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# Evaluation of the effectiveness of entomopathogens for the management of wireworms (Coleoptera: Elateridae) on spring wheat

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ABSTRACT

Wireworms, the larval stage of click beetles (Coleoptera: Elateridae), are serious soil dwelling pests of small grains, corn, sugar beets, and potatoes. *Limonius californicus* and *Hypnoidus bicolor* are the predominant wireworm species infesting wheat in Montana, particularly in the 'Golden Triangle' area of north-central Montana. Wireworm populations in field crops are increasing, but currently available insecticides provide only partial control, and no alternative management tools exist. In our study, three entomopathogenic fungi were tested for their efficacy against wireworms in spring wheat at two field locations (Ledger and Conrad, Montana, USA) in 2013. The three fungi (*Metarhizium brunneum* F52, *Beauveria bassiana* GHA, and *Metarhizium robertsii* DWR 346) were evaluated as seed-coat, in-furrow granular, and soil band-over-row drench applications in addition to imidacloprid (Gaucho® 600) seed treatment (as a chemical check), the approach currently being used by growers. Wireworm damage in these treatments was evaluated as standing plant counts, wireworm population surveys, and yield. The three fungi, applied as formulated granules or soil drenches, and the imidacloprid seed treatment all resulted in significantly higher plant stand counts and yields at both locations than the fungus-coated seed treatments or the untreated control. Significant differences were detected among the application methods but not among the species of fungi within each application method. All three fungi, when applied as granules in furrow or as soil drenches, were more effective than when used as seed-coating treatments for wireworm control, and provided an efficacy comparable or superior to imidacloprid. The fungi used in this study provided significant plant and yield protection under moderate wireworm pressure, supporting their value in the management of this pest.


## 1. Introduction

Wheat is the principal grain produced for human consumption in the United States (United States Department of Agriculture [USDA], 2012). Spring and winter wheat (*Triticum aestivum* L.) are the major cereal crops grown in Montana. Wheat production employs approximately 15,000 people in the state and accounts for approximately 25% of Montana's total agricultural revenue (Montana Wheat and Barley Committee, 2005). In recent years, wireworms, the larval stage of click beetles (Coleoptera: Elateridae), have caused increased damage to spring wheat in Montana and neighboring states. Wireworms inflict damage to many important crops around the world, primarily by feeding on roots and tubers (Parker and Howard, 2001).

Historically, wireworms have been severe pests with few or no effective management techniques. Effective control was not achieved until inexpensive and broad-spectrum insecticides became available in the 1950s. Lindane was used as a seed treatment against wireworms in many crops for more than 30 years (Toba et al., 1985). The availability of inexpensive, effective control with such insecticides created a disincentive for development of integrated methods (IPM) for wireworm control for nearly 40 years (Vernon et al., 2013). The resurgence of wireworms in recent decades can be attributed in large part to the cancelation of the registrations of many conventional insecticides that were formerly used for wireworm control, and the ineffectiveness of their second-generation replacements (Vernon et al., 2009). Producers, industry, agriculturalists, and scientists all recognize wireworms as an increasing threat to the sustainable production of many field crops, of which at least 40 have been noted in the literature. Now, producers must contend

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with a serious pest without effective management tools or the fundamental knowledge to develop new tools. In recent years, wireworm damage has become an increasing problem for growers, so the demand for meaningful risk assessment and useful methods to restrict damage is increasing (Andrews et al., 2008). However, due to the cryptic habitat of the wireworms, pest control is difficult and leads to unsatisfactory results (Blackshaw and Vernon, 2006). Although many wireworms are pests of wheat, a recent survey indicates that *Limonius californicus* (Mannerheim) and *Hypnoidus bicolor* (Eschscholtz) (Coleoptera: Elateridae) are the dominant wireworm species found in Montana, particularly in the Golden Triangle area (Kevin Wanner, unpublished data).

Wireworm damage is often confined to certain areas of a field, and stand losses can vary from zero to 60% or 70%. In addition, wireworm eggs are seldom seen, because they are deposited among soil particles (Parker and Howard, 2001). Eggs generally are laid singly and are widely scattered. Newly hatched larvae are about 1.5 mm long while fully developed larvae are about 14–19 mm long. Wireworms seen feeding on damaged plants are from 0.56 to 16.8 cm long, and are yellowish brown and cylindrical, with three pairs of legs (Parker and Howard, 2001). Pupae, which are rarely seen, are white and about 1.27 cm long.

