

AN ABSTRACT OF THE THESIS OF

Joyce Takeyasu for the degree of Master of Science in Entomology presented on November 10, 1994. Title: Control of Mint Root Borer, *Fumibotys fumalis*, with the Entomopathogenic Nematode, *Steinernema carpocapsae*.

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Field experiments were conducted to investigate the potential of using the entomopathogenic nematode, *Steinernema carpocapsae* (Weiser) strain All to control the mint root borer (MRB), *Fumibotys fumalis* (Guenée). Single and multiple applications of *S. carpocapsae* ranging from 1.2 to 7.4 billion infective juveniles (IJs) per hectare were evaluated in small plots and in large field plots.

Single applications were made either pre- or post-harvest. Pre-harvest applications ranging from 1.2 billion to 7.4 billion IJs/ha reduced MRB numbers significantly from the control, but not always below the treatment threshold of 2-3 MRBs per 929 cm<sup>2</sup>. Post-harvest nematode application rates equal to or above 2.5 billion IJs/ha reduced MRB numbers below the treatment threshold and performed as well or better than chlorpyrifos applied at 2.24 kg active ingredient (a.i.) per hectare. Multiple nematode applications using lower rates were as effective as a single application at a higher rate.

Application timing is an important consideration for successful reduction of MRB populations. Under pre-harvest conditions, nematode recovery from soil decreased significantly from 1 day to 14 days post-treatment. Limited nematode persistence, in combination with prolonged adult emergence, can lead to pre-harvest applications being applied too early. Post-harvest, fields can be treated too late. In a laboratory study, *S.*

*carpocapsae* was ineffective against MRB prepupae; therefore, fields need to be treated prior to prepupal formation. Variability in MRB development between fields further narrows the treatment window. As an alternative to precisely timing a single application, multiple nematode applications appear promising.

Because application timing requires the identification of MRB infestations in a timely fashion, studies were conducted to develop guidelines in diagnosing fields. Using Berlese funnels, fields monitored in 1993 were correctly identified as infested or non-infested in mid-August. Berlese funnel extraction offers advantages over hand-sorting because it is less labor intensive and more accurate. Efforts to correlate adult density with larval infestation were inconclusive using pheromone trap catches; however, a modified sweep search correctly ranked infestation levels in fields monitored in 1994.

Control of Mint Root Borer, *Fumibotys fumalis*, with the  
Entomopathogenic Nematode, *Steinernema carpocapsae*

by

Joyce Takeyasu

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# Control of Mint Root Borer, *Fumibotys fumalis*, with the Entomopathogenic Nematode, *Steinernema carpocapsae*

## CHAPTER 1

### INTRODUCTION

The mint root borer (MRB), *Fumibotys fumalis* (Guenée), is a serious pest of peppermint, *Mentha piperata* L., in the Pacific Northwest (Pike et al., 1988). Crop damage is caused by the larval stage which feeds on the underground stems or rhizomes. Early instars tunnel into the rhizomes, eventually hollowing out the interior as they grow in size. This feeding injury weakens the stand, making the plants more vulnerable to stresses such as low winter temperatures and propane flaming to control Verticillium wilt (*Verticillium dahliae* Kleb.) and rust (*Puccinia menthae* Pers.) (Pike et al., 1988). Left untreated, an infestation can progressively worsen each year to cause economic loss.

MRB population monitoring consists of a two-step program as follows (Berry and Fisher, 1993). First, sweep net samples during the growing season detect the presence of adult MRBs. Adult presence is an indicator of potential larval infestation later in the season. Second, soil samples are collected to determine larval density. One 929 cm<sup>2</sup> sample to a depth of 5.1 to 7.6 cm is taken every hectare (R. Berry and G. Fisher, personal communication), and treatment is recommended when an average of 2-3 larvae are found per sample. Since hand-sorting is commonly used to process soil samples, fields should not be evaluated too early because larvae will be small and difficult to detect.

Chlorpyrifos (Lorsban), applied post-harvest at a rate of 2.2 kg active ingredient (a.i.) per hectare, is currently the only chemical insecticide registered for control of MRB larvae. Because it cannot be applied pre-harvest, substantial crop damage may occur in heavily infested fields before harvest. Chlorpyrifos efficacy is affected, among other

things, by formulation and application technique. Slower dissipation rates were seen with chlorpyrifos formulated as granules than an emulsifiable concentrate, and by incorporating it into the soil as opposed to applying it as a surface spray (Getzin, 1985). In peppermint, chlorpyrifos formulated as an emulsifiable concentrate is registered for use, and is applied as a surface spray or "chemigated" through irrigation systems.

Inconsistent results observed with chlorpyrifos are attributed to difficulties encountered in bringing the insect in contact with the insecticide. Because of its low water solubility (Brust, 1966), chlorpyrifos has little vertical movement. After two hectare-cm of sprinkler irrigation, nearly all (>99%) of the chlorpyrifos residue was found in the top 2.5 cm layer of soil (Pike and Getzin, 1981). Also, chlorpyrifos loss in soil can affect efficacy through volatilization, chemical hydrolysis, microbial degradation, and clay-catalyzed hydrolysis.

Soil moisture affects chlorpyrifos persistence in soil. Irrigation immediately following treatment reduces chlorpyrifos loss, most likely due to a decrease in clay-catalyzed hydrolysis which occurs on dry soil surfaces (Pike and Getzin, 1981). In moist soils, chlorpyrifos is lost primarily through volatilization, chemical hydrolysis and microbial degradation (Getzin, 1985). Enhanced biodegradation, the increased rate of microbial degradation associated with soils receiving repeat applications of a particular pesticide, does not seem to occur with chlorpyrifos (Racke et al., 1990) although enhanced biodegradation occurs for other organophosphate insecticides (Sikora et al., 1990; Mojtahedi et al., 1991). Soil moisture may affect chlorpyrifos efficacy by influencing the bioavailability of chlorpyrifos more so than chlorpyrifos degradation (Tashiro and Kuhr, 1978).

Soil temperature and texture also affect chlorpyrifos persistence in soil. Higher soil temperatures increase the rate of chlorpyrifos degradation (Getzin, 1981a, 1981b, 1985). On dry soil surfaces, soil texture affects chlorpyrifos loss due to clay-catalyzed hydrolysis. The ability of soils to degrade chlorpyrifos depends on the type of clay

involved, but clay-catalyzed hydrolysis increases with increasing clay content (Getzin, 1981b). Organic matter has little or no ability to degrade chlorpyrifos (Getzin, 1981b); however, organic matter can adsorb to chlorpyrifos. Adsorption of chlorpyrifos by carbon accumulation in the soil is a concern in the Willamette Valley where peppermint fields are usually flamed in the spring and fall for disease control. In grass seed fields, the carbon residue resulting from burning straw and stubble adsorbed to chlorpyrifos and greatly reduced its efficacy (Kamm and Montgomery, 1990). Berry et al. (1991) reported similar results in peppermint, with lower toxicity seen with increasing field age, i.e. higher carbon and organic matter accumulation.

Other insecticides, for example ethoprop (Mocap) and carbofuran (Furadan), are known to have activity against MRB. Ethoprop is a nematicide currently in the registration process for use on mint; carbofuran is an insecticide previously used under Section 18 emergency exemption against root weevils. However, because of tighter regulations and the cost of re-registration, pesticides are becoming increasingly unavailable or more difficult to register. In addition, manufacturers have been reluctant to register new insecticides on minor crops like peppermint. Because of concerns over the impact of some pesticides on non-target organisms and the environment, there is a need for alternative control measures.

Cultural control methods for MRB control include the preventative practice of planting clean rootstock. MRB hibernacula resemble small clumps of soil and can easily pass undetected clinging to rootstock material from infested fields. Another cultural control method is tillage in the fall or early spring while MRB prepupae are overwintering within the hibernacula. Tillage was shown to result in approximately 80% reduction in adult emergence (Pike and Glazer, 1982; Talkington and Berry, 1986). Unfortunately, tillage can spread *Verticillium* wilt (Horner and Dooley, 1965; Schnathorst, 1981) and lead to soil compaction, so this practice has not been widely adopted.

Entomopathogenic nematodes in the genera *Steinernema* and *Heterorhabditis* offer a natural biological control alternative. These nematodes attack a wide range of insect pests and are particularly effective against soil-inhabiting insect pests (Klein, 1990). Pyralids known to be susceptible to these nematodes include the Mexican rice borer, *Eoreuma loftini* (Dyar) (Ring and Browning, 1990), the cranberry girdler, *Chrysoteuchia topiaria* (Zeller) (Georgis and Hague, 1991) and the greater wax moth, *Galleria mellonella* (L.), whose high susceptibility (Bedding and Akhurst, 1975) makes it an ideal laboratory bioassay organism. Preliminary field experiments (M. Morris and K. Smith, personal communication; Berry et al., 1990) suggest entomopathogenic nematodes can be used post-harvest for MRB reduction.

To investigate the potential of using the entomopathogenic nematode, *Steinernema carpocapsae* (Weiser) strain All, experiments were conducted to address the following objectives. First, to evaluate whether entomopathogenic nematodes would provide an effective and economic alternative, post-harvest experiments were conducted comparing nematode and chlorpyrifos efficacy. Second, the feasibility of applying the nematodes pre-harvest was evaluated. One advantage of using entomopathogenic nematodes is that, unlike chlorpyrifos, they can be applied pre-harvest to minimize feeding damage. Third, new information on MRB development indicated the importance of properly timing control measures. Since field diagnosis is a prerequisite to treatment, studies were conducted to evaluate methods for identifying MRB infestations. Finally, in order to circumvent the difficulties of precisely timing control measures, multiple nematode applications using lower rates were investigated.



