Persistence of Select Introduced Entomopathogenic Nematodes in the US Southwest as Potential Biological Control for Whitefringed Beetle\(^1\) in Alfalfa

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Abstract. Early stand decrease in alfalfa (Medicago sativa L.) throughout New Mexico is increasingly being attributed to root systems damaged by larvae of whitefringed beetle, Naupactus leucoloma Dejean. Entomopathogenic nematodes (Steinernema carpocapsae Weiser, Steinernema feltiae Filipjev, and Heterorhabditis bacteriophora Poinar) effectively control other species that inflict similar damage to alfalfa and other crops, but it is not known if they occur naturally or can persist if released in irrigated semiarid lands. Persistent strains of entomopathogenic nematodes were released in May 2017 by the New Mexico State University Rex E. Kirksey Agricultural Science Center in Tucumcari into an irrigated alfalfa field not known to be infested with whitefringed beetle. Treatments were mixtures of nematode species Steinernema carpocapsae (NY 001) + S. feltiae (NY 04) or S. feltiae (NY 04) + Heterorhabditis bacteriophora (Oswego) in a randomized complete block design with four replications near nontreated check plots. Twenty soil cores were collected per plot in autumn 2017 and 2018 to bioassay for establishment of entomopathogenic nematodes. After inoculation, S. carpocapsae was not found in any sample either year, while H. bacteriophora was found only in 4% of samples from S. feltiae + H. bacteriophora plots in 2017. Similar percentages of S. feltiae were found each year (~20% of cores in S. feltiae + S. carpocapsae and S. feltiae + H. bacteriophora plots). No entomopathogenic nematode was detected in nontreated check plots, suggesting lack of native entomopathogenic nematodes and lack of spread from treated plots. The multiyear persistence of S. feltiae ‘NY 04’ and perhaps H. bacteriophora ‘Oswego’ in the arid Southwestern US was not reported previously. Because entomopathogenic nematodes can control whitefringed beetle and a plethora of other crop pests with similar larval root-feeding behavior on many economic crops, establishing locally adapted sources of entomopathogenic nematodes for widespread distribution would be appropriate.

Introduction

Alfalfa (Medicago sativa L.) is a significant crop in the US Southwest, with 600,000 acres harvested in June 2018 (NASS 2019). In 2017, alfalfa hay was the second-most valuable cash crop in the State of New Mexico alone, with an estimated annual gross of $171 million (NM AgStats 2018). Additionally, hay yields reflected an 8.7% increase that, coupled with a 9% price increase, led to a revenue increase of

\(^{1}\)Naupactus leucoloma (Coleoptera: Curculionidae)
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more than $26 million for growers in New Mexico, compared to 2016. The overall value of alfalfa hay also is magnified by its essential contributions to livestock production (e.g., meat, milk, and textiles), which continues to lead New Mexico in overall agricultural commodities. According to New Mexico Agricultural Statistics for 2017 (NMAGStats 2018), the dairy industry contributed more than $1.3 billion in total milk sales, with the beef industry contributing almost $825 million in total sales for New Mexico. However, early stand decrease in alfalfa throughout New Mexico is increasingly being associated with root systems damaged by larvae of whitefringed beetle, *Naupactus leucoloma* Dejean (Barnes and De Barro 2009, OEPP/EPPO 1999, Sutherland and Lauriault 2012).

Whitefringed beetle has been associated with more than 385 host plant species (Barnes and De Barro 2009, OEPP/EPPO 1999), with rapid increase in abundance in pure stands of legumes (Barnes and De Barro 2009). Adult whitefringed beetles live above ground, hide in plant debris, and feed by cutting small notches in leaf margins of various plants that causes minimal damage (Barnes and De Barro 2009, OEPP/EPPO 1999, Shields et al. 2009). The flightless adults are transported accidentally from one location to another on any material the beetles crawl onto, into, or under for shelter. During adult life, a whitefringed beetle also disperses in pursuit of host plants and places to lay eggs (Barnes and De Barro 2009).

A single insect can infest an entire field over time because all whitefringed beetles are very fertile females; no males have been found in the USA (Barnes and De Barro 2009, OEPP/EPPO 1999). Larvae complete development feeding on and inside roots, tubers, underground stems, rhizomes, peanut pods, etc. below ground (De Jager et al. 1989, Barnes and De Barro 2009, OEPP/EPPO 1999). Pupation occurs from late April through late July, usually 5 to 15 cm deep in the soil, although some pupae and larvae may be found as deep as 30 cm (Barnes and De Barro 2009, OEPP/EPPO 1999, L. Lauriault personal observation).

