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ORIGINAL ARTICLE

Field efficacy of insect pathogen, botanical, and jasmonic acid for the management of wheat midge *Sitodiplosis mosellana* and the impact on adult parasitoid *Macroglenes penetrans* populations in spring wheat

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Abstract The wheat midge, *Sitodiplosis mosellana*, is a serious pest of wheat worldwide. In North America, management of *S. mosellana* in spring wheat relies on the timely application of pesticides, based on midge adults levels caught in pheromone traps or seen via field scouting during wheat heading. In this context, biopesticides can be an effective alternative to pesticides for controlling *S. mosellana* within an Integrated Pest Management program. A field study using insect pathogenic fungus *Beauveria bassiana* GHA, nematode *Steinernema feltiae* with Barricade polymer gel 1%, pyrethrin, combined formulations of *B. bassiana* GHA and pyrethrin, Jasmonic acid (JA) and chlorpyrifos (chemical check) was performed to determine to which extent they affect midge larval populations, kernel damage levels, grain yield, and quality, and the impacts on adult parasitoid *Macroglenes penetrans* populations. The results indicated that biopesticides JA and *S. feltiae* were the most effective in reducing larval populations and kernel damage levels, and produced a higher spring wheat yield when compared to the water control at both study locations (East Valier and North Valier, Montana, USA). Increased test weight in wheat had been recorded with two previous biopesticides at East Valier but not for North Valier, when compared over water control. These results were comparable in efficacy to the chlorpyrifos. This study also suggested that *B. bassiana* and pyrethrin may work synergistically, as exemplified by lower total larval populations and kernel damage levels when applied together. This study did not demonstrate the effect of any treatments on *M. penetrans* populations.

Key words biological control; biopesticides; entomopathogen; Integrated Pest Management

Introduction

The wheat midge (also called the orange wheat blossom midge), *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), a wheat (*Triticum* spp.) specific-herbivore, is Palearctic in origin and that was introduced accidentally in North America in the 1800s (Felt, 1912; Olfert *et al.*, 2009). Over the last few decades, it has become a wheat chronic pest in the Northern Great Plains, including Minnesota, Idaho, North Dakota, Washington and Montana (Knodel & Ganehiarachchi, 2008; Stougaard *et al.*, 2014). Also, this pest is distributed widely in many other parts of the world (Olfert *et al.*, 2009). In Montana, *S. mosellana* was first reported in 1990s, but damage to the wheat crop in this region initially remained low, with only periodic minor outbreaks. However, in 2006, an outbreak occurred in north western Montana on spring wheat in the Flathead County with estimated wheat losses over $1.5 million in this county alone (Stougaard *et al.*, 2014).

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Wheat midge infestations generally reduce wheat yield from 30%–40%; however, if the infestation is severe, the yield loss can reach up to 100% (Blodgett, 2007). Unfortunately, in recent years, the presence of *S. mosellana* appears to be expanding with outbreaks occurring in other parts (such as northcentral and eastern) of Montana.

Wheat midge is typically univoltine insect pest species, with mature larvae overwintering in the soil inside cocoons. When temperatures begin to increase in the spring, larvae leave their cocoons, pupate and emerge from soil (Doane *et al.*, 2002; Shanower, 2005). Immediately after emergence, female adults release sex pheromone (2S, 7R)-2, 7-nonadiyl dibutyrate) which attract males for mating (Gries *et al.*, 2000). Mated females fly to find wheat host plants for oviposition and they lay eggs on wheat heads, usually in the evening and early morning. Eggs hatch in 4–7 d, and larvae feed on the surface of newly developing kernels for 2–3 weeks, causing them to shrivel, crack, or become distorted (Dexter *et al.*, 1987). Third instar larvae drop from wheat heads to the soil, where they burrow in and form cocoons, usually when rainfall occurs (Olfert *et al.*, 1985).

To date, chemical insecticides are the main control method used against *S. mosellana* in North America. Insecticides (e.g., organophosphate or pyrethroid) are usually applied to control adults since larvae are well protected inside wheat kernels. Insecticide applications are made when the crop is at the heading stage, considered the most susceptible stage to wheat midge damage (Stougaard *et al.*, 2014). It is recommended to spray at the economic threshold of one *S. mosellana* adult per 4–5 wheat heads as seen in the evening, or when a pheromone trap catch exceeds 120 midges/trap/d (Elliott, 1988; Gaafar, 2010; Chavalle *et al.*, 2014; Stougaard *et al.*, 2014).

However, demand for alternative methods to control *S. mosellana* populations has been recently stimulated due to the increased risk of insecticide resistance development from the repeated/heavy use of insecticides, and the concerns associated with the environment and human health (Koureas *et al.*, 2012; Kim *et al.*, 2017). Potential alternative methods for wheat midge control include the use of resistant wheat varieties (Blake *et al.*, 2014; Chavalle *et al.*, 2014) and natural enemies such as parasitoids, for example, *Macroglenes penetrans* (Kirby) (Hymenoptera: Pteromalidae), *Euxestonotus error* (Fitch) and *Platygaster tuberosula* (Kieffer) (Hymenoptera: Platygastridae) (Olfert *et al.*, 2003; Shanower, 2005; Thompson & Reddy, 2016), and predators (Floate *et al.*, 1990; Holland *et al.*, 1996). Wheat midge resistant wheat varieties have been developed in many parts of the world (e.g., Canada, Europe and US) and shown great potential for suppressing *S. mosellana* population (Lamb *et al.*, 2002; Blake *et al.*, 2014; Chavalle *et al.*, 2017). Wheat variety resistance to *S. mosellana* is linked to antixenosis (oviposition deterrent activity) or antibiosis (larval death occurrence due to presence of *Sm1* gene) mechanisms (Lamb *et al.*, 2002; Blake *et al.*, 2014; Chavalle *et al.*, 2017). A further potential alternative method is the use of biopesticides including insect pathogens, botanicals and jasmonic acid (JA) which can offer a safe and effective alternative to chemical insecticides for controlling *S. mosellana* within an Integrated Pest Management (IPM) program (El-Wakeil *et al.*, 2010; El-Wakeil & Volkmar, 2012).

