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ORIGINAL PAPER

Impact of granular carriers to improve the efficacy of entomopathogenic fungi against wireworms in spring wheat

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Abstract

Wireworms are a major concern for wheat growers and several other crops around the globe. Environmentally friendly management strategies are needed because the present conventional chemical seed treatments can be ineffective and pose environmental risks. While biological control of wireworms in a general sense has not been practical, use of entomopathogenic fungi (EPF) is one environmentally friendly solution for this problem. In 2017, granular formulations of three EPFs, on polenta and millet spent substrate carriers, were applied in furrow at planting, at two rates, against a water control and imidacloprid seed treatment in spring wheat in Montana, USA. The selected EPFs were *Beauveria bassiana* GHA, *Metarhizium robertsii* DWR356, *M. robertsii* DWR2009, applied as granular formulations at 11 kg ha^{-1} or 22 kg ha^{-1}. In 2017, at Valier, DWR356, DWR2009 on millet carrier at 22.4 kg ha^{-1} provided greater yield, but all the treatments at lower rate were still cost-effective. In 2018, *B. bassiana* GHA and *M. robertsii* DWR2009 were retested along with *B. bassiana* ERL836 and *M. brunneum* F52. Millet carrier alone, GHA and ERL836 on millet carrier obtained cost-effective results at irrigated and non-irrigated sites in 2018. However, these were less cost-effective than imidacloprid as a seed treatment. The overall cost–benefit ratio of using EPF granules was higher in both the years compared to control. Millet on which the fungi were grown worked better than the other carriers. Further evaluation of the effect of the carrier while applying EPFs in furrow as granules is required.

Keywords *Beauveria bassiana* · *Metarhizium robertsii* DWR356 · *Metarhizium robertsii* DWR2009 · Millet carrier · Biological control · Bioinsecticides

Key message

- Entomopathogenic fungi applied as granules on nutritive carries were tested to manage wireworms in wheat.
- *Metarhizium robertsii* DWR 2009, *Beauveria bassiana* GHA and ERL836 on millet carrier were effective.
- Cost–benefit ratios for fungal granules were estimated in comparison to untreated control and imidacloprid.
- *Metarhizium robertsii* DWR 2009 granules on millet carrier at 11 kg ha^{-1} found to be cost-effective.
- Cost–benefit ratio was higher for entomopathogenic fungi compared to the untreated control.

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Introduction

Wireworms, the larval stages of click beetles (Coleoptera: Elateridae), are serious soil-dwelling pests of small grains and several other plant families including vegetables and fruits globally. About 10,000 described species in 400 genera are known today globally, and about 885 species within 60 genera are identified from North America alone (Morales-Rodriguez et al. 2014). Due to their cryptic behavior (variation in bionomics, lesser known vertical and horizontal movement habits), assessment and management of wireworms is complicated (Jackson et al. 2000; Vernon 2010; Esser et al. 2015; Traugott et al. 2015). Lately, no-till farming practices and reduced use of chemical pesticides are contributing to the increasing population of this pest throughout the world including the Northern Great Plains of the USA. (Comstock and Slingerland 1891; Morales-Rodriguez et al. 2014; Jedlička and Frouz 2007). Loss due to wireworms depends on the infestation level; in general, in the US Pacific Northwest, up to 70% yield losses have occurred on individual farms, while in North America an overall crop loss of about 25% has been reported for different crops (Jansson and Seal 1994; Staudacher et al. 2013; Morales-Rodriguez et al. 2014; Higginbotham et al. 2014). Management strategies are mainly concentrated on crop protection (Vernon et al. 2009; Vernon and van Herk 2012; van Herk and Vernon 2013). Use of neonicotinoids (imidacloprid, clothianidin and thiamethoxam) and the pyrethroid tefluthrin in cereal crops is reported to cause prolonged wireworm intoxication, whereas fipronil can provide significant protection to non-grain crops (Vernon et al. 2009, 2013) and is recently reported to provide effective management for wireworms in spring wheat as well (van Herk et al. 2018). Cultural control is another avenue that is being explored to manage wireworms, through applying soil amendments, employing different planting and harvest dates and trap cropping (Andrews et al. 2008; Landl and Glauninger 2011; Esser et al. 2015; Adhikari and Reddy 2017; Sharma et al. 2018).

Bacteria, fungi and nematodes have been repeatedly isolated from field populations of wireworms (Leclerque et al. 2013; Kleespies et al. 2013; Parker and Howard 2001). *Beauveria bassiana s.l.* (Bals.-Criv.) Vuill. (Cordycipitaceae), *Metarhizium* Sorokin spp. (Clavicipitaceae), *Cordyceps* spp. (Cordycipitaceae), *Tolypocladium* spp. W. Gams (Ophiocordycipitaceae) and *Zoophthora* spp. A. Batko (Entomophthoraceae) (Jansson and Seal 1994; Keller 1994; Mietkiewski and Balazy 2003; Kabaluk et al. 2013) have been reported from natural populations of wireworms. Entomopathogenic fungi (EPF) are known as natural antagonists of wireworms (Ritter and Richter 2013) and although one species, *Metarhizium anisopliae s.l.* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae), was first used in 1932 (Thomas 1932), determined efforts to use isolates or strains of EPFs against wireworms are only recent. *Beauveria* and *Metarhizium* have been commercialized as mycoinsecticides in the USA and elsewhere for use against a variety of pest insects. A survey in 2007 identified many commercial products based on both fungi (Faria and Wraight 2007), whereas a more recent update reported additional commercial products for just *Beauveria* (Mascarin and Jaronski 2017). The effect of any EPFs can be different on adults (Keller 1994; Ester and Huiting 2007) than on larvae (Ericsson et al. 2007; Kabaluk et al. 2007; Reddy et al. 2014; Antwi et al. 2018). Specific strains of these fungi, *Metarhizium* in particular, have been tested against wireworms in the field (Kabaluk et al. 2005, 2007; Ester and Huiting 2007; Reddy et al. 2014) as well as in the laboratory (Kabaluk et al. 2005; Ansari et al. 2009), alone (Kabaluk et al. 2007) and in combinations with other biopesticides (biologicals and entomopathogenic nematodes) and seed treatments (Kabaluk and Ericsson 2007; Ericsson et al. 2007; Reddy et al. 2014; Antwi et al. 2018). Application of EPFs as a soil drench and as granular formulations in soil has also been studied for the management of wireworms under field conditions (Ester and Huiting 2007; Reddy et al. 2014). In Austria and Germany, a *Metarhizium* in the form of an alginate-based attractive granule, and a *Beauveria* dispersible oil formulation have received temporary, emergency registration for use against wireworms (Biocare 2018).

For management of wireworms in spring wheat, *M. brunneum* F52, *B. bassiana* GHA and *M. robertsii* DWR 346 were found effective when applied as granules in furrow or as soil drenches compared to seed-coating against *Limonius californicus* (Mannerheim) and *Hypnoidus bicolor* (Eschscholtz) (Coleoptera: Elateridae) (Reddy et al. 2014). A 2-year study at two Montana locations in 2015–2016 evaluated nine biopesticides and their impact on spring wheat stand protection, populations of wireworms and yield. In 2015, at one location applications of *B. bassiana* and a combination of *M. brunneum* F52 with imidacloprid and at another location a combination of *B. bassiana* with *M. brunneum* F52 provided greater protection to plants. In addition, combination of imidacloprid with *M. brunneum* F52 and *B. bassiana* with azadirachtin produced significantly greater yields (Antwi et al. 2018).

In 2017 and 2018, we evaluated the efficacy of several EPFs at different rates and on different nutritive, granular carriers in the spring wheat cultivar Duclair using an at-planting, 'in-furrow' application. Our objective was to evaluate the effectiveness of selected EPFs at different rates and on different carriers for management of wireworms in spring wheat in Montana. Polenta and couscous represent small nutritive spheres coated with conidia that can be applied with commercial planters because of their size and shape, and both support fungus germination, outgrowth and sporulation in soil, the key aspect of using such carriers. With a nutritive carrier, the number of conidia applied to a crop multiplies as the fungus germinates colonizes the granule and then resporulates, creating a focus of millions of new conidia (Jaronski 2007, 2010). A very heterogeneous distribution of conidia results with a soil drench and many conidia are sequestered in soil pores. An insect, moving through treated soil, has to slowly acquire enough conidia on its cuticle to receive an infectious dose. In addition, conidia percolate poorly through most soils. In contrast, an insect has only to encounter such a focus around a sporulated granule to acquire more than a lethal dose of spores. There is also some evidence that such granules of actively metabolizing fungus may be attractive to wireworm via release of CO_2 (Vemmer et al. 2016). Millet is a slightly different story. Millet as a substrate for production of *Beauveria* and *Metarhizium*, and its subsequent use as a granular formulation for use against soil stages of thrips, has been commercialized in South Korea. There is a commercial product in the USA, Met52G (Novozymes Biologicals, Salem VA) based on spent broken rice substrate colonized with *M. anisopliae* F52 and targeted for use in greenhouse potting mixes, but the size and shape of the granules preclude use of at-planting granular pesticide applicators. If efficacious against wireworm millet colonized by an EPF opens another avenue for exploiting these fungi against wireworm.