Several entomopathogenic fungi, *Metarhizium anisopliae sensu lato* Sorokin in particular, have been recorded attacking field populations of wireworms (e.g., Fox and Jaques, 1958; Furlan et al., 2009), and attempts have been made to explore use of this fungus as a biological control agent for wireworms (Kabaluk et al., 2001, 2007a,b; Kabaluk and Ericsson, 2007a). *Beauveria bassiana* (Vuill.) Sorokin has also been evaluated in the laboratory and the field, albeit to a much lesser extent (Ester and Huiting, 2007). Concerns about the insufficient efficacy of these fungi have led to the idea of combining them with sub-lethal rates of selected agricultural chemicals, both to increase efficacy and to reduce insecticide resistance. Ericsson et al. (2007) noticed that synergism of *M. anisopliae* and Spinosad® resulted in higher mortality of *Agriotes lineatus* (L.) and *A. obscurus* (L.) in Canada. This fungus also showed good persistence following application to field soils (Kabaluk et al., 2007b; Kabaluk and Ericsson, 2007b). The potential to use these fungi as seed treatments against wireworms was demonstrated by Tharp et al. (2005) and Kabaluk et al. (2007b) in potatoes and maize, respectively. A commercial *B. bassiana* strain, when applied either alone or as part of an IPM strategy, significantly reduced populations of *Agriotes* spp. wireworm in potatoes and significantly reduced tuber damage compared to the untreated control in European tests (Ladurner et al., 2009), but additional work by Kolliker et al. (2011) had the opposite results. Even so, this fungus can be a valuable tool for the control of wireworms in both organic farming and integrated pest management.

In our study, we evaluated three pathogens in the field. Two were commercially available fungi – *B. bassiana* GHA and *Metarhizium brunneum* F52 (formerly *M. anisopliae* F52), while the third was an unregistered species, *Metarhizium robertsii* DWR 346 (=ARSEF8367). All were tested as control agents of wireworms in spring wheat.

## 2. Materials and methods

### 2.1. Fungi

Conidia of *B. bassiana* strain GHA were supplied as unformulated technical grade powder by Laverlam International, Butte Montana. Conidial titer was $1.6 \times 10^{11}$ conidia/g, based on a hemocytometer count of conidial suspension in 0.1% Silwet L-77 (Loveland Chemicals), and viability was 98%, based on conidial germination on potato dextrose yeast extract agar after incubation for 18 h at 27 °C. Cultures of *M. brunneum* F52 and *M. robertsii* DWR346 (=ARSEF 8367) were obtained from Novozymes Biologicals Inc., Salem, Virginia and D.W. Roberts, Utah State University, respectively. The two *Metarhizium* isolates were passaged through grasshoppers, *Melanoplus sanguinipes* (Orthopetera: Acrididae), to restore any lost infectivity and the resulting conidia stored in 30% glycerol at −80 °C. Conidia of these two fungi were produced using biphasic liquid–solid fermentation methods as described in Jaronski and Jackson (2012), and the resulting spores used in the trial represented the fourth in vitro passage from an insect host, ensuring good general infectivity. The resulting *M. brunneum* conidial powder had a titer of $5 \times 10^{10}$ conidia/g, while that of *M. robertsii* was $6.1 \times 10^{10}$/g. Conidial viability, determined as described earlier, was 88% and 90% for *M. brunneum* and *M. robertsii*, respectively. Conidial powders were stored dry (water activity, $A_w < 0.3$) at 4–5 °C until formulation and use.

### 2.2. Entomopathogen formulation

Two kg of untreated seed were coated with conidia of each fungus, to achieve a titer target of $3 \times 10^6$ conidia/g seed, as follows. The seed was first sprayed with emulsifiable vegetable oil (Golden Pest® Oil, Stoller Manufacturing, Houston Texas), at the rate of 1% v/w, using a Paasche H airbrush, then mixed in a V-cone mixer for 10 min. The appropriate amount of conidial powder to achieve the desired number of conidia per seed was then sprinkled onto the seed with frequent hand mixing, after which the seed was further mixed in the V-cone blender for 10 min. Granular carrier for the fungi consisted of commercial, 14/30 mesh, degermed yellow corn grits (Bunge North America, St Louis Missouri). The carrier (500 g) was first coated with 1% v/w of an emulsifiable vegetable oil (Golden Pest Oil, as above). The oil was applied to a thin layer of corn grits using a Paasche H airbrush, while the carrier was being gently mixed by hand. The coated granules were then mixed in a V-cone blender for 15 min to further distribute the oil through the granules. The oil-coated granules were then placed in a thin layer on a 46 × 61 mm tray and the fungal conidia were applied with a culinary flour shaker, after which the treated granules were again mixed in a V-cone blender for 15 min. Conidia of each fungus were applied in sufficient quantity to achieve a target titer of $5.6 \times 10^{11}$ viable conidia/kg carrier, based on the conidial titer and viability of each fungus. The seeds and granules were prepared at USDA-ARS, Sidney Montana, based on previous work with granular mycoinsecticide formulations for controlling sugarbeet root maggot *Tetanops myopaeformis* Röder (Diptera: Ulidiidae) with these fungi (Jaronski and Campbell, 2006; Jaronski et al., 2007). A commercial formulation of *B. bassiana*, (BotaniGard® ES by Laverlam International, Butte, Montana), and of *M. brunneum* F52 (Met52® EC by Novozymes Biologicals, Salem, Virginia) were used. An emulsifiable oil formulation of *M. robertsii* DWR 346 was prepared by mixing conidial powder of that fungus with Stoller Golden Pest Oil to an end titer of $5 \times 10^9$ viable conidia/ml. In addition, imidacloprid (Gaucho® 600, Bayer Crop Science), which is the control method currently used by growers, was used as a seed treatment to provide a chemical control.

