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Short Communication

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Efficacy of Naturally Occurring and Commercial Entomopathogenic Nematodes Against Sugar Beet Wireworm (Coleoptera: Elateridae)

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Abstract

Wireworms are the larval stage of click beetles (Coleoptera: Elateridae), and some of their species are serious pests of many crops. In the present study, we evaluated the efficacy of naturally occurring and commercial entomopathogenic nematode species against the sugar beet wireworm, *Limonius californicus* (Mannerheim), in the laboratory. First, efficacies of *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) collected from an irrigated (*S. feltiae*-SSK) and a dryland (*S. feltiae*-SSC) field and the two commercial entomopathogenic nematode species, *S. carpocapsae* (Weiser) (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae), were examined. Efficacies of the two field-collected *S. feltiae* isolates were also compared against a commercial *S. feltiae* strain. In the first bioassay, *S. feltiae*-SSK caused 63.3% wireworm mortality, followed by 30% caused by *S. carpocapsae*, 23.3% by *S. feltiae*-SSC, and 6.7% by *H.bacteriophora*. In the second assay, *S. feltiae*-SSK killed 56.7% of the wireworms, ≈2.1- and ≈5.7-fold higher than *S. feltiae*-SSC and the commercial isolate, respectively.

Key words: biological control, click beetle, integrated pest management, subterranean pest, natural enemy

Wireworms are the subterranean larval stage of click beetles (Coleoptera: Elateridae), herbivores that feed on underground plant tissues of a wide range of crops. The damage they inflict causes delayed growth, seedling death, yield and quality loss, and facilitates secondary pathogenic infections (Traugott et al. 2015). Historically, wireworm populations were suppressed by broad-spectrum conventional organochlorine insecticides, but those chemistries are no longer registered in the United States, due to human health and environmental concerns (Toba et al. 1985, Vernon et al. 2008). The lack of effective insecticides in small grains and the increased popularity of cultural practices that favor wireworm survival (e.g., reduced tillage) probably contribute to a resurgence of wireworms (Parker and Howard 2001, Jedlička and Frouz 2007, Vernon et al. 2008, Adhikari and Reddy 2017).

Wireworms are exposed to soil-dwelling natural enemies including entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae (Grewal et al. 2005). Application of *Steinernema feltiae* (Filipjev), *Steinernema carpocapsae* (Weiser), and *Steinernema riobrave* Cabanillas (Rhabditida: Steinernematidae), Poinar & Raulston, can reduce damage from sugar beet wireworm, *Limonius californicus* (Mannerheim), under laboratory conditions (Toba et al. 1983, Sandhi et al. 2020a). Although *S. feltiae* was ineffective against the wireworm, *Agriotes* spp. (Coleoptera: Elateridae), *Heterorhabditis bacteriophora* Poinar proved to be effective against them (Ester and Huiting 2007, Ansari et al. 2009). Differences in entomopathogenic nematode efficacies might be related to variation in their foraging strategies and species-specific differences in the ecology of the targeted wireworms (Milosavljević et al. 2017). *Heterorhabditis* spp. and *S. glaseri* Steiner (Rhabditida: Steinernematidae) actively search for hosts using host cues (Lewis et al. 1992, Griffin et al. 2005), whereas *S. carpocapsae* tend to remain stationary until opportunistically attaching to a mobile host (Gaugler and Campbell 1993).

Some species, such as *S. feltiae* and *S. riobrave*, have an intermediate foraging strategy enabling them to be effective against mobile and sedentary hosts (Lewis 2002, Lewis et al. 2006). The efficacy of entomopathogenic nematodes is also influenced by environmental variables (Kung et al. 1991, Kaya and Gaugler 1993, Lewis et al. 2006, Kaspi et al. 2010, Ensafi et al. 2018); hence, naturally occurring entomopathogenic nematodes that are constantly exposed to adverse soil environments and different endemic hosts might be more effective against wireworms compared with commercial laboratory-reared nematodes (Campos-Herrera and Gutiérrez 2009, Rojht et al. 2009, Barsics et al. 2013, Sandhi et al. 2020b).

Limonius californicus is the most damaging and common wireworm species in Idaho, known to be active in wheat, *Triticum aestivum* L. (Poales: Poaceae), and barley, *Hordeum vulgare* L. (Poales: Poaceae), fields throughout the growing season (Rashed et al. 2015, Milosavljević et al. 2016). *Steinernema feltiae* was detected in wireworm-infested fields of southeastern Idaho. We evaluated the virulence of *S. feltiae* collected from an irrigated and a dryland wheat field against *L. californicus* compared with selected commercially available entomopathogenic nematode species.

