Incorporating the Use of Biological Control Organisms Into Integrated Pest Management Tactics in Forest Nurseries

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Abstract

Integrated pest management uses multiple techniques to control pest organisms. Incorporating the use of biological control agents against economically important insect pests that occur in nursery production sites, such as lygus bug and black vine weevil, can be beneficial. Typical biological control agents include parasitic wasps and flies, invertebrate predators, and entomopathogenic nematodes and fungi. Two tests were conducted to determine the usefulness of entomopathogenic organisms against black vine weevil larvae. In the first test, virtually all larvae in nematode-treated soil and 50 percent of those in fungal-treated soil died within 16 days. In the second test, 85 percent of the tested larvae survived for 14 days in the control treatment, while only 5 percent survived in the nematode treatment. The high levels of mortality demonstrate the potential of using biological control as part of an integrated approach to pest management of nursery pests.

Introduction

Pest management strategies in forest nurseries usually involve implementing suppression (reactive) or prevention (proactive) practices. Suppression practices are aimed at regulating populations of pest species that cause economically intolerable levels of crop damage. Prevention practices are aimed at maintaining pest damage below economically intolerable levels.

Integrated Pest Management (IPM) considers the entire ecosystem that is being managed and constitutes a broad-based approach to reduce or maintain pest populations below a level at which they cause economic damage. IPM requires that potential pest species be monitored regularly and uses strategies that are both suppressive and preventive.

As the name implies, IPM does not rely on any single pest-management technique, but instead can integrate a combination of techniques (i.e., mechanical, biological, and chemical) against a pest (any organism that interferes with our intended goal) to manage and maintain its population level below where it causes economic damage (this does not necessarily imply zero damage). Mechanical management techniques include using barriers or traps or manually removing the pest to protect a crop. Biological techniques include using microorganisms (i.e., bacteria, fungi, and viruses), invertebrate predators (e.g., ladybird beetles and lacewings), and/or insect parasitoids (e.g., parasitic flies and wasps). Chemical techniques include applications of pesticides (insecticides, fungicides, and herbicides) using foliar, granular, or drench products. Given the potential to use a combination of management techniques, individual IPM strategies can be developed for specific crop systems and conditions and may range from simple to complex, but all require that the pest species be monitored through time.

Some major tenets of IPM are:.

- There is no silver bullet. IPM does not depend on a one-size-fits-all strategy, but instead requires that the manager tailors the practices to fit the overall goal.
- It is important to treat the causes, not just the symptoms. Pest outbreaks do not simply happen; there is usually a root cause. By addressing the cause, the manager can lessen the time and effort required to control a pest outbreak.

- Pest presence does not always mean there is a pest problem. If a pest species is found at low density, it may not be causing economically important damage.
- If you kill the natural enemies, you inherit their work. In other words, if pest management tactics cause mortality to biological enemies of the pest, then the manager must also eliminate the pests that would have been killed by the natural enemies.
- Pest management strategies can be "just in time" or "just in case." IPM depends upon monitoring pest populations (sampling and forecasting) to allow the manager to determine and schedule appropriate treatments.

In this article, I have included a review of two insect pests: tarnished plant bug and black vine weevil. Both have common biological enemies that growers may be able to incorporate into their IPM practices. To further understand this, I conducted two biocontrol trials with black vine weevil which are also described in this article.

Tarnished Plant Bug, *Lygus hesperus* Knight (Hemiptera: Miridae)

Lygus hesperus is native to Western North America, including both the Western United States and southwestern Canada. This insect is one of the most important true bug pests present in both horticultural and agricultural crops where it feeds on numerous economically important host species (Scott 2012). Feeding damage typically occurs on reproductive structures and terminal buds, causing stem lesions, distorted needles, and multiple tops. This damage results in decreased plant health and quality. Widespread damage by *L. hesperus* occurs on nursery crops throughout the United States and Southern Canada (South 1991), with damage occurring on both hardwood and conifer species (Sapio et al. 1982, Schowalter et al. 1986). Common nursery hosts include Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco), true fir (*Abies* spp.), pine (*Pinus* spp.), spruce (*Picea* spp.), and hybrid poplar (*Populus* spp.).