### 2.3. Entomopathogen application

Trials were conducted at two field locations: Ledger (N48°15.872′W111° 53.332′) and Conrad (N48°10.521′W111 °58.666′) in the 'Golden Triangle' area of Montana. Experiments were carried out from May–September 2013 at both locations. Treatment plots were 8 m × 4 m and separated from each other by a 1 m buffer to avoid cross contamination from spray drift. Each plot was comprised of 12 rows, spaced 0.3 m apart. The wheat

**Table 1**
Materials, rates, and methods of application for treatments applied in study of wireworm control in Montana, 2013. Spray applications were made in 93.5 L/ha. Formulated product rates are indicated in parentheses.

| Treatment | Material | Rate | Application method |
|---|---|---|---|
| T1 | Untreated control | – | – |
| T2 | Imidacloprid (Gaucho® 600) | 2 ml/100 kg seed | Seed treatment |
| T3 | *M. brunneum* F52 granules | $5.04 \times 10^{12}$ conidia/ha (9 kg/ha) | Applied in furrow |
| T4 | *M. brunneum* F52 emulsifiable oil suspension (Met52® EC) | $3.85 \times 10^{11}$ conidia/ha (0.08 L/ha) | Band over row soil drench |
| T5 | *M. brunneum* F52 conidia | $5.5 \times 10^{12}$ conidia/ha | Seed treatment |
| T6 | *B. bassiana* GHA granules | $5.04 \times 10^{12}$ conidia/ha (9 kg/ha) | Applied in furrow |
| T7 | *B. bassiana* GHA emulsifiable oil suspension (BotaniGard® ES) | $3.85 \times 10^{11}$ conidia/ha (0.08 L/ha) | Band over row soil drench |
| T8 | *B. bassiana* GHA conidia | $5.5 \times 10^{12}$ conidia/ha | Seed treatment |
| T9 | *Metarhizium robertsii* DWR 346 granules | $5.04 \times 10^{12}$ conidia/ha (9 kg/ha) | Applied in furrow |
| T10 | *M. robertsii* DWR 346 emulsifiable oil suspension | $3.85 \times 10^{11}$ conidia/ha (0.08 L/ha) | Band over row soil drench |
| T11 | *M. robertsii* DWR 346 conidia | $5.5 \times 10^{12}$ conidia/ha | Seed treatment |

variety 'Duclair' was seeded at both locations at a rate of 22 seeds per 30 cm using a four-row plot drill. The herbicide glyphosate (Roundup® Powermax Company) was applied before seeding at the rate of 2.5 L/ha for weed control, following regional farming practice. Fertilizer with an N, P, K ratio of 224.2, 0, and 22.4 kg/ha was broadcast while planting, and an additional application of 12.3, 25.2, and 0 kg/ha, respectively, of the three nutrients was placed through the seed plot drill. The trials were conducted under overhead irrigated conditions typically applying 5 cm of water as needed, with first irrigation applied 30 days after treatments.

The three fungi were applied either as a seed coat, as granules, or through a soil drench applied in a band over each row. Both conidia-coated seeds and granules were delivered to the field using an on-site manufactured drill. The surface drench was applied using a four-wheel All-Terrain Vehicle (ACAT® 300) equipped with a four-nozzle (SJ3-06) sprayer. Filters were removed from the nozzle during application. The volume of spray carrier was 93.52 L/ha. Material and rate used in each treatment are listed in Table 1. For the soil drench treatment, the wetting agent Silwet L-77® was added to the liquid emulsifiable formulations of the three fungi at 0.1% (volume/volume) to improve conidial dispersion in water.