Materials and Methods

Wireworms

Wireworms used in our study were collected from a dryland wheat field located in Bonneville County, ID (43.585175, −111.547660), using solar bait traps. Each trap consisted of approximately 236 cm^3 of soaked wheat and barley seed buried in soil to the depth of 15 cm and covered with dark plastic to retain heat and moisture (Rashed et al. 2015). The collected wireworms were placed individually in 5 × 5 × 10 cm (w × l × h) plexiglass containers filled with moist sand, two barley seeds were provided as food, and the containers were kept at room temperature for 1 wk before the bioassays began.

Entomopathogenic Nematodes

Three commercial strains and two field-collected entomopathogenic nematode isolates were evaluated in two bioassays. The three commercial entomopathogenic nematodes, *S. carpocapsae*, *H. bacteriophora*, and *S. feltiae*, were obtained from ARBICO Organics (Oro Valley, OR). The field-collected nematode isolates were identified as *S. feltiae* based on morphological characteristics and were later confirmed using a molecular technique (Ensafi 2018).

Originally, the entomopathogenic nematodes were collected from one dryland (42.765041, −111.682655) and one irrigated (42.702469, −111.564643) wheat field in Caribou Co., ID, in April 2017. First, five soil samples were removed from each field with a 400 cc auger (AMS, American Falls, ID) and mixed in a 5-liter plastic bag. Three subsamples, 100 g each, were placed into a 12 × 6 cm (diameter × height) plastic deli container with seven greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) larvae (Bedding and Akhurst 1975), obtained from Speedy Worm, Alexandria, MN. Containers were kept in darkness at room temperature for 3 d, and then *G. mellonella* larvae were rinsed with distilled water and individually moved onto a White trap (White 1927) to recover infective entomopathogenic nematode juveniles. Hereafter, the *S. feltiae* collected from the dryland and irrigated fields are referred to as *S. feltiae*-SSC (GenBank: MK131018.1) and *S. feltiae*-SSK (GenBank: MK131021.1), respectively. Additional details on field samplings and species identification are provided in Ensafi (2018). A recent areawide survey, using three solar bait traps/field, confirmed that the irrigated field was heavily infested with wireworms *L. californicus* and *Hypnoidus bicolor* (Eschscholtz) (Coleoptera: Elateridae), where a total of 259 wireworms were collected between May and July 2016. The dryland field had low-density infestations with a total of only nine wireworms, belonging to the three species *H. bicolor*, *Hadromorphus glaucus* (Germar) (Coleoptera: Elateridae), and *L. infuscatus* Motschulsky (Coleoptera: Elateridae), collected between May and July 2016.

Soil

The soil was a mixture of 75% sand and 25% peat moss (Sun Gro Horticulture Canada, Seba Beach, Alberta, Canada). The soil also contained 112 g of fertilizer (15-9-12 [N-P-K]; Osmocote, Scott-Sierra Horticultural Products, Marysville, OH) and 228 g of vermiculite (Therm-O-Rock West., Chandler, AZ) per 22 kg of the mix. The peat moss and vermiculate were sterilized at 82°C for 30 min and the sand at 120°C for 4 h in metal trays before mixing. Sand-dominated soils are associated with both increased wireworm damage (Hermann et al. 2013, Rashed et al. 2017) and improved entomopathogenic nematode performance against wireworms (Ensafi et al. 2018).

Efficacy Bioassays

Entomopathogenic nematode efficacies were evaluated in 4.2 × 20.3 cm (diameter × height) cone-shaped plastic pots (375 cc, volume). Each pot was filled with moist soil mix with a single *L. californicus* placed 7 cm below the surface. Three wheat seeds were buried in each pot to stimulate wireworm movement and feeding.