In 2017, the selected EPFs were the commercially available *B. bassiana* GHA and several other experimental fungi, *M. robertsii* DWR356 (ARSEF9617) and *M. robertsii* DWR2009 (ARSEF10343) being developed by USDA-ARS as potential mycoinsecticides for other insects. In 2018, *B. bassiana* ERL836 was included among the treatments on couscous and millet. In the present study, we are comparing the efficacy and cost efficiency of different carriers (millet, couscous and polenta) to analyze the possibility of using fungal granules and facilitate the dissemination of information about fungal granules on various carriers to manage wireworms and other soil-dwelling insect pests.

Materials and methods

Study sites

In 2017, fields previously determined to be heavily infested with wireworms were selected in Golden Triangle Area of north central Montana. Ledger (N48°16.334′ W111°53.175′) and Valier (N48°18.454′ W111°55.524′) were irrigated sites, with moderate to high wireworm pressure. In 2018, four sites were selected. Two irrigated and two non-irrigated sites were selected in Pondera and Teton counties. The two irrigated sites were at Ledger (N48°16.334′ W111°53.175′) and Choteau (N47°90.238′ W112°23.802′), and two non-irrigated sites were at Pendroy (N48°56.009′ W111°40.565′; hereafter named as Pendroy-1) and (N48°04.206′ W112°20.099′; hereafter named as Pendroy-2). The selected sites had a history of wireworms with moderate to high pressure. To determine the wireworm pressure, stocking traps were used to collect wireworms. (The elaborate method is explained under section 'Sampling for wireworm density'). Four–five wireworms per trap were considered as high wireworm pressure, and two–three wireworms per trap were considered as moderate wireworm pressure). In general, the soils were Scobey soil series (NRCS 1999). At the 2017 Ledger location, soils were classified as fine-loamy, mixed, Aridic Argiboroll, Assiniboine fine sandy loam. Soil at the Valier location was classified as fine, montmorillonitic, frigid Aridic Ustochrepts, Marias-Nunemaker complex. In 2018, the two non-irrigated sites, Pendroy-1 had Rothiemay-Niart clay loams soil, with 0–4% slopes and Pendroy-2 had Niart-Crago gravelly loams soil with 0–4% slopes. The two irrigated sites, Ledger soils had Fine-loamy and Choteau site had Niart-Crago gravelly loams soil with 0–4% slopes (NRCS 1999).

Experimental design

The experiments were established in a randomized complete block design in each field. Altogether there were 48 plots at each location, including four replicates of 12 treatments at each site (Table 1). An individual plot size was 3.6 m × 1.2 m with a buffer zone of 0.6 m between the plots.

Test materials

Of the selected EPFs in these trials, *B. bassiana* strain GHA is commercially available. The other EPFs used in this study in 2017 were experimental: *M. robertsii* DWR356 (ARSEF9617), *M. robertsii* DWR356 (ARSEF9621) and *M. robertsii* DWR2009 (ARSEF10343). In 2018, *B. bassiana* ERL836 and the commercial *M. brunneum* F52 were included and DWR356 was omitted [hereafter only strain names are used to identify the fungus, *M. robertsii* DWR356– DWR356; *M. robertsii* DWR2009–DWR2009; *B. bassiana* ERL836–ERL836; *M. brunneum* F52–F52; *B. bassiana* strain GHA–GHA]. The fungi were applied as granules on maize (corn) polenta, culinary couscous and/or on fungus-colonized millet.

The fungus formulations were prepared by USDA-ARS, Sidney MT. The *B. bassiana* strain GHA was originally provided by Certis USA LLC., Columbia MD as dry conidia. Conidial viability was 98%, based on conidial germination on potato dextrose yeast extract agar after incubation for 18 h at 27 °C. Cultures of DWR356 and DWR2009 were originally obtained from D. W. Roberts, Utah State University, while ERL836 was provided by B.

Table 1 Materials, rates and methods of application for treatments applied in study of wireworms control at Valier and Ledger, in 2017 and at Pendroy-1, Pendroy-2, Choteau and Ledger in 2018

Treatments 2017	Rate	Application method
Control (water)		
Gaucho® (Imidacloprid)	0.157 ml l^{-1} (120 ml kg^{-1})	Seed treatment
Beauveria bassiana GHA granules (BB)	11.21 and 22.42 kg ha^{-1}	In furrow
Metarhizium robertsii DWR356 granules (MR356)	11.21 and 22.42 kg ha^{-1}	In furrow
Metarhizium robertsii DWR356 on millet (MRM356)	11.21 and 22.42 kg ha^{-1}	In furrow
Metarhizium robertsii DWR2009 Granules (MR2009)	11.21 and 22.42 kg ha^{-1}	In furrow
Metarhizium robertsii DWR2009 on millet (MRM2009)	11.21 and 22.42 kg ha^{-1}	In furrow
Treatments 2018	Rate	Application method
Control (water)		
Gaucho® (Imidacloprid)	0.157 ml l^{-1} (120 ml kg^{-1})	
Millet (M)	11.21 kg ha^{-1}	In furrow
Couscous (C)	11.21 kg ha^{-1}	In furrow
Beauveria bassiana GHA millet (BBM)	11.21 kg ha^{-1}	In furrow
Beauveria bassiana GHA couscous (BBC)	11.21 kg ha^{-1}	In furrow
Beauveria bassiana ERL836 millet (ERLM)	11.21 kg ha^{-1}	In furrow
Beauveria bassiana ERL836 couscous (ERLC)	11.21 kg ha^{-1}	In furrow
Metarhizium brunneum F52 millet (F52M)	11.21 kg ha^{-1}	In furrow
Metarhizium brunneum F52 couscous (F52C)	11.21 kg ha^{-1}	In furrow
Metarhizium robertsii DWR2009 millet (MRM)	11.21 kg ha^{-1}	In furrow
Metarhizium robertsii DWR2009 couscous (MRC)	11.21 kg ha^{-1}	In furrow

Parker, University of Vermont Entomological Research Laboratory, Burlington, VT. In 2018, the commercial *M. brunneum* F52 was substituted for DWR356. This strain, F52, was derived from a culture supplied to USDA by Taensa Corp. in 2002 and had been maintained in 30% glycerol at − 80 °C. The three *Metarhizium* isolates and ERL836 had been previously passaged twice through grasshoppers, *Melanoplus sanguinipes* (Fabricius) (Orthoptera: Acrididae), to restore possible lost infectivity due to successive in vitro culturing, and the resulting conidia stored in 30% glycerol at − 80 °C. Conidia of all but GHA were produced using biphasic liquid–solid fermentation methods as described in Jaronski and Jackson (2012), and the resulting conidia that were used in the trial represented the fourth in vitro passage from an insect host, ensuring good general infectivity. Conidial viability, determined as described earlier, was > 90% for all fungi. Conidial powders were stored dry [water activity, a_w, of ~ 0.25, measured with an Aqualab® water activity meter (Decagon Devices, Pullman WA)] at 4–5 °C until formulation and use.

The granular carriers for the fungi consisted of either commercial polenta, a coarse corn meal (Bob's Red Mill, Milwaukee OR) or culinary couscous (Bob's Red Mill, Milwaukee OR). Either carrier was first coated with a solution of 1% aqueous carboxymethylcellulose to a final 8% v/w. The binder was applied as a fine spray using a DeVilbiss 163 atomizer onto 2.5 kg granules in a rotating concrete mixer. Conidia of each fungus were then slowly applied in sufficient quantity to the rotating carrier to achieve a target titer of 2×10^8 viable conidia g^{-1} of carrier, based on the conidial titer and viability of each fungus. Treated granules were air-dried on flat trays in a laminar air flow for 24 h, to a water activity end point of < 0.3 a_w units, as measured with the Aqualab® meter, then packaged and refrigerated until use.