### 2.4. Plot design and data collection

A randomized complete block design with five replicates was used, with 8 × 4 m treatment plots separated from other plots by 1 m buffer zones to prevent any overlap of treatment effects. To assess treatment effectiveness, both the number of standing plants and the grain yield in each plot were recorded. The number of standing plants in each plot was evaluated by assessing stand counts in randomly selected 1 $m^2$ quadrats (Reddy, 2011). A Hege 140 plot combine was used to thrash the wheat plots to collect grain kernels for assessing the yield.


**Fig. 1.** The wireworm sampling device "stocking traps". See text for description of trap assembly and use.

### 2.5. Larval wireworm sampling

Stocking traps (disposable foot socks) were used for assessing wireworm presence and estimating their density (Fig. 1). Approximately 180 cc of wheat seed was placed in a nylon stocking, which was then tied shut with string, leaving a tail end of about 38.1 cm. Just before use, the traps were immersed in water for 24 h so that the grain started to germinate, making it attractive to wireworms. A hole was dug in the soil about 7.62–15.24 cm deep and 20.32–25.4 cm wide with a shovel. The nylon stocking was pressed down at the bottom of the hole to spread the grain mixture across as wide an area as possible. The string was left above the soil to help relocate the stocking later, and the stockings were covered with 1–2 inches of soil. Two stocking traps per replicate plot were deployed one day after sowing, and one of these traps was collected after 14 days while the second was collected after 28 days. Larvae caught inside the stocking mesh were counted in the laboratory.

### 2.6. Statistical analyses

All analyses were conducted using SAS version 9.3 (SAS Institute, 2011). Data on number of larvae and standing plants were pooled within the treatments and analyzed using two-way ANOVA, and differences among the treatments were tested using Fisher's Least Significant Difference (LSD) *Test* ($\alpha = 0.05$). Linear regression was used to analyze the correlation between yield loss and mean number of standing plants.

## 3. Results

### 3.1. Effect of treatments on wireworm population

At the Ledger site, we found a significant difference in wireworm numbers between the untreated plots and the treatments with different entomopathogens or regular seed treatment at both two weeks ($F = 2.08$, df = 10, $P = 0.0467$) and four weeks after planting and treatment ($F = 2.15$, df = 10, $P = 0.04$) (Fig. 2). When data collected at the two sampling times were compiled for analysis, a significant reduction was detected in the wireworm population from two weeks to four weeks after application ($F = 15.26$, df = 1, $P = 0.0002$); a significant reduction in wireworm population was also detected in the treated plots compared to the untreated control ($F = 3.57$, df = 10, $P = 0.0005$). Meanwhile, no significant interactions between treatments and sampling at different times after application were found ($F = 0.62$, df = 10, $P = 0.7929$) (Two-way ANOVA). Treatments with various agents applied by different methods were equally effective at both two and four weeks after application ($F = 1.24$, df = 10, $P = 0.0423$).

