Evaluation of Persistent Entomopathogenic Nematodes for Biological Control of Plum Curculio

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The plum curculio, *Conotrachelus nenuphar*, is a native pest of apples and stone fruits in eastern North America. The adult damages fruit by its feeding and oviposition. This insect has adapted well to cultivated tree fruits throughout its range in eastern North America and is a key pest in cherry, apple and peach production. Most apple orchards in the northeastern US and Canada are treated every year with insecticides to control this pest; growers in northeastern apple orchards typically apply 2–3 insecticide treatments to manage plum curculio each year, but fruit damage levels and fruit yield losses in commercial apple orchards can range from 0.5–3%, even with a complete insecticide control program. In a recent survey of commercial apple growers in New England, the plum curculio was identified as the pest that required the greatest pest management effort. The plum curculio is also considered to be one of the primary pests limiting organic apple production the eastern US, as currently available pesticides and other methods approved for use in organic orchards have limited effectiveness.

> **"Incorporation of biological control agents such as entomopathogenic nematodes into orchard management pest management systems has been proposed as a way to control plum curculio in apple. Although this tactic would be useful for all commercial apple producers, we feel that it would be of particular value in organic apple plantings, which tend to have greater PC infestations because of fewer effective management options. An established, persistent entomopathogenic nematode population could help reduce the number of plum curculio larvae residing in the orchard floor that originate from dropped infested fruits."**

Historically, organophosphate insecticides have been the primary means of control for plum curculio. The Food Quality Protection Act of 1996 required the U.S. Environmental Protection Agency to develop more stringent tolerances on organophosphate insecticides along with other classes of broad-spectrum materials. Although a number of reduced-risk pesticides have been developed to replace some of the older materials used on tree fruit, the high cost and sophisticated information needed to ensure their efficacy has limited their use by commercial growers. Some newer insecticides, primarily neonicotinoids, are being evaluated for efficacy against plum curculio. Over-reliance on one or a few classes of insecticides in agricultural systems raises concerns about the development of resistance in the target insect and the impacts of those insecticide treatments on non-target species such as honey bees.

Biological Control of Plum Curculio with Nematodes

Incorporation of biological control agents such as entomopathogenic nematodes into orchard management pest management systems has been proposed as a way to reduce the potential for resistance development (Lacey & Shapiro-Ilan 2008). In its lifecycle, plum curculio has four duff and soil-dwelling stages that are vulnerable to attack by entomopathogenic nematodes present in the soil profile. These stages are: the overwintering adult in the duff, the last instar larvae entering the soil to pupate, the pupa and the emerging adult.

In laboratory bioassays, entomopathogenic nematodes, particularly strains of *Steinernema riobrave*, *S. feltiae*, *S. carpocapsae*, and *Heterorhabditis bacteriophora* have shown efficacy against last instar plum curculio larvae (Shapiro-Ilan et al. 2002, Alston et al. 2005, Kim and Alston 2008). In the field, various strains of these species have reduced populations by as much as 70–97%, depending on the nematode strain, insect stage, treatment timing and field conditions. Effective control of larval populations has been achieved if the correct nematode species and strain was selected and effectively introduced into the system.

The majority of current research has been directed toward the inundative release (or "biopesticide approach") using commercially reared nematode strains. However, commercial nematode strains tested under NY field conditions have not persisted at any effective population level after 6 months, and the use of these commercial strains would require an annual application. Over the last two years, we have been testing nematode strains that are native to NY, adapted to persist under NY conditions, and have been explicitly propagated to avoid some of the problems with persistence displayed by commercially available nematodes.

NY native strains of the nematode species *S. carpocapsae* (strain 'NY 001') and *H. bacteriophora* (strain 'Oswego') were isolated during the 1990s and used extensively during the past 20 years in field research in NY alfalfa plantings (Shields et al. 2009). In 2004, *S. feltiae* (strain 'NY 04') was isolated from snout beetle-infested fields in northern NY and used in field trials since that time. These NY native nematode strains have been shown to kill mature larvae of Japanese beetle, *Popillia japonica,* and European chafer, *Rhizotrogus majalis*, at varying levels of effectiveness, with *H. bacteriophora* 'Oswego' killing 50% of late-instar Japanese beetle larvae and 8% of European chafer larvae. The native strains show excellent pathogenicity because late-instar larvae are the most difficult life stage for nematodes to attack and kill. These strains have been documented to persist in field sites for many years after inoculation (Shields et al. 2009).

