

## Soil Type Mediates the Effectiveness of Biological Control Against *Limoni* *californicus* (Coleoptera: Elateridae)

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### Abstract

Wireworms, the larval stage of click beetles (Coleoptera: Elateridae), are a considerable threat to cereal and vegetable production in the Pacific Northwest and Intermountain regions of the United States. As insecticides are generally ineffective, alternative controls are needed to improve wireworm management. Wireworms are continuously exposed to a wide range of subterranean pathogenic organisms in the soil; identifying these organisms and determining their impact would contribute to the development of biological control for wireworms. Here, we evaluated the efficacy of an entomopathogenic nematode, *Steinernema carpocapsae* (Weiser) (Rhabditida: Steinernematidae), and a fungus, *Metarhizium brunneum* Petch (strain F52) (Hypocreales: Clavicipitaceae), for control of the Pacific Northwest predominant wireworm species *Limoni* *californicus*, in two different soil media. We also examined whether diatomaceous earth (DE) increases the efficacy of entomopathogens through facilitating their penetration into the host integument. Treatments containing *M. brunneum* (F52) resulted in the highest rates of wireworm mortality, indicating that the fungus may be more effective than the nematode at reducing population size. However, results were impacted by soil media. In peatmoss-dominated medium, *M. brunneum*-containing treatments were more effective in reducing feeding damage than treatments containing *S. carpocapsae*. However, in sand-dominated medium, treatments with *S. carpocapsae* provided relatively better seedling protection. No consistent effect of DE was detected. Our results suggest that the effectiveness of wireworm biological control agents depends on soil media, such that the application of biological control against wireworms must be made with knowledge of field soil type.

**Key words:** interspecific interaction, cereal, soil texture, emergence success, biological control, integrated pest management

The importance of developing integrated pest management (IPM) strategies for pest control is particularly important when conventional chemical approaches fail to offer a solution. Wireworms are one such example of an ongoing pest problem with limited management options in the Pacific Northwest and Intermountain regions of the United States. Wireworms are the larval stage of click beetles (Coleoptera: Elateridae) and have been a major challenge to a wide range of crops. This is because they live in the soil for several years, continuing to feed on underground plant tissues and moving as deep as 1.5 m into the soil profile when environmental conditions are not favorable (Andrews et al. 2008). In cereals, damage to the sprouting seed can result in emergence failure, while later stage plants can experience delayed growth and yield.

The resurgence of wireworms is largely attributed to bans on persistent conventional pesticides (Vernon et al. 2008). Currently,

neonicotinoid seed treatments are the only pesticide option registered in cereal crops; however, such seed treatments often fail to provide acceptable levels of protection (Vernon et al. 2008, 2009). Moreover, there is uncertainty about the future of neonicotinoid seed treatments due to their potential link with increased bee mortality (Godfray et al. 2014). Thus, while developing IPM strategies for wireworms was historically a low priority given the availability of a diverse set of inexpensive conventional insecticides, there is now an urgent need to rigorously investigate alternative management approaches.

As soil dwelling organisms, wireworms are exposed to a wide range of natural enemies that inhabit the same subterranean environment. Wireworm mortality from entomopathogenic fungi, bacteria, and nematodes has been documented (Furlan et al. 2009, Barsics

et al. 2013, Traugott et al. 2015); studies have also investigated the effectiveness of *Metarhizium* spp. (Kabaluk and Ericsson 2007a,b; Reddy et al. 2014), *Beauveria bassiana* (Ester and Huiting 2007, Ladurner et al. 2009, Kölliker et al. 2011, Sufyan et al. 2017), and the parasitic nematode *Steinernema* spp. (Toba et al. 1983, Ester and Huiting 2007, Morton and Garcia-del-Pino 2017) for managing wireworms. While some of these studies demonstrated substantial wireworm mortality, or reduced crop damage (e.g., Ladurner et al. 2009, Reddy et al. 2014, Morton and Garcia-del-Pino 2017), others have proven less promising (Ester and Huiting 2007, Kölliker et al. 2011); environmental variables such as temperature, food availability, and exposure time may influence the efficacy of entomopathogenic agents (Kabaluk and Ericsson 2007b).