The millet-based granular was prepared by growing the respective fungi on culinary hulled millet (*Panicum miliaceum* L.) solid substrate that was prepared following Kim et al. (2011). Each kg of millet (Bob's Red Mill, Milwaukee OR) was mixed with 500 ml 0.16% citric acid solution within a plastic bag and cooked in a hot water bath at 90–95 °C for 1 h, after which it was transferred to a vented mushroom spawn bag (Unicorn Industries, Commerce TX), and autoclaved 55 min at standard temperature and pressure. After the millet substrate had cooled, it was inoculated with liquid blastospore cultures of the respective fungi (inoculum volume being 10% of original dry substrate weight). The *Beauveria* inoculum was produced in a glucose–yeast extract–potassium nitrate medium (20–30 g glucose, 15–20 g yeast extract, 4 g KNO_3 per liter), while the *Metarhizium* strains were produced in a medium consisting of 30 g glucose, 20 g yeast extract, 8.5 g corn steep liquor solids and 4 ml monosorbitan oleate (Tween® 80) per liter. The liquid cultures, inoculated with conidia from agar media, were incubated on a rotary shaker at 280 rpm and 25° ± 1 °C for 3–4 days and then were used to inoculate the millet. The sealed bags of inoculated millet were incubated for 2 weeks with intermittent mixing and then transferred to heavy weight, kraft paper bags (Jaronski and Jackson 2012) for drying. The cultures were dried at 15–20% relative humidity and 20–24 °C for 5 days at which time moisture content was < 0.3 a_w. Excess conidia were removed from the millet by agitation in a vibratory sieve shaker through successive 20- and 100-mesh sieves. Final moisture of the dried millet was 0.30–0.35 a_w.

As a quality control check both types of granules were sprinkled onto water agar after formulation and incubated at 24–25 °C. All granules showed fungal outgrowth and robust sporulation characteristic of the appropriate fungus after 5–6 days. The millet granules were stored refrigerated until used.

Application of materials

Before seeding, the herbicide glyphosate (RT3®, Monsanto Company, St. Louis, MO), was applied at the rate of 2.5 l/ha for pre-emergent weed control, following regional farming practice. Fertilizer (N, P and K) was also applied at a ratio of 224.2, 0 and 22.4 kg/ha in a band 2–3 cm from the seed through Morris® double-shoot, no-till openers during planting. The materials were applied at 12 kg ha^{-1} and 25 kg ha^{-1}. Spring wheat cultivar, Duclair, was seeded at the rate of (~ 230 seeds/m^2). In 2017, all EPF treatments were applied in furrow with the seed at the rate of 5 g per plot (11.2 kg ha^{-1}) and 10 g per plot (22.4 kg ha^{-1}); (Table 1). Imidacloprid (Gaucho® 600, Bayer Crop Science), a seed treatment commonly used by growers, was used as a chemical control (120 ml kg^{-1}). The Valier site was seeded on May 4, 2017, and the Ledger site was seeded on May 10, 2017.

In 2018, all EPF treatments were applied with in furrow at planting at the rate of 5 g per plot (11.2 kg ha^{-1}). The fields were seeded at Pendroy on May 14 and May 16, 2018, Choteau and Valier on May 24, 2018, and Ledger on May 29, 2018. The experimental plots received 5 cm of water via overhead irrigation once a week. The first irrigation was done within 30 days of planting in irrigated sites. Non-irrigated sites only received natural rain. Harvesting was done in August 2017 and September–October 2018. Soil moisture and temperature in the top 12 cm of the soil profile were recorded during every plant stand count (once in 2 weeks). Both parameters were recorded from furrows with a soil moisture meter (Spectrum Technologies Inc., Illinois, USA) and soil thermometer (Taylor, Illinois, USA).

Sampling for plant damage

To determine the damage to wheat seedlings due to wireworm populations, the numbers of seedlings in each plot were counted randomly using 1 m line-intercept method. From an individual plot, two counts were taken, one each from the two middle rows ($n = 2$; 1 m apart). The first count was taken 2 weeks after planting, after the seeds had germinated. The starting and ending points of the sample areas were labeled with wooden stakes, so the same seedlings could be recounted every time. The subsequent counts were taken at the interval of 2 weeks at both sites. At the time of harvesting, plant heights were also measured using a meter ruler (Washington, USA), selecting the same marked plants.

Sampling for wireworm density

To determine the density of wireworm larvae, traps were established in each plot, following soil sampling bait trap method of Reddy et al. (2014). In summary, stocking traps were prepared with mixture of wheat and barley seeds. The traps were soaked in water for 24 h to make the seeds sprout. Wireworms are attracted to the germinating seeds due to the release of CO_2 by the germinating seeds. The traps were buried in 8–15-cm-deep holes and were covered with soil and covered with black plastic to provide an environment amenable for wireworms. Traps were collected at 2 and 4 weeks, separately from each plot. The traps were established twice during the growing season. Traps with wireworms were brought to the Western Triangle Agricultural Research Center (WTARC), Conrad, Montana, and processed in Berlese Funnels (Bioquip products, California, USA, with wooden stands for the Berlese Funnels built at WTARC), and wireworms were separately collected from each plot. Later, they were identified using the Etzler (2013) key for wireworm identification.

Post-harvest data collection

Before harvest, each plot was measured and its area calculated for accurate yield data. Grain harvested from each plot was brought to the WTARC facility and was cleaned by using seed cleaning machine (Almaco, Allan Machine Company, Iowa, USA). Later, plot weight and test weight was measured by using a laboratory balance (Ohaus, Adventure™). Wheat samples were processed through a grain analyzer (Perten Instruments IM9500; Hägersten, Sweden) for grain moisture and protein. About 300 g of sample for each plot was processed to obtain protein and moisture content. Plot weight and moisture were used to calculate yield.

Cost–benefit analysis (CB ratio)

Cost of the material was calculated per treatment and then converted into per hectare. Cost included cost of seeds (certified Duclair), fertilizers (potash, urea and monoammonium phosphate), herbicides (RT3® glyphosate), conventional insecticide (imidacloprid), cost of irrigation and cost of EPFs. Cost of EPF granules was estimated at \$11 kg^{-1} based on the commercial experience of the second author. In non-irrigated fields, cost of irrigation was not included. For benefit calculation, present market rate of Duclair wheat @ \$5.50 per bushel was considered for January 2019 price for spring wheat with 14% protein.

Statistical analysis

A mixed model analysis was done. Data were pooled for each replicate, and treatments were considered as fixed, while block was considered as random effect. Normality of data was tested with univariate procedure within PROC MIXED. The effect of treatments on the number of plants, seed test weight, seed yield, seed protein and number of wireworms was analyzed through a two-way ANOVA by using PROC MIXED procedure in SAS 9.4 (PROC MIXED, SAS Institute 2018). These estimates of least-square means (LSM) and differences of least-square means were evaluated (Type 3 test of fixed effects *F* test). Multiple comparison among the treatments was made using Fisher's least significant test (LSD) at $\alpha = 0.05$ by using the standard error generated in ANOVA. The mean and variance of wireworm populations was calculated in Microsoft Excel 2013.

Results

Three wireworm species, *L. californicus*, *H. bicolor* and *Aeolus mellillus* Say, were collected from the study sites in both years. In 2017 and 2018, the most common species was *L. californicus* (~55% of total population), followed by *H. bicolor* (~25% of total population) and *A. mellillus* (~20% of total population). In the selected fields, at irrigated sites, the major population comprised *L. californicus* (60%) and *H. bicolor* (40%), whereas at non-irrigated sites the population was of *L. californicus* (80%) and *H. bicolor* (20%). At all the sites, population of *A. mellillus* was about 1% (Table 5).

In May–June 2017, the Valier site had a higher mean soil moisture (volumetric water content of 70 ± 3%) and temperature (18 ± 2 °C) than the Ledger site (53 ± 3%; 14 ± 2 °C), but in July–August 2017 moisture did not differ between the two sites (range of 35 ± 5%). Valier still had higher soil temperature (45 ± 2 °C) compared to Ledger (23 ± 2 °C). In 2018, at non-irrigated sites, soil moisture decreased from May to August (from 35 ± 3 to 15 ± 3%) and soil temperature decreased (from 42 ± 2 to 30 ± 2 °C). Of the two irrigated sites, the Ledger site had moisture and temperature remain same throughout May–August (43 ± 3% and 20 ± 3 °C). At Choteau, moisture increased (12 ± 2 to 17 ± 2%) and temperature decreased from 40 ± 2 to 25 ± 2 °C.