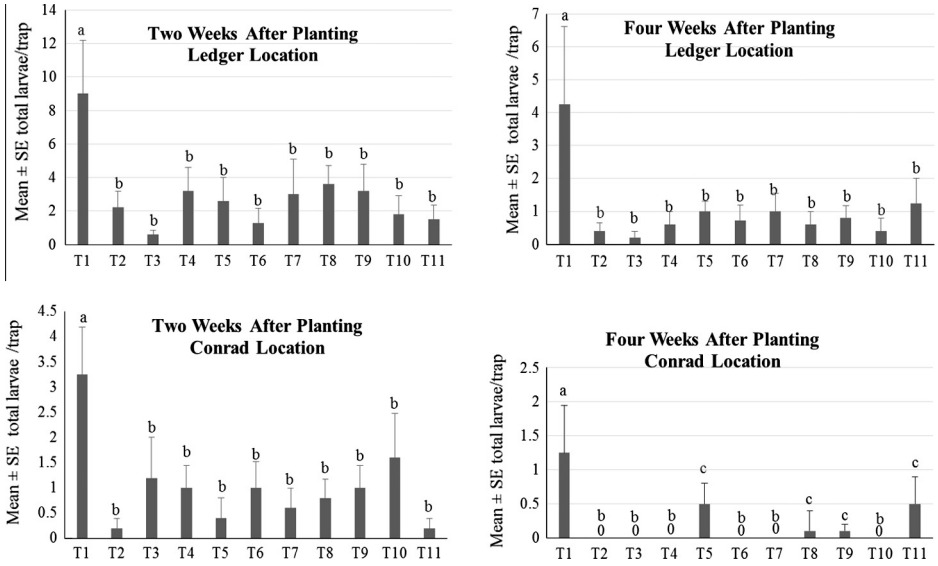


**Fig. 2.** Number of wireworm larvae recorded in stocking traps in each treatment (mean ± SE). Different letters above the bars indicate significant differences (Two-way ANOVA, Tukey HSD, $\alpha = 0.05$). T1: Untreated control; T2: Imidacloprid seed treatment; T3: *Metarhizium brunneum* F52 granules applied in furrow; T4: *M. brunneum* F52 soil drench; T5: Seed treatment with *M. brunneum* F52; T6: *Beauveria bassiana* GHA granules applied in furrow; T7: *B. bassiana* GHA soil drench; T8: Seed treatment with *B. bassiana* GHA; T9: *Metarhizium robertsii* DWR346 granules applied in furrow; T10: *M. robertsii* DWR346 soil drench; T11: Seed treatment with *M. robertsii* DWR346.

A similar trend was also found at the Conrad site. Significant differences were detected in wireworm numbers among various treatments ($F = 3.13$, df = 10, $P = 0.0019$). Significantly lower wireworm numbers were found four weeks after treatment compared to two weeks ($F = 15.74$, df = 1, $P = 0.0001$). Treatments and sampling time after their application had no significant interactions ($F = 0.97$, df = 10, $P = 0.4725$) (Two-way ANOVA). Treatments with various control agents significantly reduced wireworm numbers at two weeks after application ($F = 2.14$, df = 10, $P = 0.0409$). Again, there were no significant differences among various treatment agents and application methods ($F = 1.02$, df = 10, $P = 0.582$). A significant difference was detected in wireworm numbers among treatments at four weeks after application ($F = 2.01$, df = 10, $P = 0.0545$), and a trend of lower wireworm numbers was found in the various treatments compared to the untreated control (Fig. 2). The plots with seed treatments of *M. brunneum* and *M. robertsii* appeared to have numerically but not significantly lower wireworm numbers than other fungal treatments two weeks after application, but were the least effective at four weeks. This may indicate that these seed treatments were only effective at the earlier stage of wheat growth.

### 3.2. Plant stand density

At Ledger, significant differences were observed among the treatments in stand counts ($F = 3.28$, df = 10, $P = 0.003$). Except for seed treatments with the three fungi, the number of standing plants in the treated plots were significantly higher than in the control plots ($F = 1.62$, df = 10, $P < 0.023$) (Fig. 3). Significant differences in the number of standing plants were detected among the application methods of *M. brunneum, M. robertsii,* and *B. bassiana* ($F = 8.70$, df = 2, $P = 0.0008$), but not among the fungal species ($F = 0.42$, df = 2, $P = 0.6625$), and there was no significant interaction between fungal species and application methods ($F = 0.50$, df = 4, $P = 0.7348$) (Two-way ANOVA). All three fungi applied as formulated granules in the furrow and as a soil drench were associated with significantly greater stand counts than the seed treatment ($F = 0.85$, df = 4, $P < 0.031$), whereas no significant difference was found between fungal application as granules vs. as a soil drench ($P > 0.05$). However, the seed treatment with imidacloprid provided significant control of wireworms while seeds treated with fungi failed to do so ($F = 1.24$, df = 4, $P < 0.621$) (Fig. 3).