Insect pathogens, for example, fungi and nematodes, natural pathogens of insects, have been formulated and commercialized as biopesticides. Fungi infect insect hosts by direct penetration of host cuticles, invade hemocoel and eventually kill the hosts within 6–7 d (Vega *et al.*, 2012). Nematode infective juveniles (IJs) enter insect hosts through natural openings (mouth, spiracles and anus), penetrate into the hemocoel and then release symbiotic bacteria that will kill the hosts within 2 d (Grewal *et al.*, 2006). Insect pathogens have been successfully examined or used against a variety of insect pest species and considered as components of an IPM program (Chandler *et al.*, 2011; Shrestha *et al.*, 2015; Portman *et al.*, 2016; Shapiro-Ilan *et al.*, 2016). However, the effect of insect pathogens on *S. mosellana* has received little attention, except for the study by Keller and Wilding (1985), reporting that naturally occurring fungus *Entomophthora brevinucleate* Nov. (Zygomycota: Entomophthorales) was pathogenic to adults in winter wheat fields in Switzerland.

Several botanical biopesticides have been developed from plant extracts, especially from species of Rutaceae, Lamiaceae, Meliaceae, and Asteraceae that can be toxic and/or repellent to insect pests (Isman, 2006). The most widely used or tested botanical products against a variety of insect pests are pyrethrin and azadirachtin, extracted respectively from chrysanthemum flowers and neem trees (Isman, 2006). These two products have been tested against *S. mosellana* in Germany and Finland but reported with only limited success (Kurppa & Husberg, 1989; El-Wakeil *et al.*, 2013).

Jasmonic acid, a natural plant hormone derived from linolenic acid via the octodecanoid pathway, is released by plants when they are attacked by insect herbivores, which yields increased production of compounds involved in resistance to herbivores (Thaler, 1999a,b; Pickett *et al.*, 2006). Exogenous application of JA has been shown to induce resistance to various insect pests in crops such as cotton (against cotton aphids *Aphis gossypii* [Glover]) (Omer *et al.*, 2000), tomato (against potato aphid, *Macrosiphum euphorbiae* [Thomas]) (Cooper & Goggin, 2005), rice (against brown planthopper,

Nilaparvata lugens [Stål]) (Senthil-Nathan *et al.*, 2009), and wheat (against *Rhopalosiphum padi* [L.]) (Quiroz *et al.*, 1997). In Germany, JA application has shown to induce resistance against *S. mosellana* in fields of winter wheat plants, thereby protecting kernels from damage and enhancing yield (El-Wakeil *et al.*, 2010).

In this field study, we evaluated several commercially available biopesticides for their abilities to reduce *S. mosellana* larval population, kernel damage levels and improve yield and quality of spring wheat. This effort is a first step in an attempt to identify suitable biopesticide products for wheat midge control. The biopesticides selected for this field study were: (1) the insect pathogenic fungus *Beauveria bassiana* (Bals.) Vuill. GHA (Mycotrol ESO®) (Ascomycota: Hypocreales), (2) the nematode *Steinernema feltiae* (Nematoda: Rhabditida) (Scanmask®), (3) JA, (4) pyrethrin (PyGanic EC® 1.4), and (5) combined formulations of *B. bassiana* GHA and pyrethrin (Xpectro OD®). The Xpectro product was considered for this study since it has demonstrated some synergistic effects on control of other insect pests, for example, alfalfa weevils *Hypera postica* Gyllenhal (Coleoptera: Curculionidae) and wheat head armyworms *Dargida diffusa* Walker (Lepidoptera: Noctuidae) (Reddy *et al.*, 2016; Reddy & Antwi, 2016). Chlorpyrifos (Lorsban®) was included as a reference pesticide chemical because this pesticide is widely used in spring wheat by growers in Montana and other parts of world to control *S. mosellana* populations (Chavalle *et al.*, 2014; Stougaard *et al.*, 2014). In addition, the impact of these biopesticides on adult population levels of the parasitoid *M. penetrans* was examined.

Materials and methods

Locations of spring wheat field trials

The experiments were conducted at three locations: North Valier (N 48° 35.192, W112° 21.169), East Valier (N 48° 30.206, W112° 14.350) and East Conrad (N 48° 14. 403, W111° 60.119), in the Golden Triangle area of Montana, USA. This area is an important cereal growing region in Montana and the experiment locations were known to have had high levels of *S. mosellana* infestation in previous years (Pestweb Montana, 2017). The common cropping system in this area is cereal crops grown year after year or a fallow (noncrop) period following 1–2 years cereal crop rotation, and the crop lands are mostly nonirrigated (McVay *et al.*, 2010). Winter wheat is usually seeded in September with seeding rate of 100–150 kg seeds/ha, while spring wheat is seeded from April to May with 150–220 kg seeds/ha (McVay *et al.*, 2010). Average grain yields recorded from 2005 to 2015 were: 2000–2800 kg/ha for winter wheat and 1500–2600 kg/ha for spring wheat dryland (National Agricultural Statistics Service, 2016). Further information regarding cereal crops management practices can be obtained from McVay *et al.* (2010).

