidacloprid) alone.

[c] Expected additive proportional percentage leaf area damage for the nematode–Gaucho (imidacloprid) combinations.

[d] Observed proportional percentage leaf area damage for the nematode–Gaucho (imidacloprid) combinations.

[e] Interaction between treatments: Antagonistic (Mni - Me = a negative value), Nonadditive effect (synergistic or antagonistic; $\chi > 3.841$), (Synergistic (Mni - Me = a positive value).

yields that were significantly better than Gaucho the chemical seed treatment (Table 3).

Discussion

In general, *P. cruciferae* fed less on plots treated with Gaucho + Scanmask, and Gaucho 600 at 7 to 14 DPT. Moreover, the interaction of Gaucho + Scanmask was synergistic (Table 4). This agrees with observations that EPNs and imidacloprid interacted synergistically on *Popillia japonica* Newman (white grub) (Coleoptera: Scarabaedae) mortality (Koppenhöfer and Kaya 1998; Koppenhöfer et al. 2000a, 2002), where a general reduction in mobility appeared to be a major factor responsible for this synergistic interaction (Koppenhöfer et al. 2000b). A general disruption of nerve function from imidacloprid seems to enhance the attachment of juvenile EPN to the host and subsequent penetration (Koppenhöfer et al. 2000a).

Under moderate, high, and extremely high *P. cruciferae* feeding pressure in our study, Gaucho + Scanmask was the only treatment that had leaf area injury rates on par with Gaucho 600 (imidacloprid). EPNs applied alone or in combination with other EPNs were not effective, possibly due to ultraviolet radiation or desiccation (Shapiro-llan et al. 2002), and that above-ground application of EPNs could be improved by protecting the EPNs from harmful environmental conditions (Glazer et al. 1992, Baur et al. 1997, Head et al. 2004, Schroer and Ehlers 2005, Shapiro-llan et al. 2006). Moreover, the nematode products were obtained from different companies, and hence, production method and formulation might also be among the factors that affected their efficacy. Notwithstanding this, it is reasonable to use EPNs from different producers, as the growers will also purchase EPNs from different sources.

Under moderate *P. cruciferae* feeding pressure, Gaucho 600 resulted in the highest yield of 843.2 kg/ha at WTARC, while Barricade (1%) + Scanmask resulted in the highest yield of 1020.8 kg/ha at Sweet Grass and 670.2 kg/ha at Cutbank. Under high and extremely high *P. cruciferae* feeding pressure at Sweet Grass and Cutbank, the Barricade (1%) + Scanmask treatment had the highest yields, suggesting that when the seed treatment protection period is exceeded, Barricade and Scanmask (*S. feltiae*) can be used as a complement to Gaucho to reduce *P. cruciferae* feeding and subsequent yield losses. Based on yield, EPNs applied alone as single control agent or in combination with other EPNs were not effective, especially under high and extremely high *P. cruciferae* feeding pressure. Similar to our findings, Shapiro-llan et al. (2010, 2015, 2016) found that the sprayable gel Barricade significantly enhanced the efficacy of EPNs (*S. feltiae, S. carpocapsae*) for controlling *S. pictipes* and *Synanthedon exitiosa* (Say) (Lepidoptera: Sesiidae).

The data indicate that Barricade (1%) + Scanmask can serve as alternative to the seed treatment. Moreover, Scanmask can be used to complement the seed treatment when the protection period is exceeded. However, net returns on the use of these biopesticides need to be ascertained.

Acknowledgments

We would like to thank (United States Department of Agriculture) USDA-National Institute of Food and Agriculture Hatch (Accession#MONB00859) and Professional Development Program of the USDA-Western Sustainable Agriculture Research and Education project #2014-38640-22175/Utah State University sub award # 140867038 for funding. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the National Institute of Food and Agriculture (NIFA) of the United States Department of Agriculture (USDA). We would also like to thank John Miller, Amber Ferda, Julie Prewett, Judisch Krystal, and Dowson Berg for assistance with field work.

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Toxicology Reports 3 (2016) 473–480

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Evaluation of toxicity of biorational insecticides against larvae of the alfalfa weevil

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ARTICLE INFO

Article history:
Received 14 April 2016
Received in revised form 4 May 2016
Accepted 4 May 2016
Available online 5 May 2016

Keywords:
Low risk insecticides
Insect pathogenic fungi
Efficacy
Lethal concentration
Mortality rate