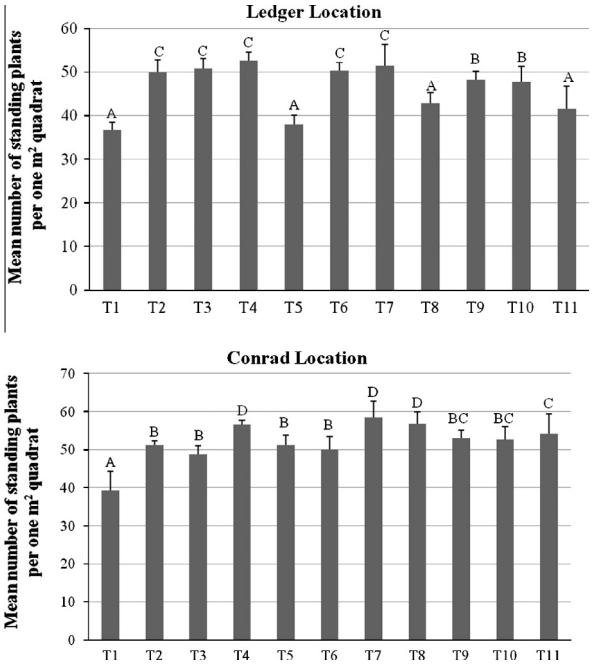


**Fig. 3.** Number of standing plants in different treatments at two locations (mean ± SE). Different letters above the bars indicate significant differences (Two-way ANOVA, Tukey HSD, $\alpha = 0.05$). T1: Untreated control; T2: Imidacloprid seed treatment; T3: *Metarhizium brunneum* F52 granules applied in furrow; T4: *M. brunneum* F52 soil drench; T5: Seed treatment with *M. brunneum* F52; T6: *Beauveria bassiana* GHA granules applied in furrow; T7: *B. bassiana* GHA soil drench; T8: Seed treatment with *B. bassiana* GHA; T9: *Metarhizium robertsii* DWR346 granules applied in furrow; T10: *M. robertsii* DWR346 soil drench; T11: Seed treatment with *M. robertsii* DWR346.

At the Conrad location, marginal significance was found among various treatments ($F = 2.01$, df = 10, $P = 0.0565$). However, the wireworm population at Conrad was much lower than at Ledger. Thus wireworm numbers at Conrad were just at the treatment threshold based on Montana State University recommendations, while pressure at Ledger was quite severe. The untreated control

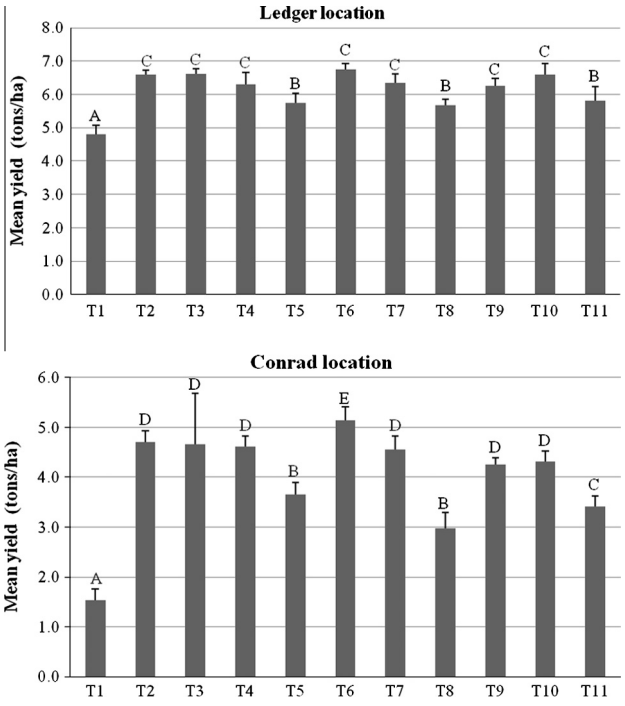


**Fig. 4.** Wheat yield produced in different treatments at two locations (mean ± SE). Different letters above the bars indicate significant differences (Two-way ANOVA, Tukey HSD, $\alpha = 0.05$). T1: Untreated control; T2: Imidacloprid seed treatment; T3: *Metarhizium brunneum* F52 granules applied in furrow; T4: *M. brunneum* F52 soil drench; T5: Seed treatment with *M. brunneum* F52; T6: *Beauveria bassiana* GHA granules applied in furrow; T7: *B. bassiana* GHA soil drench; T8: Seed treatment with *B. bassiana* GHA; T9: *Metarhizium robertsii* DWR346 granules applied in furrow; T10: *M. robertsii* DWR346 soil drench; T11: Seed treatment with *M. robertsii* DWR346.

had the smallest number of standing plants. The banded soil drench treatments with *M. brunneum* and *B. bassiana* and the seed treatment with *B. bassiana* had a greater number of standing plants than the untreated control and other treatments ($F = 087$, df = 10, $P = 0.121$) (Fig. 3). There were no significant differences among fungal species ($F = 0.48$, df = 2, $P = 0.6249$), or application methods ($F = 1.58$, df = 2, $P = 0.2194$), and no significant interaction between the two factors was detected ($F = 0.55$, df = 4, $P = 0.7006$) (Two-way ANOVA).