While the entomopathogenic fungi (EPF) have generally been effective for wireworm (larva) control (Campos-Herrera and Gutiérrez 2009, Půža and Mráček 2010, San-Blas et al. 2012, but see Toba et al. 1983, Morton and Garcia-del-Pino 2017), *M. brunneum* has also shown efficacy against the adult stage of wireworms, especially when applied with pheromone (Kabaluk et al. 2013, Kabaluk et al. 2015). Moreover, improved effectiveness of the entomopathogenic nematodes (EPN) when they are applied in combination with EPF has also been reported in lepidopterans (Barbercheck and Kaya 1991, Acevedo et al. 2007, Schulte et al. 2009) and coleopterans (Choo et al. 2002, Anbesse et al. 2008). Fungal infections might enhance nematode efficacy by impairing avoidance behaviors of host organisms, or by increasing the rate of host respiration, which can attract nematodes (Ansari et al. 2008). However, the underlying mechanisms of such synergistic interactions remain poorly understood. Moreover, differences in soil characteristics may explain variability in the observed efficacies of various biological control against wireworms. For instance, wireworm responses to environmental cues are known to be influenced by soil characteristics such as porosity and texture (Jones and Shirck 1942, Parker and Seeney 1997, van Herk and Vernon 2006, Hermann et al. 2013), which could subsequently affect their movement, behavior, and related ecological interactions with natural enemies in the soil. In addition, the thick integument of the later larval instars in most wireworm species can pose a physical barrier, limiting the ability of the entomopathogenic organisms to penetrate their host. Therefore, presenting wireworms with an environment which increases the likelihood of physical damage to their integument may facilitate entomopathogenic infections. The natural dust particle, diatomaceous earth (DE), is known for its efficacy in damaging waxy cuticles of insects, which would ultimately result in mortality due to desiccation (Ebeling 1971, Korunić 1998). However, the effectiveness of DE in facilitating entomopathogenic infections in wireworms has yet to be determined.

Here, we evaluated efficacies of biological control against one of the most damaging wireworm species in the Pacific Northwest (Esser et al. 2015, Milosavljević et al. 2015) and Intermountain (Morales-Rodriguez et al. 2014, Rashed et al. 2015) regions of the United States, *Limonius californicus* (Mannerheim) (Coleoptera: Elateridae). Our objectives were to: 1) evaluate effectiveness of two commercially available biological control organisms, the EPN *S. carpocapsae* and the EPF *M. brunneum* (starin F52), in protecting wheat plants; 2) determine whether a combined EPF–EPN application offered synergistic protection against wireworms; and 3) examine whether the addition of DE improved the effectiveness of the biocontrol agents. As both the extent of wireworm damage (Rashed et al. 2017), and the efficacy of EPN (Edit and Thurston 1995), can be influenced by soil texture, all evaluations were conducted in both sand-dominated and peatmoss-rich media.

## Materials and Methods

### Wireworm Collection and Study Location

The sugar beet wireworm *L. californicus* was collected in June 2017 from a heavily infested dryland wheat field near Ririe, ID, using solar bait traps as described in Rashed et al. (2015). In summary, each trap consisted of germinating wheat and barley seeds placed at approximately 15-cm depth, topped with soil, and then covered by a dark plastic. Collected wireworms were maintained individually in 5 × 5 × 10 cm (W × L × H) plexiglass containers filled with field soil and two barley seeds. Wireworms were kept in the containers until being used in the experiment within 10 d of collection. The study was carried out in the University of Idaho, Aberdeen Research and Extension Center greenhouses in Aberdeen, ID, from June to July 2017. The average daily temperature in the greenhouse was 26.8°C (SE: 0.3°C).

