Can Entomopathogenic Nematodes Applied Against Plum Curculio Larvae Survive the Winter in New England?

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Entomopathogenic nematodes (EPNs) are small round worms that lack color, body segments, and appendages. This type of tiny worm lives in the soil and causes disease to the soil-dwelling stages of many species of arthropods; hence its name 'entomopathogenic' (= insect-killing). EPNs are highly effective against many pests, are non-toxic to humans and other mammals, have been shown to have reduced impact on beneficial insects and are typically organically certified. In New England apple orchards, EPNs have been evaluated in the field since 2013. Nearly all studies conducted in New England (for two examples, see Fruit Notes articles in the winter 2019 and summer 2020 issues), have shown good to excellent performance. Some EPN species have been shown to be able to overwinter in New England's weather. If this can be confirmed for Massachusetts, this would act as a method of pest control that allows growers to apply EPNs fewer times, saving them time and financial resources.

The purpose of this study was to determine to what extent the EPNs *Steinernema carpocapsae* strain All (SC) and *Steinernema riobrave* 355 strain (SR) that were applied to the soil in July 2020 at two locations in the UMass Cold Spring Orchard in Belchertown, Massachusetts, survived through the winter.

Materials & Methods

2020 Field Research: *Steinernema carpocapsae* (SC) strain All and *Steinernema riobrave* (SR) strain 355 were applied to the soil at two locations in UMass Amherst's Cold Spring Orchard (Belchertown, Massachusetts). The first location was X-block where EPNs were applied by hand on 16 July, 2020. A total of 7 different treatments, each replicated 4 times, were evaluated. In all, there were 24 areas with EPN applications and 4 control sites. The treatments were: SR and SC applied singly at low application rate, SR and SC applied singly at high application rate, SR and SC combined at low application rate, SR and SC combined at high application rate, and a control where water but no EPNs were applied.

The second location was Rock Mountain where a single EPN species (*S. riobrave* 355 strain) was applied on 17 July, 2020. One billion EPNs (donated by BASF) were applied using a tractor-mounted sprayer with the nozzle removed. The entire perimeter and 2 inner rows were sprayed with EPNs. For a 1-minute video showing the application of EPNs, click HERE.

On 25 May, 2021, 10 months after the original EPN application, soil samples were collected from the same sites where the EPNs had been applied. In X-block, soil was gathered from the same 28 spots that received particular combinations of EPN species and application rates in 2020. In Rock Mountain, 16 samples were taken from the perimeter and from 2 rows in (both areas received EPNs), and 6 samples were taken from the center of the block, were no EPNs were applied. Each soil sample was placed inside a 500 ml plastic container and was taken back to a lab at UMass Amherst.

Lab Work: From May 5,2021 through June 23, 2021, the lab work started. We placed 20 wax moth larvae on top of each soil sample (Figure 1A). Wax moth larvae were used because this insect is highly susceptible to EPNs. The larval mortality was checked at 24, 48, and 72 hours. On June 4, 2021 White traps (Figure 1B) were set up to test Koch's Postulates which is a way of confirming that the wax moth larvae had indeed died from EPNs and that the EPNs could effectively reinfect and kill subsequent hosts.

The White traps involved a small petri dish with a piece of filter paper draped over it inside a larger Petri dish. Then, 20 mL of water was added to the larger Petri dish. From each sample a black (symptoms of cadavers either not infected by EPNs or initially infected by EPNs but then taken over by something else) and tan cadaver (symptoms characteristic of SC and SR infections) was chosen to be placed in a White trap (Figure 1B). Twelve days later the water was collected from each Petri dish and placed on the centrifuge for 10 seconds (to help concentrate any EPNs). Two mL of this water was poured onto a piece of filter paper and 20 new, live wax moth larvae were added. On June 21, 2021, up to 5 black larvae were dissected to check for the presence of nematodes. If EPNs were found, then that cadaver was placed in a Petri dish on top of a damp piece of filter paper. Then, 15 new, live wax moth larvae were added (Figure 1C). This was done to see if the EPN's would continue to re-infect and kill wax moth larvae. EPN presence was identified through dissection in the lab on June 23, 2021(Figure 1D).

Figure 1 (A): Twenty wax moth larvae placed on top of each soil sample, **(B)** White trap set up for wax moth larvae suspected to be infected with EPNs (left: tan larva; right: black larva), **(C)** Reinfection of wax moth larvae from the cadaver that was found to have EPNs upon dissection, **(D)** EPNs in a plum curculio larva, as seen under stereomicroscope.