ABSTRACT

The alfalfa weevil, *Hypera postica* (Coleoptera: Curculionidae), is a major pest of alfalfa *Medicago sativa* L. (Fabaceae). While *H. postica* usually causes the most damage before the first cutting, in summer of 2015 damaging levels of the pest persisted in Montana well after the first harvest of alfalfa. Although conventional insecticides can control *H. postica*, these chemicals have adverse effects on non-target organisms including pollinators and natural enemy insects. In this context, use of biorational insecticides would be the best alternative options, as they are known to pose less risk to non-target organisms. We therefore examined the six commercially available biorational insecticides against *H. postica* under laboratory condition: Mycotrol® ESO (*Beauveria bassiana* GHA), Aza-Direct® (Azadirachtin), Met52® EC (*Metarhizium brunneum* F52), Xpectro OD® (*B. bassiana* GHA + pyrethrins), Xpulse OD® (*B. bassiana* GHA + Azadirachtin) and Entrust WP® (spinosad 80%). Concentrations of 0.1, 0.5, 1.0, and 2.0 times the lowest labelled rates were tested for all products. However, in the case of Entrust WP, additional concentrations of 0.001 and 0.01 times the lowest label rate were also assessed. Mortality rates were determined at 1–9 days post treatment. Based on lethal concentrations and relative potencies, this study clearly showed that Entrust was the most effective, causing 100% mortality within 3 days after treatment among all the tested materials. With regard to other biorational, Xpectro was the second most effective insecticide followed by Xpulse, Aza-Direct, Met52, and Mycotrol. Our results strongly suggested that these biorational insecticides could potentially be applied for *H. postica* control.

1. Introduction

Alfalfa weevil *Hypera postica* (Gyllenhall) (Coleoptera: Curculionidae), is the most destructive insect pest of alfalfa *Medicago sativa* L. (Fabaceae) in the intermountain west of the United States [1]. *H. postica* not only decreases yield and quality of the first cutting, but can also harm subsequent cuttings [2]. Both larvae and adults damage terminals, foliage and new crown shoots, thereby lowering crop yield and quality [3]. However, the larvae caused the most damage [4]. During severe infestations, larvae can cause substantial defoliation, resulting in severe first cutting losses [5]. Heavily infested fields may appear silver or white, with most leaves skeletonized or consumed entirely [1]. If large numbers of adults or larvae survive until harvest, they damaged stems and crown buds, retarding regrowth [6]. A decrease in stem elongation occurred at a density of 30–100% of the smallest larval density [7]. Residual effects from severe damage decrease plant vigor, resulting in lower stand density and poor yields in subsequent harvests [2].

Although *H. postica* is native to Europe it was inadvertently introduced into the western United States in the early 1900s [8], and into the eastern United States in the late 1940s [9]. In Montana, alfalfa is the second most important crop after small grains [10]. Alfalfa growers in Montana first began to notice *H. postica* during spring 2013 when the weevil caused considerable damage and yield losses [10]. In addition, alfalfa weevils caused economic damage in irrigated fields in the Yellowstone and Missouri river valleys in Montana [10]. Insecticidal treatment are economical when a larval population average between 1.5–2.0 larvae/stem, or 20 larvae/sweep [11]. In 2014 and 2015, *H. postica* outbreak occurred in Valier, Montana. Even though *H. postica* does the most damage before the first cutting [12], considerable damage was also noticed even after the first harvest.

To date, other than classical biological control, insecticide applications and early harvesting are the most common management strategies for alfalfa weevil [13]. However, most of the chemical insecticides used to manage this pest are extremely

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http://dx.doi.org/10.1016/j.toxrep.2016.05.003

Table 1
Materials and application rates used for the laboratory bioassays against *Hypera postica* larvae.

Treatment	Chemical name	Trade name	Concentrations (ml/l)	Source
T1	Untreated control	–	–	–
T2	spinosad (*Saccharopolyspora spinosa*)	Entrust® WP	0.000091, 0.00091, 0.0091, 0.0455, 0.091, and 0.182	Dow Agro Science LLC, Indianapolis, IN
T3	*Metarhizium brunneum* F52	Met52® EC	0.072, 0.36, 0.72, and 1.44	Novozymes Biologicals, Salem, VA
T4	*Beauveria bassiana* GHA	Mycotrol ESO®	0.072, 0.36, 0.72, and 1.44	LAM International, Butte, MT
T5	Azadirachtin (extracts from *Azadirachta indica*)	Aza-Direct®	0.144, 0.72, 1.44, and 2.88	Gowan Company, Yuma, AZ
T6	*B. bassiana* GHA + pyrethrins	Xpectro® OD	0.25, 1.25, 2.5, and 5.0	LAM International, Butte, MT
T7	*B. bassiana* GHA + cold pressed Neem extract	Xpulse® OD	0.072, 0.36, 0.72 and 1.44	LAM International, Butte, MT

hazardous to bees [14,15], and other beneficial insects like the parasitoids *Bathyplectes curculionis* (Thomson) (Hymenoptera: Ichneumonidae) and *Oomyzus incertus* Ratzburg (Hymenoptera: Eulophidae) [16]. Increasing concerns for environmental safety and insecticide resistance arising from a frequent use of synthetic insecticides affect the long-term feasibility of the current strategy of alfalfa weevil management [17]. Consequently, many alfalfa growers in north central and central Montana are looking for more environmental friendly control methods for managing this destructive pest.