Prior to each bioassay, all entomopathogenic nematodes were reared in *G. mellonella* larvae (Bedding and Akhurst 1975). Newly emerged infective juveniles were stored in distilled water at 7°C for 1 wk, and then they were acclimated to 23°C for 1 h and their mobility was confirmed under a stereomicroscope (Wild Heerbrugg, M3Z, Heerbrugg, Switzerland). The infective juveniles were suspended in 100 ml of distilled water and poured onto the soil surface at a rate of 33 infective juveniles/cm^2 (3,100 infective juveniles/pot, 33 × 10^8 infective juveniles/ha). Control pots were treated with distilled water. The pots were maintained in the laboratory at 23 ± 2°C and 16:8 h (L:D) photoperiod, and wireworms were inspected at 12 d. Dead wireworms were removed and dissected under a stereomicroscope (Wild Heerbrugg, M3Z, Heerbrugg, Switzerland) to determine the presence of entomopathogenic nematode infection. Surviving wireworms were placed individually in containers filled with autoclaved sand and were monitored for one additional week to further assess mortality. Each bioassay was a completely randomized design with three temporal blocks. In each of three temporal blocks, there were 10 replicates for each of the treatments and the controls.

The first bioassay was conducted August 2017 and June 2018 and included four entomopathogen treatments: *S. feltiae*-SSK, *S. feltiae*-SSC, and commercial *S. carpocapsae* (Nemastar) and *H. bacteriophora* (Nematop). The second bioassay was conducted during May and June 2019 to assess the efficacies of the field-collected *S. feltiae*-SSK and *S. feltiae*-SSC, and the commercial *S. feltiae* (Nemaplus).

Statistical Analyses

Generalized linear mixed model (IBM Corp. 2017), assuming a binomial distribution, with a logit link function, was used to compare wireworm mortality among treatments. The model included the

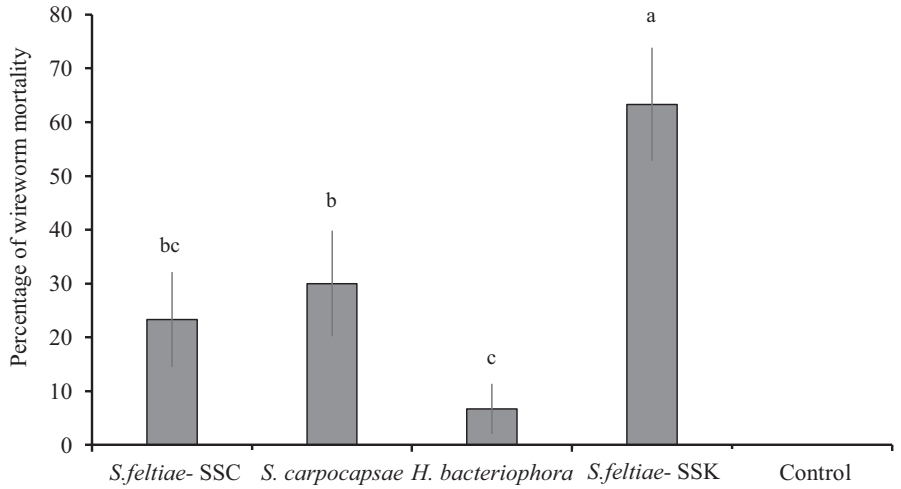

Fig. 1. Percentage of wireworm mortality caused by each entomopathogenic nematode species, across the three temporal blocks. The field collected isolate, *S. feltiae*-SSK, caused significantly higher mortality than the commercial entomopathogenic nematode strains. Different letters over bars indicate significant differences according to least significant difference pairwise comparisons, which followed a generalized linear mixed model analysis, and error bars represent standard errors (±1 SE) of the estimated mean probabilities. No wireworm mortality was recorded in the noninoculated control.

fixed effect of treatment (nematode isolate) and the random effect of temporal block. Least significant difference was used for pairwise comparisons (Carmer and Walker 1985). No mortality occurred in the nontreated controls, and they were excluded from our statistical analyses.

Results

In the first bioassay, the efficacy of the entomopathogenic nematodes against *L. californicus* varied across treatments (F = 6.44; df = 3, 116; P < 0.001) and *S. feltiae*-SSK caused 2.7-, 2.1-, and 9.5- fold more mortality than *S. feltiae*-SSC, and commercial *S. carpocapsae* and *H. bacteriophora*, respectively (Fig. 1). No wireworm mortality occurred in control treatments.

Treatment effects were also detected in the second bioassay (F = 6.67; df = 2, 87; P = 0.002) and *S. feltiae*-SSK caused 2.1- and 5.7-fold more wireworm mortality than *S. feltiae*-SSC and the commercial *S. feltiae*, respectively (Fig. 2). There was no difference between the lethality of the commercial *S. feltiae* and *S. feltiae*-SSC. No wireworm mortality occurred in the controls.