### 3.3. Grain yield in different treatments

Grain yield at the Ledger site was significantly higher for treatments with the three fungi applied as formulated granules or as a soil drench (averaging 6.3–6.8 tons/ha), and the imidacloprid seed treatment (6.6 tons/ha) compared to the treatments with fungus-coated wheat seed (5.8 tons/ha) or the untreated control (4.8 tons/ha) ($F = 2.36$, df = 10, $P = 0.0246$) (Fig. 4). Consistent with the stand counts, significant differences were found among fungal application methods ($F = 7.30$, df = 2, $P = 0.0021$), but not among fungal species ($F = 0.02$, df = 2, $P = 0.9816$), and there was no significant interaction between fungal species and application methods ($F = 0.63$, df = 4, $P = 0.6458$) (Two-way ANOVA). Fungal applications made as granules in the furrow or as a banded soil drench were superior to fungus-coated seed treatments ($F = 0.76$, df = 4, $P = 0.624$), and there was no significant difference in yield between the two application methods of granular or drench ($F = 1.84$, df = 4, $P = 0.085$).

Similarly, at the Conrad site, improved yield production was also found in various treated plots (3.0–5.0 tons/ha on average) over the untreated control (averaging 1.5 tons/ha), and significance differences were found in yield from different treatments ($F = 2.05$, df = 10, $P = 0.0506$) (Fig. 4).

### 3.4. Correlation between grain yield and plant stand count

A positive correlation was detected between grain yield levels and the number of standing plants at both experimental sites (Ledger: $R^2 = 0.3298$; Conrad: $R^2 = 0.1243$, $P < 0.05$).

## 4. Discussion

Wireworm populations are increasing in Montana, in large part because the current insecticidal seed treatments are less effective than previously available insecticides. Wireworms have a long life cycle in the soil, during which they feed on and damage the seed and seedlings of most field crops (Vernon et al., 2009). Control of wireworms has proven difficult, and even neonicotinoid seed treatments, the current standard, give only partial control (Barsics et al., 2013). Patchy wireworm distribution (Smith et al., 1981) and the position of the larvae in the soil have limited the importance of predators and parasitoids. As a consequence, the potential for control with entomopathogenic fungi, especially the two U.S.-registered strains, remains one of the few biological control options.

The entomopathogenic fungi *B. bassiana* and *M. anisopliae s.l.* naturally exist in the soil, and are widely recognized as promising biological control agents for use against insect pests (McCoy, 1990). Germinating fungal spores penetrate the insect's cuticle and proliferate, making them functionally analogous to contact insecticides (Jaronski, 2007). Both *Metarhizium* spp. and *Beauveria* spp. produce insecticidal toxins (Zimmermann, 2007a,b), such as destruxins, secreted by *Metarhizium* spp. and oosporein, beauvericin, oxalic acid and bassianolide produced by *B. bassiana*. Spores are produced on the surface of cadavers (Whitten and Oakeshott, 1991; Starnes et al., 1993), and are subsequently available to infect other susceptible hosts (Bateman et al., 1996). However, many fungal propagules typically on the order of $10^6$ conidia/g soil, are needed to infect and kill hosts (Kabaluk et al., 2001, 2007a). Both *B. bassiana* and *M. anisopliae s.l.* have been tested as biological control agents for wireworm control with mixed success in other studies in maize and potatoes. Kabaluk et al. (2007a) reported that broadcasting a granular formulation of *M. anisopliae* before planting reduced wireworm feeding holes in potato tubers by 33% in six independent field trials, but this reduction was not statistically significant because of high variability. Ericsson et al. (2007) found that synergism between *M. anisopliae* and Spinosad® resulted in higher mortality of *A. lineatus* and *A. obscurus* in Canada. Similarly, Kabaluk et al. (2007a,b) also observed a synergy between spinosad and *M. anisopliae* (when applied as seed treatments) that significantly increased fresh weight. In addition, Ester and Huiting (2007) observed a significant reduction of wireworm damage in potatoes in the Netherlands when plots were treated with *B. bassiana* or the combined application of *B. bassiana* (furrow application) with imidacloprid (tuber drench). However, as far as we know there has been little research on using different fungal isolates combined with different application methods for managing wireworms in wheat. In the current study, *M. brunneum*, *B. bassiana,* and *M. robertsii* applied using the same methods were equally effective in reducing wireworm damage, but fungal efficacy varied significantly with application method.