In this context, as a green alternative to synthetic insecticides, use of biorational insecticides would be the best alternative options because these insecticides are usually considered low-risk agents having the features of low mammalian toxicity as well as less impact on non-target organisms [18]. The biorational insecticides include the use of naturally derived compounds from plants or microbes such as spinosyns and azadirachtin, living organisms (insect pathogenic fungi) such as *Beauveria bassiana* (Bals.) Vuill (Ascomycota: Hypocreales) and *Metarhizium brunneum* (*anisopliae*) (Metsch.) Sorokin (Ascomycota: Hypocreales) or the combined formulation of these insecticides [18]. In recent years, a number of biorational insecticides are commercially available and have been used or tested against variety of pest species such as aphids [19], thrips [20], and coleopteran pests [21,22]. No attempts have been made so far to study the effects of these insecticides on *H. postica* control except the studies by Hedlund and Pass [23] and Sakurai et al. [24], who showed the infection of *H. postica* with *B. bassiana*, and *M. brunneum*. This study therefore aimed to evaluate the toxicity of biorational insecticides against *H. postica* under the laboratory conditions.

2. Materials and methods

2.1. Rearing of insects

H. postica larvae were collected from alfalfa fields in Valier, Montana, USA, using sweep nets in July 2015 and taken to the laboratory. Larvae were placed in collapsible cages (12 × 10 × 10 cm), fed alfalfa foliage, and held at 22 ± 2 °C, 70–80% RH and an approximately 14:10 h L:D photoperiod. Field-collected larvae were separated by instar as described by Harcourt [25]. The instars ranged from first to fourth instars. The first instar is light yellow or tan in color with a darker head and about 1 mm long while the second instar is yellowish-brown with their head deepening to black, third and

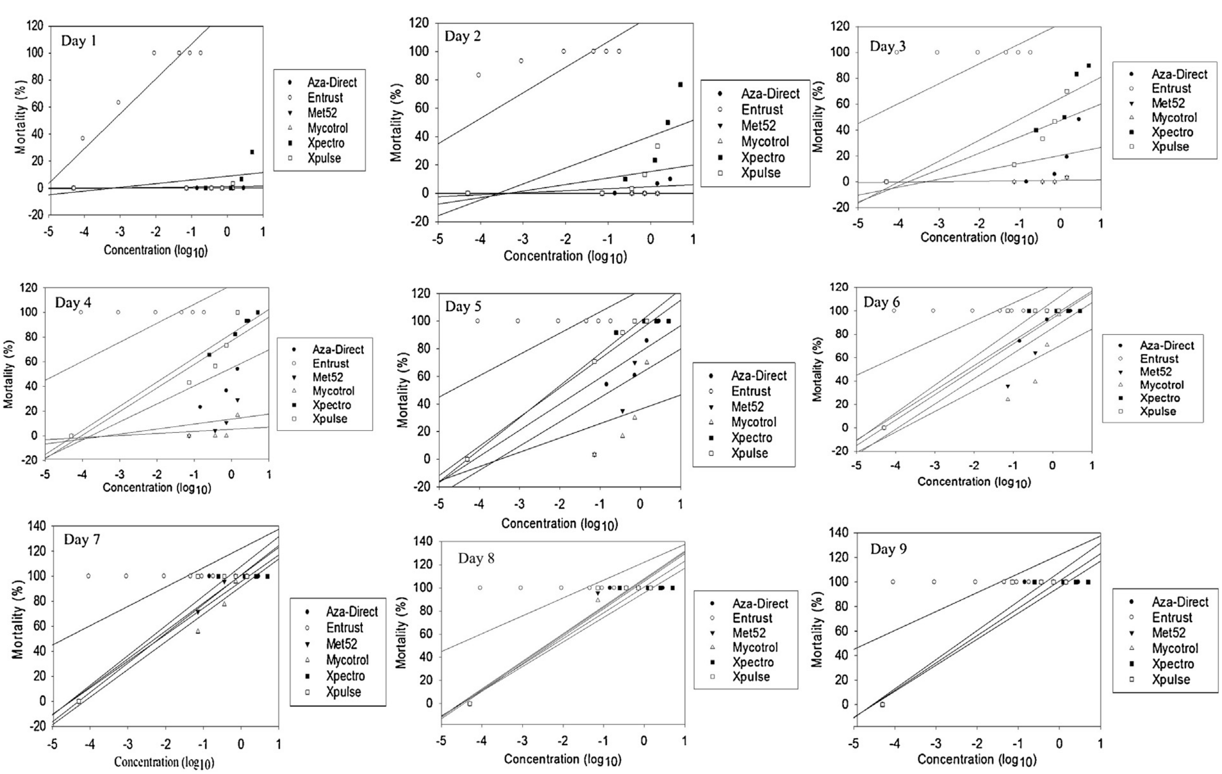

Fig. 1. Percentage mortality of 2nd instar larvae of *Hypera postica* treated with different concentrations (log) of biorational insecticides: Mycotrol® ESO (*Beauveria bassiana* GHA), Aza-Direct® (Azadirachtin), Met52® EC (*Metarhizium brunneum* F52), Xpectro OD® (*Beauveria bassiana* GHA + pyrethrins), Xpulse OD® (*Beauveria bassiana* GHA + Azadirachtin) and Entrust WP® (spinosad 80%) at days 1–9.

Table 2
Lethal concentrations and relative potencies of *Hypera postica* larvae to biorational insecticides.