Once established, the pests can be managed for some crops but usually not eliminated from a farm (OEPP/EPPO 1999). No currently available insecticides are labeled to control whitefringed beetle larvae in alfalfa, and there are no resistant alfalfa varieties. In New Mexico, alfalfa growers rotate infested fields into permanent perennial grass pastures because of whitefringed beetles, with no hope of replanting alfalfa. Because of the importance of alfalfa to growers in New Mexico and the Southwestern US, development of management strategies for whitefringed beetles is critically important.

Three species of entomopathogenic nematodes (*Steinernema carpocapsae* Weiser, *Steinernema feltiae* Filipjev, and *Heterorhabditis bacteriophora* Poinar) effectively control alfalfa snout beetle, *Otiorhynchus ligustici* (L.), a species related to whitefringed beetle that damages alfalfa roots in a similar way (Shields et al. 1999, 2009; Neumann and Shields 2008, 2011; Shields and Testa 2017). The species prey on other relatives of whitefringed beetles that inflict similar damage to alfalfa (Long et al. 2000). The entomopathogenic nematode strains used against alfalfa snout beetle were locally isolated and reared to protect their native ability to persist in the field. Shields et al. (2018) found the strains persisted for multiple years across soil type and crop rotation from a single low-dose inoculation.

Consequently, the entomopathogenic nematodes might have value in controlling whitefringed beetle in the Southwest US, based on previously reported successes (Barnes and De Barro 2009). Risser et al. (2016) thoroughly reviewed entomopathogenic nematodes when reporting their research. Localized natural strains of the entomopathogenic species of interest (*S. carpocapsae*, *S. feltiae*, and
*H. bacteriophora* are found in natural environments in Oklahoma (Risser et al. 2016), as well as elsewhere throughout the world (Barnes and De Barro 2009). Soil moisture seems to determine the presence and amount of activity with a lower threshold of 55 cm precipitation necessary to sustain entomopathogenic nematodes (Risser et al. 2016). Zepeda-Jazo et al. (2014) reported isolating some native entomopathogenic nematodes only from cultivated sites while others were also isolated from perennial croplands and noncultivated sites in areas with much precipitation in Mexico.

The objectives of the study were to: 1) evaluate the potential of local adaptation by NY entomopathogenic nematode strains effective on alfalfa snout beetle by releasing them into an irrigated alfalfa field and 2) track multiyear persistence of any released strains suggesting local adaptation.

### Materials and Methods

Entomopathogenic nematodes are exempt from EPA pesticide regulation and require no protective equipment for application (Tofangsazi et al. 2018). A local producer who suffered stand loss from whitefringed beetle provided an approximately 14.6-ha irrigated alfalfa field sown the previous summer to evaluate local adaptation of the New York entomopathogenic nematode strains. The soil was Redona fine sandy loam (fine-loamy, mixed, superactive, thermic Ustic Calciargids).

Entomopathogenic nematode species mixtures were chosen based on preferred soil niches by each species. *S. carpocapsae* prefers the top 5 cm of the soil profile, *S. feltiae* prefers the top 20 cm, and *H. bacteriophora* ranges from the surface to 35 cm deep in the profile (Ferguson et al. 1995). Strains used in the study were *S. carpocapsae* 'NY 01’, *S. feltiae* 'NY 04’, and *H. bacteriophora* 'Oswego’. Modified rearing techniques were used to retain genetic encoding for persistence in the field (Shields 2015).

Nematodes were released by the New Mexico State University Rex E. Kirksey Agricultural Science Center at Tucumcari into an area of the alfalfa field of the producer. Treatments were two entomopathogenic nematode species mixtures (*S. feltiae* + *S. carpocapsae* or *S. feltiae* + *H. bacteriophora*) and a nontreated check. Plots were 18.3 x 18.3 m arranged in a randomized complete block design with four replications at the inner edge of the outer span of the three-span sprinkler system. An area near the outer edge of the middle span at least 15.3 m from the treated plots was four replications of nontreated plots. The experimental design was necessary because of the documented ability of persistent entomopathogenic nematodes to move with harvest equipment and grazing animal hooves.