## CHAPTER 2

### REVIEW OF LITERATURE

#### Peppermint Production

Peppermint, *Mentha piperata* L., in the family Labiatae, is grown commercially for its oil. The oil is produced in glands located primarily on the lower leaf surface and is used to flavor chewing gum, candy, toothpaste and medicinals. Peppermint is a perennial crop propagated by rhizomes (underground stems), not by seed. The rhizomes are planted in the spring, initially in rows; however, lateral growth via runners results in a solid stand the following year. Peppermint grows to a height of approximately 0.8 m before flowering. Although oil yields increase until full bloom (Bullis et al., 1948), harvest is recommended at early bloom to obtain the best oil quality. Lacy et al. (1981) recommend harvesting prior to 10% bloom while White et al. (1987) found harvesting at 10-20% bloom to be optimal. Once cut, the hay is left in windrows for 24-36 hours to cure. It is then chopped, loaded into distillation tubs, and steam distilled to separate the oil.

Because peppermint needs a midsummer daylength of at least 15 hours for satisfactory oil yields, production is limited to areas north of the 40th parallel (Green, 1960). The Pacific Northwest (Oregon, Washington, Idaho and Montana) and the Midwest (Indiana, Wisconsin and Michigan) are the main peppermint growing areas in the United States. Oregon currently ranks first in the nation in peppermint oil production with over three million pounds of oil or 45.7% of the total United States production in 1992 (USDA Oregon Agricultural Statistics Service, 1993). Peppermint acreage in Oregon is primarily divided between western Oregon (54%) and central Oregon (43%). A small portion (3%) of the state's acreage is in the northeastern part of the state. For a complete

history on the development of peppermint cultivation in the United States and Oregon, see Landing (1969) and Matzat (1981).

Peppermint grows best in rich, well-drained soils with pH ranging from 6 to 7.5 (Martin et al., 1976; Jackson et al., 1983). Because of its shallow root system, peppermint has high moisture requirements. In the Pacific Northwest, fields are irrigated every 4-7 days with approximately 2.5 cm of overhead irrigation. Sprinkler irrigation systems (hand or wheel lines) are most common although there are fields with center pivots, linear track systems and water cannons. The irrigation system also can be used to apply fertilizer during the growing season as well as pesticides that have chemigation labels.

Weed control is an important factor in peppermint production not only because weeds compete for water, nutrients and space, but also because certain weeds can discolor and impart off-flavors to the oil (Green, 1960; Martin et al., 1976). Weed control consists of preventative measures such as fallowing or growing a weed-free crop prior to planting peppermint. After peppermint has been planted, weed control is primarily accomplished with chemical herbicides. Although tillage is an effective weed control method (Green, 1960), it has not been widely adopted because it can spread *Verticillium* wilt (Horner and Dooley, 1965; Schnathorst, 1981).

*Verticillium* wilt, a disease caused by the fungus *Verticillium dahliae* Kleb., is the limiting factor in growing peppermint. *Verticillium* wilt causes a characteristic curling of the upper leaves accompanied by stunting and chlorosis (or bronzing) of infected plants before they eventually die. In heavily infested fields, peppermint may no longer grow. Because the microsclerotia of *V. dahliae* can survive in the soil for approximately 13 years (Schnathorst, 1981), *Verticillium* wilt is a long term problem. Management tactics include planting wilt tolerant varieties such as Todd's Mitcham or Murray Mitcham and rotating to a non-susceptible crop. Propane flaming, used to control mint rust (*Puccinia menthae* Pers.), also was found to reduce *Verticillium* wilt (Horner and Dooley, 1965). As a result,

flaming in the spring for rust control and again in the fall for wilt control have become established practices.

Plant parasitic nematodes feed on peppermint roots, leading to stunted plants with reddish foliage. In addition, plant parasitic nematodes can exacerbate *Verticillium* wilt severity (Faulkner et al., 1970). Although several species have been recovered from peppermint (Bergeson and Green, 1979; Ingham, 1992), the root lesion nematode, *Pratylenchus penetrans* (Cobb) Chitwood and Otiefa, is the predominant nematode pest in Oregon peppermint fields (Pinkerton, 1983). Nematode management includes pre-plant soil fumigation, use of clean planting stock, and application of the nematicide, oxamyl (Vydate).

The insect pest complex in peppermint varies from geographic areas to individual fields. For example, the mint stem borer, *Pseudobaris nigrina* (Say), is found only in Idaho and eastern Oregon. Root weevils are more cosmopolitan, but since adults are brachypterous and unable to readily disperse, they occur more on a field-to-field basis. Other insect pests, e.g. aphids, rarely cause damage. In contrast, cutworm and looper populations can cause damage that warrants an insecticide application. Because of the negative impact of insecticides on non-target organisms, control measures are recommended only when pest density exceeds a threshold level. For example, two-spotted spider mites are considered secondary pests, achieving pest status when chemical insecticides disrupt their natural enemies (M. Morris, personal communication).

Several insect species occasionally feed on peppermint but are of little or no economic importance. Of particular interest in relation to the MRB are two other pyralid moths: the false celery leaf-tier (FCL), *Udea profundalis* (Packard), and the orange mint moth (OMM), *Pyrausta orphisalis* Walker. Adult FCLs are very similar in appearance to MRB moths and are frequently mistaken for them. Unlike the MRB, however, the FCL is of little or no economic importance in peppermint. Adult OMMs also may be confused with MRB moths. In this case, correct identification is especially important since the

OMM is considered beneficial. OMM larvae feed on the terminal growth of peppermint plants which encourages lateral branching, and in some cases, leads to higher oil yields (Pike et al., 1987).

In addition to the MRB, other pests that attack the below-ground portions of peppermint include the garden symphylan, *Scutigera immaculata* (Newport), the mint flea beetle (MFB), *Longitarsus ferrugineus* (Foudras), and several species of root weevils of which the strawberry root weevil (SRW), *Otiorhynchus ovatus* (Linn.) is the most common. Like the MRB, the larval stage is the damaging stage for MFBs and root weevils. The garden symphylan is not a true insect, possessing six pairs of legs as newly hatched nymphs and 12 pairs as adults. Both nymphs and adults feed on small roots and root hairs. Berry and Fisher (1993) provide population threshold levels and management guidelines for these and other peppermint pests.

### **Mint Root Borer**

The MRB is a light brown moth in the family Pyralidae with a wingspan of approximately 22 mm. It has distinctive line markings on the forewings that are used in identification. According to Munroe (1976), Guenée first described the MRB in 1854 and named it *Ebulea fumalis*. Subsequently, Walker and Grote independently described the MRB as *Scopula orasusalis* and *Botis badipennis*, respectively. Forbes (1923) referred to the MRB as *Phlyctaenia fumalis* (Guenée); however, when the MRB was discovered in Oregon, it was identified as *Pyrausta fumalis* (Guenée) (Berry, 1974). Munroe (1976) placed the MRB in the genus *Fumibotys* where it remains today as *Fumibotys fumalis* (Guenée).

The MRB is a relatively recent pest of peppermint and spearmint. The first documentation of MRB damage was in 1971 in a peppermint field in Umatilla county, Oregon (Berry, 1974). Since then, it has been found in the mint production areas of

Oregon, Washington, Idaho and Montana. For a number of years, the MRB was not found in central Oregon, but recently has been discovered there (M. Morris, personal communication). Similarly, Scotch spearmint (*Mentha cardiaca* G.) was a known host of the MRB, but the host status of native spearmint (*Mentha spicata* L.) has only recently been determined (R. Schneider, personal communication).

The following information on MRB biology is taken from Berry (1974) and Pike et al. (1988) unless otherwise indicated. MRB adults emerge from the soil over a two month period beginning in June with peak emergence normally in mid-July. There is one generation per year with adults living for 8-10 days at 20° C. Twelve hours after emerging, female moths emit a sex pheromone to attract males for mating (Davis et al., 1984). Once mated, females begin laying eggs on the foliage. The flat, slightly oval eggs are 1.0-1.5 mm in length and are translucent white. Usually five eggs are deposited in an overlapping fashion and hatch in 9-10 days at 20° C.

Larvae are cream-colored with a brown head capsule reaching a maximum body length of approximately 19 mm. First instars feed on the foliage for several days before dropping to the soil surface and tunneling into the rhizomes. As the larvae grow, they completely hollow out the interior of the rhizome. Although MRB larvae are usually found inside rhizomes, late instars can be found in soil as they leave damaged rhizomes in search of other rhizomes to enter. In late summer/early fall, larvae exit the rhizomes and begin constructing silk-lined earthen cells called hibernacula as they prepare to overwinter. The MRB overwinters as a prepupa. Prepupae are approximately 9 mm in length, contracted in appearance with distinct segmentation. Pupation occurs in the spring followed once again by adult emergence.

### Entomopathogenic Nematodes

Rhabditid nematodes in the families Steinernematidae and Heterorhabditidae are effective biological control agents against many soil insect pests of agricultural crops (Klein, 1990). The larval stage of the insect is usually targeted since it causes direct damage to the crop. For lepidopteran larvae, susceptibility to the nematodes is age-dependent, with early instars more susceptible than late instars (Kondo, 1987; Glazer and Navon, 1990; Glazer, 1992). Susceptibility also depends on species, both that of the insect and nematode (Bedding et al., 1983). Furthermore, lepidopteran prepupae (Kaya and Hara, 1980; Kaya and Grieve, 1982), pupae (Kaya and Hara, 1980, 1981; Hara and Kaya, 1983b), and adults (Triggiani and Poinar, 1976; Kaya and Grieve, 1982) are susceptible to varying degrees.