According to a study by Kabaluk and Ericsson (2007b), *M. anisopliae* seed treatment enhanced crop yield and development in maize. Also, Kabaluk et al. (2007a) showed that corn seed treated with *M. anisopliae (brunneum)* isolate F52 conidia resulted in a significant increase in stand density (77.9% – F52-treated vs. 66.7% – no F52) and yield (9.6 t/ha – F52-treated vs. 7.6 t/ha – no

F52). In addition, Kabaluk et al. (2007b) noticed good control of wireworms with F52 applied as seed coat on maize. In our study, the seed treatment with fungal coating did not achieve satisfactory control, especially at the Ledger location, where wireworm pressure was considerable. Meanwhile, the fungi applied as formulated granules or as soil drench achieved an efficacy comparable or superior to the imidacloprid seed treatment both in terms of the number of plants and yield levels. The mode of action of the fungi might account for the difference in effectiveness of application methods. In contrast to bacterial, viral, or protozoan entomopathogens, which need to be ingested by their hosts to cause an infection, fungi can infect hosts without being ingested, by penetrating the cuticle wall. Application of the fungi on granules applied in furrow or band-over-row drench may have created somewhat more even distribution of fungal conidia through the developing wheat rhizosphere than did seed treatment. Additionally, use of a nutritive granular carrier for a fungus results in a multiplication of the original titer of conidia in a tight focus around each granule, and thus an increase in the effective rate of fungus (Jaronski, 2010). Rhizosphere colonization by *Metarhizium* and *Beauveria* has been reported (Klingen et al., 2009; Fisher et al., 2011),which situation would further spread fungus through the wireworm feeding zone, especially when conidia are spread through the soil to be colonized by wheat roots, in contrast with conidia as a seed coated. Our results are similar to those of Filipchuk et al. (1995), who found that by drenching the soil with a conidial suspension of *Metarhizium* control of the tobacco wireworm, *Conoderus vespertinus* Fabricius (Coleoptera: Elateridae), was comparable to that achieved with an organophosphate insecticide.

Surprisingly, counts of wireworm did not correspond directly with stand counts or yields, as this might be due to a low number of wireworms attracted to the sampling units. Another possible explanation is that due to the slow speed of kill by these fungi, a longer time might be needed to reduce the population. Even so, before actually killing the host, fungal infection may have inhibited insect feeding on the wheat seeds and roots, thus enhancing seed germination and seedling growth. Similar reduced feeding due to infection with entomopathogenic fungi has been reported in several insects (Arthurs and Thomas, 2000; Ekesi and Maniania, 2000; Tefera and Pringle, 2003; Maehara et al., 2007). The exact species composition of the wireworm species complex in wheat might be another reason why we failed to detect an impact on wireworm numbers, as fungal pathogenicity may vary with host species. For example, Kabaluk et al. (2007b) noted that *M. anisopliae* F52 (currently *M. brunneum* F52) was more virulent toward the Great Basin wireworm *Ctenicera pruinina* (Horn) found along the Columbia River between Washington and Oregon, than *A. obscurus* and *A. lineatus,* the wireworms found in south coastal British Columbia.

Entomopathogenic fungi, similar to many other biological control agents, have significant implications for the development of sustainable cropping ecosystems. Due to such environmental concerns as insecticide resistance and the impact on pollinators and natural enemies arising from the intense and repeated use of insecticides, the desirability of more environmentally friendly agents such as entomopathogenic fungi is increasing for pest management strategies. These fungi are able to persist in the field for months to years depending on environmental conditions, and have good potential for providing sustainable pest control (Scheepmaker and Butt, 2010). However, in practical use, field efficacy and persistence of entomopathogenic fungi are often limited by various ecological factors, and a better understanding of environmental variables at the site of application would optimize the field use of these fungi (Jaronski, 2010). However, high product costs and high application rates may limit the practical use of the fungi as a simple substitute for chemical insecticides (Hluchy and Samsinakova, 1989; Adane et al., 1996; Rice and Cogburn, 1999; Bourassa et al., 2001; Lord, 2001; Meikle et al., 2001; Padin et al., 2002; Jaronski, 2010). Furthermore, although infection with entomopathogens appears to be relatively common and is sometimes effective at reducing adult populations, its effect on subsequent larval populations is still uncertain.

Further efforts to develop entomopathogens as biopesticides for managing *L. californicus* and *H. bicolor* are needed to screen new, potentially more virulent isolates, including several that have shown good laboratory efficacy and some field efficacy. Finally, enhanced pest control may be achieved by appropriate incorporation of entomopathogenic fungi into an integrated pest management scheme.

## 5. Conclusions

The entomopathogenic fungi used in our study, particularly the two commercial strains, had significant effects on wireworms in the wheat plots, in terms of increase in both plant stand count and yield. Overall, biological control of wireworms using entomopathogenic fungi such as *M. brunneum F52*, *B. bassiana* GHA. and *M. robertsii* DWR 346 is a promising option, especially in the absence of effective chemical agents and given the potential for incorporating their use into an integrated pest management program.

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