A low-labor rearing method was used at the Cornell University Entomology Laboratory to rear infective juvenile nematodes in parasitized larvae of greater wax moth, *Galleria mellonella* (L.), in containers of sawdust (Testa and Shields 2017). Each container of *S. carpocapsae* or *H. bacteriophora* contained ~25 million or *S. feltiae* ~17 million infective juveniles. The containers were received by next-day delivery on the morning of application and prepared by repeatedly rinsing the entomopathogenic nematode-laden sawdust and greater wax moth larvae cadavers through a 2x layer of window screen with 37.85 L water. A small plot sprayer (3.05-m boom) with the nozzles and screens removed was used to dribble streams 20 inches apart at a rate of approximately 3 liters per 100 m² across the center 12.2 m of each treated plot. Approximately 510 million *S. carpocapsae* or *H. bacteriophora*, or 174 million *S. feltiae* were applied per acre.
Applications were between 1900 and 2030 hours (Neumann and Shields 2008) on 25 May 2017, when beginning and ending air temperatures were 29 and 24°C, respectively; the afternoon warm 10-cm soil temperature was 27°C and the cool temperature the following morning was 17°C. The first treatment applied was S. feltiae + H. bacteriophora. The sprayer was thoroughly rinsed before S. feltiae + S. carpocapsae was applied. Plot areas were sprinkler-irrigated with approximately 1.25 cm within 12 hours of application to facilitate movement of entomopathogenic nematodes into the soil (Shields et al. 1999).

Irrigation was applied from early April through October each year through a center-pivot system to prevent observable moisture stress. The field was grazed by horses during the winter of 2017-2018. In spring 2018, 225 kg/ha of 11-52-00 were applied uniformly to the field based on soil test recommendations. The horses had not been allowed to graze before samples were collected in 2018; however, there was evidence of moderate grazing by mule deer.

From 31 October to 2 November 2017 and 28-29 November 2018, the center 12.2 x 12.2 m was sampled for nematodes. Samples were collected from bare ground. In 2017, the field had been harvested recently with little regrowth. Soil moisture at sampling time was near field capacity, having been irrigated right before the recent harvest, and soil temperatures at 10 cm averaged 16.7 and 9.4°C and air temperatures averaged 17.8 and 5.0°C, for the daily warm and cool, respectively. In 2018, the alfalfa had been harvested about 4 weeks earlier and was in a semi-rosette growth phase with patches of prostrate rosettes and upright growth comingled. Soil moisture at sampling time was near field capacity, despite not having been irrigated for several weeks, soil temperatures at 10 cm averaged 8.3 and 2.2°C and air temperatures averaged 21.1 and 3.3°C, for the daily warm and cool, respectively.

Plots were sampled by treatment, beginning with the nontreated check followed by S. feltiae + H. bacteriophora and then S. feltiae + S. carpocapsae, and the core sampler was rinsed after each treatment. Twenty, 2.5-cm-diameter soil cores were collected from each plot to 15 cm on a 4 x 5 grid uniformly spaced across the plot. Each core was separated into the top 5 cm and the next 10 cm and placed in separate containers to be bioassayed at the Cornell University Entomology Laboratory for entomopathogenic nematodes by species. Samples were bioassayed in the laboratory by exposing the soil in the sample to indicator host insect larvae (greater wax moth) and incubated at room temperature for 7 days. Results were expressed as a percentage of the 20 samples in a plot having nematodes as indicated by parasitized greater wax moth larvae (Shields et al. 1999). A separate soil sample representing the entire field was collected for fertility each year.

Data from 2017 and 2018, as a percentage of each entomopathogenic nematode species released, were analyzed using the Mixed procedure of SAS (SAS Institute 2010) to determine if differences existed between years (2017 and 2018) and among treatments (nontreated check, S. feltiae + S. carpocapsae, and S. feltiae + H. bacteriophora) and for year x treatment interaction. Treatments and years were considered fixed effects (2018 had to follow 2017 and the environment and percentage of a given entomopathogenic nematode species in 2017 could have had an effect on the results in 2018), and replications were considered random. When differences among treatment means or within the interaction were significant ($P \leq 0.05$), they were separated by least significant difference using the PDMIX800 SAS macro (Arnold M. Saxton, University of Tennessee, Knoxville, 2000). Means were separated by protected least significant difference ($P < 0.05$).
Results

Year and year x treatment interaction effects were not significant for any entomopathogenic nematode (Table 1). *S. carpocapsae* was not in any sample, and statistics were not determinable. *H. bacteriophora* was found only in about 4% of *S. feltiae* + *H. bacteriophora* samples in 2017, when it was not different from zero of the nontreated check and *S. feltiae* + *S. carpocapsae* plots (data not shown). The percentages of samples with *S. feltiae* in *S. feltiae* + *S. carpocapsae* and *S. feltiae* + *H. bacteriophora* were significantly greater than zero and consistent across years as indicated by the non-significant interaction.