Initial control efforts were directed at foliar-feeding insects (Gaugler, 1981). Under the right environmental conditions, the nematodes can reduce infestations to an acceptable level; however, foliar applications often yield disappointing results because exposure to UV light, high ambient temperatures and desiccation adversely affect nematode survival (Begley, 1990). Focusing on those species that occur in the soil or in cryptic habitats has met with greater success. Commercial markets for entomopathogenic nematode products include agricultural crops such as turf, berries, mint, citrus, ornamentals and mushrooms in both the U.S. and abroad (Georgis, 1990; Georgis and Hague, 1991; Georgis, 1992). Efficacy against such lepidopteran pests as the cranberry girdler, *Chrysoteuchia topiaria* (Zeller) (Georgis and Hague, 1991), the carpenterworm, *Prionoxystus robiniae* (Peck) (Lindgren and Barnett, 1982; Lindgren et al., 1981), and various cutworms (Poinar, 1986) are encouraging. Interest in these nematodes has resulted in a large volume of research which has been compiled in an extensive bibliography (Smith et al., 1992).

Among the attributes that make entomopathogenic nematodes successful from a pest management perspective are 1) a wide host range, 2) environmental and worker safety, 3) ability to kill the host rapidly, usually within 24-48 hours, 4) commercial availability, 5) ease of application, and 6) EPA exemption. A wide host range can be both a desirable and undesirable trait (Kaya and Gaugler, 1993). The concern is that these nematodes will indiscriminantly kill any insect, including beneficials. Although entomopathogenic nematodes are capable of killing hosts from nearly all insect orders and non-insect hosts that include other invertebrates (Arachnida, Symphylida, Gastropoda, Diplopoda and Crustacea) and tadpoles (Amphibia) (Poinar, 1989), the bulk of these data were obtained from laboratory infections in petri plates. In field tests, there is no evidence that nematodes adversely affect non-target organisms (Akhurst, 1990; Georgis et al., 1991). As a result, they have been exempted from registration by the U. S. Environmental Protection Agency (Gorsuch, 1982). Perhaps most important from a commercial perspective is the ability to economically mass produce a control agent. Entomopathogenic nematodes can be reared *in vitro*, and the steinernematids are especially amenable to production on a commercial scale (Friedman, 1990).

*Steinernema carpocapsae* Weiser is one of 15 recognized species in the genus *Steinernema* (Hom, 1994). For a number of years, steinernematid nematodes were placed in the genus *Neoaplectana* which has resulted in some confusion in the literature. Both *Steinernema* and *Neoaplectana* were accepted as valid genera until they were synonymized and assigned the genus name *Steinernema* (Wouts et al., 1982). Taxonomy at the species level has resulted in further confusion. The synonym *feltiae* for *carpocapsae* has been problematic since *S. feltiae* also is a recognized species. To minimize confusion, Poinar (1990) suggests providing strain information with the species name as standard nomenclature. Nematode strains are actually geographic isolates that have yet to be properly characterized; therefore, they are not true taxonomic designators, i.e. subspecies. Molecular techniques (Curran, 1990) can be used to provide taxonomic

information below the species level. In particular, the polymerase chain reaction (PCR) technique has been successfully used in this regard (Joyce et al., 1994; Liu and Berry, 1995).

The nematode life cycle consists of adults, eggs and four juvenile stages (Kaya, 1985). The sexes are separate in steinernematids with adult males one-third to one-eighth the size of females (Poinar, 1979). All developmental stages occur inside the insect cadaver except for the infective juvenile (IJ), a free-living form of the third juvenile stage. Produced in response to unfavorable conditions such as diminishing food supply (Sulgostowska, 1979; Popiel et al., 1989), IJs exit the cadaver in search of new hosts. IJs retain the second juvenile cuticle which may play a role in protecting them from natural enemies (Poinar and Jansson, 1986; Epsky et al., 1988; Timper and Kaya, 1989). Also, the IJs do not feed, having a collapsed gut with the mouth and anus closed (Poinar and Leutenegger, 1968). Consequently, IJs have a limited time to locate and enter a host, relying on stored lipid reserves which comprise 32-38% of the total body weight (Selvan et al., 1993).

Until a host is found, abiotic environmental conditions play an important role in nematode survival. For instance, exposure to UV radiation is detrimental to nematode survival (Gaugler and Boush, 1978; Gaugler et al., 1992). Most infective juveniles of *S. carpocapsae* (94.9%) exposed to direct sunlight lost their pathogenicity in 60 minutes (Gaugler and Boush, 1978). Soil texture also affects nematode survival in that survival decreases with increasing clay content (Kung et al., 1990a). Compared to sandy soils, clay soils have smaller pore sizes and higher water potentials. In addition to restricting nematode movement, clay soils have poor aeration and low oxygen concentrations which negatively affects nematode survival (Kung et al., 1990b). Although too much water can create anaerobic conditions, nematodes need moisture for survival and mobility (Georgis and Gaugler, 1991).



Optimum soil moisture for nematode survival is quite low. For example, *S. carpocapsae* survived best at a soil moisture content of 2% (Kung et al., 1991). Drier soils may enhance nematode survival, but host-finding ability and infectivity may be compromised (Kaya, 1990). Infective juveniles enter an anhydrobiotic state in response to desiccation (Womersley, 1990) during which time they are inactive and unable to infect hosts. Similarly, temperatures favoring long-term nematode survival may not necessarily be ideal for host-finding and infectivity. The respiration rate is temperature dependent (Lindgren et al., 1986); therefore, lower temperatures would favor survival albeit in a less active state that would limit host-finding. The ability to survive at a particular temperature varies with nematode species. *S. carpocapsae* survives best at temperatures ranging from 5-25°C (Kung et al., 1991). Its pathogenicity and survival are adversely affected at 35°C (Schmiege, 1963; Kung et al., 1991) perhaps as a result of its temperate origins (Molyneux, 1985). In contrast, survival and pathogenicity of *S. glaseri*, a nematode of tropical/subtropical origin, are greater at higher temperatures (15-35°C) than at 5°C (Kung et al., 1991).

Host-finding strategies divide entomopathogenic nematodes into cruisers and ambushers (Lewis et al., 1992) although several species show both traits. Cruisers actively seek a host by active migration. When a host cue is encountered, they shift search patterns from ranging (linear movement) to localized searching (Lewis et al., 1992). Possible host cues include CO<sub>2</sub> (Gaugler et al., 1980), host excretory products (Schmidt and All, 1979), temperature (Burman and Pye, 1980; Byers and Poinar, 1982), host plasma (Khlibsuwan et al., 1992), the symbiont bacteria and various chemical gradients (Pye and Burman, 1981). CO<sub>2</sub> appears to be a particularly important host cue (Lewis et al., 1993). Cruisers are more adept at finding sedentary hosts whereas ambushers are better adapted to finding active hosts. Ambushers use an energy conserving approach, relying on host mobility to establish contact with the host. Ambushers are less influenced by host cues (Lewis et al., 1992 and 1993); however, this is not to say that they are not

attracted to hosts. *S. carpocapsae* is a typical ambusher, yet exhibits attraction to insect larvae (Schmidt and All, 1978). As is characteristic of an ambushing strategy, IJs of *S. carpocapsae* have little downward movement (Moyle and Kaya, 1981; Georgis and Poinar, 1983). Instead, they remain near the soil surface where they often nictate. Nictation, the behavior of waving the body from side to side while the posterior end of the nematode is anchored to the substrate, is thought to orient nematodes to approaching hosts (Ishibashi and Kondo, 1990).

Once a host is found, IJs enter the host through natural openings, i.e. the mouth, anus and spiracles (Mráček et al., 1988). The primary route into lepidopteran larvae appears to be through the mouth (Poinar and Himsworth, 1967; Kondo and Ishibashi, 1986). Entry via wounds also can occur. In addition, some heterorhabditids possess a terminal tooth and can gain entry through the cuticular sutures and intersegmental membrane (Bedding and Molyneux, 1982; Peters and Ehlers, 1994). Some insects, however, have a number of adaptations to resist nematode infection. Scarabaeid larvae, for example, have 1) a high defecation rate, 2) low CO<sub>2</sub> output, 3) sieve plates covering the spiracles, and 4) the ability to groom or push nematodes away from the mouth with the anterior legs (Gaugler, 1988). In *Spodoptera litura* (Fabricius), dense spines line the spiracles which appear to discourage nematode entry (Kondo and Ishibashi, 1989). The low susceptibility of *Leptinotarsa decemlineata* (Say) to entomopathogenic nematodes is attributed to repellent feces and their ability to encapsulate the nematodes (Thurston et al., 1994).

The final destination of IJs that have successfully entered a host is the hemocoel. The nematode can be detected in the hemocoel in as little as four hours after exposing the host to IJs, increasing with time so that after 12-24 hours, more nematodes are found in the hemocoel than in the alimentary tract (Kondo and Ishibashi, 1988). IJs gaining entry through the mouth and anus must penetrate the gut epithelium; those entering through the spiracles must penetrate the tracheal tubes. Because IJs have a firm head framework,

penetration is thought to occur by mechanical pressure (Kondo and Ishibashi, 1989). In the hemocoel, IJs release a gram-negative bacterium which they carry in their intestinal tract (Poinar, 1966). Steinernematid and heterorhabditid nematodes are associated with bacteria in the genus *Xenorhabdus* and *Photorhabdus*, respectively (Hom, 1994). The particular bacterial species carried by a nematode depends on the nematode species (Akhurst, 1983). *S. carpocapsae* is associated with the bacterium *Xenorhabdus nematophilus* Thomas and Poinar subspecies *nematophilus*.