A randomized complete block design (RCBD) with four replicates per treatment was used. Plots were 8 m × 4 m and separated from each other by 1 m buffer zones to avoid cross contamination of treatments. The experiments were performed in fields planted with the wheat midge susceptible spring wheat cultivar "Duclair" (Lanning *et al.*, 2011) in 2016.

Monitoring wheat midge flight behavior with pheromone traps

To determine the best date for biopesticide application, the abundance of *S. mosellana* adult males was monitored using pheromone traps, as per Bruce *et al.* (2007). Delta traps were baited with pheromone lures ([2S, 7S]-nonadiyl dibutyrate) (Great Lakes IPM, Inc., Vestaburg, MI, USA) and attached above the sticky card inserts (Scentry®). They were installed in experimental fields at a rate of one trap per field to monitor *S. mosellana* adult populations. Traps were painted green to decrease nontarget insects catch, placed 20 m inside from the field edges, and the height was adjusted weekly to match the height of the wheat canopy (Thompson & Reddy, 2016). The traps were set on June 10, 2016 at each experimental location and monitored nearly every day from Monday to Friday until the first week of August.

Application of biopesticide products

Commercial formulations of five biopesticide products were used for the study. Mycotrol ESO® and Xpectro OD® were obtained from Lam International (Butte, MT, USA), Scanmask from Sierra Biological Inc. (Pioneer, CA, USA), jasmonic acid from Sigma–Aldrich (St. Louis, MO, USA) and PyGanic EC® 1.4 (pyrethrin) from McLaughlin Gormley King (Minneapolis, MN, USA). Biopesticide product rates were based on the manufacturer's recommendations (Table 1).

All biopesticide products were thoroughly mixed with tap water to obtain the desired concentrations (Table 1). However, for JA and Scanmask preparations, 1 mg JA was dissolved in 1 mL acetone and then dispersed in water to give a solution of 1 mL JA per liter of water (El-Wakeil *et al.*, 2010), while 1% Barricade polymer gel (Barricade

Table 1 Biopesticide products and rate of application in each treatment.

Treatment	Active ingredient	Concentration	Amount of each product
Water (control)	–	–	–
PyGanic EC®	Pyrethrin 1.4% (w/v)	4.167 ml/L	1.69 L/ha
Mycotrol ESO®	*Beauveria bassiana* GHA 10.9%	2.50 ml/L	1.02 L/ha
Xpectro® OD	(2.11×10^{10} viable spores/mL) (w/v) *B. bassiana* GHA (1%–2%) + Pyrethrin (0.75%) (w/v)	2.5 ml/L	1.02 L/ha
Barricade and Scanmask	Barricade polymer gel and *Steinernema feltiae*	Barricade polymer gel 1% + 300 000/m^2 nematode	3×10^9 nematodes/ha
Jasmonic acid	Jasmonic acid (w/v)	1 mg/L	408 mg/ha
Lorsban® (chemical check)	Chlorpyrifos 48% (w/v)	4. 00 mL/L	1.63 L/ha

International, Inc., FL, USA) was added to mixture of Scanmask and tap water (Table 1). This percentage of gel mixed with nematode *S. feltiae* improved control of other foliar insect pests such as: wheat stem sawflies *Cephus cinctus* Norton (Hymenoptera: Cephidae) and flea beetles *Phyllotreta cruciferae* Goeze (Coleoptera, Chrysomelidae) (Antwi & Reddy, 2016; Portman *et al.*, 2016). Two controls were included in the study. A water treatment served as a negative control (control) and chlorpyrifos (Lorsban®) (Dow Agro Science LLC, Indianapolis, IN, USA) served as a chemical check.

All biopesticide products, plus the two controls, were applied on the same date at all field experimental trial locations. However, the East Conrad location was not used for this study in 2016 due to very low incidence of *S. mosellana* adult populations based on a pheromone trap count and the spring wheat was no longer at the susceptible stage (G. Shrestha personal observation). Treatments were applied using a SOLO backpack sprayer (SOLO, Newport News, VA, USA). The sprayer was calibrated to deliver ca. 408 L mixture/ha based on nozzle flow and walking speed. The plots were sprayed on June 29, 2016, when the wheat plants were at a susceptible stage to midges (early boot) and coincided with the peak emergence of wheat midge adults. Scouting was performed to determine wheat midge threshold levels for treatment applications. Spraying was carried out from 7 to 9 pm, when midge adult activity appeared to be high in the fields.

Wheat midge larvae in white traps

White traps were used to assess the wheat midge larval populations in the treatment plots, using a method adapted from El-Wakeil *et al.* (2010). The traps, constructed of plastic dishes (diameter 125 mm; height 65 mm), were placed in the soil at the base of wheat tillers or stands in each plot. Each trap was partly filled with tap water (100–150 mL) and three to four drops of soap detergent. Four days after treatment, two traps were placed in each treatment plot. Samples were collected from traps every week, brought immediately to the laboratory and examined under a binocular or stereomicroscope to determine the presence of *S. mosellana* larvae.

Midge-damaged wheat kernels

Wheat midge-damaged kernels in the biopesticide treatments and the control plots were assessed when the wheat kernels were almost ready to harvest. Ten wheat heads were randomly sampled from each treatment plot, placed in a brown paper bag, and transported immediately to the laboratory. Wheat heads were subsequently threshed individually by hand to determine the total number of wheat kernels and the number of midge-damaged kernels per wheat head. Midge-damaged kernels were characterized based on criteria (such as shriveled, cracked or deformed kernels) reported by Knodel and Ganehiarachchi (2008) and Stougaard *et al.* (2014).

