Evaluation of Entomopathogenic Nematodes Against Plum Curculio: Effects of Nematode Species, Application Rates, and Persistence in the Soil

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The use of biological control agents such as entomopathogenic (= insect-killing) nematodes for insect pest control is gaining interest among fruit growers. EPNs are very small, soft bodied, non-segmented roundworms that are parasites of insects. EPNs are commercially available and are used to kill a wide variety of economically important insect pests. EPNs occur naturally in soil environments and locate their prey in response to carbon dioxide, vibration, and other chemical cues. In general terms, EPNs are considered environmentally friendly non-chemical alternatives to controlling pests. For example, they are safe for humans and the environment and are not considered threats to beneficial insects or other non-target organisms. EPNs can be used in organic production systems. In addition, they can be applied using standard pesticide equipment (for a short video showing EPN application at the UMass Cold Spring Orchard, click HERE), and there is no need for personal protective equipment and re-entry restrictions.

In New England, EPNs have been evaluated against plum curculio larvae in multiple farms for nearly a decade. Combined results from multiple published studies indicate that (1) *Steinernema riobrave* and *S. carpocapsae* have emerged as the EPNs species that are most effective at killing the immature stages of plum curculio in the soil, and (2) EPNs can be applied to the soil in areas underneath the canopies of odor-baited trap trees (see Piñero et al., 2020), areas that are expected to hold greater densities of plum curculio compared to any other trees in the orchard. The economic feasibility of using EPNs applied underneath the canopies of trap trees is very promising because, even if high rates of nematodes are applied, such applications would only need to be made to a small proportion of the acreage.

Here, we compared the performance of the EPNs *S. riobrave* and *S. carpocapsae*, evaluated at various application rates, at killing plum curculio larvae in the soil. A secondary objective was to estimate EPN persistence by exposing EPN-treated soil to wax moth larvae (*Galleria mellonella*) about 8 weeks after initial EPN application in the field.

Materials & Methods

Field study. The field component of this study took place at the University of Massachusetts Cold Spring Orchard (Belchertown, MA) from 16 July to 30 September, 2020. In early July 2020, we collected apple fruitlets presumably infested with plum curculio from unmanaged trees in the Amherst/Belchertown areas. Upon collection, the fruit was stored at ambient

Table 1. Entomopathogenic nematode (EPN) treatments applied to the soil at the UMass Cold Spring Orchard (Belchertown, MA) against the immature stages of plum curculio.

Treatments	Nematode application rate
Steinernema riobrave (low rate)	500,000 IJ/m^2
S. riobrave (high rate)	2 million IJ/ m^2
S. carpocapsae (low rate)	500,000 IJ/m^2
S. carpocapsae (high rate)	2 million IJ/ m^2
S. riobrave +*S. carpocapsae* (low rate)	250,000 IJ S.r. + 250,000 IJ S.c.) /m^2
S. riobrave +*S. carpocapsae* (high rate)	1 million IJ S.r. + 1 million IJ S.c.)/ m^2
Control	Water only

temperature for about 10 days to allow plum curculio larvae to continue developing. On 16 July, the fruit was transported to an unsprayed section of the orchard.

EPN treatments**.** *Steinernema riobrave* and *S. carpocapsae*, were evaluated alone and in combination, at two application rates (low and high; Table 1). Water was used as a control. Sixty fruitlets and 33 plum curculio larvae were placed underneath the canopies of each of 28 apple trees, within a 1 m^2 area. EPNs were applied at the rates described in Table 1, using 3.78 L of water, and the same amount of water alone was applied to the control. After treatment application, the emergence cages were placed on the ground, covering the fruit, and the edges of the cages were buried in the soil to ensure the emerged adults would not escape. To preserve soil moisture, we added 3.78 L of water to each experimental area three days after initial EPN application. As soon as the first adult plum curculio was captured in the topping device of the cages, small pieces of apple were placed inside the device as an attractant. The emergence of adult plum curculios from the experimental cages was recorded twice a week for 5 weeks starting on 3 August,

View of the experimental site showing the pyramidal emergence traps used for the quantification of adult plum curculio emergence after the application of the EPNs *Steinernema riobrave* and *S. carpocapsae*, at various application rates, or water (control). Trap dimensions: 1 m x 1 m at the base.

2020. Total weevil emergence from each of the 28 experimental cages (7 treatments * 4 replications) over a 5-week period was used for the statistical analyses.

Evaluation of EPN persistence in the soil. A follow-up evaluation was conducted at UMass campus. Greater wax moth, *Galleria mellonella*, a highly susceptible host, was used to evaluate the virulence of the EPN treatments approximately 8 weeks after original application in the field. The moth larvae were purchased online from Bestbait. On September 15, 2020, 2 lb-samples of soil were retrieved from each of the 28 experimental units at the Cold Spring Orchard field site. The soil was transported to the lab in 1 quart plastic containers with lid. Upon arrival to the laboratory, each container received 15 ml of distilled water and 15 wax moth larvae were placed inside each container,