Lygus hesperus spend the winter as adults (figure 1), often in plant debris and transplant beds (South 2012). After hatching, the insect's development is temperature dependent and it goes through multiple nymphal instars before reaching the adult stage (Cooper and Spurgeon 2012). The adults can be active fliers but spend much of their time on their host plants (Blackmer et al.

Figure 1. The tarnished plant bug (*Lygus hesperus*) (adult pictured) is an important pest in nursery crops. (Photo by USDA Agricultural Research Service)

2004). Both adults and nymphs feed on plant juices using piercing/sucking mouthparts. In the Northern States there are three to four generations per year, and damage to 1-0 conifer seedlings usually occurs from June to September (South 2012). Populations can be monitored by examining nearby weedy species, using visual damage of the nursery crop, and confirming the identification by catching and examining individual specimens (South 2012).

While more important in traditional agriculture, multiple biocontrol options are available against *Lygus hesperus*. These biocontrol agents include naturally occurring parasitoids such as the wasps *Anaphes iole* Girault (Hymenoptera: Mymaridae) and *Peristenus relictusis* Loan (Hymenoptera: Braconidae) that use *L. hesperus* eggs as hosts (Haye et al. 2007, Zhu and Williams 2002). Naturally occurring predators can also be biocontrol agents such as bigeyed bugs (*Geocoris* spp. [Hemiptera: Geocoridae]), damsel bugs (*Nabis* spp. [Hemiptera: Nabidae]), minute pirate bugs (*Orius tristicolor* White [Hemiptera: Anthocoridae]), and several species of spiders that feed on *L. hesperus* during its nymphal stages (Zalom et al. 2018). Similar to the parasitoids, predators are more important mortality agents in traditional agricultural settings than in nurseries.

Black Vine Weevil, *Otiorhynchus sulcatus* Fabricus (Coleoptera: Curculionidae)

Otiorhynchus sulcatus is native to Europe and was introduced into North America in the early 1900s (Drooz 1985). The adult weevil (figure 2) is matte

Figure 2. Black vine weevils (*Otiorhynchus sulcatus*) (adult pictured) feed on more than 100 host plants. (Photo by USDA Agricultural Research Service)

black in color, approximately 9 to 12 mm long, and consists entirely of females that reproduce parthenogenetically (without mating) (Moorhouse et al. 1992). The front wings (elytra) of adult black vine weevil are fused, making them incapable of flight and limiting the distance they are capable of dispersing (Maier 1978).

Black vine weevils feed on over 100 host species with the adults feeding nocturnally on foliage and leaving a distinct notching pattern (Warner 1975). Most of the damage to plants occurs from larval root feeding (Nielsen and Dunlap 1981). The weevils primarily overwinter as mature larvae (for up to several months), but some adults may also survive the winter (Moorhouse et al. 1992). Larvae are creamy white, legless grubs and appear curved in appearance. Larvae inhabit the soil where they feed on small roots, but as they mature, they can feed on larger roots and girdle seedlings (Moorhouse et al. 1992). Common nursery hosts include yew (*Taxus* spp.), spruce, rhododendron (*Rhododendron* spp.), hemlock (*Tsuga* spp.), and grape (*Vitis* spp.) (Drooz 1985). These insects have also been found in containers in which Douglas-fir and oak (*Quercus* spp.) were growing (personal observations).

Because adult black vine weevils are strongly nocturnal in their movements, direct observation is not adequate to assess weevil presence. Assessment must include a variety of boards, cardboard, pitfall traps, and sticky bands (Smith-Fiola 2001). In addition, foliage can be examined for adult feeding.

Multiple insecticides are registered for use against black vine weevil (Frank et al. 2020). In addition, several predators are natural enemies of black vine weevil (Moorhouse et al. 1992). Entomopathogenic nematodes and fungi are frequently used to control the soil-inhabiting larvae in nurseries (Bruck 2005, Klingen et al. 2015).

Biocontrol Trials

Two trials at the Franklin H. Pitkin Forest Nursery (Moscow, ID) were conducted to test the effects of nematode and fungal applications on black vine weevil larvae. The objectives were to confirm the presence of black vine weevil, evaluate biocontrol potential in a nursery greenhouse setting, and generate data on the efficacy of biocontrol treatments on black vine weevil.