Macroglenes penetrans adult populations

This study examined that biopesticide treatments and controls had a significant impact on *M. penetrans* adult populations, a wheat midge parasitoid which has recently been reported in the Golden Triangle area of Montana (Thompson & Reddy, 2016). A sweep net was used to estimate the adult parasitoid populations. Sweeping was conducted with a standard sweep net, and 20 sweeps were made from each treatment plot. The sampling was performed the day before treatment and, 3, 7, and 15 d after treatment.

Yield and quality of wheat kernels

A Hege 140 plot combine was used to harvest the wheat grains from treatment plots. The precaution was used to avoid the borders and any overlap of treatment effects on wheat yield and quality. Each plot was trimmed from edges, plot length was measured and the wheat grain threshed from the center of each plot. Wheat grains were cleaned with a seed processor (Almaco, Nevada, IA, USA) and weighed on a scale to determine yield. Test weight was measured on a Seedburo test weight scale. The protein and moisture percentages of seed was determined with NIR grain analyzer IM 9500 (Perten Instruments, Springfield, IL, USA).

Statistical analysis

One-way analysis of variance (ANOVA) was performed to examine the effect biopesticide treatments had on wheat midge kernel damage levels, yield and quality (test weight, protein % and moisture %) of spring wheat compared to the water and chlorpyrifos controls at each study location. A normal quantile–quantile plot was performed to confirm normality of data and equality of variance. No transformation of data was required to achieve normal distribution. Tukey's *post hoc* test was used for multiple comparisons among the treatment means. Likewise, for the sweep net data set, one-way ANOVA was performed to examine the effect of treatments on total populations of *M. penetrans* adults at each study location.

The water traps data were found to be nonnormally distributed even after the log transformation, and the nonparametric one-way analysis of variance (Kruskal–Wallis test), was consequently used to examine treatment effects on wheat midge larval populations per sampling time across the treatments on each sampling date or on total midge larval populations. A Mann–Whitney *U*-test was used as a *post hoc* test for multiple comparisons between the means followed by a Bonferroni correction to adjust the probability ($\alpha = 0.01$). The data were analysed using the software statistical package R 2.15.1 (R Development Core Team, 2017).

Results

Wheat midge adult activity based on pheromone trap catch and scouting

At all three field locations, the flight activity of wheat midge adults began at about the same date, June 15–21, in 2016 (Fig. 1). Within 2 weeks, adult activity accelerated sharply at East Valier, increased gradually at North Valier and remained very low at East Conrad (Fig. 1). The economic threshold level of adult activity that warranted the application of pest control measures in relation to susceptible stages of spring wheat occurred at only two (East Valier and North Valier) of the three locations (Fig. 1). During scouting, the numbers of *S. mosellana* adults recorded were >2 flying adults per four–five wheat heads both in East Valier and North Valier locations while nearly zero at the East Conrad location. The total cumulative numbers of *S. mosellana* male adults captured in pheromone traps at East Valier, North Valier, and East Conrad locations were: 2397, 855, and 121, respectively (Fig. 1).

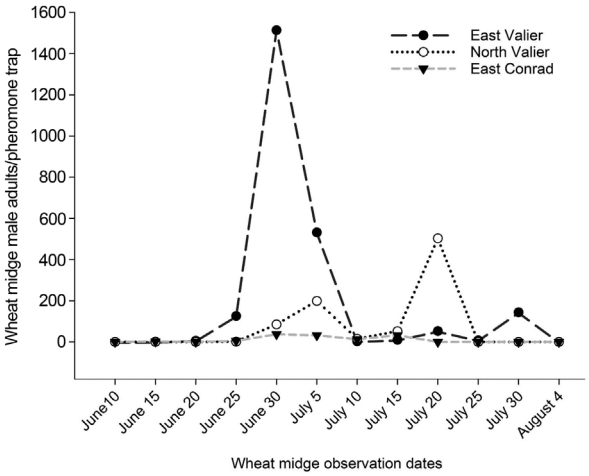

Fig. 1 Adult wheat midge *Sitodiplosis mosellana* populations captured by pheromone traps at the three study locations in Montana.

Larval populations

Regardless of treatments or study locations, no *S. mosellana* larvae were caught in white traps for the first three sampling dates, with the exception of a few larvae (0.25–0.50) in the chlorpyrifos and *S. feltiae* treatments at the East Valier location, but these differences were not significant ($\chi^2 = 9.36$; df = 6; $P > 0.05$, Kruskal–Wallis test) (Table 2). However, on the fourth and fifth sampling dates, wheat midge larvae were observed in all treatment plots in both trial locations. Significant differences in *S. mosellana* larvae were recorded between treatments at the fourth ($\chi^2 = 23.42$; df = 6; $P < 0.001$, Kruskal–Wallis test) and fifth sampling ($\chi^2 = 18.43$; df = 6; $P < 0.01$, Kruskal–Wallis test) dates on the East Valier location. In contrast, significant differences in larvae

Table 2 Effects of biopesticides application on total wheat midge *Sitodiplosis mosellana* larval populations (mean ± SE), recorded in white water traps (two traps per plot) in spring wheat fields at the two study locations of Montana.