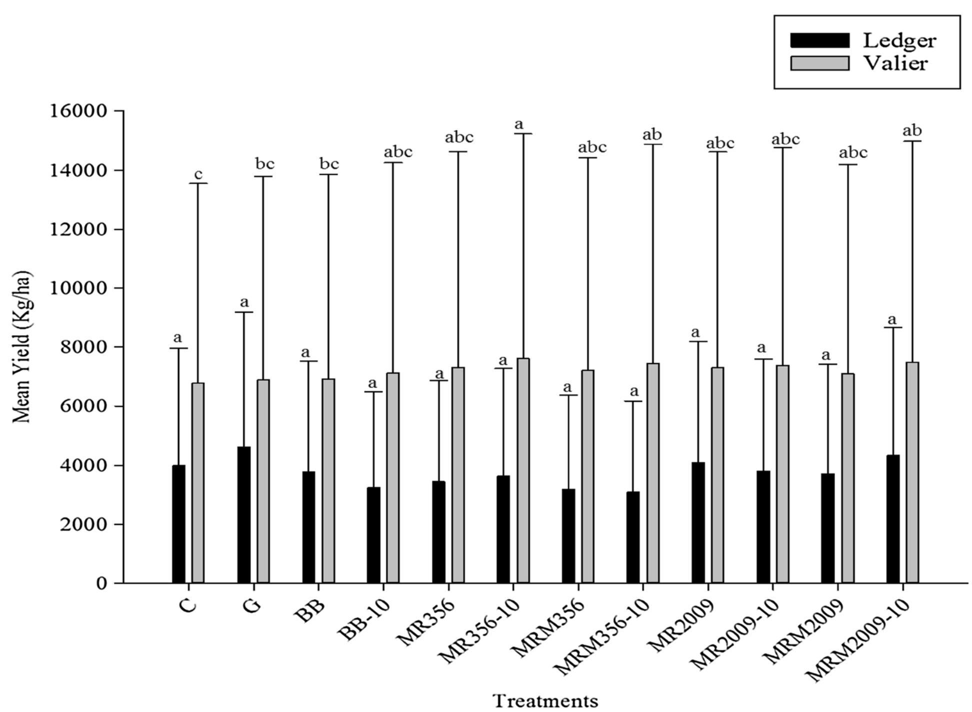

Fig. 1 Mean yield (mean yield + SE) ($kg\ ha^{-1}$) of spring wheat (Duclair), at Valier and Ledger sites in Golden Triangle Area of Montana in 2017. *x* axis indicates the twelve treatments ($n = 4$). *C*, control; G, imidacloprid; BB, *Beauveria bassiana* GHA granules on polenta; MR356, *Metarhizium robertsii* DWR 356 on polenta; MRM356, *Metarhizium robertsii* DWR 356 on millet; MR2009, *Metarhizium robertsii* DWR 2009 on polenta; MRM2009, *Metarhizium robertsii* DWR 2009 on millet (10 lb $acre^{-1}$, 11.21 kg ha^{-1} or 5 g $plot^{-1}$); BB-10, MR356-10, MRM356-10, MR2009-10, MRM2009-10, treatments at double rate (20 lb $acre^{-1}$, 22.42 kg ha^{-1} or 10 g $plot^{-1}$)

Table 2 Impact of entomopathogenic fungus treatments on spring wheat 'Duclair' performance in 2017 in terms of plant count, yield and test weight expressed as actual mean and S.E

	Plant count	Yield (kg ha^{-1})	Test weight (g)	No. of wireworms
Treatments Valier				
Control	29.03 ± 0.94^{ab}	6776 ± 228^{c}	77.99 ± 354^{ab}	0.5 ± 0.82^{a}
Imidacloprid	29.72 ± 0.94^{ab}	6897 ± 228^{bc}	77.41 ± 354^{b}	0.5 ± 0.82^{a}
B. bassiana polenta (BB)	29.97 ± 0.94^{ab}	6929 ± 228^{bc}	77.55 ± 354^{ab}	2.25 ± 0.82^{a}
M. robertsii DWR356 polenta (MR356)	30.91 ± 0.94^{ab}	7310 ± 228^{abc}	77.48 ± 354^{ab}	0.75 ± 0.82^{a}
M. robertsii DWR356 millet (MRM356)	28.97 ± 0.94^{b}	7207 ± 228^{abc}	77.81 ± 354^{ab}	0.001 ± 0.82^{a}
M. robertsii DWR2009 polenta (MR2009)	31.59 ± 0.94^{a}	7311 ± 228^{abc}	78.44 ± 354^{a}	1.25 ± 0.82^{a}
M. robertsii DWR2009 millet (MRM2009)	29.87 ± 0.94^{ab}	7099 ± 228^{abc}	77.55 ± 354^{ab}	0.25 ± 0.82^{a}
B. bassiana polenta (BB-10)	31.06 ± 0.94^{ab}	7127 ± 228^{abc}	77.97 ± 354^{ab}	1.75 ± 0.82^{a}
M. robertsii DWR356 polenta (MR356-10)	29.19 ± 0.94^{ab}	7616 ± 228^{a}	78.05 ± 354^{ab}	0.75 ± 0.82^{a}
M. robertsii DWR356 millet (MRM356-10)	31.25 ± 0.94^{ab}	7441 ± 228^{ab}	77.39 ± 354^{b}	0.5 ± 0.82^{a}
M. robertsii DWR2009 polenta (MR2009-10)	30.25 ± 0.94^{ab}	7377 ± 228^{abc}	77.97 ± 354^{ab}	2 ± 0.82^{a}
M. robertsii DWR2009 millet (MRM2009-10)	30.41 ± 0.94^{ab}	7492 ± 228^{ab}	77.86 ± 354^{ab}	0.75 ± 0.82^{a}
F (*P*)	2.67 (0.55)	611 (0.19)	1 (0.63)	2.3 (0.67)
Treatments Ledger				
Control	14.03 ± 2.78^{ab}	3985 ± 672^{a}	75.11 ± 0.97^{a}	3.75 ± 5.2^{a}
Imidacloprid	20.78 ± 2.78^{a}	4600 ± 672^{a}	76.08 ± 0.97^{a}	1.25 ± 5.2^{a}
B. bassiana polenta (BB)	18.12 ± 2.78^{ab}	3767 ± 672^{a}	75.72 ± 0.97^{a}	2 ± 5.2^{a}
M. robertsii DWR356 polenta (MR356)	14.59 ± 2.78^{ab}	3441 ± 672^{a}	75.2 ± 0.97^{a}	1.5 ± 5.2^{a}
M. robertsii DWR356 millet (MRM356)	13.12 ± 2.78^{ab}	3187 ± 672^{a}	75.04 ± 0.97^{a}	12.25 ± 5.2^{a}
M. robertsii DWR2009 polenta (MR2009)	16.06 ± 2.78^{ab}	4089 ± 672^{a}	75.91 ± 0.97^{a}	7.25 ± 5.2^{a}
M. robertsii DWR2009 millet (MRM2009)	18.28 ± 2.78^{ab}	3711 ± 672^{a}	75.12 ± 0.97^{a}	5 ± 5.2^{a}
B. bassiana polenta (BB-10)	15.16 ± 2.78^{ab}	3239 ± 672^{a}	75.62 ± 0.97^{a}	1.25 ± 5.2^{a}
M. robertsii DWR356 polenta (MR356-10)	13.12 ± 2.78^{ab}	3642 ± 672^{a}	75.65 ± 0.97^{a}	5.25 ± 5.2^{a}
M. robertsii DWR356 millet (MRM356-10)	12.22 ± 2.78^{b}	3086 ± 672^{a}	75.06 ± 0.97^{a}	8.25 ± 5.2^{a}
M. robertsii DWR2009 polenta (MR2009-10)	16.87 ± 2.78^{ab}	3801 ± 672^{a}	75.77 ± 0.97^{a}	12.5 ± 5.2^{a}
M. robertsii DWR2009 millet (MRM2009-10)	19.19 ± 2.78^{ab}	4330 ± 672^{a}	76.14 ± 0.97^{a}	11.75 ± 5.2^{a}
F (*P*)	8 (0.51)	1934 (0.90)	2.8 (0.99)	12.6 (0.48)

Standard error and least significant differences (superscripts) are calculated with the means generated by PROC MIXED and Fisher's least significant test analysis ($\alpha = 0.05$)