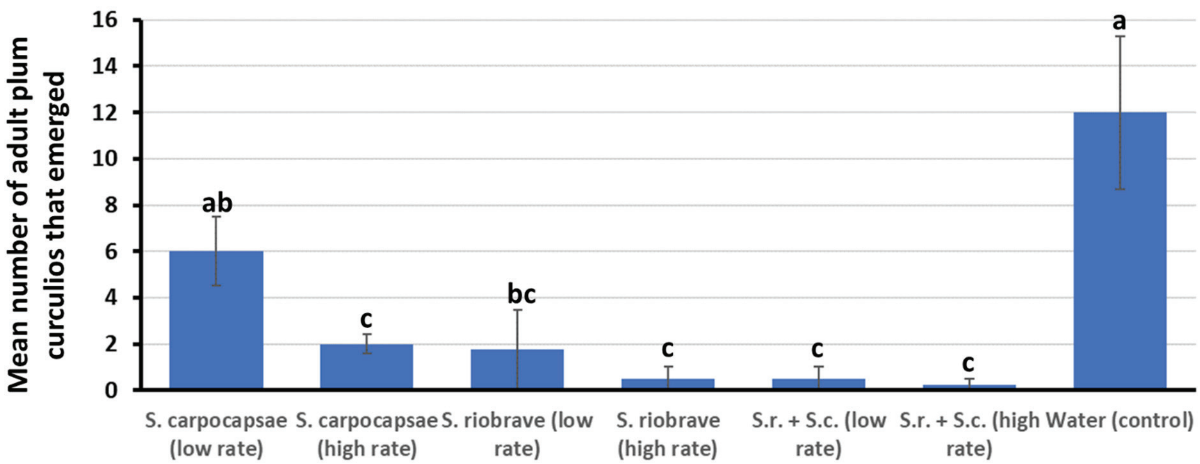

Figure 1. Number (mean ± standard error of the mean, a measure of how precise the estimate is) of adult plum curculios that were recovered from emergence cages following application of EPN treatments or water (control). Different letters above bars denote statistically significant differences between treatments at odds of 19:1.

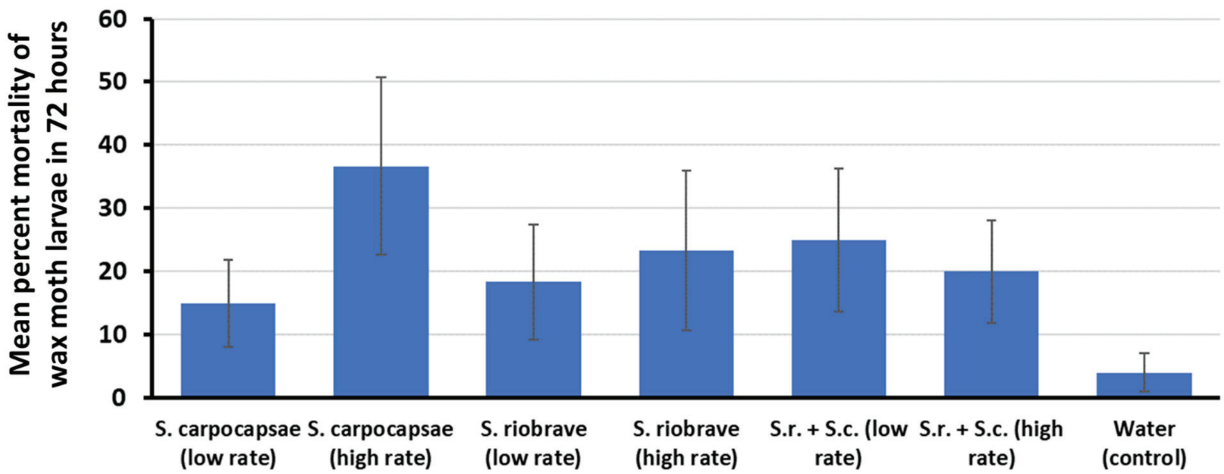

Figure 2. Mortality values (mean ± standard error of the mean) of wax moth (*Galleria mellonella*) larvae that were exposed to soil samples collected (on 15 September, 2020) from each of the 28 experimental units at Cold Spring Orchard (Belchertown, MA). Soil samples corresponded to the EPN treatments or water (control) that were applied against plum curculio on 16 July, 2020.

on top of the soil. Mortality of wax moth larvae was recorded at 24, 48, and 72 hours after exposure.

Results

Field study. Overall, 92 adult plum curculios were recovered from the 28 emergence cages. As shown in Figure 1, the most effective EPN treatments (the ones that resulted in the lowest levels of plum curculio emergence) were *S. carpocapsae* and *S. riobrave* (both at the high rates) and the two rates of *S.r.* + *S.c.* When compared to the control, *S.r.* + *S.c.* (high rate) resulted in a 48-fold reduction in the number of adult PCs that emerged from the soil whereas *S. riobrave* (high rate) and *S.r.* + *S.c.* (low rate), showed a 24-fold reduction. *Steinernema riobrave* at the high and at the low rates and *S. carpocapsae* at the high rate performed similarly well.

Evaluation of EPN persistence. Mortality levels of wax moth larvae caused by EPN treatments ranged from 15% (*S. carpocapsae*, low rate) to 38% (*S. carpocapsae*, high rate) when soil was taken from Cold Spring Orchard in mid-September, from the same areas where EPNs were applied in mid-July. However, the statistical analyses showed no significant differences in the levels of mortality of wax moth larvae among EPN treatments and the control (Figure 2).

Conclusions

The results from this study indicated that *Steinernema carpocapsae* and *S. riobrave* (both at the high application rates evaluated) and the two rates of both EPN species combined performed best at killing plum curculio larvae in the soil. The follow-up study that sought to assess the persistence of EPNs in the soil showed some positive results, but variability among samples likely prevented us from detecting statistical differences when compared to water control. This investigation will be conducted again in 2021 to confirm our results. Overall, this study shows, once more, than EPNs are effective at killing plum curculio larvae in the soil. Biological control involving the application of EPNs targeting the soil-dwelling stages of plum curculio has the potential to manage this pest more sustainably in a reduced-spray environment, including organic systems.

Acknowledgments

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