Treatment	Wheat midge larvae (Mean ± SE)					
	Jul 7	Jul 14	Jul 21	Jul 28	Aug 5	Total larvae
North Valier						
Water (control)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	5.50 ± 0.65a	1.25 ± 0.48a	6.75 ± 0.85a
Steinernema feltiae	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.41b	0.75 ± 0.25a	1.75 ± 0.48c
Jasmonic acid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.25 ± 0.48bc	0.75 ± 0.25a	3.00 ± 0.70bc
Beauveria bassiana GHA	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.75 ± 0.63a	1.25 ± 0.48a	6.00 ± 0.91ab
B. bassiana GHA + pyrethrin	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.75 ± 0.25b	0.75 ± 0.25a	2.50 ± 0.29c
Pyrethrin	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.50 ± 0.29a	2.25 ± 0.48a	6.75 ± 0.63a
Chlorpyrifos (chemical check)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.50 ± 0.29bc	0.50 ± 0.50a	1.00 ± 0.70c
P value	NS	NS	NS	0.001	NS	0.001
East Valier						
Water (control)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	8.25 ± 0.63a	4.25 ± 0.48a	12.50 ± 1.04a
Steinernema feltiae	0.00 ± 0.00	0.00 ± 0.00	0.50 ± 0.29	2.50 ± 0.28b	1.25 ± 0.62ab	4.25 ± 0.25bc
Jasmonic acid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.50 ± 0.29b	0.75 ± 0.25b	3.25 ± 0.25c
Beauveria bassiana GHA	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	7.50 ± 1.19a	2.50 ± 0.65ab	10.00 ± 0.91ab
B. bassiana GHA + pyrethrin	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.25 ± 0.48ab	2.25 ± 0.48ab	6.50 ± 0.29bc
Pyrethrin	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.75 ± 0.65a	3.50 ± 0.29a	8.25 ± 0.48ab
Chlorpyrifos (chemical check)	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.25	1.75 ± 0.62b	0.75 ± 0.48b	2.75 ± 0.75c
P value	NS	NS	NS	0.001	0.01	0.001

Note: NS indicates the no significant. Mean values within columns bearing the different letters within each location are significantly different (Mann–Whitney *U*-tests followed by Bonferroni correction [$\alpha = 0.01$]).

numbers were found only on the fourth sampling date ($\chi^2 = 22.82$; df = 6; $P < 0.001$, Kruskal–Wallis test) but without effect on the fifth sampling date ($\chi^2 = 8.70$; df = 6; $P > 0.05$, Kruskal–Wallis test) in the North Valier location.

On the fourth sampling date at the East Valier location, among biopesticide treatment plots, significantly fewer *S. mosellana* larvae were recorded for the treatments with *S. feltiae* (2.50 ± 0.28) and JA (2.50 ± 0.29), while the remaining treatments showed no significant differences compared to the water control (8.25 ± 0.63) (Table 2). On the fifth sampling date, however, significantly fewer *S. mosellana* larvae were found only in the JA treatment (0.75 ± 0.25) compared to the water control (4.25 ± 0.48) (Table 2).

Similarly, at the North Valier location, significantly fewer *S. mosellana* larvae were recorded for the treatments with *S. feltiae* (1.00 ± 0.41) and JA (2.25 ± 0.48) compared to the water control (5.50 ± 0.65) on the fourth sampling date (Table 2). Moreover, the combined application of *B. bassiana* and pyrethrin also reduced larval populations, but this effect was not observed when *B. bassiana* or pyrethrin was applied individually (Table 2).

With respect to the total larval populations, the study showed that biopesticide treated plots with JA, *S. feltiae* and combined application of *B. bassiana* and pyrethrin had significantly fewer larvae than the water treatment at both study locations; East Valier ($\chi^2 = 24.75$; df = 6; $P < 0.001$, Kruskal–Wallis test) and North Valier ($\chi^2 = 21.67$; df = 6; $P < 0.001$, Kruskal–Wallis test). Other biopesticide treatments were not significantly different from water control (Table 2).

Midge-damaged wheat kernels

In overall, higher wheat kernel damage inflicted by *S. mosellana* larvae was observed at East Valier in contrast to the North Valier location (Fig. 2). Mean levels of kernel damage in biopesticides/controls treated plots ranged from 20% to 48% for East Valier location and from 11% to 23% for North Valier location (Fig. 2). However, this study showed that biopesticide treatments had significant impact on wheat midge kernels damage at both study locations: East Valier (df = 6,258; $F = 11.7$; $P < 0.001$) and North Valier (df = 6,267; $F = 7.40$; $P < 0.001$). Interestingly, among the biopesticide treatment plots, the significantly lower kernel damage percentages were

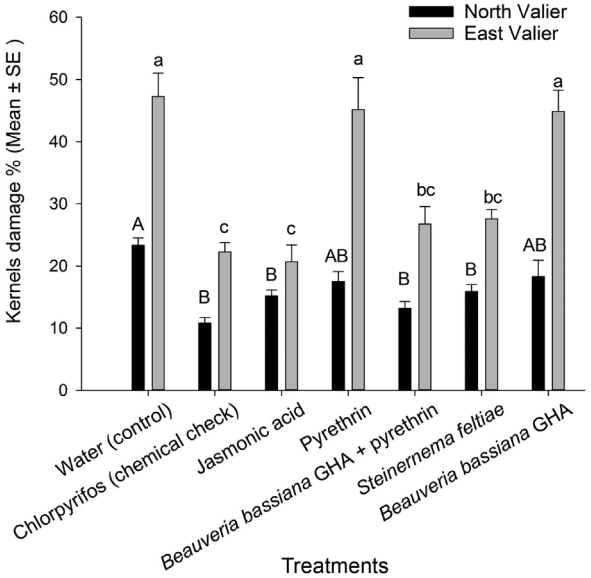

Fig. 2 Effect of biopesticides application on the percentage of damaged kernels inflicted by wheat midge *Sitodiplosis mosellana* larvae in spring wheat (cv. Duclair) at the two study locations in Montana. Bars bearing the same uppercase or lowercase letters are not significantly different (Tukey's test, $P > 0.05$).