Table 1. Percentage of Entomopathogenic Nematodes by Species in Soil Cores in Alfalfa at Tucumcari, NM, after Being Applied on 27 May 2017

<table>
<thead>
<tr>
<th></th>
<th><em>Steinernema carpocapsae</em></th>
<th><em>S. feltiae</em></th>
<th><em>Heterorhabditis bacteriophora</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>November 2017</td>
<td>0.0</td>
<td>14.9</td>
<td>1.3</td>
</tr>
<tr>
<td>November 2018</td>
<td>0.0</td>
<td>12.2</td>
<td>0.0</td>
</tr>
<tr>
<td>SED</td>
<td>----</td>
<td>1.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not treated</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Sf + Hb</td>
<td>0.0</td>
<td>18.8</td>
<td>1.9</td>
</tr>
<tr>
<td>Sf + Sc</td>
<td>0.0</td>
<td>21.9</td>
<td>0.0</td>
</tr>
<tr>
<td>SED</td>
<td>----</td>
<td>1.7</td>
<td>0.7</td>
</tr>
</tbody>
</table>

| P-value  | Year       | ND | 0.1876 | 0.1380 |
|          | Treatment  | ND | <0.0001 | 0.1196 |
|          | Year x treatment | ND | 0.3380 | 0.1196 |

aData are means of four replications as the percentage of 20 soil cores per plot collected in November 2017 and 2018, in which the nematode species was present. bSED and ND signify standard error of the difference and not detectable statistically. cNontreated plots were in an adjacent area and not within the statistical randomization of *S. feltiae* + *H. bacteriophora* and *S. feltiae* + *S. carpocapsae*, to minimize likelihood of incidental contamination. Data for the nontreated check were included in the statistical analyses.

Discussion

Application rates of infective juvenile nematodes were similar to those used by Neumann and Shields (2008) for *H. bacteriophora* and *S. carpocapsae* in two-species mixtures, but only about 1/3 for *S. feltiae*. Shields et al. (1999) reported a difference in soil distribution 7 days post-application between application of 1 and 6 billion infective juveniles of *H. bacteriophora*, but the treatments were similar by 708 days post-application. The climate at the location of the study was semiarid, subtropical continental with annual average temperatures of 14.4°C and 40.5 cm total annual precipitation of which approximately 80% falls between April and October. The environmental conditions were described as good for development of larvae of whitefringed beetles.
(Barnes and De Barro 2009), as well as entomopathogenic nematodes (Long et al. 2000). Risser et al. (2016) reported that soil moisture might determine the presence of specific entomopathogenic nematodes, and Barnes and De Barro (2009) reported the same for whitefringed beetle. Irrigation used to supplement precipitation in the study was sufficient to maintain soil moisture above the amount necessary to sustain entomopathogenic nematodes reported by Risser et al. (2016). Shields et al. (1999) and Neumann and Shields (2008) reported greater numbers of positive samples collected about 1 week after release when release was later, allowing for more optimum soil temperatures in New York (14 May versus 29 June).

Consistent with results of the study that no entomopathogenic nematodes were found in nontreated plots 6 and 18 months after release (Table 1), Shields et al. (1999) and Neumann and Shields (2008) reported no detection of naturally occurring entomopathogenic nematodes before release. Lack of prior presence was not surprising in the region because of scarce precipitation (Risser et al. 2016). The study area was not part of the Ogallala or any other major aquifer used for irrigation, and irrigation using surface water has been available only for a little more than 60 years. Additionally, that entomopathogenic nematodes were still not found in nontreated plots 18 months after release when the field had been grazed during the intervening winter suggested that transfer by hoof action did not occur, which was reported as being possible (P. Porter personal observation). Finally, lack of spread of entomopathogenic nematodes to nontreated plots might have been because of few hosts in the field. Neumann and Shields (2008) reported the spread of entomopathogenic nematodes to nontreated plots after 2 years in a field very infested by alfalfa snout beetle; however, the plot size was smaller than those used in the present study and nontreated plots were randomized together with treated plots. Shields et al. (2009) reported movement from research plots to throughout the farm within 5-10 years after release.

While presence of *S. carcocapsae* in northern New York has been about 10% in samples (Neumann and Shields 2008, Shields et al. 2009), none was found in the present study (Table 1). That was attributed to the environment in which the top 5 cm of soil where *S. carcocapsae* are active (Shields et al. 1999, 2009; Neumann and Shields 2008, 2011) are characterized by warm temperatures and dry conditions.