Results

X-Block: The results from the X-block location show that the species *Steinernema carpocapsae* in a high concentration exerted the highest level of mortality (60.9%) of wax moth larvae when compared to the control (20.0%), followed by *S. riobrave* low (34.1%) and high (39.4%) and *S. carpocapsae* low (20.3%) (Figure 2). These results resemble those recorded in the field study with plum curculio, conducted in July 2020. The high mortality rate in the *Steinernema carpocapsae* at high application

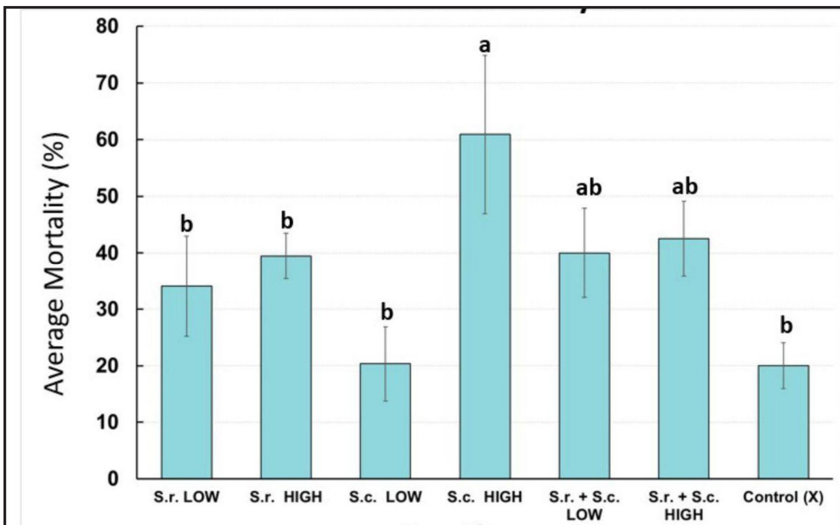

Figure 2. Average mortality of wax moth larvae in soil samples taken from X-block. S.r. = *Steinernema riobrave* strain 355; S.c. = *Steinernema carpocapsae* strain All. High and low refer to EPN application rates. EPNs were applied in early July 2020 and soil samples were taken on May 25, 2021.

rate achieved 10 months after initial application can be attributed to that EPN species. Because all the other species and concentration levels were not statistically different from the control, then the mortality rates could be explained by other factors such as native nematode species, or other insect pathogens.

Rock Mountain: The results from Rock Mountain suggest that *S. riobrave* may have survived the winter in that area because of the high mortality rate of wax moth larvae in the exterior (24.4%) and in the 2 inner rows (20.9%) vs. the control (2.5%) (= no EPNs applied), which was from the center of the block (Figure 3). Although the perimeter had the highest mortality, we acknowledge there could have been other factors in the soil such as a higher concentration of native nematode species or other insect pathogens in the perimeter.

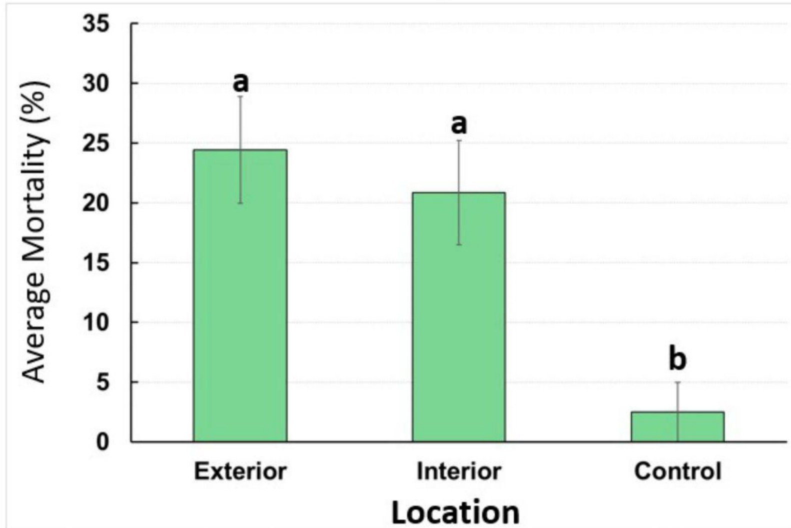

Figure 3. Average mortality of wax moth larvae in soil samples taken from Rock Mountain. The EPN *Steinernema riobrave* strain 355, donated by BASF, was applied in July 2020. Soil samples were taken on May 25, 2021. Exterior = Samples taken from perimeter-row trees; Interior = samples taken from 2 rows in; Control = Samples taken from the block center. Both exterior and interior samples received EPNs in the 2020 application.