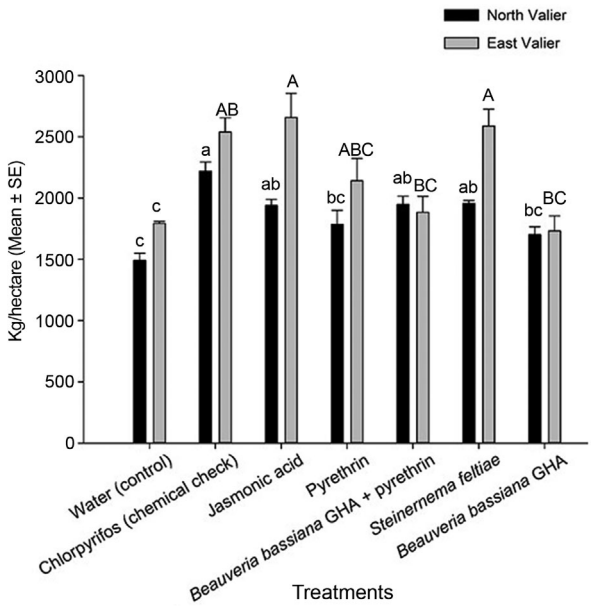

Fig. 3 Effect of biopesticides application on yield of wheat midge *Sitodiplosis mosellana* infested spring wheat (cv. Duclair) at the two study locations in Montana. Bars bearing the same uppercase or lowercase letters are not significantly different (Tukey's test, $P > 0.05$).

observed when wheat plots were treated with JA, *S. feltiae* or combined application of *B. bassiana* and pyrethrin over water control plots at both study locations (Fig. 2). In contrast, the other two biopesticide treatments; pyrethrin and *B. bassiana* did not protect the wheat kernels from wheat midge larval damage and the kernel damage levels were similar to water treated plots (Fig. 2).

Yield

To assess the impact of biopesticide treatments on wheat grain yield, the obtained yield data of each biopesticide treatment plot was compared with yield from the water (control) and chlorpyrifos (chemical check) treatment plots. The results showed that biopesticide treatments had a significant impact on wheat grain yield at both study locations: East Valier (df = 6,21; $F = 8.03$; $P < 0.001$) and North Valier (df = 6,21; $F = 11.27$; $P < 0.001$). Grain yield at the East Valier location was significantly higher for treatments with the *S. feltiae* or JA as compared to the treatment with water control (Fig. 3). Moreover, the yield of these two biopesticide treatments was similar with chlorpyrifos treatment yield, without significant difference (Fig. 3). In contrast, wheat plots treated with *B. bassiana*, pyrethrin or their combined treatments had not produced higher grain yield when compared over water sprayed plots (Fig. 3).

Concerning yield results from North Valier location, similarly the treatments with JA and *S. feltiae* produced the higher grain yields compared to water control treatment (Fig. 3). In addition, higher grain yields were further recorded when *B. bassiana* and pyrethrin applied together in comparison to when they applied individually (Fig. 3).

Quality

Test weight, protein % and moisture % were examined as a part of wheat kernel quality to determine whether the biopesticide treatments had an effect on these parameters compared to the water and chlorpyrifos controls. This study demonstrated that treatments had a significant impact in a test weight at the East Valier location (df = 6,21; $F = 8.96$; $P < 0.001$) while without effect at North Valier (df = 6,21; $F = 2.26$, $P > 0.05$) (Table 3). The biopesticide treatments with JA (796.81 ± 2.25) and *S. feltiae* (790.33 ± 6.66) had significantly higher test weights while the remaining treatments had no significant difference, when compared to the water control (748.81 ± 4.03). Overall, test weight across treatments varied from 731 to 796 (kg/cubic meter) and 762 to 800 (kg/cubic meter) respectively at East Valier and North Valier locations (Table 3).

Table 3 Effect of biopesticides application on some quality parameters of wheat midge *Sitodiplosis mosellana* infested spring wheat (cv. Duclair) at the two study locations of Montana.

Treatment	Quality parameters (Mean ± SE)		
	Test weight (kg/m^3)	Protein %	Moisture %
North Valier			
Water (control)	762.01 ± 14.71a	16.72 ± 0.22a	10.25 ± 0.01a
Steinernema feltiae	799.74 ± 7.34a	17.09 ± 0.28a	10.32 ± 0.03a
Jasmonic acid	794.65 ± 6.01a	17.05 ± 0.26a	10.27 ± 0.04a
Beauveria bassiana GHA	780.57 ± 6.51a	17.09 ± 0.28a	10.26 ± 0.03a
Beauveria bassiana GHA + pyrethrin	791.58 ± 5.35a	16.72 ± 0.32a	10.25 ± 0.04a
Pyrethrin	789.30 ± 6.65a	16.90 ± 0.27a	10.30 ± 0.03a
Chlorpyrifos (chemical check)	789.40 ± 4.54a	16.95 ± 0.16a	10.26 ± 0.03a
East Valier			
Water (control)	748.14 ± 4.03c	16.61 ± 0.13a	10.57 ± 0.02a
Steinernema feltiae	790.33 ± 6.66ab	16.36 ± 0.48a	10.61 ± 0.03a
Jasmonic acid	796.81 ± 2.25ab	16.52 ± 0.37a	10.53 ± 0.04a
Beauveria bassiana GHA	731.13 ± 13.32bc	16.75 ± 0.64a	10.49 ± 0.05a
Beauveria bassiana GHA + pyrethrin	752.01 ± 9.08bc	17.23 ± 0.15a	10.51 ± 0.03a
Pyrethrin	760.90 ± 11.56abc	17.10 ± 0.46a	10.50 ± 0.03a
Chlorpyrifos (chemical check)	794.13 ± 8.71ab	17.45 ± 0.27a	10.50 ± 0.02a

Note: Mean values within columns bearing the same letters within each location are not significantly different (Tukey's test, $P > 0.05$).