Methods

In mid-February 2019, weevil larvae were collected from infested containers containing Douglas-fir or oak seedlings. These larvae were transported to the University of Idaho's forest entomology lab and maintained for 24 h at 20 °C in 500-ml-plastic containers filled with dampened potting mix used at the nursery. The next day, larvae were transferred into pots (2 larvae in each of 36 pots). Larvae were covered with approximately 2.5 cm of potting mix. Containers were maintained at 24 °C in the laboratory with a 12:12 h (light:dark) regimen throughout the trial period.

Three treatments were each randomly assigned to 12 containers: (1) control (no organisms added), (2) nematode application (one mealworm infected with the nematode *Heterorhabditis bacteriophora* placed just beneath the medium surface) (figure 3), or (3) fungal application (*Isaria fumosorosea* was suspended in water and applied to the medium surface at 1.6 ml/L).

Larvae were examined 4, 8, 12, and 16 days after treatment (using four, two, two, and four containers, respectively, from each treatment) to determine if they were alive and appeared healthy. At each sampling time, larvae from each container were removed, separated from one another, touched with a dull probe to determine if they would respond to touch, and then maintained individually in small petri dishes with damp potting mix to determine if (and

Figure 3. Mealworm larvae infected with *Heterorhabditis bacteriophora* were used in the nematode treatments to test biological control of black vine weevils. (Photo by Stephen Cook)

when) they were infected with one of the entomopathogens. All larvae were alive when transferred and subsequently classified as dead when they did not respond to touch during the examinations or showed signs of infection as described below.

Dead larvae were dissected to determine if nematodes were present in the cadaver. Larvae that showed obvious infection with one of the entomopathogens were counted as infected and discarded. Larvae infected by nematodes turn reddish in color prior to death, while larvae infected with fungus turn a brownish color prior to death (figure 4). Larvae that had successfully pupated were counted as healthy since they had completed development. Because a large percentage of individuals had pupated, the trial concluded before eclosion to the adult stage and emergence from the containers occurred. Because of the limited replication, no statistical tests were conducted.

The second experiment was conducted in February and March 2020 to provide a statistically defensible study on larval mortality caused by the nematode. Black vine weevil larvae were collected from the Franklin H. Pitkin Forest Nursery and handled in the same fashion as described for the first trial. Four larvae were randomly assigned to each of 10 pots. Two treatments (five pots each) were included: (1) control

Figure 4. These (right) healthy, (middle) fungus-infected, and (left) nematode-infected black vine weevil larvae were removed from potting soil 12 days following treatment. (Photo by Stephen Cook)

and (2) the same nematode treatment used in the first trial. Larvae were removed from the pots after 10 days to determine overall survival and nematode infection. A students-t-test was conducted using the Statistix 10 software package (Analytical Software 2013) to compare survival of the larvae between the two treatments.

Results

In the first trial, larval survival declined over time, especially for those in the nematode or fungal treatments (figure 5). No black vine weevil larvae in the nematode-treated containers survived to pupate, while 75 and 50 percent of larvae had pupated in the control and fungal-treated containers, respectively. The nematode treatment caused mortality faster than the fungal treatment. This result may be because nematodes were introduced in infected mealworms, and thus the density and timing of dispersal may have given them an advantage over the fungus treatment in a short-term trial. Commercially available nematodes are typically applied suspended in a water solution which may disperse more slowly and be at lower density than used in this trial. Although not analyzed statistically, this trial indicated that both biocontrol treatments were effective.

In the second trial, larvae in the control treatment had higher survival than those in the nematode treatment ($t = 7.16$; 8 df; $[P > t] = 0.0001$) (figure 6). Some of the larvae in the control treatment died, but none were determined to be infected with nematodes.