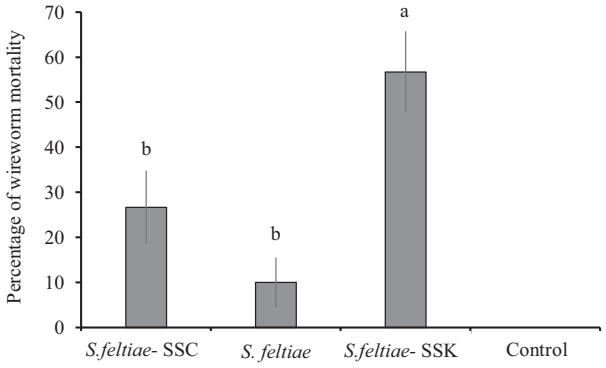

Fig. 2. Percentage of wireworm mortality caused by each field collected *S. feltiae* isolate and a commercial strain of *S. feltiae*. The field collected isolate, *S. feltiae*-SSK, caused significantly higher mortality than both commercial strain and the field collected *S. feltiae*-SSC. Different letters over bars indicate significant differences according to least significant difference pairwise comparisons, which followed a generalized linear mixed model analysis, and error bars represent standard errors (±1 SE) of the estimated mean probabilities. No wireworm mortality was recorded in the noninoculated control.

Discussion

The efficacies of *S. feltiae*, *S. carpocapsae*, and *H. bacteriophora* against *L. californicus* differed, with *H. bacteriophora* being the least effective. The relatively low efficacy of *H. bacteriphora* against *L. californicus* has also been reported by other researchers (Sandhi et al. 2020a, b), as well as against *A. obscurus* (Morton and Garcia-del-Pino 2017). In contrast, Ansari et al. (2009) demonstrated that *H. bacteriophora* can cause up to 67% mortality in lined click beetle, *Agriotes lineatus* (L.) (Coleoptera: Elateridae) larvae. Differences in wireworm species, age, and experimental conditions likely underlie inconsistencies among reports.

Although our first bioassay revealed between-species differences in the efficacies of the evaluated entomopathogenic nematodes against *L. californicus*, the second bioassay confirmed the existence of efficacy variability within *S. feltiae*, including between the two field-collected isolates, with *S. feltiae*-SSK being the most effective. Lethality of *S. feltiae*-SSK is supported by Sandhi et al. (2020b), who reported 48–50% *L. californicus* mortality, in the laboratory, caused by *S. feltiae* isolates from Montana. Sandhi et al. (2020b) also reported a substantial decline in native *S. feltiae* efficacy in 'shade house' experiments. Differences in soils are associated with different levels of wireworm damage (Hermann et al. 2013, Rashed et al. 2017) and with different entomopathogenic nematode efficacies against wireworms and other insect pests (Moyle and Kaya 1981, Kung et al. 1990, Kaspi et al. 2010, Ensafi et al. 2018). Hence, the entomopathogenic nematode efficacies we observed might be altered in accordance with different environmetnal conditions and different strains of entomopathogenic nematodes.

Differences in nematode pathogenicity within species might be influenced by variations among geographic isolates and strains (Kaya and Gaugler 1993), and our two field collected *S. feltiae* isolates differed in terms of infectivity to *L. californicus*. Genetic variation is unlikely to adequately explain the observed difference between *S. feltiae*-SSK and *S. feltiae*-SSC efficacies against *L. californicus*; Ensafi (2018) found variability between the two isolates, determined through partial ribosomal RNA gene complex

sequencing (internal transcribed spacer; ITS1 and ITS2) and D2D3 expansions of 28S, was negligible. It is important to note that sometimes weak entomopathogenic nematode efficacy has been attributed to poor adaptation to the target insect (Kaya and Gaugler 1993, Koppenhöfer and Fuzy 2006); the field from which *S. feltiae*-SSK was collected was infested with *L. californicus*, whereas the field from which *S. feltiae*-SSC was collected was not infested by *L. californicus*. The importance of entomopathogenic nematode adaptation to target insects warrants further investigation.

Different entomopathogenic nematodes can vary in their efficacy against *L. californicus*. In the present study, one of the two field-collected entomopathogenic nematode isolates performed better than selected commercial strains. Our results suggest the importance of preserving naturally occurring entomopathogenic nematodes in field crops and identifying ecological approaches that can maximize their efficacy under field conditions.

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