There were no significant differences among biopesticide treatments or controls in protein % or moisture % at either study location: East Valier (protein: df = 6,20; $F = 0.52$; $P > 0.05$; moisture: df = 6,20; $F = 0.95$; $P > 0.05$) and North Valier (protein: df = 6,20; $F = 0.74$; $P > 0.05$ and moisture: df = 6,20; $F = 0.60$; $P > 0.05$). The average protein and moisture were 16%–17% and 10%–11%, respectively, across treatments or locations (Table 3).

Macroglenes penetrans adult populations

Regardless of locations, biopesticide or chlorpyrifos treatments had no significant impact on total population of *M. penetrans* adults: East Valier (df = 6,21; $F = 0.54$; $P > 0.05$) and North Valier (df = 6,21; $F = 2.15$; $P > 0.05$) locations. The total mean number of parasitoid adults per treatment plot ranged from 1 to 3 at both study locations (Fig. 4).

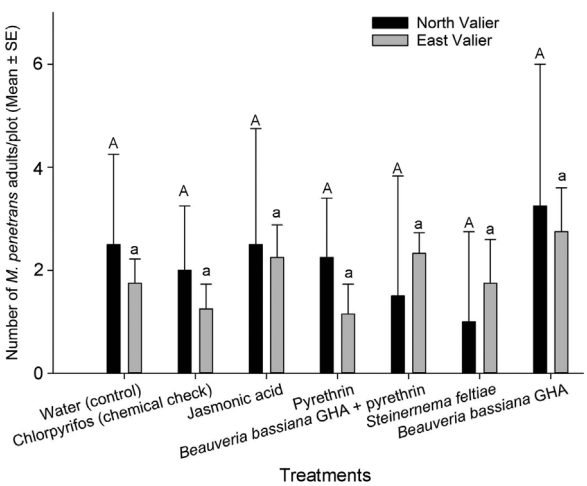

Fig. 4 Effect of biopesticides application on total *Macroglenes penetrans* adult populations. Post application data (3, 7, and 15 d after treatments) were merged together for statistical analysis. Bars bearing the same uppercase or lowercase letters are not significantly different (Tukey's test, $P > 0.05$).

Discussion

The results of this field based study indicated that biopesticide products JA and *S. feltiae* with 1% Barricade polymer gel have the ability to reduce *S. mosellana* larval populations, kernel damage levels and to increase grain yield of spring wheat when compared to the water control treatment at both study locations East Valier and North Valier. Increased test weight in wheat grains were also recorded for the plots treated with JA and *S. feltiae* at the East Valier location but not at the North Valier location, when compared over water control treatment. The JA and *S. feltiae* results were comparable in efficacy to the

standard pesticide, chlorpyrifos. This study also suggested that *B. bassiana* and pyrethrin may work synergistically as exemplified by lower total larval population and higher kernel protection when they were both used together, but without effects when applied individually. This study did not conclude the effect of any treatments including chlorpyrifos on wheat midge parasitoid adults *M. penetrans* population levels.

Various methods have been employed to estimate the larval populations of *S. mosellana* in spring or winter wheat crop fields when examining the efficacy of chemical insecticides, biopesticides, or pest pressure in the following year (Doane *et al.*, 1987; El-Wakeil *et al.*, 2010; Gaafar *et al.*, 2011; Chavalle *et al.*, 2014). For example, Chavalle *et al.* (2014) determined the impact of several chemical insecticides on the larval populations of *S. mosellana* based on the dissection of wheat heads followed by counting the larvae before they dropped from wheat heads onto the soil. Another method used was to collect soil samples from *S. mosellana*-infested fields before (spring) or after (fall) crop harvest and wash samples to determine the number of larvae in fields to better predict pest pressure following year (Doane *et al.*, 1987). In addition, white traps have also been effectively used to estimate larval populations of *S. mosellana* while ascertaining the efficacy of chemical or biopesticide products (El-Wakeil *et al.*, 2010; Gaafar *et al.*, 2011; El-Wakeil & Volkmar, 2012). White traps can be installed at the soil surface near the base of wheat tillers or stands to catch larvae migrating from wheat heads to the soil at the end of the growing season.

Our data support previous studies that have found white traps could be used to estimate larval populations in wheat midge-infested fields. As predicted, *S. mosellana* larvae were recorded mostly at the fourth and fifth sampling dates regardless of locations or treatments since rainfall occurred 2–3 d prior to both samplings (NRCS, 2016) and the precipitation is known to trigger larvae to fall onto the soil from wheat heads at the end of cropping season (El-Wakeil *et al.*, 2010; Thompson & Reddy, 2016).

In our study, comparatively higher *S. mosellana* larval populations were recorded in white traps at the East Valier than at the North Valier. This observation was supported by the pheromone trap data, with sixteen hundred male adults recorded at the East Valier, but only two hundred at the North Valier during the most susceptible stages of wheat. The treatments clearly showed the significant impacts on total larval populations of *S. mosellana* on spring wheat and the significantly fewer larvae were found for plots treated with JA, *S. feltiae* and combined formulation of *B. bassiana* and pyrethrin when compared to the water treatment at both study locations. Our results for JA resemble the findings of El-Wakeil *et al.* (2010), who reported significantly lower numbers of *S. mosellana* larvae in winter wheat fields sprayed with JA compared to the untreated plot. No previous reports are available on the impact to *S. mosellana* larval populations of the other biopesticides examined in our trial. The lack of individual effects of *B. bassiana* or pyrethrin applications on larval populations or other studied parameters could be due to mode of action and presumably with these biopesticides inability to reduce the daily fecundity of wheat midge adults. Furthermore, abiotic factors (e.g., sunlight, temperature) may have contributed to their lack of effect, since the previous studies have shown that fungus or pyrethrin half-life can decline rapidly (2 h to 3 d) in outdoor environments (Inglis *et al.*, 1995; Angioni *et al.*, 2005; Jaronski, 2010).