In 2017, the Ledger site had greater wireworm pressure (> 5 per trap) compared to the Valier site (< 1 per trap) and thus yield also varied at both locations (Fig. 1). Because both sites had different soil texture (Ledger, sandy loam; Valier, sandy) and the Ledger site also had wild oats [*Avena fatua* L. (Poaceae)], which were hand-managed but still believed to effect the wheat yield, we are not comparing the two sites for the different variables. However, yield and test weight of wheat were significantly different between both sites ($F = 5$; $df = 11$; $P < 0.01$). In 2017, at both locations, plant counts, yield (kg ha^{-1}), test weight (g) and number of wireworms collected from each plot did not vary significantly (Table 2). In a post hoc test, however, significant variation in plant count, yield and test weight was observed at Valier; at Ledger only the plant count differed. At Valier, pairwise comparison showed a significant difference in height and test weight in the DWR 2009 treated plots. Compared to yield from control plots, yield of plots treated with DWR 356 on polenta at 11 kg ha^{-1}, DWR356 on millet at 11 kg ha^{-1}, DWR 2009 on millet at 11 kg ha^{-1} were greater at the Valier site. At Ledger, plots treated with Gaucho and DWR 2009 on millet had greater plant stand count and yield (Table 2). At Ledger, the number of wireworms collected in each trap after 2 weeks was significantly higher than the number of wireworms collected after 4 weeks, ($F = 3.59$; $df = 21$; $P < 0.01$). However, at the Valier site a significantly greater number of wireworms were collected after 4 weeks from the plots treated with imidacloprid, GHA (at both rates) and DWR 2009 (on polenta and millet).

In 2018, sites were not compared because of variation in soil type and wireworm pressure. Out of the five selected sites, significant variation in yield due to treatment was only found at one site (Pendroy-2; $F = 772$; $df = 33$;

Table 3 Impact of entomopathogenic fungus treatments on spring wheat 'Duclair' performance in 2018 in terms of plant count, yield and test weight as expressed as the actual mean and S.E

Non-irrigated sites					Irrigated sites			
Treatments	Plant count	Yield (kg ha^{-1})	Test weight (g)	No. of wireworms	Plant count	Yield (kg ha^{-1})	Test weight (g)	No. of wireworms
Pendroy site 1					Choteau			
Control	8.37 ± 0.7^{a}	1805 ± 142^{c}	74.5 ± 0.35^{a}	0 ± 0.58^{a}	7.5 ± 0.7^{a}	311 ± 73^{b}	72 ± 0.8^{ab}	2.5 ± 0.76^{ab}
Imidacloprid	9 ± 0.7^{a}	2194 ± 142^{abc}	73.4 ± 0.35^{b}	2.75 ± 0.58^{a}	7.7 ± 0.7^{a}	629 ± 73^{a}	74 ± 0.8^{a}	2.5 ± 0.76^{ab}
Millet	8.37 ± 0.7^{a}	2041 ± 142^{abc}	74.2 ± 0.35^{ab}	1 ± 0.58^{a}	8.5 ± 0.7^{a}	515 ± 73^{ab}	73 ± 0.8^{ab}	0.75 ± 0.76^{b}
Couscous	9 ± 0.7^{a}	2190 ± 142^{abc}	74.4 ± 0.35^{a}	1 ± 0.58^{a}	8.5 ± 0.7^{a}	476 ± 73^{ab}	72 ± 0.8^{ab}	1.75 ± 0.76^{b}
B. bassiana millet	9.37 ± 0.7^{a}	2114 ± 142^{abc}	74.25 ± 0.35^{ab}	1 ± 0.58^{a}	8 ± 0.7^{a}	541 ± 73^{a}	73 ± 0.8^{ab}	1.5 ± 0.76^{b}
B. bassiana couscous	9.25 ± 0.7^{a}	2162 ± 142^{abc}	74.26 ± 0.35^{ab}	0.5 ± 0.58^{a}	8.2 ± 0.7^{a}	553 ± 73^{a}	72 ± 0.8^{ab}	2.3 ± 0.76^{ab}
B. bassiana ERL836 millet	8.87 ± 0.7^{a}	2226 ± 142^{ab}	74.91 ± 0.35^{a}	1.25 ± 0.58^{a}	9.2 ± 0.7^{a}	440 ± 73^{ab}	73 ± 0.8^{ab}	3.75 ± 0.76^{a}
B. bassiana ERL836 couscous	8.75 ± 0.7^{a}	2038 ± 142^{abc}	74.51 ± 0.35^{a}	1 ± 0.58^{a}	7.6 ± 0.7^{a}	507 ± 73^{ab}	72 ± 0.8^{ab}	1.75 ± 0.76^{b}
M. brunneum F52 millet	8.87 ± 0.7^{a}	2081 ± 142^{abc}	74.37 ± 0.35^{a}	1.5 ± 0.58^{a}	8.7 ± 0.7^{a}	465 ± 73^{ab}	71 ± 0.8^{b}	1.5 ± 0.76^{b}
M. brunneum F52 couscous	8.87 ± 0.7^{a}	1877 ± 142^{bc}	74.52 ± 0.35^{a}	0.5 ± 0.58^{a}	8.5 ± 0.7^{a}	492 ± 73^{ab}	73 ± 0.8^{ab}	0.75 ± 0.76^{b}
M. robertsii DWR2009 millet	8 ± 0.7^{a}	2236 ± 142^{ab}	74.46 ± 0.35^{a}	1 ± 0.58^{a}	8.8 ± 0.7^{a}	430 ± 73^{ab}	73 ± 0.8^{ab}	1 ± 0.76^{b}
M. robertsii DWR2009 couscous	9.12 ± 0.7^{a}	2293 ± 142^{a}	74.42 ± 0.35^{a}	0.75 ± 0.58^{a}	8.8 ± 0.7^{a}	455 ± 73^{ab}	74 ± 0.8^{a}	1.2 ± 0.76^{b}
F (*P*)	1.9 (0.97)	403.53 (0.39)	0.88 (0.29)	1.6 (0.23)	1.78 (0.66)	205 (0.33)	2.3 (0.53)	2 (0.19)
Pendroy site 2					Ledger			
Control	2 ± 0.88^{b}	0 ± 277^{d}	0 ± 13^{d}	9 ± 3^{abc}	13.5 ± 1.4^{b}	3710 ± 348^{b}	74 ± 0.5^{bc}	1.5 ± 1.5^{a}
Imidacloprid	9 ± 0.88^{a}	2194 ± 277^{a}	72 ± 13^{a}	10 ± 3^{abc}	16.8 ± 1.4^{ab}	4857 ± 348^{a}	75 ± 0.5^{a}	2.75 ± 1.5^{a}
Millet	2 ± 0.88^{b}	1103 ± 277^{b}	52 ± 13^{ab}	7 ± 3^{abc}	14.8 ± 1.4^{ab}	4457 ± 348^{ab}	74 ± 0.5^{abc}	3 ± 1.5^{a}
Couscous	2 ± 0.88^{b}	606 ± 277^{bcd}	35 ± 13b^{cd}	9 ± 3^{abc}	13 ± 1.4^{b}	4742 ± 348^{a}	74.6 ± 0.5^{abc}	2 ± 1.5^{a}
B. bassiana millet	2 ± 0.88^{b}	244 ± 277 ^{cd}	16 ± 13 ^{cd}	11 ± 3^{abc}	14.9 ± 1.4^{ab}	4819 ± 348^{a}	74.8 ± 0.5^{ab}	3 ± 1.5^{a}
B. bassiana couscous	1 ± 0.88^{b}	0 ± 277^{d}	0 ± 13^{d}	15 ± 3a	15 ± 1.4^{ab}	4497 ± 348^{ab}	74.4 ± 0.5^{abc}	2.5 ± 1.5^{a}
B. bassiana ERL836 millet	3 ± 0.88^{b}	898 ± 277^{bc}	50 ± 13^{abc}	6 ± 3^{bc}	13.2 ± 1.4^{b}	4048 ± 348^{ab}	74 ± 0.5^{abc}	2.75 ± 1.5^{a}
B. bassiana ERL836 couscous	1 ± 0.88^{b}	0 ± 277^{d}	0 ± 13^{d}	7 ± 3^{abc}	15.3 ± 1.4^{ab}	3707 ± 348^{b}	73.6 ± 0.5^{c}	4.75 ± 1.5^{a}
M. brunneum F52 millet	2 ± 0.88^{b}	330 ± 277 ^{cd}	16 ± 13 ^{cd}	5 ± 3^{c}	15.6 ± 1.4^{ab}	4112 ± 348^{ab}	74 ± 0.5^{abc}	2.5 ± 1.5^{a}
M. brunneum F52 couscous	2 ± 0.88^{b}	284 ± 277 ^{cd}	17 ± 13^{d}	6 ± 3^{bc}	17.8 ± 1.4^{a}	4175 ± 348^{ab}	74 ± 0.5^{abc}	5.5 ± 1.5^{a}
M. robertsii DWR2009 millet	1 ± 0.88^{b}	0 ± 277^{d}	0 ± 13^{d}	12 ± 3^{abc}	15.2 ± 1.4^{ab}	4157 ± 348^{ab}	74 ± 0.5^{abc}	3 ± 1.5$_{a}$
M. robertsii DWR2009 couscous	1 ± 0.88^{b}	213 ± 277 ^{cd}	16 ± 13 ^{cd}	14 ± 3^{ab}	13.6 ± 1.4^{b}	4163 ± 348^{ab}	73.9 ± 0.5^{bc}	2.5 ± 1.5^{a}
F (*P*)	2.5 (< 0.0001)	772 (< 0.0001)	36 (0.0018)	8.4 (0.3)	4 (0.43)	933 (0.19)	1 (0.29)	4.2 (0.85)