The nematode and bacterium have a symbiotic relationship. The bacterium alone is not pathogenic when ingested orally, but multiplies rapidly and kills the insect when introduced into the hemocoel (Poinar and Thomas, 1967; Milstead, 1979). Without the nematode, the bacterium cannot access the hemocoel since the bacterium is not free-living. In fact, symbiont bacteria have only been isolated from the intestinal tract of entomopathogenic nematodes and from insects that have been attacked by the nematodes (Poinar, 1979). In addition to transporting the bacteria, the nematode appears to impair the host immune system, allowing the bacteria to establish. Burman (1982) detected toxin production by the nematodes to coincide with the presence of third and fourth stage juveniles and adults. Moreover, Götz et al. (1981) found a substance secreted by the nematodes that destroys host immune proteins. Initially, the nematodes enter the host undetected (Dunphy and Webster, 1985, 1986, 1988a), leaving the host immune system intact to repair tissue damage caused by the nematodes and to eliminate foreign bacteria that may have been introduced. Approximately five hours after nematodes have entered the host (Matha and Mráček, 1984; Dunphy and Thurston, 1990), the symbiont bacterium is released. Although the host produces hemocytes in an attempt to eliminate the bacterium, the bacterium is able to withstand the host cellular response. The bacterium produces a lipopolysaccharide hemocytotoxin (Dunphy and Webster, 1988b) which eventually destroys the hemocytes and results in a bacterial septicemia that kills the host.

The symbiont bacteria benefit the nematodes by providing a favorable environment and a food source for the nematodes. Without the bacteria, the nematode cannot complete its life cycle as illustrated by axenic nematodes (those reared without the symbiont bacteria) which are able to kill the host but cannot reproduce (Poinar and Thomas, 1966). In addition to providing nutrition, the bacterium also produces antibiotics (Paul et al., 1981; Akhurst, 1982; Richardson et al., 1988) to prevent other microorganisms from colonizing the insect cadaver, thus optimizing conditions for nematode development. Nematode development, however, is affected by the existence of two forms of the bacterium called the primary and secondary forms. The two forms are distinguished from each other by colony morphology and the uptake of dyes in the plating medium (Akhurst, 1980, 1983). Although both forms are equally pathogenic to insects (Akhurst, 1980; Dunphy and Webster, 1984; Akhurst and Boemare, 1990), only the primary form provides optimum conditions for nematode reproduction and is selectively retained by the IJ (Akhurst, 1980). Low nematode yields associated with the secondary form is attributed to the lack of antibiotic production (Nealson et al., 1990). In contrast, the primary form produces antibiotics (Akhurst, 1982). The primary form converts to the secondary form but usually not the other way around (Akhurst, 1980; Wouts, 1990). A bacteriophage may be involved in the conversion from the primary to secondary form (Poinar et al., 1989); however, Wouts (1990) suggests another bacterial species is involved. Associated with the primary form but not with the secondary form, the other bacterial species may optimize nematode yields by providing the cue for IJ formation through the production of substances disliked by the nematode.

### CHAPTER 3

## Control of Mint Root Borer in Peppermint with Post-Harvest Applications of the Entomopathogenic Nematode, *Steinernema carpocapsae*

### ABSTRACT

Field experiments were conducted to investigate the potential of post-harvest applications of the entomopathogenic nematode, *Steinernema carpocapsae* (Weiser) strain All to control the mint root borer (MRB), *Fumibotys fumalis* (Guenée). Application rates equal to or above 2.5 billion IJs/ha were as effective as chlorpyrifos applied at 2.24 kg active ingredient (a.i.) per hectare. A bioassay using greater wax moth, *Galleria mellonella* (L.), larvae showed nematode persistence to decrease significantly from one day to seven days post-application. In a laboratory experiment, MRB prepupae were not susceptible when exposed to 10,000 IJs, indicating that fields need to be treated prior to prepupal formation in late summer/early fall. Variability in MRB development between fields was observed and further complicates application timing.

### INTRODUCTION

The mint root borer (MRB), *Fumibotys fumalis* (Guenée), is a serious pest of peppermint (*Mentha piperata* L.) and spearmint (*M. cardiaca* G. and *M. spicata* L.) in the commercial production areas of Oregon, Washington, Idaho and Montana. There is one generation per year with adults emerging over a two month period beginning in late spring (Berry, 1974). Eggs are laid on the foliage but following eclosion, early instars drop to the soil surface and tunnel into the rhizomes or underground stems. Although larval feeding occurs throughout the growing season, crop damage is especially severe at the end of the season when larvae have reached their maximum size. Late instars weaken the

stand by hollowing out the interior of rhizomes. This feeding damage results in increased winter injury and yield reduction (Pike et al., 1988; Berry and Fisher, 1993). Treatment is recommended when 2-3 MRBs are found per 929 cm<sup>2</sup> soil sample (Berry and Fisher, 1993).

Strategies for managing the MRB include the preventative measure of using clean roots when planting new fields. Once a field is infested, control usually consists of post-harvest treatment with chlorpyrifos (Lorsban) directed at late instars. An alternative is to till in the fall or early spring which reduces adult emergence by approximately 80% (Pike and Glazer, 1982; Talkington and Berry, 1986); however, tillage has not been widely adopted because it can spread *Verticillium* wilt. Currently, chlorpyrifos is the only chemical insecticide registered for use against the MRB, but inconsistent control necessitates finding an alternative control measure to better manage this pest.

The entomopathogenic nematode, *Steinernema carpocapsae* (Weiser) strain All, is an effective biological control agent, especially against insect pests found in cryptic habitats and in the soil (Begley, 1990; Klein, 1990). The nematode, in association with the bacterium *Xenorhabdus nematophilus* Thomas and Poinar, is capable of killing a wide range of insects. From a pest management perspective, the nematode possesses several desirable traits. In addition to killing hosts rapidly (usually within 48 hours), it does not appear to harm non-target organisms (Akhurst, 1990; Georgis et al., 1991). Furthermore, the nematode is compatible with many agrichemicals (Hara and Kaya, 1983a; Forschler et al., 1990; Rovesti and Deseo, 1990) and can be applied with conventional liquid spray equipment.

Because the soil is the natural medium for the infective juvenile or free-living form of the nematode, soil insect pests are successfully reduced with *S. carpocapsae*. Results from a preliminary field experiment suggested that *S. carpocapsae* would effectively control MRB larvae when applied post-harvest at a rate of 7.4 billion IJs per hectare (M. Morris and K. Smith, personal communication; Berry et al., 1990). Although good

control efficacy is the driving force behind implementing an alternative control measure, availability and affordability are additional considerations. The All strain of *S. carpocapsae* is commercially available (BioVector<sup>®</sup>) and advances in mass production technology have priced the nematode competitively with chemical insecticides. To minimize treatment cost, it is of interest to determine the lowest rate that will provide economic reduction.

Late instar MRBs are the intended target of a post-harvest application, but a delay in treatment will result in prepupal formation. MRB larvae begin constructing silk-lined earthen cells (hibernacula) in late summer/early fall and enter the overwintering prepupal stage. If *S. carpocapsae* is capable of killing both larvae and prepupae, the treatment window widens considerably; however, if MRB prepupae are unaffected by the nematode, application timing becomes critical. Prepupae of other lepidopteran species are highly susceptible to *S. carpocapsae* (Kaya and Hara, 1980; Kaya and Grieve, 1982); however, the susceptibility of MRB prepupae is not known. Also, the role of the hibernaculum in possibly protecting prepupae from the nematode is not known. Pupal cells constructed out of soil particles may act as physical barriers to nematode infection (Hara and Kaya, 1983b; Gaugler, 1988).

In these studies, the post-harvest potential of *S. carpocapsae* as a biological control agent for MRB was evaluated. Objectives were three-fold: 1) compare nematode efficacy with that obtained with chlorpyrifos and find the minimum rate needed for control, 2) determine nematode persistence in the soil, and 3) investigate prepupal susceptibility to *S. carpocapsae*. Field efficacy experiments in small plots and on a large scale basis were conducted from 1991 to 1993 to address the first objective. A nematode persistence study was conducted using areas of the 1992 large scale experiment to address the second objective. Finally, a bioassay was conducted in 1992 to address the third objective.

## MATERIALS AND METHODS

### Efficacy Studies

Small plot experiments were conducted in commercial peppermint fields in 1991 and 1992. The 1991 experiment compared the following treatments: 0, 1.8, 3.7 and 7.4 billion IJs/ha of *S. carpocapsae* strain All (biosys, Palo Alto, CA). Treatments were replicated five times using a randomized complete block design (RCBD) in 14.9 m<sup>2</sup> plots. In 1992, nematode rates of 0, 2.5 and 4.9 billion IJs/ha were compared with 2.24 kg a.i./ha chlorpyrifos. A RCBD was used with treatments replicated four times in 22.3 m<sup>2</sup> plots. For both experiments, treatments were applied using a CO<sub>2</sub> driven backpack sprayer delivering approximately 281 liters/ha at 2.1 kg/cm<sup>2</sup> pressure. On the evening of September 10, 1991 and August 28, 1992, the plots were sprinkler irrigated with approximately 0.3 cm of water to moisten the ground prior to application. Treatments were then applied with the irrigation running, and immediately followed with approximately 2.5 cm of irrigation to move the nematodes down into the soil. The plots were evaluated 7 days (1991) and 14 days (1992) post-treatment. For each plot, six soil samples (464.5 cm<sup>2</sup> to a depth of six cm) were collected. Rhizomes were separated from the soil and placed in Berlese funnels equipped with 75 watt bulbs to extract live larvae. The extraction process took four days or until the rhizomes were completely dry. The soil was sifted through a screen (mesh size 0.6 cm) and carefully inspected for larvae and hibernacula.