Determining the ability of chemical insecticide or biopesticide products to protect wheat kernels from wheat midge larval damage is fundamental for determining potential control options (Elliott, 1988; El-Wakeil *et al.*, 2010; Chavalle *et al.*, 2014). Several studies have examined the ability of various chemical insecticide or biopesticide products to protect spring or winter wheat kernels from *S. mosellana* larval damage in the Europe and North America (Elliott, 1988; El-Wakeil *et al.*, 2010; El-Wakeil *et al.*, 2013; Chavalle *et al.*, 2014). For example, Elliott (1988) reported that chlorpyrifos and endosulfan protected 60%–75% of wheat kernels from *S. mosellana* larval damage. This finding is similar to our chlorpyrifos treatment results from both study locations. Chavalle *et al.* (2014) and El-Wakeil *et al.* (2013) also indicated higher wheat kernel protection from chlorpyrifos but did not quantify the level of kernel protection in their studies.

In a previous study, El-Wakeil *et al.* (2010) demonstrated that exogenous application of JA on winter wheat crop provided more than 75% protection to kernels compared to the water control treatment. Our results are in line with this finding, with more than 80% of the kernels protected from *S. mosellana* larval damage by the application of JA at both of our study locations. Although the possible mechanisms that could have impacted such enhanced protection from wheat midge larvae are fairly unknown, it was likely that the application of JA induced spring wheat plants to release volatilies or produce secondary metabolites that acted as a repellent to *S. mosellana* (Senthil-Nathan *et al.*, 2009; El-Wakeil *et al.*, 2010). Furthermore, when wheat midges were exposed to JA treated wheat plants, adult fecundity and larval feeding may have been further reduced, since it has been demonstrated with other insect pest species (Omer *et al.*, 2000; Cooper & Goggin, 2005; Senthil-Nathan *et al.*, 2009).

This study found that the insect pathogenic nematode (IPN) *S. feltiae* with 1% Barricade polymer gel effectively protected wheat kernels from *S. mosellana* larval feeding. IPNs have been most successfully used against different soil inhabiting insect pest species (Kaya & Gaugler, 1993). However, with the development of a suitable adjuvant (Barricade polymer gel) that can protect infective juveniles from ultraviolet rays or extend their survival on aboveground foliage (Antwi & Reddy, 2016; Shapiro-Ilan *et al.*, 2016); IPNs can now be considered for use against aboveground crop insect pests as well (Antwi & Reddy, 2016; Portman *et al.*, 2016; Shapiro-Ilan *et al.*, 2016). Among the several nematode species, *S. feltiae* has been recognized to have many advantages including a broad host range, high virulence, adaptability to a wide range of temperatures (10–30 °C) and an ability to seek their target host (Gaugler *et al.*, 1989). Furthermore, in Montana, *S. feltiae* has recently been found to be the most effective nematode species against a wide range of important foliar insect pests including wheat stem sawflies and flea beetles when applied in conjunction with 1% Barricade under field conditions (Antwi & Reddy, 2016; Portman *et al.*, 2016). Thus, our findings and those of these previous field studies indicate that *S. feltiae* has the potential to be used against several foliar insect pests in Montana and can be combined with many IPM programs. In contrast, this study suggests that separate application of *B. bassiana* or pyrethrin will be ineffective in reducing kernel damage levels caused by *S. mosellana* larvae. This is corroborated by the fact that no information has been found in the literature on the successful use of *B. bassiana* or pyrethrin in relation to *S. mosellana* management. Although both biopesticides can be disregarded for use in wheat midge management when applied separately, the significantly higher kernel protection provided by their combined application at the North Valier location suggests that they might have a place in IPM programs.

In addition to lower levels of wheat kernel damage, higher grain yields were found when wheat plots were treated with JA and *S. feltiae* compared over the water control treatment, at both study locations. A similar relationship between kernel damage and yield data has been reported in several previous studies (Elliott, 1988; El-Wakeil *et al.*, 2010; El-Wakeil *et al.*, 2013; Chavalle *et al.*, 2014).

With respect to the parasitoid study, our results could not conclude directly that treatments have any negative impacts on *M. penetrans* adults' population. This could be due to the low parasitoid population levels recorded irrespective of treatments/locations or because of parasitoids mobile nature that relatively small research plots could be insufficient on determining these impacts. In contrast, there was also the possibility that parasitoids could be unaffected by chlorpyrifos or biopesticide treatments since the emergence of *M. penetrans* usually occurred 10 d after the *S. mosellana* with the highest peak emergence (Thompson & Reddy, 2016). Based on this finding and the nature of wheat midge and parasitoid emergence patterns, it warrants further laboratory and long-term field investigations that determine whether the direct and indirect exposure to synthetic insecticide or biopesticide have any negative impact on the parasitoid biology and population development.

In summary, our results indicate that the biopesticides JA and *S. feltiae* with 1% Barricade polymer gel would be suitable for the management of wheat midge in spring wheat in Montana. However, further cost/benefit analysis study is needed to determine if the application of these biopesticide products is economical and sustainable for spring wheat growers in Montana.

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Disclosure

The authors disclose no potential conflicts of interest associated with this manuscript.

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