Wireworm population collected from each treated plot is also analyzed. Standard error and least significant differences (superscripts) are calculated with the means generated by PROC MIXED and Fisher's least significant test analysis ($\alpha = 0.05$). The treatments were applied in randomized block design ($n = 4$); water was used as control; and imidacloprid was used as chemical control

$P < 0.0001$) (Table 3; Fig. 2a, b). Wireworm pressure at all the five sites varied with the Pendroy-2 site having the highest wireworm pressure (> 5 per trap), followed by Ledger (< 1.5 per trap), Choteau (< 1 per trap), Pendroy-1 (0.5 per trap) and Valier (< 0.2 per trap) (Tables 4, 5). The yields were greater at the irrigated sites, but at Choteau, which was irrigated, hail damage occurred; hence, the yield was less (Table 3; Fig. 2b). Nevertheless, among

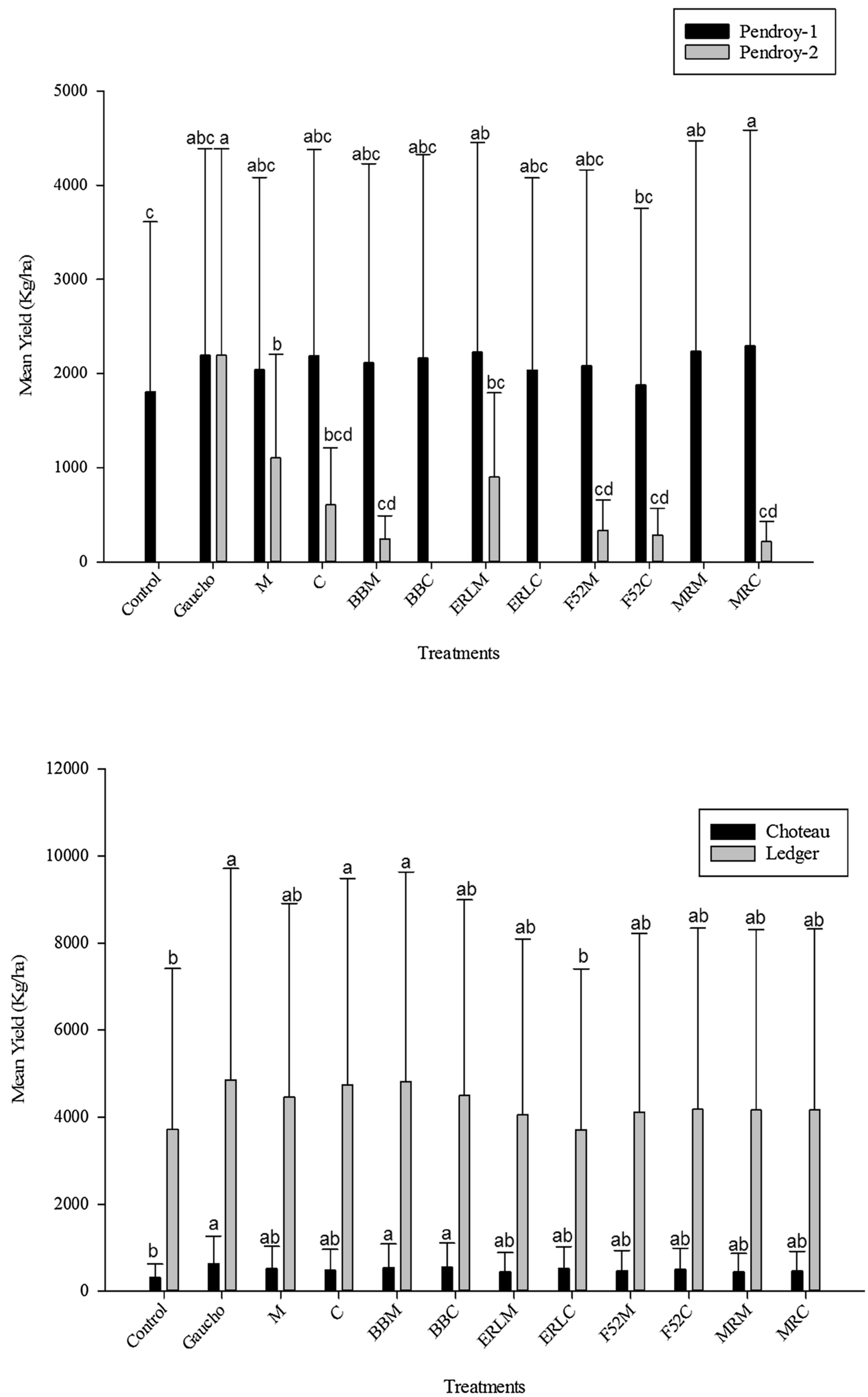

Fig. 2 Mean yield (mean yield + SE) at four sites in Golden Triangle Area of Montana in 2018. (a) Mean yield (kg ha^{-1}) of spring wheat (Duclair) at non-irrigated sites (Pondera-1 and Pondera-2). (b) Mean yield (kg ha^{-1}) of spring wheat (Duclair) at irrigated sites (Choteau and Ledger). In figures a and b, *x* axis indicates the twelve treatments ($n = 4$). M, millet; C, couscous; BBM, *Beauveria bassiana* GHA on millet; BBC, *Beauveria bassiana* GHA on couscous; ERLM, *Beauveria bassiana* ERL836 on millet; ERLC, *B. bassiana* ERL836 on couscous; F52M, *Metarhizium brunneum* F52 on millet; F52C, *Metarhizium brunneum* F52 on couscous; MRM, *Metarhizium robertsii* DWR2009 on millet; MRC, *Metarhizium robertsii* DWR2009 on couscous

Table 4 The average counts and variance in wireworm population in 15 and 30 days

Site	Day	Mean	Variance
Pendroy-1	15	0.7	0.7
Pendroy-1	30	0.3	0.4
Pendroy-2	15	2.0	5.0
Pendroy-2	30	7.0	36
Choteau	15	0.5	0.6
Choteau	30	1.0	2.0
Ledger	15	1.6	4.0
Ledger	30	1.0	3.0

Higher variance compared to the observed means indicates over dispersion of wireworms among different treatments

non-irrigated sites after post hoc analysis ($\alpha = 0.05$), at Pendroy-1, ERL836 on millet and DWR2009 on both millet and couscous carrier provided significantly greater yield than the control ($F = 403$; $df = 33$; $P < 0.3947$). At Pendroy-2, the greater wireworm pressure resulted in no yield with some treatments. Conventional seed treatment (imidacloprid) provided a significant greater yield and, hence, protection from wireworms. Treatments of ERL836 on millet carrier and the millet carrier, alone, provided a significant protection against wireworm (Table 3; $F = 772$; $df = 33$; $P < 0.0001$). These two treatments also had significantly greater test weight. At the irrigated sites, at both sites, GHA on millet and on couscous carrier and the couscous carrier alone provided a significantly greater yield than the control (Table 3; $F = 205$; $df = 33$; $P < 0.33$; $F = 933$; $df = 33$; $P < 0.19$).