Large plot experiments were conducted in 1992 and 1993 using irrigated areas as experimental units. In 1992, treatments consisted of 0, 2.5 and 4.9 billion IJs per hectare *S. carpocapsae*, and 2.24 kg a.i./ha chlorpyrifos. A RBCD replicated four times was used. Three of the blocks were located in a commercial peppermint field near Corvallis, OR (plot size 0.7 ha) while the remaining block was located in a commercial peppermint field



near Jefferson, OR (plot size 0.4 ha). Treatments were applied according to label directions between September 1-7 by injection into the irrigation line over a 30-60 minute interval to ensure even distribution. Applications were made in the late afternoon or evening except under cloudy skies on the morning of September 4. The ground was moistened with approximately 0.3 cm of water prior to application, and irrigated with approximately 2.5 cm of water immediately after treatments were applied. Evaluation of the experiment consisted of taking twenty soil samples (464.5 cm<sup>2</sup>) in each plot 14 days post-treatment. More soil samples were collected than in the small plot experiments in an attempt to reduce the large variation in count data caused by the aggregated distribution of MRB larvae. Because evaluation consists of destructive sampling, the sample size taken in the large plot experiment was prohibitive in the small plot experiments. MRB larvae and hibernacula were recovered by using Berlese funnels and soil screens as described above.

The 1993 large plot experiment compared 2.5 billion and 4.9 billion IJs per hectare *S. carpocapsae* with 2.24 kg a.i./ha chlorpyrifos in a RCBD. Treatments were replicated three times with two blocks located in a commercial peppermint field near Jefferson, OR (plot size 1.5 ha) and the remaining block located in a commercial peppermint field near Coburg, OR (plot size 2.7 ha). To obtain an estimate of the infestation level in each of the fields, pre-treatment soil samples (one 929 cm<sup>2</sup> soil sample every hectare) were taken on August 13 and September 1 for the Coburg and Jefferson fields, respectively. Treatments were then applied through the irrigation system at midday on September 7-8 for the Coburg field and September 13-14 for the Jefferson field.

Because the Jefferson field was sprinkler irrigated, the same procedure for the 1992 large scale experiment was used to apply the treatments. A different approach had to be taken in the Coburg field which had a center pivot irrigation system. For each treatment, delivering the appropriate rate involved the preparation of a nematode solution whose concentration was determined according to the injection rate and the area treated

per unit time. Treatments were injected into the irrigation system with the first pass of the pivot. The pivot was then repositioned to make a second pass over the treated area, delivering approximately 0.64 cm of irrigation with each pass. The experiment was evaluated 10 days post-treatment, taking eight soil samples (929 cm<sup>2</sup> to a depth of six cm) in each plot. Again, the number of live MRB larvae and hibernacula were determined using Berlese funnels and soil screens.

### **Nematode Persistence**

A nematode persistence study was conducted in the untreated and 4.9 billion IJs/ha treated areas of the 1992 large plot experiment using a modification of the methods described by Fan and Hominick (1991). The plots were evaluated 0, 1, 7, 14 and 28 days post-application by collecting soil at six random sites in each plot. On each sampling date, two 150 cm<sup>3</sup> samples to a depth of six cm were collected at each site and combined. The soil was passed through a screen with 2.794 mm openings (W.S. Tyler Co., Mentor, OH) and approximately 225 cm<sup>3</sup> of the screened soil was placed in a 237 ml plastic container. The soil was moistened, if dry, to obtain a soil moisture content of approximately 30% w/w. Five late instar *Galleria mellonella* (L.) (Waxworms, Cameron, WI) were placed on the soil surface. The containers were then covered and incubated in the dark at 20°C. After six days, the larvae were replaced with another five larvae and again incubated for six days at 20°C. Dead larvae were dissected, and the number of nematodes present was recorded. Cadavers with characteristic signs of nematode infection (light brown coloration and disintegration of internal organs) were assigned a count of one even though nematodes were not found upon dissection. A natural log transformation of nematode counts stabilized the variance and the data were analyzed as a RCBD.

### **Prepupal Susceptibility to Nematodes**

In April 1992, MRB hibernacula containing prepupae were collected from a peppermint field in Sunnyside, WA and kept at 4°C until the start of the experiment. A total of 160 insects were used. Prepupae were carefully removed from 80 hibernacula while the remaining 80 were left intact. Infective juveniles of *S. carpocapsae* strain All (biosys, Palo Alto, CA) was dissolved in water to make a nematode stock solution. This stock solution was further diluted to give the following concentrations: 5, 10, 25, 50, 100, 500 and 1000 IJs/ml.

To expose hibernacula and prepupae to various nematode concentrations, a single hibernaculum or prepupa was placed in the bottom of a 29.6 ml plastic cup and filled with 30 g dry weight of sterilized soil (a 50:50 mixture of sand and sandy loam soil). To each cup, 2.9 ml of deionized water was added and allowed to equilibrate for 30 minutes. This was followed by one ml of the appropriate nematode dilution or deionized water to bring the soil moisture content to 13% w/w. The cups were treated with 0, 5, 10, 25, 50, 100, 500 or 1000 IJs with treatments replicated ten times. Each cup was then covered with a plastic lid and incubated in the dark at 25°C. Mortality was assessed after six days.

A second experiment compared the relative susceptibility of variegated cutworm *Peridroma saucia* (Hubner) larvae and MRB prepupae to nematodes. Cutworm larvae were reared at 22°C on artificial diet (Bioserv, Frenchtown, NJ). The procedure was identical to the first experiment except half the cups received a cutworm larva (late 4th to early 5th instars) while the remaining cups received a MRB prepupa. Treatments consisted of 0, 1,000 and 10,000 IJs per cup, replicated ten times. Mortality was assessed after three days at 30° C. Dead larvae and prepupae were dissected to confirm nematode presence or absence.

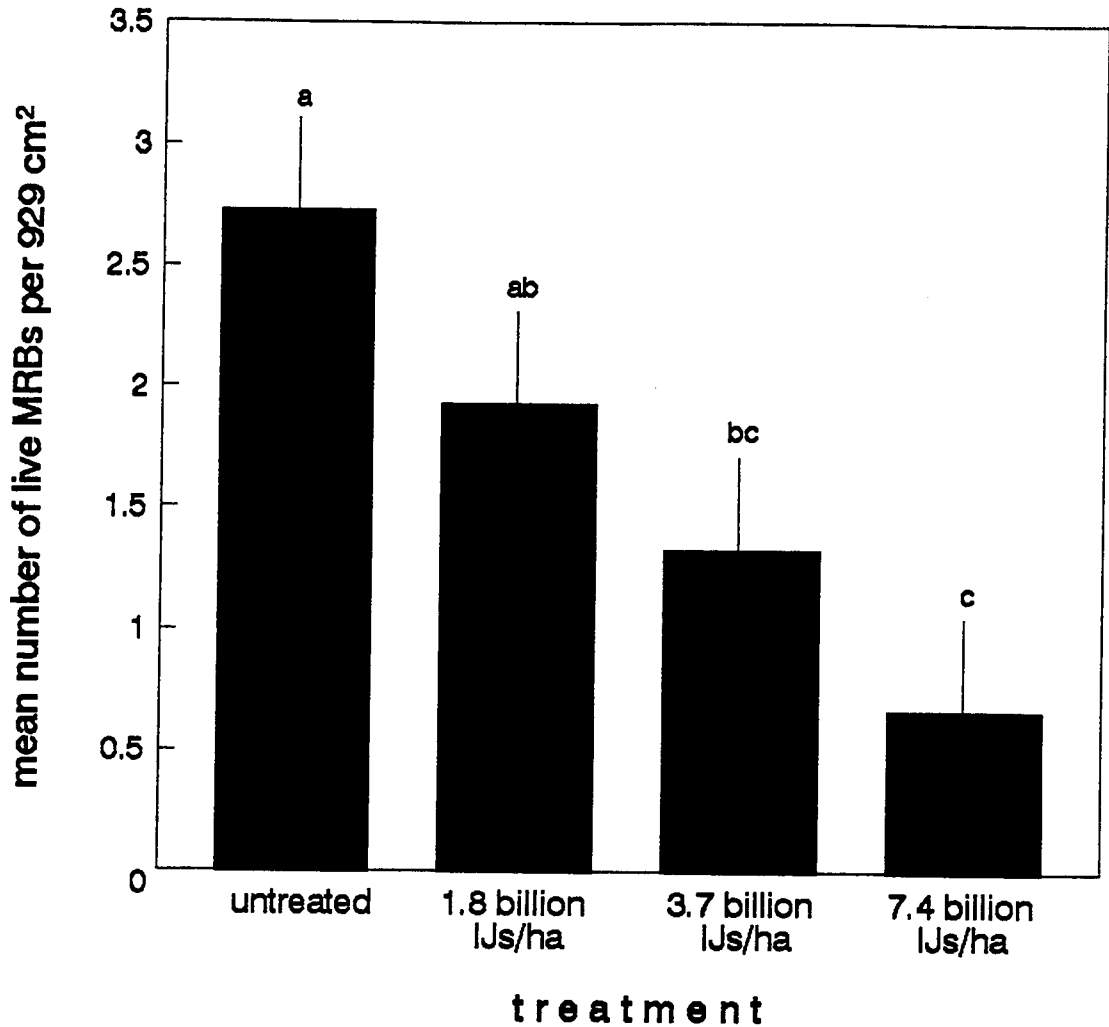
## RESULTS

### Efficacy Studies

In the 1991 small plot experiment, significant differences in the mean number of live MRB larvae recovered were not detected between the 3.7 billion and 7.4 billion IJs/ha rates; however, differences between these rates and the control were significant (Figure 3.1). Although differences were not detected between the 1.8 billion IJs/ha rate and the control, all nematode rates reduced MRB numbers below the treatment threshold of 2-3 MRBs per 929 cm<sup>2</sup>. A trend for higher MRB reduction with increasing nematode rate was seen with percent reductions of 29.3%, 51.2% and 75.6% for the 1.8 billion, 3.7 billion and 7.4 billion IJs/ha rates, respectively.