The presence of *S. feltiae* was typical of what has been found in northern New York (20-30%; Shields et al. 1999, 2009; Neumann and Shields 2008, 2011). In other experiments, *S. feltiae* had the greatest effect and the other two species help when insects are abundant; however, *S. feltiae* often was overlooked by other researchers because it is lackluster in laboratory conditions although it is abundant in the field, killing many insects.

The presence of *H. bacteriophora* in 2017 (Fig. 1) was typical of that in northern New York (5-20%; Shields et al. 1999, 2009; Neumann and Shields 2008, 2011). Because *H. bacteriophora* is a cruising entomopathogenic nematode (Neumann and Shields 2008), only limited signs were observed based on abundance of the host, which in the study was expected to be scarce, if not non-existent.

Risser et al. (2016) in a neighboring Southwest US state found no native *S. carcocapsae* or *H. bacteriophora* at two dry sites and only few at two wet sites in samples collected away from dung pats. Only *S. feltiae* was at the two drier sites and much less abundant than in the present study (Table 1). Zepeta-Jazo et al. (2014) found native *Steinernema* sp. in 85% and *Heterorhabditis* sp. in 14% of 19 samples from varied cropping systems at three municipalities in an area with much precipitation in Mexico.
Very few samples in 2017 included both *S. feltiae* and *H. bacteriophora* (data not shown). In other studies in which entomopathogenic nematodes were released (Shields et al. 1999, 2009; Neumann and Shields 2008, 2011), 1-2 years were required for sufficient establishment and dispersion of entomopathogenic nematodes. Once well-dispersed, *S. feltiae* were most active in the top 15 cm and *H. bacteriophora* most active below that (Shields et al. 2009). Consequently, *H. bacteriophora* might have been at that depth in the field before sampling time in 2018. *S. feltiae* in 18 and 22% of the samples for *S. feltiae + H. bacteriophora* and *S. feltiae + S. carcocapsae* treatments (Table 1), respectively, were scarce in the range of what typically is found in northern New York (20-30%; Shields et al. 1999, 2009; Neumann and Shields 2008, 2011), but still significantly greater than the nontreated check with 0%. While the amounts are typical of long-term persistence amounts in alfalfa fields in New York, they suggest a large number of insect hosts have not been present in the treated areas. If a large number of insect hosts had been present, entomopathogenic nematodes in the samples would range from 35-50%. However, experience suggests the current 15-21% were sufficient to respond to invasion by susceptible hosts.

The three Shielentomopathogenic nematode species tested are available commercially, but commercial strains have poor persistence (7-30 days) (Long et al. 2000) due to rearing procedures and failure to retain genes for persistence. Poorly persisting commercial strains would require annual applications to achieve pest control. The strains in the study were isolated from agricultural fields in New York, and rearing procedures were used to retain genes for persistence. Shields et al (2018) reported on persistence of the strains under agricultural conditions in New York where a single inoculation persisted in 75 agricultural fields for multiple years across crop rotations. Consequently, the degree of success of entomopathogenic nematodes for biological control of whitefringed beetle and similar pests might be enhanced by establishing local, soil-based sources from which entomopathogenic nematodes could be collected to increase in living hosts for immediate distribution to other fields (Shields et al. 2009), especially because the spread of whitefringed beetle probably cannot be controlled because of its hitchhiking nature.

In conclusion, this is the first known report of lack of natural populations of *S. carcocapsae*, *S. feltiae*, and *H. heterorhabditis* in irrigated lands of the US Southwest and the first known report of successful establishment of one of the species, *S. feltiae*, and possibly, *H. heterorhabditis*. In many cases it might not be possible to salvage alfalfa stands already damaged by whitefringed beetles; however, field reclamation might be feasible using entomopathogenic nematodes in irrigated fields in semiarid regions for future alfalfa establishment after a reasonable rotation period to avoid allelopathy (Lauriault et al. 2009). Entomopathogenic nematodes also could be applied to protect new fields before economic loss occurred (Long et al. 2000). Because the whitefringed beetle has 385 host plant species including many economic crops (Barnes and De Barro 2009) and there are many other crop pests with similar larval root feeding behavior that entomopathogenic nematodes could control, locally adapted sources might be established for widespread distribution (Shields et al. 2009, Neumann and Shields 2011).

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References Cited


NMAgStats. 2018. 2017 New Mexico Agricultural Statistics. New Mexico Department of Agriculture and USDA-NASS, Las Cruces, NM.