NY nematode strains are also active at very low temperatures. *S. carpocapsae* 'NY 001' and *S. feltiae* 'NY 04' infect >80% of Curculionidae beetles starting at 6°C and 100% at 8°C. *H. bacteriophora* 'Oswego' begin infective activity at 8°C and infect 100% of hosts at 10°C. Since adult plum curculios overwinter in the soil duff, they are susceptible to attack by all NY species for long periods of time before beginning their spring movement into the orchards.

Materials and Methods

In the laboratory, we conducted trials to determine the potential of *S. carpocapsae* 'NY 001', *S. feltiae* 'NY 04', and *H. bacteriophora* 'Oswego' as plum curculio (PC) biocontrol agents. Petri plates were filled with autoclaved soil and inoculated with water solutions at concentrations of 250–1250 nematode infective juveniles (IJs) per ml, and PC larvae and adults were confined in the plates to determine the ability of different nematode strains to kill these two life stages. We found that exposure of PC larvae to the nematodes was more effective than for PC adults, with the highest mortality obtained using a combination of *S. feltiae* and *S. carpocapsae* (up to 75%); mortality of PC larvae was seen within 14 days (Figure 1). There was no effect of increasing the concentration of IJs above 250/ml.

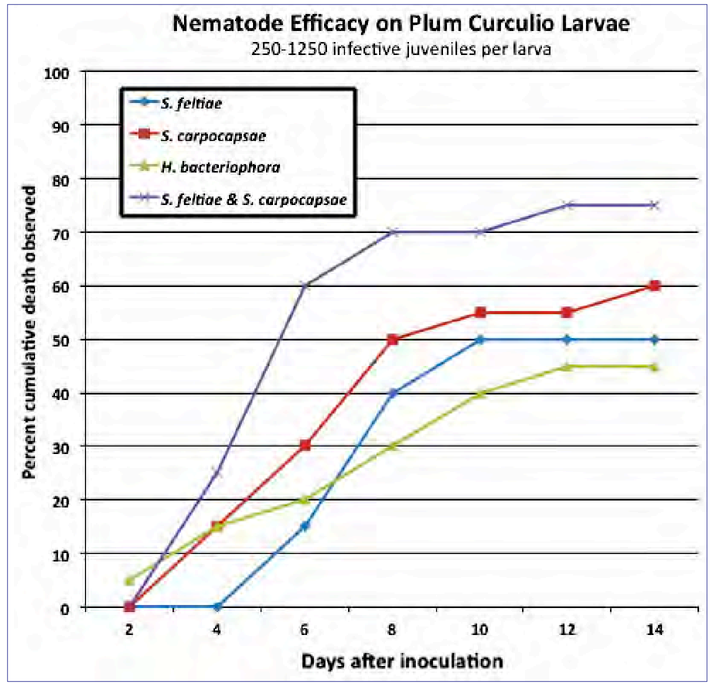

Figure 1. Efficacy of nematodes on killing plum curculio larvae.

For testing in the field, we selected a young Idared apple planting and an established block of Empire apples, both located at a NYSAES research farm, as sites for establishment of long-term entomopathogenic nematode (EPN) populations within designated treatment areas. In the spring of 2012, soil cores from each block were bioassayed for presence of EPNs prior to inoculation of the soil surface with native NY strains. No existing nematode populations were found in any of the soil cores. A population of nematode infective juveniles (IJs) of *S. feltiae* and *S. carpocapsae* was applied to the soil surface on May 30 using a modified herbicide sprayer mounted on an ATV. The nematodes were reared in a lab colony using wax moth larvae as hosts, which were brought to the field, dissolved and mixed in water in the spray tank, and applied shortly before sunset in order to avoid excessive sun or heat exposure (Figure 2).

To assess the level of nematode establishment in these plots, we conducted a larval exposure trial using laboratory-reared PC larvae. In August 2012, we dug micro-plot arenas (4.5" diam x 5" deep) using a soil corer in an inoculated row in the Idared block. In each replicate, 10 mature PC larvae were introduced into the arena, which was covered with an emergence trap top, and checked for emerged PC adults over a 5-week period (Figure 3). The same setup was repeated in an untreated row, to serve as a negative check, as well as in an untreated row in which the arenas were first hand-inoculated with laboratory-mixed nematode populations (containing 7,500-9,000 *S. feltiae* IJs per arena), to serve as a positive check. We repeated this trial in 2013, in both the Idared and Empire research plantings, and also in two commercial organic apple orchards in the Hudson Valley (Milton and Fishkill, NY) that had been inoculated in June of that year.