Conclusions

The results from this study suggest that both *Steinernema carpocapsae* strain All and *Steinernema riobrave* strain 355 were indeed able to survive the winter in the study site. It was interesting to note that in X-block, where both EPN species were applied either, alone or in combination, only the SC – high release rate treatment was statistically higher than the control. This could suggest that the SR-SC combinations were antagonistic to each other, at least in terms of persistence. To confirm these results and better evaluate EPN pathogenicity over time, a follow up study should be conducted using larvae of common pests found in the field, such as plum curculio larvae. If EPNs are found to overwinter in the soil in high levels this could lead to a form of biological control that would require fewer re-applications by the grower, thereby saving time and expenses.

Acknowledgments

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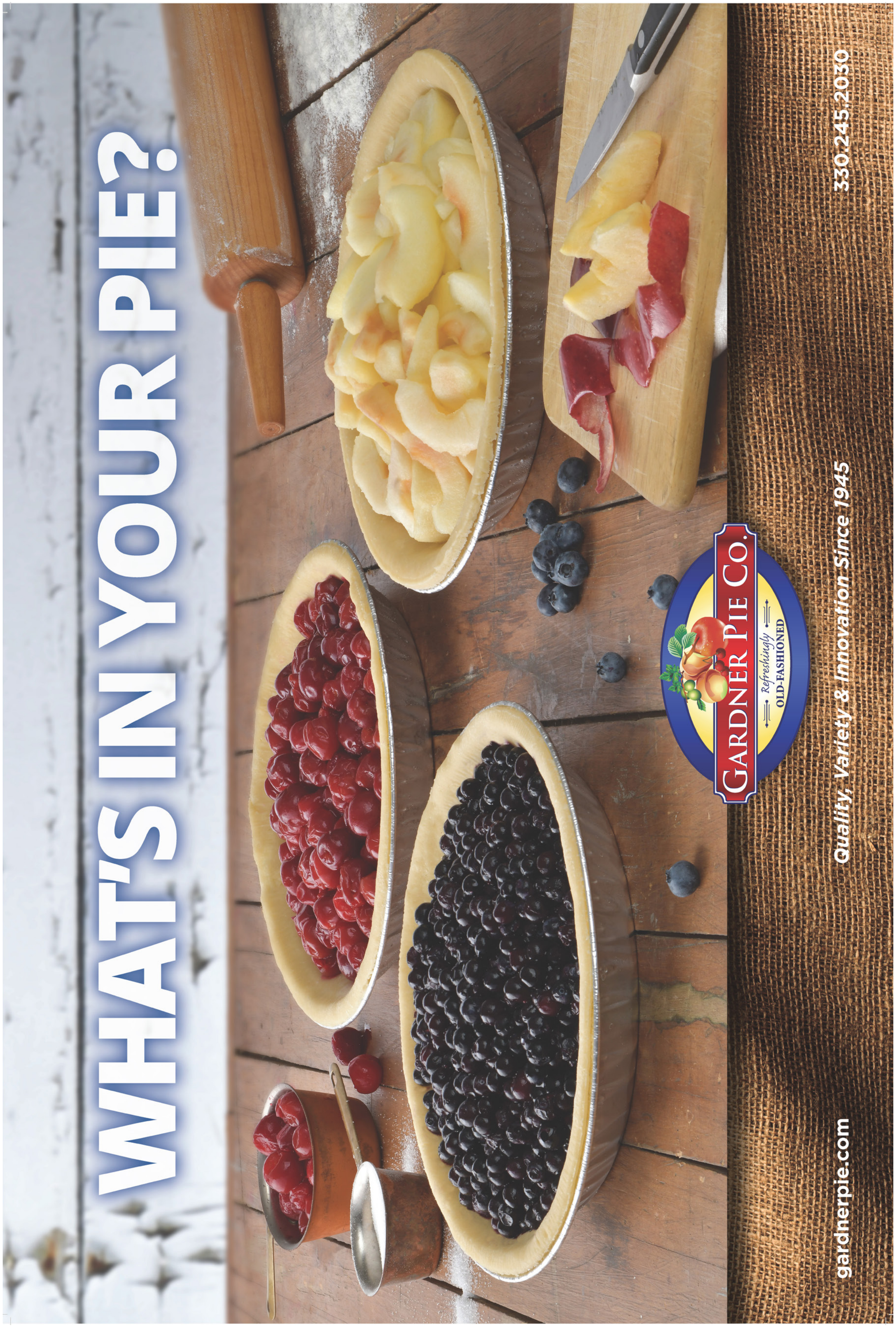
Entomopathogenic nematodes (EPNs). Inside the plump wax moth cadaver are thousands of EPNs ready to serve as biocontrols against soil-dwelling crop pests.



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