Treatments	Day	LC_{50} (g a.i./L)	C. I. (95%)	$P > \chi^2$	Relative Potency (LC_{50};S/LC_{50};T)[a]
Entrust	2	0.0000123	1.42538×10^{-5}–0.0000432	0.9054	1
Mycotrol ESO[b]	4	0.163602	0.159619–0.167686	1.0000	7.52×10^{-5}
Met52 EC[c]	4	0.23434	0.14327–1.17575	0.7271	5.25×10^{-5}
Aza-Direct	4	0.08146	0.04730–0.12666	0.0221	0.000151
Xpulse OD[d]	4	0.01417	0.00573–0.02360	0.0229	0.000868
Xpectro OD	4	0.00109	0.0001385–0.00229	0.4189	0.011284
Entrust WP	4	ND[e]	ND	ND	ND
Mycotrol ESO	5	0.10845	0.08129–0.16632	0.2299	0.000113
Met52 EC	5	0.03441	0.02316–0.04705	0.0421	0.000357
Aza-Direct	5	0.01758	0.00407–0.03324	0.0317	0.0007
Xpulse OD	5	0.00371	0.0005850–0.00663	0.5906	0.003315
Xpectro OD	5	0.00172	0.00157–0.00188	1.0000	0.007151
Entrust WP	5	ND	ND	ND	ND

[a] Ratios of the lethal concentrations of standard insecticide (Entrust WP) to the treatments at 50% mortality.
[b] 2×10^{13} viable spores per quart with weight estimate of 4.78×10^{12} grams per spore.
[c] 5×10^{10} viable conidia per gram of active ingredient and contains 5.5×10^{9} colony forming units (CFU)/gram of product.
[d] *Beauveria bassiana* Strain GHA (0.06%) contains $\geq 1 \times 10^{11}$ viable spores per quart.
[e] ND, no data due to single response values (100% mortality), and therefore could not be determined by statistical analysis as well as lethal ratios.

fourth instar size is up to 9 mm long, are bright green with shiny black head capsule, and have a white stripe down the halfway point of their rears. Second instars were used for all tests.

2.2. Biorational insecticides

Biorational insecticides tested were of commercial formulations (Table 1) and were stored dried at 4–5 °C until diluted to the desired concentrations for use. The concentrations used in the study were 0.1, 0.5, 1.0 and 2.0 times the lowest label rate. However, in case of Entrust product, we prepared additional concentrations of 0.001 and 0.01 times the lowest label rate since this product has been known for high toxicity.

2.3. Toxicity tests

Toxicity tests were performed in the laboratory from 15 July through August 2015 when larvae from field populations were available. Materials were applied via contact at the desired concentrations (see Table 1 for rates). For each replicate, five larvae were transferred onto a disk of Whatman No. 1 filter paper (9 cm diameter, Whatman quantitative filter paper, ashless, Sigma-Aldrich, St. Louis, Missouri, USA) in a 9 cm disposable Petri dish where they were topically treated with the test material.

Each Petri dish also contained three alfalfa stems about 5 cm long, each with 6–8 leaves as larval food. Six replicate Petri dishes, containing a total of 30 larvae were treated using a Sprayer

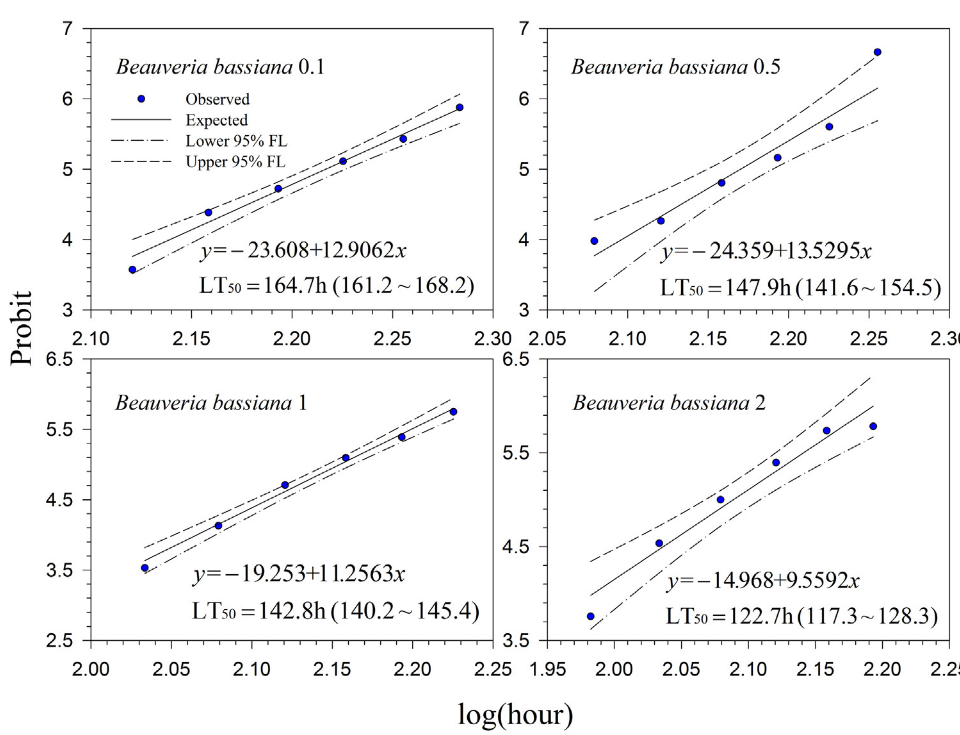

Fig. 2. Probit analysis on median lethal time (LT_{50}) of Mycotrol® ESO (*Beauveria bassiana* GHA) treated 2nd instar larvae of *Hypera postica* at different concentrations.