### Soil Media and Experimental Pots

Two soil media were prepared by manipulating sand:peatmoss (Sun Gro Horticulture Canada Ltd., Seba Beach, AB, Canada) ratios. Both media mixes contained fixed amounts of fertilizer (Osmocote, Scott-Sierra Horticultural Products Co., Marysville, OH) and vermiculite (Therm-o-Rock West Inc., Chandler, AZ). The sand-dominated medium consisted of 75% sand and 25% peatmoss; the peatmoss-rich medium was a 50:50 mix. Soils were homogenized prior to placement in pots.

### Experimental Design and Treatments

Experiments were conducted in two ‘time-blocks’, 1 wk apart, and in two separated greenhouse chambers. There was a total of nine treatments, in each soil medium per time-block (Table 1). Thiamethoxam, a commonly used neonicotinoid to manage wireworm damage, was included as a chemical control treatment. Our treatments also included a non-treated positive (wireworm, with no treatment) and a noninfested negative (no wireworm) control. The remaining treatments of *S. carpocapsae*, *M. brunneum*, and the combination of the two, with or without DE, constituted the remaining experimental treatments (Table 1). There were 5 pot-replicates per treatment per time-block (10 pot-replicates total per treatment), arranged in a completely randomized design. Each pot was 22.9 × 22.9 × 24.1 cm (W × L × H) and contained a single *L. californicus* larva that was placed 10 cm below the soil surface; larvae were selected from late instars for uniformity in size and averaged 15.1 (SE = 0.16) mm in length. After wireworms were added to pots, each pot was planted with four spring wheat seeds (var. UI Stone; Chen et al. 2013), one in each corner of the pot, at a 2.5-cm depth. All entomopathogenic mixtures were suspended in 473 ml of water and applied across the soil surface, prior to covering the dropped seeds, to simulate at-planting field applications. The 473 ml suspension volume was selected to prevent loss of natural enemies through drainage. *Metarhizium brunneum* strain F52 (Met52 EC), previously classified and incorporated as commercial product *M. anisopliae* by Novozymes Biological Inc. (Rehner and Kepler 2017), was obtained from Evergreen Growers Supply LLC., Clackamas, OR. The product was applied at the rate of 3 ml per pot (EPF), following the highest rate that is recommended for drench application in pot. *Steinernema carpocapsae* (ARBICO Organics Co., Oro Valley, OR) was applied at the rate of 19 ml (~52,258 EPN) per pot (EPN). The application rate for DE (Perma-Guard, Bountiful, UT) treatment was 0.88 g for each pot (168 kg/ha). Similar to the entomopathogens, DE powder was first suspended in water (or EPF/EPN mix, depending on

**Table 1.** Details of treatments applied in both soil media

Medium	Treatment	Replicates per block	<i>Metarhizium brunneum</i> (EPF)	<i>Steinernema carpocapsae</i> (EPN)	DE	Seed treatment	<i>Limonius californicus</i>
Sand-dominated	1	5	–	–	–	✓	✓
	2	5	✓	✓	✓	–	✓
	3	5	✓	✓	–	–	✓
	4	5	–	✓	–	–	✓
	5	5	✓	–	–	–	✓
	6	5	✓	–	✓	–	✓
	7	5	–	✓	✓	–	✓
	8	5	–	–	–	–	✓
	9	5	–	–	–	–	–
Peatmoss-dominated	1	5	–	–	–	✓	✓
	2	5	✓	✓	✓	–	✓
	3	5	✓	✓	–	–	✓
	4	5	–	✓	–	–	✓
	5	5	✓	–	–	–	✓
	6	5	✓	–	✓	–	✓
	7	5	–	✓	✓	–	✓
	8	5	–	–	–	–	✓
	9	5	–	–	–	–	–

Treatments involved combinations of entomopathogens (EPF and/or EPN), DE, and thiamethoxam seed treatment.

treatment) and then applied to the soil surface, prior to covering seeds. Thiamethoxam (CruiserMaxx, Syngenta, Greensboro, NC) treatment was applied as seed treatments at the recommended rate of 325 ml/100 kg.

### Evaluations

Emergence success, probability of plant damage, damage latency (number of days to damage), and plant biomass were recorded in each pot. Plant damage was measured as the presence of wilted or dead central leaf, the presence of point of feeding (just below the soil surface) at harvest, and/or seedling death. Above- and belowground tissues were removed 5 wk after planting and dried at 40°C for 96 h prior to biomass determination.