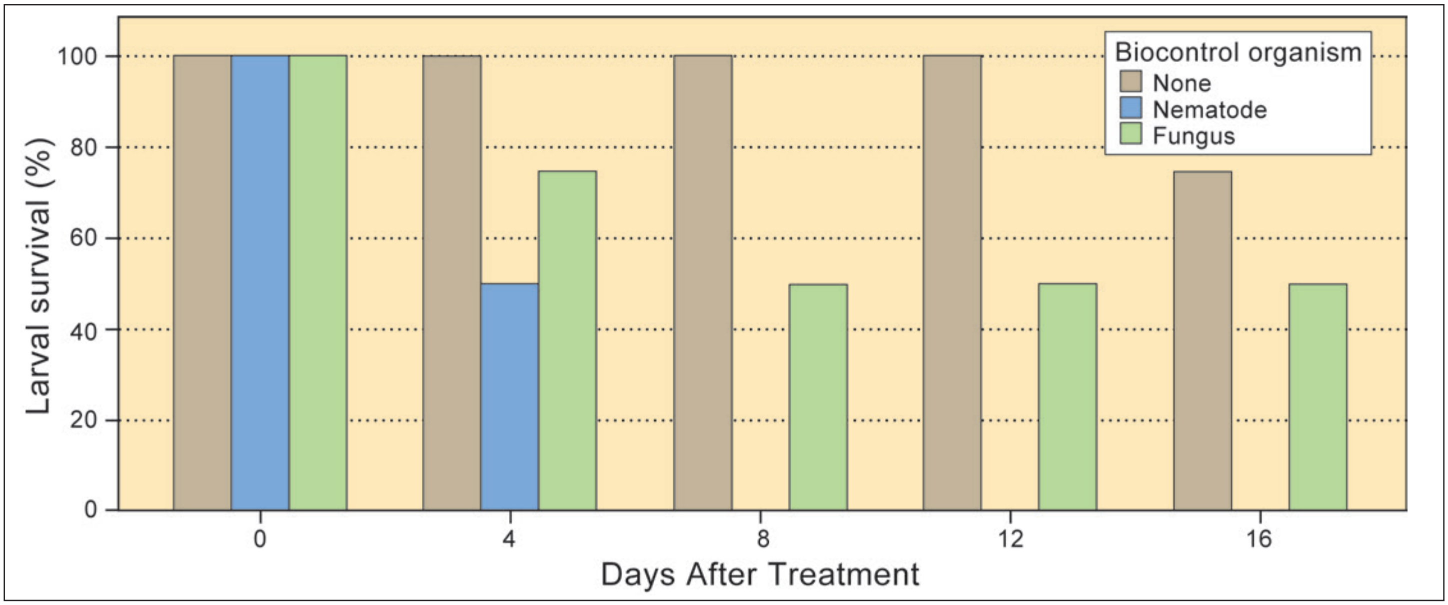

Figure 5. Larval survival varied by treatment during a 16-day assessment period for the biocontrol trial conducted in 2019.

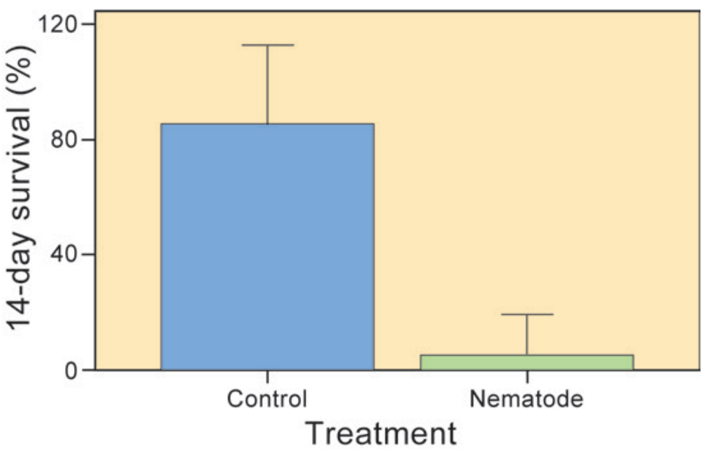

Figure 6. In the 2020 trial, larval survival for those in the control treatment was significantly greater than those in the nematode treatment based on Students' t-test comparisons ($t = 7.16$; 8 df; $[P > t] = 0.0001$).

Conclusions

IPM is a broad-based approach to management of an entire system. A combination of techniques including biological control are typical in a nursery IPM program. Some pest species, such as *Lygus hesperus,* have a large complex of natural enemies that prey upon them. For other pests, such as black vine weevil, natural enemies, such as entomopathogenic nematodes and fungi, can be effective treatments against infestation. These natural enemies can penetrate the soil mix to attack and kill root-feeding larvae. Results from the current trials further demonstrate that nematodes, such as *Heterorhabditis bacteriophora*, and fungi, such as *Isaria fumosorosea*, can be used in an IPM system to kill black vine weevil larvae. Both the nematode and the fungus are commercially available entomopathogens that infect soil invertebrates and pose no threat to plant material.

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