In the 1992 small plot experiment, the two nematode treatments significantly reduced MRB numbers compared with the control (Figure 3.2). Percent reductions of 62.5% and 81.2% for the 2.5 billion and 4.9 billion IJs/ha rates respectively reduced MRB numbers below the treatment threshold. In contrast, the chlorpyrifos treatment, with a 18.8% reduction in MRB numbers, failed to lower MRB numbers below the treatment threshold and was not significantly different from the control.

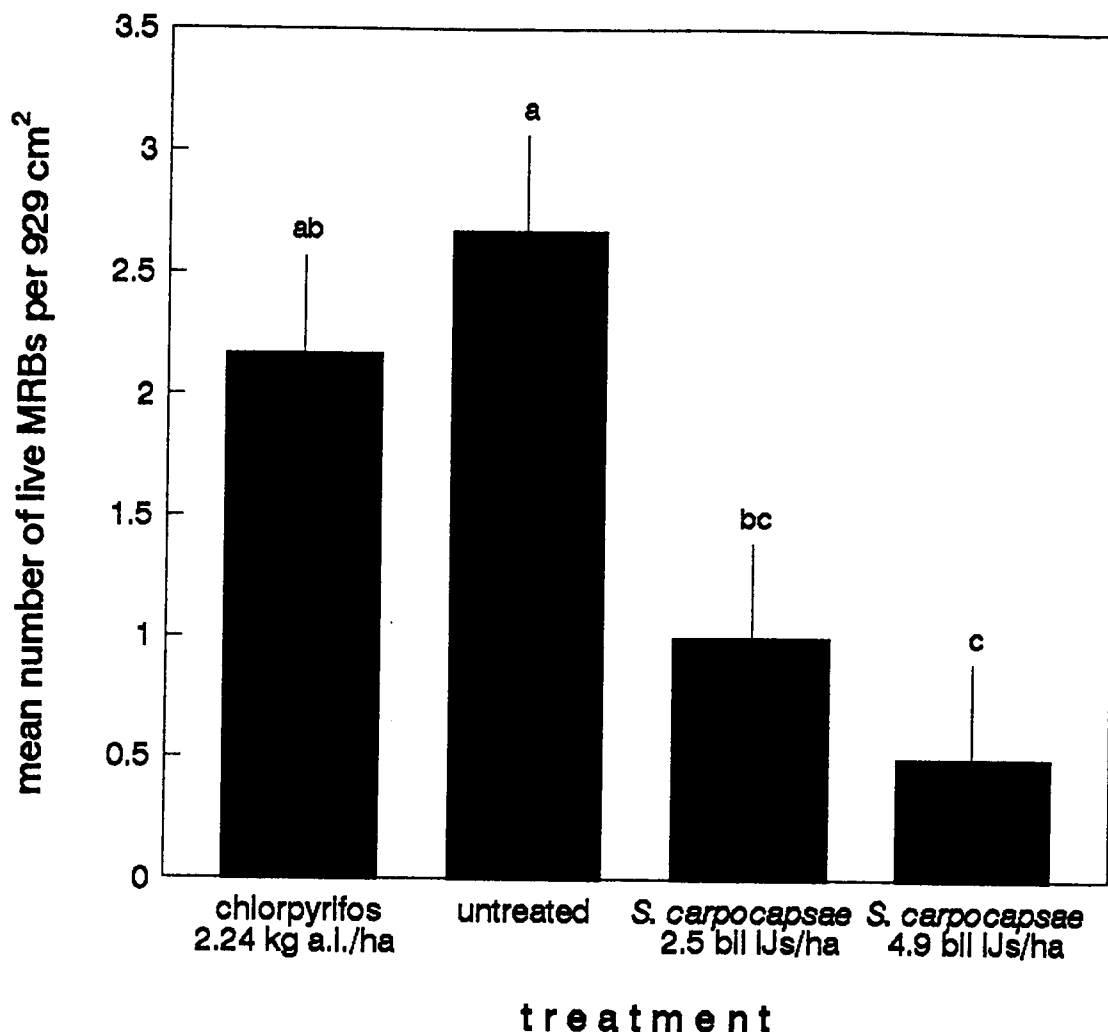
In the 1992 large plot experiment, the chlorpyrifos and nematode treatments significantly reduced MRB numbers compared to the untreated control (Figure 3.3). The chlorpyrifos treatment resulted in a 73.5% reduction in MRBs while the nematode treatments yielded 75.2% and 85.5% reductions for the 2.5 billion and 4.9 billion IJs/ha rates, respectively. Significant differences were not detected among any of these treatments as was the case for the 1993 large scale experiment which compared the same treatments (Figure 3.4).



**Figure 3.1** Mean number of mint root borers recovered from 929 cm<sup>2</sup> soil samples in 1991 post-harvest experiment (in small plots) conducted in a commercial peppermint field in Lane county, Oregon. Separation of means by Fisher's Protected LSD ( $p \leq 0.05$ ). Bars with different letters are significantly different.

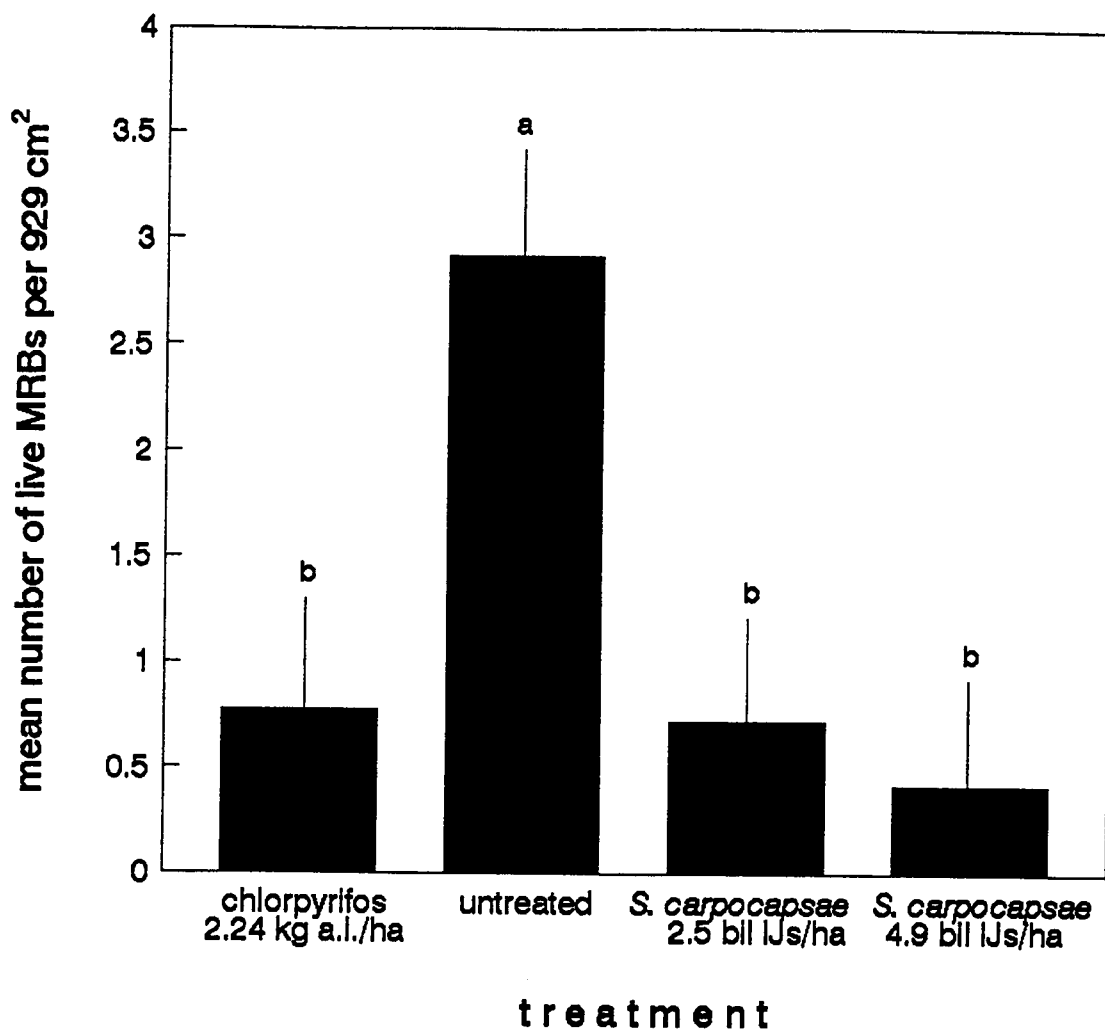
### Nematode Persistence

On the pre-treatment sampling date, most (205 out of 240) of the *G. mellonella* larvae used to bioassay the soil samples survived. Of those that died, only one was attributed to nematode infection. A single nematode was recovered from the dead larva which indicated the presence, in low numbers, of a natural population of