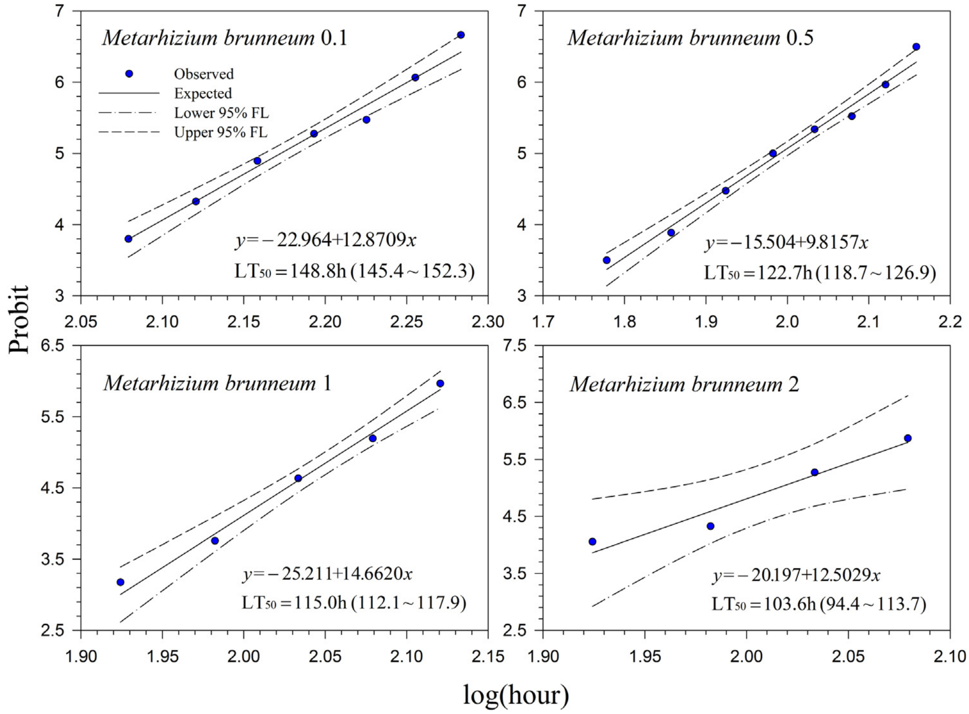

Fig. 3. Probit analysis on median lethal time (LT_{50}) of Met52® EC (*Metarhizium brunneum* F52) treated 2nd instar larvae of *Hypera postica* at different concentrations.

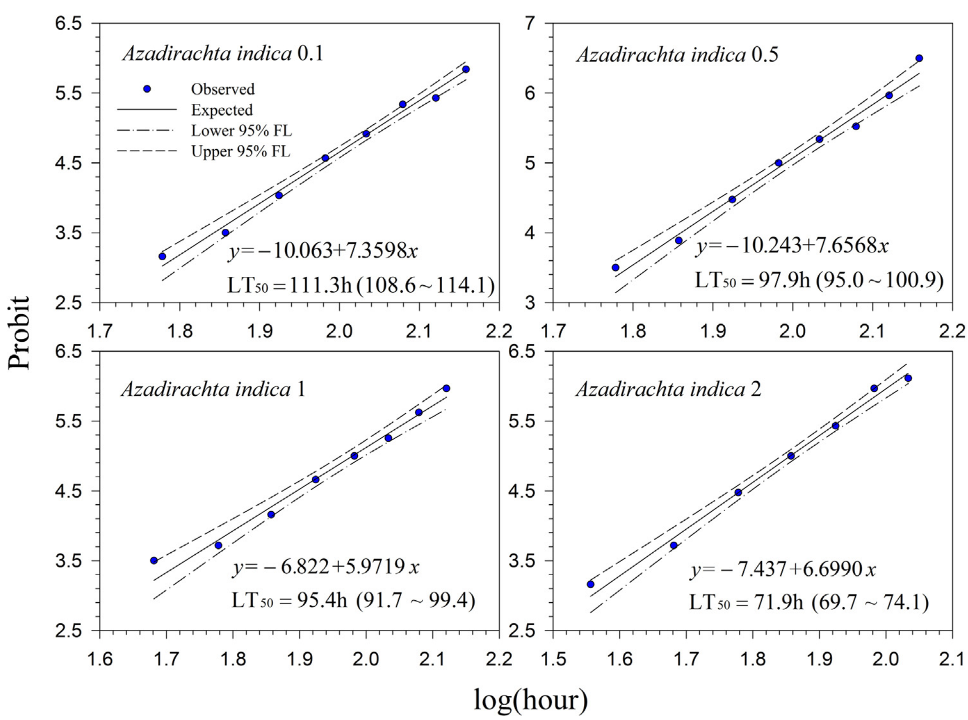

Fig. 4. Probit analysis on median lethal time (LT_{50}) Aza-Direct® (Azadirachtin) treated 2nd instar larvae of *Hypera postica* at different concentrations.