Results

In these field assays, because of very dry soil conditions beginning in late May 2012, coupled with the lack of natural hosts (other insect larvae) in the orchard soil, the establishment of EPN populations in the 2012-treated rows was significantly hampered. In the 2012 micro-plot arena assays, the average percent adult emergence in field-inoculated plots (70%) was greater than in both the hand-inoculated (31%) and check (41%) plots (Figure 4), indicating that adequate field establishment of the EPN population had not yet occurred. In the 2013 Geneva micro-plot assays, survival of larvae to the adult stage was much lower in all treatments than the levels seen in 2012 (maximum of 26.3%); however, poor survival was also seen in the checks, so that no significant treatment differences were found (Figure 5). The same trend was seen in the Hudson Valley 2013-inoculated sites, but at least in one orchard (Milton), survival in the hand-inoculated plots was significantly lower than in the checks (Fig 6.). The progression of soil core samples in each of the treated orchards taken at successive times after being inoculated shows that gradual field establishment of EPN populations is taking place at all of the sites, with greater levels being seen for *S. feltiae* (Figure 7); by the end of the 2013 season, most sites showed this species present in 20–30% of samples taken. It is anticipated that levels will ultimately stabilize at 30–40%, which would correspond to an optimal level for effective biocontrol to begin occurring.

Figure 2. Wax moth larvae (top) containing entomopathogenic nematode infective juveniles, and the ATV-mounted sprayer (bottom) used to apply them to the orchard floor.

Figure 3. Micro-plot arena used for field assays of nematodes on mature plum curculio larvae.

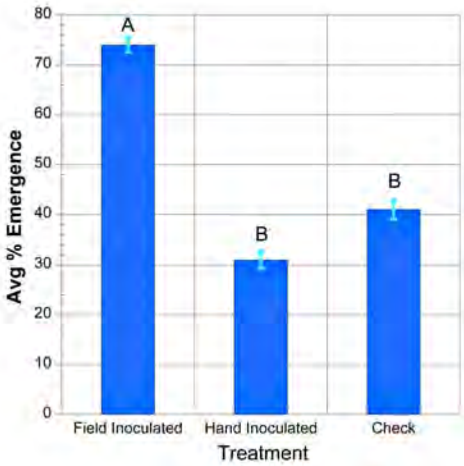

Figure 4. Plum curculio emergence in field nematode plots, Geneva 2012.

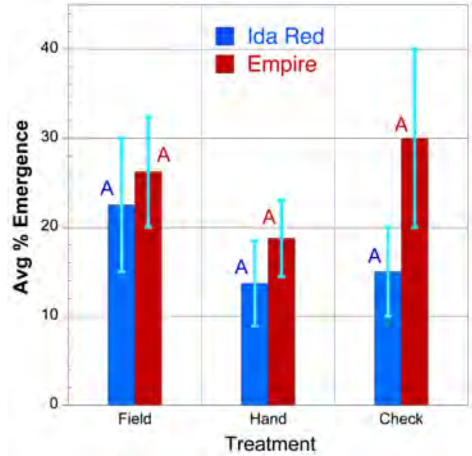

Figure 5. Plum curculio emergence in field nematode plots, Geneva 2013.

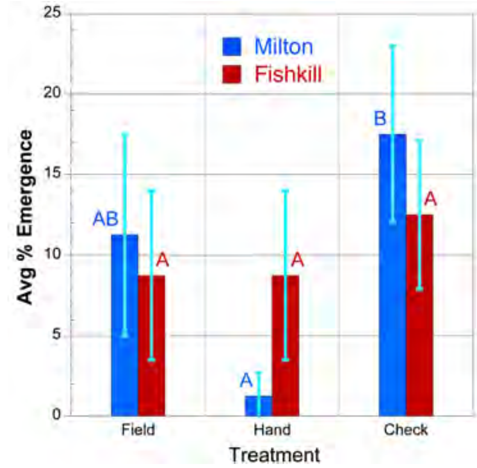

Figure 6. Plum curculio emergence in field nematode plots, Hudson Valley 2013.