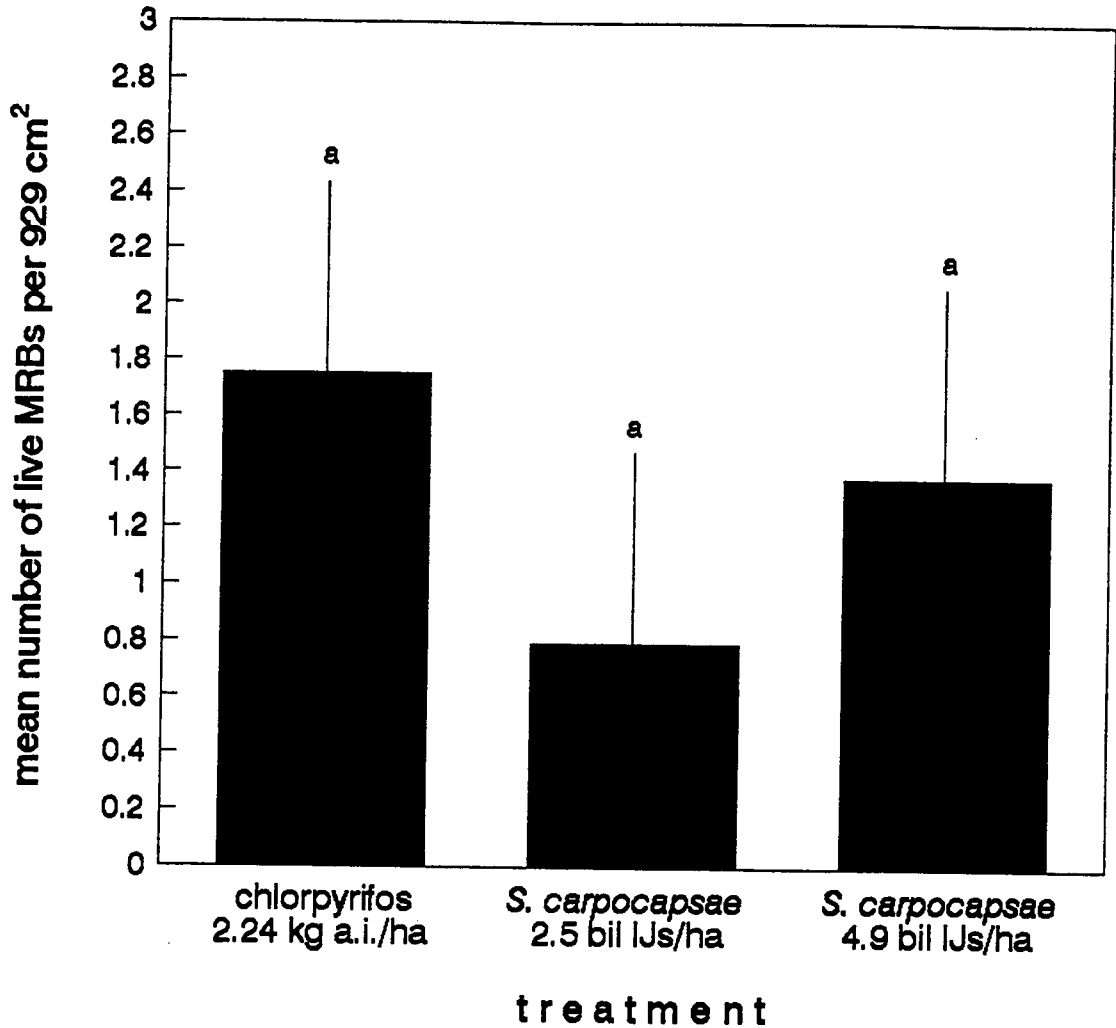
**Figure 3.2** Mean number of mint root borers recovered from 929 cm<sup>2</sup> soil samples in 1992 post-harvest experiment (in small plots) conducted in a commercial peppermint field in Benton county, Oregon. Separation of means by Fisher's Protected LSD ( $p \leq 0.05$ ). Bars with different letters are significantly different.

entomopathogenic nematodes in the Corvallis field where the sample was collected. In samples taken one day post-application, all *G. mellonella* larvae died with an average of 123.6 IJs recovered in 225 cm<sup>3</sup> of soil (Figure 3.5). There was a significant decrease in nematode recovery in the next set of samples taken seven days post-treatment. Nematode recovery in the samples taken 14 days post-treatment was not significantly different from



**Figure 3.3** Mean number of mint root borers recovered from 929 cm<sup>2</sup> soil samples in 1992 post-harvest experiment (in large plots) conducted in commercial peppermint fields in the Willamette Valley, Oregon. Separation of means by Fisher's Protected LSD ( $p \leq 0.05$ ). Bars with different letters are significantly different.

the previous sampling date due to a noticeable increase in nematode recovery in the Jefferson field. This increase may have coincided with IJ production; however, by 28 days post-treatment, nematode recovery from the soil dropped to an average of 3.1 IJs in 225 cm<sup>3</sup> of soil.

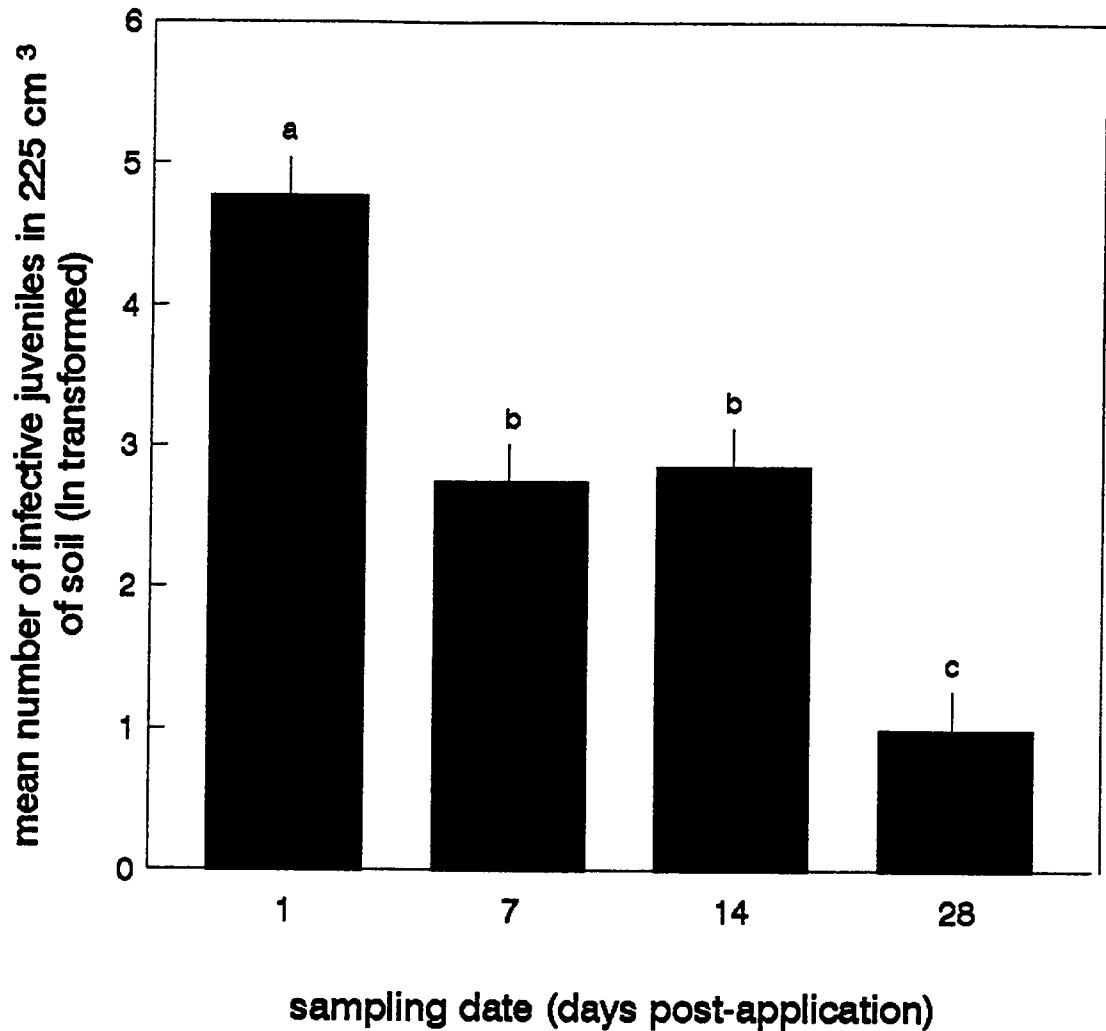


**Figure 3.4** Mean number of mint root borers recovered from 929 cm<sup>2</sup> soil samples in 1993 post-harvest experiment (in large plots) conducted in commercial peppermint fields in the Willamette Valley, Oregon. Separation of means by Fisher's Protected LSD ( $p \leq 0.05$ ). Bars with different letters are significantly different.

### Prepupal Susceptibility to Nematodes

In the first experiment, mortality was not observed in any of the treatments even at the highest nematode concentration of 1,000 IJs/ml (Table 3.1). All prepupae successfully pupated and emerged as adults. To verify that the lack of mortality was not due to non-pathogenic nematodes, a fifth instar variegated cutworm was placed in each of 12





**Figure 3.5** Mean number of infective juveniles recovered in 225 cm<sup>3</sup> of soil using *Galleria mellonella* as a bioassay organism. Separation of means by Fisher's Protected LSD ( $p \leq 0.05$ ). Bars with different letters are significantly different.

randomly selected cups treated with the 1,000 IJs/ml. After three days, all cutworm larvae were dead and dissection of the cadavers confirmed the presence of nematodes.