After harvest, soil media within each pot was screened twice through #4 and #14 sieves. If the wireworm was not recovered, the soil was inspected by two experimenters, on a white background, to find the wireworm. If wireworm was not found it was scored as dead; wireworm bodies decompose within 3 wk and they cannot be recovered after this point, especially where infected by the EPF (A.R. and P.E., personal observations).

### Statistical Analysis

Statistical analyses were performed in R (3.4.1; R Core Team). We used generalized linear mixed models (GLMMs) to explore the fixed effects of soil media, treatment, and the treatment × soil interaction, and the random effect of time-block, on latency (to damage) and plant biomass. Where a significant treatment × soil interaction was present, results from type III Wald chi-square tests are presented. These models assumed a normal distribution of the response variables based on the distributions of the observed data (Shapiro–Wilk normality tests,  $P > 0.05$  for both response variables). Total plant biomass was calculated on a per-pot basis. Similar models with the same explanatory variables were used for germination success, the probability of feeding damage, and wireworm mortality, except these models included a binomial error distribution, with a logit link, based on the distribution of the responses. ‘Noninfested’ (no wireworm) controls were not included in feeding damage analyses.

## Results

### Wireworm Mortality

Wireworm mortality was affected by treatment ( $X^2 = 14.84$ ,  $df = 7$ ,  $P = 0.038$ ; Fig. 1). The highest mortality was observed in EPF/DE (55%), followed by treatments EPN/EPF (50%), EPN/EPF/DE (50%), and EPF (44.5%), all of which included the EPF. Mortality rates in thiamethoxam (30%), EPN (14.3%), and EPN/DE (20%) treatments were not significantly different from the nontreated control (15%) (Fig. 1). No significant effects of soil medium, and soil × treatment were detected.

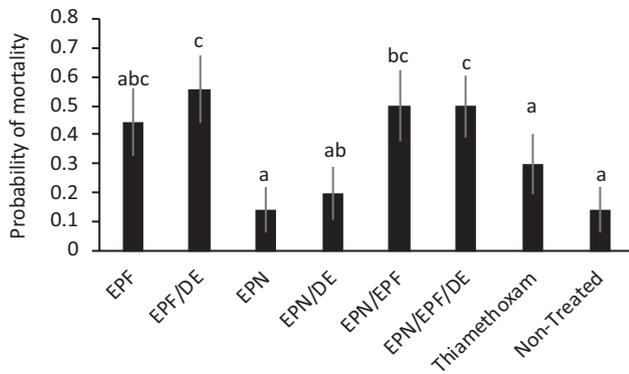
### Germination Success, Wireworm Damage Rate, and Latency

Germination success did not vary among treatments ( $X^2 = 8.63$ ,  $df = 8$ ,  $P = 0.37$ ), ranging from 85 to 98%; the highest rate of emergence occurred in the noninfested controls. No significant effects of soil medium or soil × treatment interaction were detected.

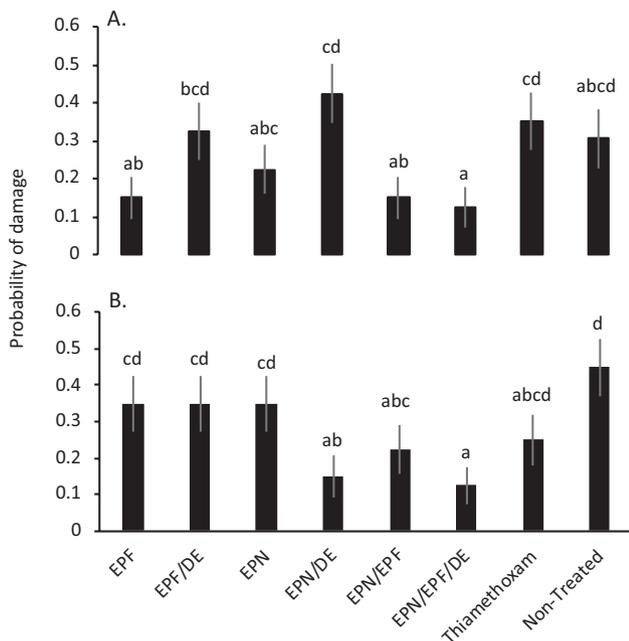
The rate of wireworm damage, however, was affected by both treatment ( $X^2 = 16.76$ ,  $df = 7$ ,  $P = 0.019$ ) and soil ( $X^2 = 4.06$ ,  $df = 1$ ,  $P = 0.043$ ), with an overall higher probability of damage associated with sand-dominated than peatmoss-dominated medium. A significant soil × treatment interaction ( $X^2 = 15.29$ ,  $df = 7$ ,  $P = 0.032$ ) was also detected and results for each soil medium are presented separately (Fig. 2).