(Sprayco, Livonia, MI) with 1 ml of a test material [26]. Controls were treated with 1.0 ml of tap water. Following application, Petri dishes were held under the same laboratory conditions as used for rearing of insect. Larval mortality was assessed daily for nine days.

2.4. Statistical analyses

SAS 9.4 was used in analyzing the data [27]. Abbott's formula was used to adjust for control mortality [28], Sigma Plot 13.0 (SPSS Inc., Chicago, IL) for plotting the graphs of mortality (%) versus log concentration, and PROC PROBIT procedure for estimating the lethal values (LC_{50}s). Comparison of the 95% confidence limits was used to determine differences in lethal values [29–31].

Among the different products, Entrust product caused 100% mortality of *H. postica* larvae within 3 days, while other products caused at 0–100% mortality at 4–9 days (Fig. 1). Based on this condition, we estimated LC_{50} of Entrust and other products at 2 days or 4 and 5 days post treatment respectively (Table 2).

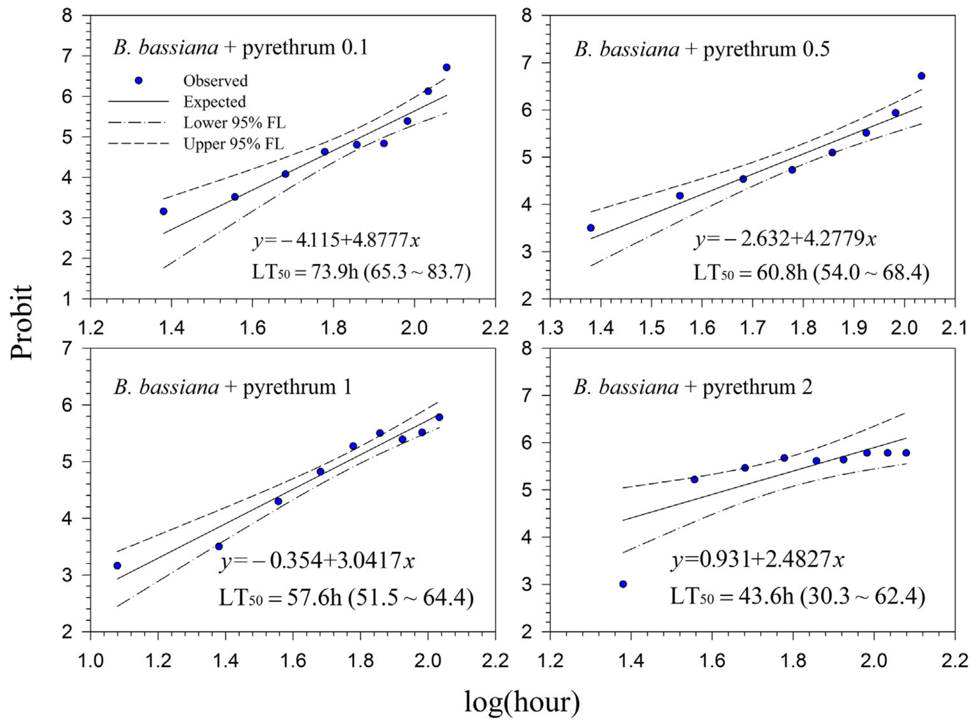

Fig. 5. Probit analysis on median lethal time (LT_{50}) of Xpectro® (*B. bassiana* GHA + pyrethrins) treated 2nd instar larvae of *Hypera postica* at different concentrations.

Extra binomial variations due to genetic and environmental influences that caused poor fit were accounted for by multiplying the variances by the heterogeneity factor (χ^2/k-2), where k is the number of concentrations [27,31,32]. Relative potencies for the treatments were compared using the lethal concentrations [30].

Because mortality rates of the all tested materials increased over time (Figs. 2–7), treatments were also analyzed for LT_{50} using the program Probit-MSChart [33]. Mortality response (in probits) was regressed against $\log_{10}$ day.