The cost–benefit (CB) ratios were lower for 2017 compared to 2018. Based on our CB analysis, in 2017, for the irrigated sites, the economic benefit was greater for conventional insecticide seed treatment compared to control but was greatest for DWR2009 on millet at the high rate of 22.4 kg ha^{-1}. In 2018, at both irrigated and non-irrigated sites, the greatest benefit was for the conventional insecticide. However, at the irrigated site, GHA on millet, and at non-irrigated sites, millet alone and ERL836 on millet gave positive benefits compared to the control (Table 6).

Table 5 Actual number of wireworms collected in 2017 and 2018 from different plots in non-irrigated and irrigated plots

Treatments 2017	Valier	Ledger		
Control	2	15		
Imidacloprid	2	5		
B. bassiana polenta (BB)	9	8		
M. robertsii DWR356 polenta (MR356)	7	5		
M. robertsii DWR356 millet (MRM356)	3	6		
M. robertsii DWR2009 polenta (MR2009)	3	21		
M. robertsii DWR2009 millet (MRM2009)	0	49		
B. bassiana polenta (BB-10)	2	33		
M. robertsii DWR356 polenta (MR356-10)	5	29		
M. robertsii DWR356 millet (MRM356-10)	8	50		
M. robertsii DWR2009 polenta (MR2009-10)	1	20		
M. robertsii DWR2009 millet (MRM2009-10)	3	47		
Treatments 2018	**Non-irrigated sites**		**Irrigated sites**	
	Pendroy site 1	Pendroy site 2	Choteau	Ledger
Control	0	35	10	6
Imidacloprid	11	42	10	11
Millet	4	26	3	12
Couscous	4	36	7	8
B. bassiana millet	4	0	6	12
B. bassiana couscous	2	0	9	10
B. bassiana ERL836 millet	5	24	15	11
B. bassiana ERL836 couscous	4	28	7	19
M. brunneum F52 millet	6	20	6	10
M. brunneum F52 couscous	2	22	3	22
M. robertsii DWR2009 millet	4	48	4	12
M. robertsii DWR2009 couscous	3	55	5	10

Table 6 Cost and benefit analysis of managing wireworms in spring wheat with entomopathogenic fungus and conventional insecticide (Gaucho®) compared to control in field experiments of 2017–2018 in Montana

Treatment 2017	Cost/ha (US $)	Mean yield (including two sites)/ha	Income (US $) from yields @$5.5/bushel	Net benefit (US $)	Benefit over unsprayed treatment (US $)	Cost–benefit ratio
Control	18.5	5380	807	788	0	0.02
Imidacloprid	21.5	5748	862	840	52	0.02
B. bassiana polenta	42.9	5348	802	759	− 29	0.05
M. robertsii DWR356 polenta	42.9	5375	806	763	− 25	0.05
M. robertsii DWR356 millet	42.9	5197	779	736	− 51	0.05
M. robertsii DWR2009 polenta	42.9	5700	855	812	23	0.05
M. robertsii DWR2009 millet	42.9	5405	810	767	− 20	0.05
B. bassiana polenta	67.42	5183	777	710	− 78	0.09
M. robertsii DWR356 polenta 10 g	67.42	5629	844	776	− 11	0.08
M. robertsii DWR356 millet 10 g	67.42	5263	789	722	− 66	0.09
M. robertsii DWR2009 polenta 10 g	67.42	5589	838	770	− 17	0.08
M. robertsii DWR2009 millet 10 g	67.42	5911	886	819	30	0.08
Treatments 2018 Irrigated	Cost/ha	Yield	Income	Net benefit	Benefit over unsprayed treatment	Cost–benefit ratio
Control	18.5	2010	3015	283	0	0.06
Imidacloprid	21.5	2743	411	389	106	0.05
Millet	42.9	2486	372	330	46	0.13
Couscous	42.9	2609	391	348	65	0.12
B. bassiana millet	42.9	2680	402	359	75	0.12
B. bassiana couscous	42.9	2525	378	335	52	0.12
B. bassiana ERL836 millet	42.9	2244	336	293	10	0.14
B. bassiana ERL836 couscous	42.9	2107	316	273	− 10	0.15
M. brunneum F52 millet	42.9	2288	343	300	17	0.14
M. brunneum F52 couscous	42.9	2333	350	307	24	0.13
M. robertsii DWR2009 millet	42.9	2293	344	301	18	0.14
M. robertsii DWR2009 couscous	42.9	2309	346	303	20	0.14
Treatments 2018 Non-irrigated	Cost/ha	Yield	Income	Net benefit	Benefit over unsprayed treatment	Cost–benefit ratio
Control	10.5	902	135	124	0	0.08
Imidacloprid	13.5	2194	329	315	190	0.04
Millet	34.9	1572	235	200	76	0.17
Couscous	34.9	1398	209	174	49	0.20
B. bassiana millet	34.9	1179	176	141	17	0.24
B. bassiana couscous	34.9	1081	162	127	2.3	0.27
B. bassiana ERL836 millet	34.9	1562	234	199	74	0.17
B. bassiana ERL836 couscous	34.9	1019	152	117	− 6.9	0.29
M. brunneum F52 millet	34.9	1205	180	145	21	0.23
M. brunneum F52 couscous	34.9	1080	162	127	2.2	0.27
M. robertsii DWR2009 millet	34.9	1118	167	132	7.8	0.26
M. robertsii DWR2009 couscous	34.9	1253	187	152	28	0.22

Cost of grain is based on the price offered in January 2019 ($5.5/bushel for 14% protein spring wheat)

Table 7 Cost and benefit analysis of managing wireworms in spring wheat with entomopathogenic fungus on three carrier, polenta, millet and couscous compared to control in field experiments of 2017–2018 in Montana, expressed by treatment means and S.E

Treatment 2017 and 2018	Irrigated				Non-irrigated			
	Ledger		Choteau		Pendroy-1		Pendroy-2	
	Yield	Cost–benefit ratio	Yield	Cost–benefit ratio	Yield	Cost–benefit ratio	Yield	Cost–benefit ratio
Millet	4457^{ab}	0.06	515^{ab}	1.2	2041^{abc}	0.12	1103^{b}	0.26
Couscous	4742^{a}	0.06	476^{ab}	1.5	2190^{abc}	0.11	606^{bcd}	0.62
B. bassiana polenta	3767^{b}	0.08	×	×	×	×	×	×
B. bassiana millet	4819^{a}	0.06	541^{a}	1.1	2114^{abc}	0.12	244^{cd}	21
B. bassiana couscous	4497^{ab}	0.06	553^{a}	1	2162^{abc}	0.12	0	0
M. robertsii DWR2009 polenta	4089^{ab}	0.07	×	×	×	×	×	
M. robertsii DWR2009 millet	3934^{ab}	0.07	430^{ab}	1.9	2236^{ab}	0.11	0	0
M. robertsii DWR2009 couscous	4163^{ab}	0.07	455^{ab}	1.7	2293^{a}	0.11	213^{cd}	−11
M. robertsii DWR356 polenta	3441^{b}	0.09	×	×	×	×	×	×
M. robertsii DWR356 millet	3187^{b}	0.09	×	×	×	×	×	×
B. bassiana ERL836 millet	4048^{ab}	0.07	440^{ab}	1.8	2226^{ab}	0.11	898^{bc}	0.3
B. bassiana ERL836 couscous	3707^{b}	0.08	507^{ab}	1.2	2038^{abc}	0.12	0	0
M. brunneum F52 millet	4112^{ab}	0.07	465^{ab}	1.6	2081^{abc}	0.12	330^{cd}	2.4
M. brunneum F52 couscous	4175^{ab}	0.07	492^{ab}	1.3	1877^{bc}	0.14	284^{cd}	4.5

Cost of grain is based on the price offered in January 2019 ($5.5/bushel for 14% protein spring wheat). Yield at Choteau was effected by hail. Only Ledger site was common in 2017 and 2018, and *Metarhizium robertsii* DWR2009 millet was used in both years; hence, yield for this treatment was averaged for 2017 and 2108. '×' sign indicates the treatments not tested in 2018. Standard error and least significant differences (superscripts) are calculated with the means generated by PROC MIXED and Fisher's least significant test analysis ($\alpha = 0.05$)

When three carriers were compared, at irrigated sites, GHA on millet and on couscous carrier provided lower CB ratio and at both non-irrigated sites ERL836 on millet provided low ratio. However, at Pendroy-1, DWR2009 on millet and couscous also provided low ratio (Table 7).