In both soil media, the lowest rate of damage was associated with the EPN/EPF/DE treatment (Fig. 2A and B). In peatmoss-dominated medium, the lowest predicted probabilities of damage were associated with EPF (0.15) and EPN/EPF/DE (0.12), but not with EPF/DE (0.32) (Fig. 2A). Overall, predicted probabilities of wireworm damage were observed to be relatively lower for EPF, EPF/DE, and EPF/EPN/DE treatments compared to the thiamethoxam (0.35) and EPN/DE (0.42) treatments (Fig. 2A).

The predicted probability of damage in sand-dominated medium was observed to be the lowest in EPN/DE (0.15), EPN/EPF (0.22), and EPN/EPF/DE (0.12), which were significantly lower than the nontreated control (0.45), but not different from the thiamethoxam seed treatment (0.25) (Fig. 2B).



**Fig. 1.** The average probability of mortality in each of the biological and chemical treatments including noninfested (no wireworm), nontreated wireworm, seed treatment (thiamethoxam), EPF, EPN, and the combination of the two, applied with (EPF/EPN/DE) and without (EPF/EPN) DE. Different letters represent the presence of significant differences. Error bars represent SEs ( $\pm 1$ ).

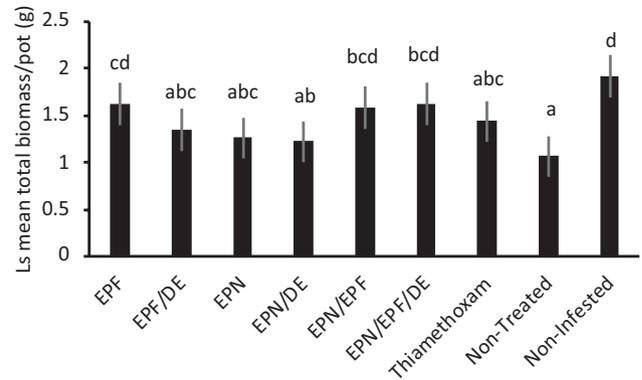


**Fig. 2.** The average probability of feeding damage in each of the biological and chemical treatments including nontreated, seed treatment (thiamethoxam), EPF, EPN, and the combination of the two, applied with (EPF/EPN/DE) and without (EPF/EPN) DE, in peatmoss-dominated (A) and sand-dominated (B) media. Different letters represent the presence of significant differences. Error bars represent SEs ( $\pm 1$ ).

Time to damage, i.e., damage latency, was not affected by treatment ( $X^2 = 11.56$ ,  $df = 7$ ,  $P = 0.12$ ) or soil media ( $X^2 = 0.61$ ,  $df = 1$ ,  $P = 0.43$ ). In the peatmoss-dominated medium, the average number of days ( $\pm$ SE) to detect wireworm feeding damage in the emerged seedlings was 19.4 (0.87) versus the 18.5 (0.79) recorded for the sand-dominated soil. No significant soil medium  $\times$  treatment interaction was detected.