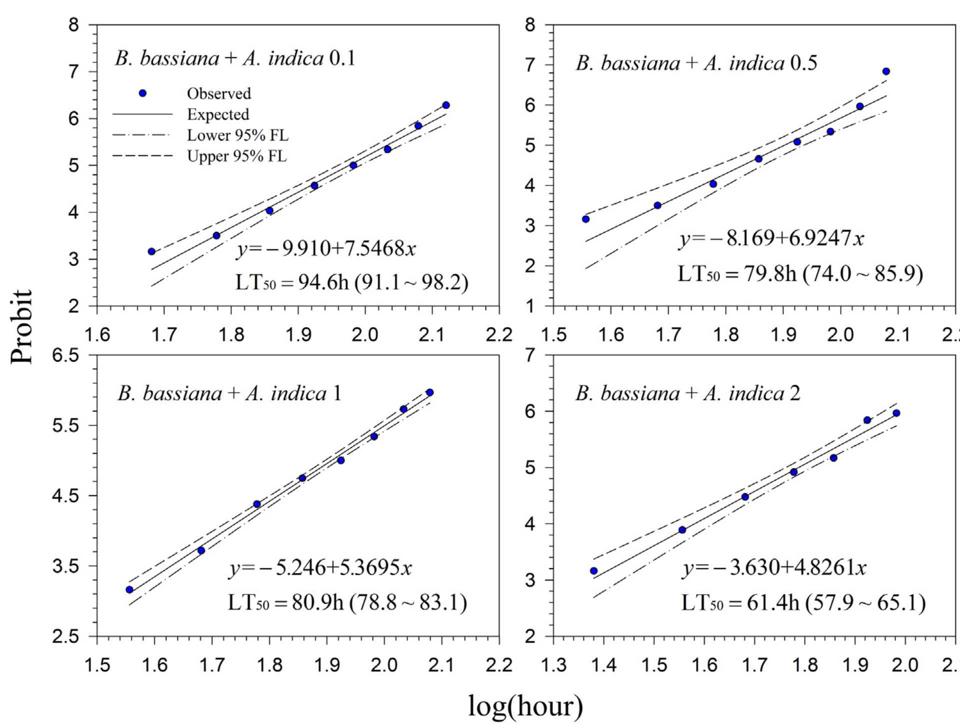

Fig. 6. Probit analysis on median lethal time (LT_{50}) of Xpulse® (*B. bassiana* GHA + azadirachtin) treated 2nd instar larvae of *Hypera postica* at different concentrations.

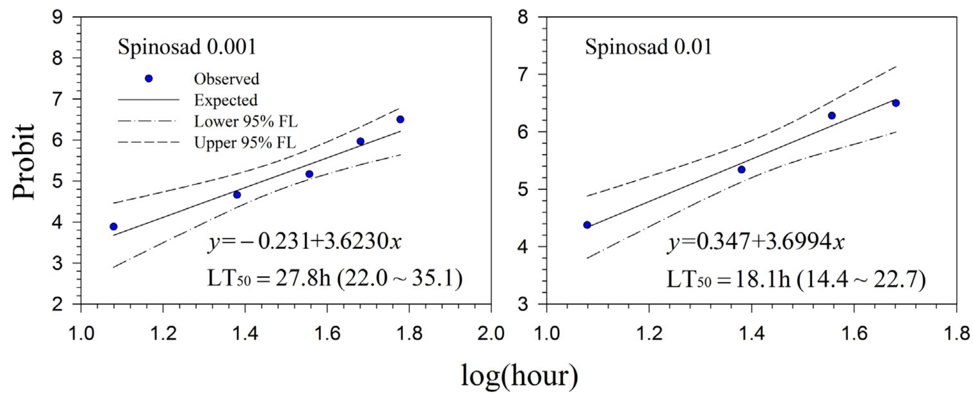

Fig. 7. Probit analysis on median lethal time (LT_{50}) of Entrust® (spinosad) treated 2nd instar larvae of *Hypera postica* at different concentrations.

3. Results

3.1. Mortality percentage

Among all tested biorational insecticides, Entrust caused high mortality to *H. postica* larvae, acting rapidly and reaching 100% mortality at day 3 across all concentrations (Fig. 1). However, for other products, such as, Aza-Direct, (naturally derived compounds from plants) and, Xpulse and Xpectro (combined formulation of insect pathogenic fungi and naturally derived compounds from plants) took 6–7 days to kill 100% of *H. postica* larvae across all concentrations (Fig. 1). Furthermore, insect pathogenic fungus products for example Met52 and Mycotrol took 5–9 days to kill 100% of *H. postica* larvae across all concentrations (Fig. 1).

3.2. Lethal time (LT_{50})

In overall, the toxicity results of contact bioassay with all tested materials against second instars of *H. postica* showed good linear regression relationship between mortality (in probit) and time ($\log_{10}$ day) after treatment (Figs. 2–7). The mortality rate (in probit) increased with $\log_{10}$ day for all examined products.

Generally, lethal time decreased with increasing concentrations for all treatments. However, the differences in lethal time were found between the products. Among the tested products, highest lethal time (122.7–164.7 h; 5.11–6.86 days) was obtained for Mycotrol (Fig. 2) in contrast to the lowest lethal time for Entrust (18.1–27.8 h; 0.75–1.16 days) (Fig. 7). For the other products, the second highest lethal time was found for Met52 (103.6–148.8 h; 4.32–6.2 days) (Fig. 3) followed by Aza-Direct (71.9–111.3 h; 2.996–4.64 days) (Fig. 4), Xpulse (61.4–94.6 h; 2.56–3.94 days) (Fig. 6) and Xpectro (43.6–73.9 h; 1.82–3.08 days) (Fig. 5).

3.3. Lethal concentration (LC_{50})

The lethal concentrations for each tested material are depicted in Table 2. Generally, there was a good fit to the model assumptions. Entrust was found the most effective biorational insecticide compared to all other tested materials, since Entrust had a lowest LC_{50} value (Table 2). Among other products, based on lethal concentrations estimated at day 4 and day 5, Xpectro was second most effective biorational insecticide followed by Xpulse, Aza-Direct, Met52, and Mycotrol (Table 2).