Table 1. Plum curculio oviposition damage to fruitlets in nematode-treated orchards

Idared Block	% Damage		Empire Block	% Damage	
	2012	2013		2012	2013
Untreated Rows	7.2	2.7	Untreated Rows	35.9	8.6
Treated Rows	3.8	1.7	Treated Rows	33.7	14.1

As another method of assessing overall impact of the nematode treatments on PC populations, fruitlet samples were taken from each of the (2012-inoculated) Geneva research orchards during June of each of the two years. In each row of each plot sampled, 100 fruits from each of 5 trees were assessed for PC oviposition damage (scars). Damage levels in 2013 were reduced at each site from those seen in 2012 (Table 1). At the Empire site, damage in the treated rows decreased 52% from 2012; however, damage in the untreated check rows decreased 76% from 2012, so damage in the check (8.6%) was roughly half of that seen in the treated rows (14.1%). At the Idared site, results were more along the trend anticipated: fruit damage in the untreated rows decreased ~63% from 2012, and in the treated rows, it decreased ~55% from 2012, with damage marginally lower in the nematode treatment (1.7%, vs 2.7% in the check).

Discussion

We believe the deployment of persistent entomopathogenic nematodes adapted to NY conditions should be effective at reducing plum curculio populations for the following reasons. The NY native nematode strains have been shown to persist in significant levels in grass areas for many years, and recycle on the wide array of other hosts which frequent the soil in the grass habitat. New York has a number of native white grub species, along with Japanese beetles that are frequently found in these grassy areas and are easily colonized by the NY nematode strains. In addition, the cold temperature activity of the native nematode strains allows the nematodes to attack the overwintering adult plum curculio before they begin their spring movement toward the orchard, and they will continue to attack any time the adults are in contact with soil during the cold temperature low insect activity periods typical of NY spring weather patterns. Behavior studies report that PC adults spend several days on the ground after emerging from overwintering diapause while in transit to the apple trees, even though the insect has the ability to fly.

The mature plum curculio larvae exiting the fruit and burrowing 2–3 inches into the soil should be vulnerable to attack by the "ambush strategy" nematode species (e.g., *S. carpocapsae* 'NY 001') that dominate the top of the soil profile. Quiescent pupae and pre-emergent adults are sitting targets for the "hunting strategy" nematode species (e.g., *H. bacteriophora* 'Oswego', *S. feltiae* 'NY 04') that follow the CO_2 gradient released by the insect. As the emerging adult burrows upward, it has to pass through the ambush nematode layer, where it is also vulnerable to attack.

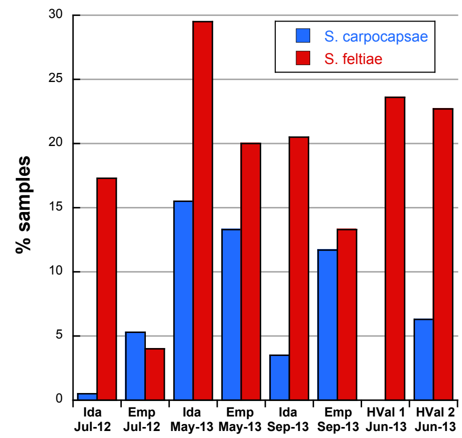

Figure 7. Progression of nematode establishment in field sites as seen from percent soil samples showing presence of nematodes over time in Geneva (Idared and Empire) and Hudson Valley orchard sites, July 2012 to September 2013.

The use of entomopathogenic nematodes focuses a mortality factor on two vulnerable plum curculio lifecycle windows. Soil inoculation with native nematode strains persisting for many years, provides the economic benefit of a single application suppressing insect populations for multiple seasons. The added benefit of suppressing Japanese beetle larvae within the orchard may also result in a reduction of the adult damage on the tree foliage. While similar results could be achieved using commercially available nematodes, the lack of persistence requires an annual application as a biopesticide. The establishment of a persistent nematode population from native cold-adapted strains significantly reduces the cost of the biocontrol strategy and removes the issue of variable nematode quality typical of commercially produced and purchased nematodes. Although this tactic would be useful for all commercial apple producers, we feel that it would be of particular value in organic apple plantings, which tend to have greater PC infestations because of fewer effective management options. An established, persistent EPN population could help reduce the number of plum curculio larvae residing in the orchard floor that originate from dropped infested fruits.

Acknowledgments

We would like to thank Steve Clark (Prospect Farm, Milton, NY), Josh Morgenthau (Fishkill Farms, Hopewell Junction, NY), and Fabio Chizzola (Westwind Orchard, Accord, NY), for allowing us to work on their farms. Invaluable technical lab and field assistance was provided by Dave Kain, Kristin McGregor, Cortni McGregor, Dylan Tussey, Tessa Lessord, Chrissy Dodge, and Danielle Carolie. We are grateful to Tracy Leskey, Starker Wright, and Torri Hancock (USDA-ARS, Kearneysville, WV) for supplying the plum curculio starter population, and to the NYS Specialty Crops Block Grants Program for its funding support.

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