### Plant Biomass

Total plant biomass was affected by treatment ( $X^2 = 22.58$ ,  $df = 8$ ,  $P = 0.004$ ) but not by soil media ( $X^2 = 1.27$ ,  $df = 8$ ,  $P = 0.26$ ) or the interaction between the two factors ( $X^2 = 11.26$ ,  $df = 8$ ,  $P = 0.19$ )



**Fig. 3.** Least square means of total plant biomass, per pot, in the evaluate treatments that included noninfested (no wireworm), nontreated, seed treatment (thiamethoxam), EPF, EPN, and the combination of the two, applied with (EPF/EPN/DE) and without (EPF/EPN) DE. Different letters represent the presence of significant differences. Error bars represent SEs ( $\pm 1$ ).

(Fig. 3). Average plant biomasses in EPF ( $1.95 \pm 0.19$ ), EPN/EPF ( $1.90 \pm 0.22$ ), and EPN/EPF/DE ( $1.96 \pm 0.21$ ) were not statistically different from the noninfested ( $2.21 \pm 0.14$ ) pots, but were significantly higher than the nontreated controls ( $1.24 \pm 0.14$ ). Although the observed total plant biomasses in EPF/DE ( $1.57 \pm 0.17$ ), EPN ( $1.47 \pm 0.16$ ), and EPN/DE ( $1.48 \pm 0.16$ ) treatments were relatively higher than that of the nontreated controls, the improvement was not significant (Fig. 3).

### Discussion

Our results showed variability in the probability of postmergence damage between the EPF- and EPN-containing treatments, and between sand-dominated and peatmoss-dominated media. Wireworm mortality was primarily associated with EPF-containing treatments, regardless of the soil media. However, no synergistic effect on mortality was detected in EPN/EPF, since no statistical difference was present among EPF, EPF/EPN, and EPF/EPN/DE; this also indicates that no antagonism between natural enemies was observed.

While the highest rate of mortality was achieved in the EPF/DE treatment, all EPF-containing treatments resulted in relatively higher mortality rates than the nontreated, EPN, EPN/DE, and thiamethoxam treatments. This supports a study by Ansari et al. (2009), who reported *Agriotes lineatus* mortality rates between 10 and 100% following direct exposure to inoculum of 10 different strains of *M. anisopliae* in laboratory trials. Increases in plant biomass were also associated with the majority of EPF-containing treatments when compared to the nontreated wireworm controls. The observed 30% mortality in our thiamethoxam treatment, and the lack of difference from our nontreated control, is in line with previous studies that show neonicotinoids do not cause considerable wireworm mortality but rather reduce feeding damage by inducing morbidity (Van Herk et al. 2007, Vernon et al. 2008).

Soil physical characteristics are known to affect the extent of wireworm damage to crops (Milosavljević et al. 2016, Rashed et al. 2017), with greater damage often associated with sandy soil (Hermann et al. 2013, Rashed et al. 2017). Similarly, 48.6% and 33.2% of seedlings in our study were affected by wireworms in sand-dominated and peatmoss-rich media, respectively. Quick water depletion in the porous sand has been suggested to stimulate wireworm searching for moisture in succulent underground plant tissues such as

potato tubers (Hermann et al. 2013). It is also possible that increased plant residue and organic matter in the peatmoss-dominated medium provided wireworms with an alternate food source (Hemerik and de Fluiter 1999), which reduced feeding on the wheat seeds. However, Traugott and colleagues (2007, 2008) indicated soil organic contents only constitute a negligible portion *Agriotes* spp. diet, making this unlikely. Moreover, wireworm damage latency was not influenced by soil media, indicating that the presence of plant residue in peatmoss-dominated soil did not inhibit wireworm foraging.