Furthermore, we computed relative potencies at day 2 for Entrust and at days 4 and 5 for Mycotrol, Met52, Aza-Direct, Xpulse, and Xpectro, using Entrust as the standard insecticide at 50% mortality (Table 2). The result showed that none of the treatments had potencies, which were at par when compared with Entrust (Table 2).

4. Discussion

H. postica rapidly became the most devastating pest of alfalfa in the United States following its invasion in the 1940s, [34] largely due to an absence of specialized natural enemies. The USDA carried out a large-scale biological control program against this pest in the late 1950–1970s [35]. Seven parasitoid species were employed [36] in addition to the fungus *Zoophthora phytonomi* (Arthur) (Zygomycetes: Entomophthorales) [37]. Although natural enemies have brought the *H. postica* population below the economic threshold level in other places, it is still a serious pest in many parts of Montana. This pattern may be due to a lack of natural enemies in these areas. Exploring the potential of biorational insecticides to manage *H. postica* may protect these same natural enemies from the adverse effects of conventional insecticides.

In this study, Entrust (spinosad) caused 100% mortality of *H. postica* within 3 days after treatment. Based on the relative potencies, Entrust was the most effective among the treatments. While Entrust (spinosad) was effective against *H. postica*, this chemical is known to be harmful to natural enemies, particularly parasitoids. Spinosad, the active ingredient in Entrust, has been observed to be intrinsically toxic to pollinators especially bees, though it has low toxicity to many beneficial insects [38]. Williams et al. [39] reported that hymenopteran parasitoids are significantly more susceptible to spinosad than predatory insects, with 78% of laboratory studies, and 86% of field studies reporting a moderately harmful, or harmful results. While further laboratory and field studies examining the effect of Entrust (spinosad) on the parasitoids of *H. postica* would be helpful, the need for these parasitoids may be low, since Entrust causes high mortality to *H. postica*. Although many insect growth regulators have been tested and found to be effective against *H. postica* [40,41], further cost-benefit analyses of these products are needed as they seem expensive to use given the level of crop loss.

In this study, Xpectro® (*B. bassiana* + pyrethrins) and Xpulse® (*B. bassiana* + azadirachtin) mixture products were effective in causing mortality in *H. postica*. Although the tested fungal pathogens Mycotrol (*B. bassiana*), and Met52 (*M. brunneum*) have delayed effect, they both caused 100% mortality within 9 days of treatment. Over a thousand pathogens have been isolated from insects [22]. Pathogens associated with major insect pests are potential candidates for development into microbial insecticides [42]. Fungal pathogens have a different mode of action than synthetic insecticides, killing their hosts through infection that leads to the subsequent production of insecticidal toxins, such as *destruxins* [43,44]. Harcourt et al. [45] reported that *H. postica* larvae were found to be infected by a fungal entomopathogen (*Entomophthora phytonomi* Arthur) which significantly reduced the weevil population in Canada. However, Millstein et al. [46] reported the importance of conidial discharge and relative humidity in *Erythrina* sp. infecting *H. postica* in Kentucky, USA. Mustafa et al. [47] reported that conidial suspensions of entomopathogenic fungi *B. bassiana, M. anisopliae*, *Lecanicillium lecanii* (Zimm.) Zare and W. Gams and *Clonostachys rosea* (Link: Fr.) isolates had significant effects on *H. postica* adult mortality, with isolates of *B. bassiana*, *M. anisopliae*, and *L. lecanii* being more effective on adults than *C. rosea*.

Aza-Direct (extracts from *Azadirachta indica*) also caused 100% mortality 7–9 days after treatment in this study. These results agree with Oroumchi and Lorra [48] who reported that aqueous extracts of neem seed kernels and leaves, and chinaberry *Melia azedarach* L. (Meliaceae) leaves applied to alfalfa leaves in the laboratory caused high mortality and strong growth-disturbing effects in the larval stages of *H. postica,* with most larvae dying before or during molting. Yardim et al. [49] reported that neem (azadirachtin) had significant effects on the larvae of another alfalfa weevil, *Hypera variabilis* Hbst. (Coleoptera: Curculionidae) but insignificant effects on the total number of predators in alfalfa fields in Turkey.

In general, our study showed that the tested materials including various entomopathogenic fungi can be used to manage *H. postica*. However, it remains to be seen if similar levels of control can be obtained under field conditions. Most of the naturally derived insecticides used in this study are currently commercially available in the United States, and could be adopted by growers. Further research is needed to determine the impact of these insecticides on non-target insects and natural enemies (e.g. bees and parasitoids).

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This work was supported by Professional Development Program of the USDA-Western Sustainable Agriculture Research and Education project #2014-38640-22175/Utah State University sub award # 140867038; and USDA National Institute of Food and Agriculture, Multistate Project W3185, The Working Group Biological Control of Pest Management Systems of Plants [Accession number# 231844]. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the National Institute of Food and Agriculture (NIFA) or the United States Department of Agriculture (USDA). We also thank Dr. Hsin Chi for the help with some aspect of statistical analysis of the data.

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