Discussion

Much of exploration of EPFs is reported on various *Agriotes* spp. Eschscholtz, especially in potato crops since *Agriotes* is a common genus in Europe, America and Asia (Kabaluk et al. 2005; Ericsson et al. 2007; Ansari et al. 2009). In-furrow application of EPFs granules has gained attention in last few years and is supposed to work more efficiently compared to other application methods like seed treatment and topical application due to efficient transfer of infectious dose of conidia to the insect that encounters the fungi and can produce comparable results to the chemical treatment for wireworm management (Filipchuk et al. 1995; Ester and Huiting 2007; Jaronski 2007; Reddy et al. 2014). Furthermore, the granules based on a nutritive carrier which also can increase the efficacy of EPFs by virtue of regrowth and conidiation after application (Jaronski 2010). In 2017 and 2018, we have tested EPFs at different rates and on the different nutritive carrier. In 2017, DWR 356 at double rate gave greater yield at one site. In 2018, at irrigated sites *B. bassiana* GHA on millet and couscous performed comparable to imidacloprid and better than other EPF treatments, whereas at non-irrigated sites, ERL836 on millet, DWR2009 on millet and couscous gave greater yield compared to other EPF treatments and equivalent to imidacloprid. However, while comparing CB ratio in 2017 they were lower compared to the 2018 ratios and CB ratios for EPF at low rates (5 g/plot) were lower. In 2018, CB ratios were lower for irrigated fields compared to non-irrigated sites, in spite of one irrigated site getting damaged by hail and producing much lesser yield. At irrigated sites, millet, couscous, BB on millet and couscous had lower CB ratio and at non-irrigated sites, millet, ERL836 on millet and DWR2009 on millet and couscous had low CB ratio.

In 2017, higher yield was observed with DWR 356 on polenta, DWR 356 on millet and DWR 2009 on millet at 22.4 kg ha^{-1} at Valier compared to the untreated control. Greater acquisition of EPF conidia occurs in wireworms when a higher dose of EPF is applied and that also increases the mortality in wireworms (Butt and Ansari 2011). In the present study, greater yield in the plots treated with the higher rate of EPFs indicated a similar phenomenon. However, the number of wireworms collected from the same plots unexpectedly did not decrease in 4 weeks. This situation indicates that these higher rates of EPFs might have inhibited the insect feeding and enabled the enhanced seed germination and seedling growth but might not have killed the wireworms quickly. Repellency effect of EPFs toward termites (Isoptera) and grasshoppers (Orthoptera) and beetle larvae (*Popillia japonica;* Coleoptera: Scarabaeidae) is reported (Jaronski 2013; Mburu et al. 2011; Fry et al. 1997); however for wireworms, only a slight deterrent effect of *M. anisopliae* granules is reported against *Agriotes* species (Kabaluk et al. 2005). High concentrations of the EPFs in soil can cause repellency, but if a food source is present then this repellent effect is reduced (Kabaluk et al. 2005). That is why we also believe that use of stocking traps to collect wireworms mainly indicates the distribution of wireworms; hence, number of wireworms cannot directly be related to the efficiency of EPFs (Sharma et al. 2018). Similar results are reported by Reddy et al. (2014) in similar environmental conditions. On the other hand in 2017, at Ledger, the site with a greater wireworm pressure, although not significantly but Gaucho and DWR 2009 on millet carrier on a higher rate protected the wheat plants and enabled a higher yield. This shows that at the higher rate, DWR 2009 on millet carrier was able to provide protection to the wheat plants, which was of similar intensity as was provided by imidacloprid seed treatment. Nevertheless, the nonsignificant difference also raises the point of whether these higher rates being beneficial enough in achieving greater savings and profit for farmers. Moreover, as expected, the CB ratios were lower for EPFs applied at 11.2 kg ha^{-1}.

In 2018, results indicated that both millet and couscous have an impact on wireworms along with EPFs. The ability of an insect to detect EPF also depends on the carrier on which the fungus is deposited (Meyling and Pell 2006; Ormond 2007). Selection of carrier for fungal formulations depends on availability, price, and efficiency of the carrier to provide a nutritional base to the fungal conidia but would fungal conidial carrier also attract or deter the insect is an interesting aspect to study. Nevertheless, in terms of CB ratio, in 2018, the use of EPFs did have lower CB ratio compared to control. The important factor raised in 2018 experiment is that millet and couscous alone as carriers (without incorporation of EPFs) were able to perform well. Millet at non-irrigated sites and couscous at irrigated sites were able to generate better protection from wireworms and hence greater yield. When three carriers, polenta, millet and couscous are compared in both years, at irrigated sites, GHA on both millet and couscous found to be equally cost-effective, whereas at non-irrigated sites DWR 2009 on millet and couscous and ERL836 on millet carrier were cost-effective. Among the carriers, it seems that at the sites where wireworm pressure is high, millet provides slightly better results in terms of its effectiveness and cost efficacy. Also in 2018, at Pendroy-2, the site with maximum wireworm pressure in both the years, where some EPFs did not provide any protection, ERL836 on millet and millet alone were able to provide some protection to the wheat stand. Recently, in Germany, attract and kill strategy is reported to reduce the damage by 37–75% caused by *Agriotes* spp. in potato crop when baker's yeast is applied in rows along with *M. brunneum* (Brandl et al. 2017). Presence of polenta, millet and couscous as a nutrient carrier of EPFs should be explored further to analyze the attractiveness of these carriers for wireworms.

Landscape and soil texture play a major role in wireworm population, their movement in the soil and extent of damage caused by wireworms (Milosavljević et al. 2016; Ensafi et al. 2018; Hermann et al. 2013). Greater damage of wireworms occurs in sandy type soils (Hermann et al. 2013, Rashed et al. 2017). Although not much impact of soil type is associated with the efficacy of EPFs, EPFs are reported to reduce the damage to wheat seedlings in sand dominating soils (Ensafi et al. 2018). In the present study at non-irrigated sites, millet-based DWR 2009 and ERL836 showed better results. At both non-irrigated sites, the soil was gravel/sandy clay loam.

EPF efficacy can also be affected by soil moisture and temperature. Soil moisture of 80–100% and temperature 30 °C are considered best for increasing efficacy of EPFs as loose conidia (Mishra et al. 2015). Jaronski and Jackson (2008) reported that *Metarhizium* on polenta germinated, grew out and resporulated at soil moistures as low as the permanent wilting point (water activity of 0.983), 15% water holding capacity of their clay soil. EPF on granules alter the dose acquisition process, whereby intense focii of conidia result with granules, and contact of an insect with such focii results in the insect acquiring a very high number of conidia. In our 2017 study, soil moisture remained high (55–80%), but temperature remained less than favorable temperature (15–20 °C). In 2018, the temperature was higher (25–45 °C), but soil moisture level was much lower (15–45%). We believe these variations in soil moisture and temperature have played a major role in effecting the efficacy of EPFs in the present study. Yields at irrigated sites in 2018 also got affected due to loss of 50% of yield at Choteau site due to hail damage. This situation also impacted the overall benefit obtained from irrigated sites in 2018 compared to 2017 along with the fact that soil moisture levels were lower in 2018. CB ratios were much higher for non-irrigated fields compared to irrigated fields, most probably due to greater efficacy of EPFs at irrigated sites with stable moisture levels.

Conclusions

In conclusion, in 2017, although significant differences were not observed, DWR 356 and DWR 2009, at 22.4 kg ha^{-1}, enabled greater yields and the protection from wireworm damage. However, lower EPF rates obtained lower CB ratio and hence are proved to be more cost-effective. In 2018, where only low rates were used for all EPFs, irrigated and non-irrigated sites showed variations in results. At irrigated sites, GHA on millet and couscous, and at non-irrigated sites, DWR 2009 on millet, ERL836 on millet and millet alone provided effective protection and also obtained lower CB ratio due to better yields. In terms of cost-effectiveness, 2017, had lower CB ratio compared to 2018, due to better yields. Millet turned out to be a comparatively more effective carrier. Moisture played a major role in terms of negatively affecting the efficacy of EPFs in 2018. Although EPFs were less cost-effective than using imidacloprid as a seed treatment, further studies are required to lower the cost of EPFs for a low price crop such as wheat. Progress in increasing the cost-effectiveness of EPFs will provide us the environmentally friendly option to combat the wireworm problem in wheat production.

Author contributions

GR and AS conceived and designed research. SJ produced the fungus formulations. AS conducted research, analyzed data, interpreted data and wrote the manuscript. All authors edited and approved the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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