Although no effects of soil media or treatment were detected on germination success, significant variations in postemergence feeding damage were present. In the sand-dominated medium, the combination of EPN and EPF, either with (EPN/EPF/DE) or without (EPN/EPF) DE, and the combination of EPN and DE (EPN/DE) significantly reduced the rate of damage to wheat seedlings compared to the nontreated wireworm controls and applications of either EPN or EPF alone. However, a synergistic interaction between EPN and EPF is unlikely to explain the effectiveness of EPN/EPF and EPN/EPF/DE, since the EPN/DE treatment was also effective in reducing damage. Although nonsignificant, the probability of damage in sand-dominated medium was also relatively lower in EPN-treated pots than the nontreated controls (Fig. 2). The effectiveness of the EPN in the sand-dominated medium might be due to improved dispersal and survival in more porous sandy soils (Moyle and Kaya 1981, Kaya and Gaugler 1993). Although a previous potted field trial concluded nearly 50% *A. obscurus* mortality following *S. carpocapsae* application (Morton and Garcia-del-Pino 2017), in our study the average mortality rate due to *S. carpocapsae* did not exceed 20% in the absence of the EPF. The difference between these results might be explained by variation in wireworm species or by the use of primarily late instar larvae in our study; late instar wireworms are hard-bodied and less vulnerable to nematode infestation than early instars. Since no considerable mortality was detected for EPN-only treatments (EPN and EPN/DE), the overall reduced damage in nematode treatments in the sand-dominated medium might have been the result of manipulated wireworm behavior following infestation. While nematode infestation could eventually lead to wireworm mortality over time, the nonlethal effect(s) of nematodes on wireworm behavior and feeding warrants additional studies.

The EPF *M. brunneum* (strain F52) appeared to play a key role in reducing wireworm damage. In the peatmoss-dominated medium, damage rates in the presence of *M. brunneum* (EPF) and both *M. brunneum* and *S. carpocapsae*, with (EPN/EPF/DE) or without (EPN/EPF) the addition of DE, were significantly lower than pots treated with a mixture of *S. carpocapsae* and DE as well as those which were treated with thiamethoxam. Damage rates in EPF, EPN/EPF, and EPN/EPF/DE treatments were consistently, but nonsignificantly, lower than the nontreated control. The improved efficacy of EPF-containing treatments in peatmoss-dominated medium can be attributed to its relatively higher moisture retention than the sand-dominated medium. This supports a previous report of increased *M. anisopliae* (= *brunneum*) incidence in soil with relatively higher organic content (Quesada-Moraga et al. 2007).

Our results indicate that soil texture and composition may mediate the effectiveness of *S. carpocapsae* and *M. brunneum* (strain F52). While *M. brunneum* was more effective in protecting plants in the peatmoss-rich soil, *S. carpocapsae* treatments were more effective in the sand-dominated medium. The addition of DE did not appear to improve the efficacy of either natural enemy. However, the reduced damage observed in EPN/DE and EPN/EPF/DE treatments in sandy media suggests that DE might facilitate nematode infestation of wireworms or interfere with

wireworm movement within the sandy soil, where water depletion happens quickly, thus improving DE efficacy. As the mode of action of this product is primarily through damaging the protective cuticle of pests, elevated moisture levels can interfere with DE function (Ebeling and Wagner 1959, Ebeling 1971, see Korunić 1998, for a review).

Overall, *M. brunneum* (F52) appeared the more effective natural enemy in terms of ability to reduce *L. californicus* numbers. However, soil application of both *S. carpocapsae* and *M. brunneum* (F52) showed potential in reducing *L. californicus* damage to wheat seedlings, and in some cases were more effective than the chemical seed treatment. Our findings are consistent with those of a field study by Reddy et al. (2014) reporting efficacies of *B. bassiana*, *M. brunneum*, and *M. robertsii*, against *L. californicus*, as comparable to imidacloprid seed treatment. The observed efficacy of the biological control organisms in reducing feeding damage was not consistent between soil media, indicating that recommendation of biological control agent must be made with knowledge of field soil type. Moreover, in this we focused on late instars of *L. californicus* which is one of the most difficult species of wireworm to manage in Western, Pacific Northwest, and Intermountain regions of the United States.

While application of the EPN and EPF against wireworms in potted greenhouse trials appeared promising, evaluating efficacies of EPN and EPF under field circumstances would be an essential step as differences in application dose and other environmental variables may impact outcomes. Thus, future field studies in various soil types, and with different species of wireworms, will improve our understanding of entomopathogenic organism ecology and their overall efficacy against wireworms.

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