In the second experiment, there was no nematode mortality due to nematodes observed in the controls for either the VC or MRB (Table 3.2). One cutworm larva died in the control treatment, but dissection of the cadaver revealed no nematodes. Because it

**Table 3.1** Susceptibility of mint root borer prepupae and hibernacula to *Steinernema carpocapsae*

<b>treatment</b>	<b>% mortality due to <i>S. carpocapsae</i></b>	
	<b>prepupa</b>	<b>hibernacula</b>
0 IJs	0	0
5 IJs	0	0
10 IJs	0	0
25 IJs	0	0
50 IJs	0	0
100 IJs	0	0
500 IJs	0	0
1000 IJs	0	0

**Table 3.2** Susceptibility of mint root borer prepupae and variegated cutworm larvae to *Steinernema carpocapsae*

<b>treatment</b>	<b>% mortality due to <i>S. carpocapsae</i></b>	
	<b>MRB prepupa</b>	<b>VC larva</b>
0 IJs	0	0
1,000 IJs	10	100
10,000 IJs	0	100

was smaller in size, it is presumed that this larva died of starvation since the larvae were incubated for 6 days without food. The other larvae exhibited signs of starvation but recovered when given artificial diet. At 1,000 IJs/ml, all ten cutworm larvae died from nematode infection while only one MRB prepupa died. One cutworm larva was alive when the experiment was evaluated. It was clinging to the lid, out of contact with the soil; however, it eventually died two days later and dissection of the cadaver confirmed nematodes as the cause of death. At 10,000 IJs/ml, all the VC larvae died from nematode infection while all the MRB prepupae were still alive. The prepupae eventually pupated

and emerged as adults. Because the MRB prepupa that died occurred at the lower nematode concentration, there is reason to believe that this prepupa was injured in some way while being taken out of its hibernaculum. An injury would have provided an entryway for nematode infection.

## DISCUSSION

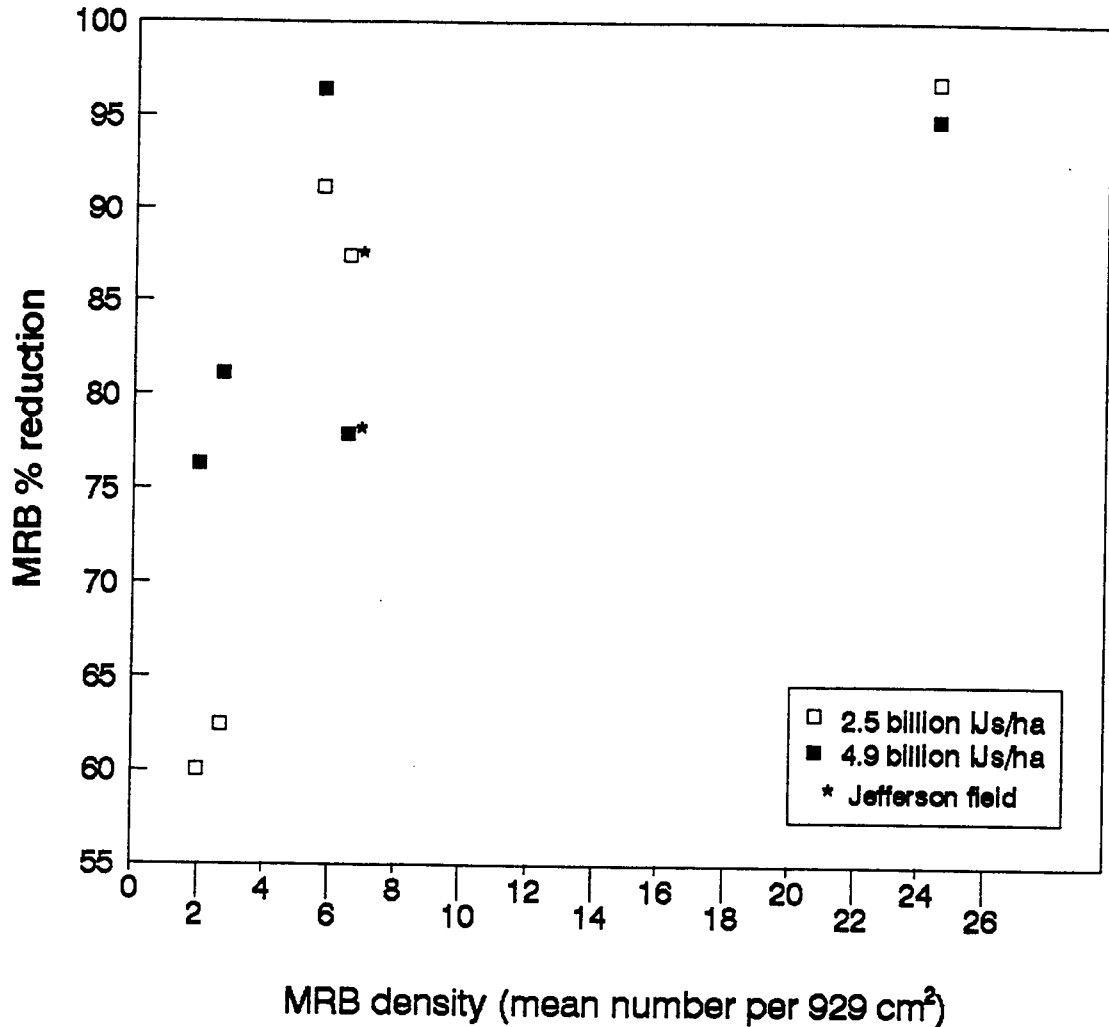
These experiments demonstrate that a post-harvest nematode application can effectively control the MRB. Compared to untreated controls, nematode rates equal to or above 2.5 billion IJs/ha resulted in significant MRB reduction. Only the 1.8 billion IJs/ha rate in the 1991 small plot experiment failed to reduce MRB numbers significantly from the control. This experiment, however, was evaluated only 7 days after treatments were applied because the plots were going to be oversprayed with chlorpyrifos. The nematodes may not have had sufficient time to provide maximum population reduction, resulting in an underestimation of nematode efficacy.

Significant reduction in MRB numbers were not detected between the 2.5 billion and 4.9 billion IJs/ha rates; however, there is reason to believe that control may be sacrificed by reduced rates. The 1991 small plot experiment displayed a trend for higher MRB reduction with increasing nematode rate. Six days after nematode treatment, Purcell et al. (1992) observed significant differences in larval mortality of *Helicoverpa zea* (Boddie) among three nematode concentrations, but no trend was observed eleven days after treatment. Additional mortality between the first and second evaluation of the experiment (with comparatively higher larval mortality for the lowest nematode concentration) and large variation in larval mortality in the treatments are cited as the cause of non-significant results on the second evaluation. Evaluating an experiment at different points in time may influence what is observed. In the 1991 small plot experiment,

it is unclear whether significant differences actually exist between application rates or whether this is an artifact from sampling the experiment too early. Further studies are needed to address this issue.

Both the 2.5 and 4.9 billion IJs/ha rates of *S. carpocapsae* performed as well or better than chlorpyrifos at a rate of 2.24 kg a.i./ha. Under current prices, the cost to the grower to apply nematodes at 2.5 billion IJs/ha is approximately the same as a chlorpyrifos treatment. Chlorpyrifos demonstrated variable results, performing poorly in the 1992 small plot experiment but better in the large plot experiments. Different application methods were used and may explain why variable results were obtained. Chlorpyrifos may provide better control when chemigated as in the large plot experiments rather than applied as a ground spray as in the small plot experiment. Chlorpyrifos efficacy also is affected by factors such as soil type (Getzin, 1981b), microbial degradation (Getzin, 1981a), and organic matter and carbon residue accumulation (Kamm and Montgomery, 1990; Berry et al., 1991).

Curran (1992) observed a negative relationship between percent mortality and pest density in experiments evaluating entomopathogenic nematodes against the black vine weevil, *Otiorhynchus sulcatus* (F.). He hypothesized that the nematodes accumulate around the first insect encountered, leaving other insects uninfected. For the MRB, percent mortality and pest density appear to be a positive rather than a negative relationship. Data from each of the fields involved in the post-harvest experiments showed that percent reduction increased with increasing MRB density until an upper limit was reached (Figure 3.6). It should be noted that the Jefferson field used in the 1993 large plot experiment was limed prior to applying the treatments which may have affected the percent reduction. Closer examination of the relationship between percent mortality and MRB density reveals a possible difference in efficacy between the 2.5 billion and the 4.9 billion IJs/ha rates. Substituting the reciprocal of MRB density along the x-axis to linearize the graph, the regression line for the 2.5 billion IJs/ha rate appears to have a



**Figure 3.6** Relationship between mint root borer percent reduction and mint root borer density for two application rates of *Steinernema carpocapsae*

steeper slope than that for the 4.9 billion IJs/ha rate (Figure 3.7). The slopes of the regression lines were not statistically significant ( $p=0.10$ ); however, the graph suggests that the higher rate is less influenced by MRB density and that nematode efficacy is higher at high pest densities. Further studies are needed to determine how pest density affects nematode efficacy.

Under post-harvest conditions, persistence of *S. carpocapsae* appears to be short-lived. Nematode recovery in soil decreased substantially over a four week period. This is

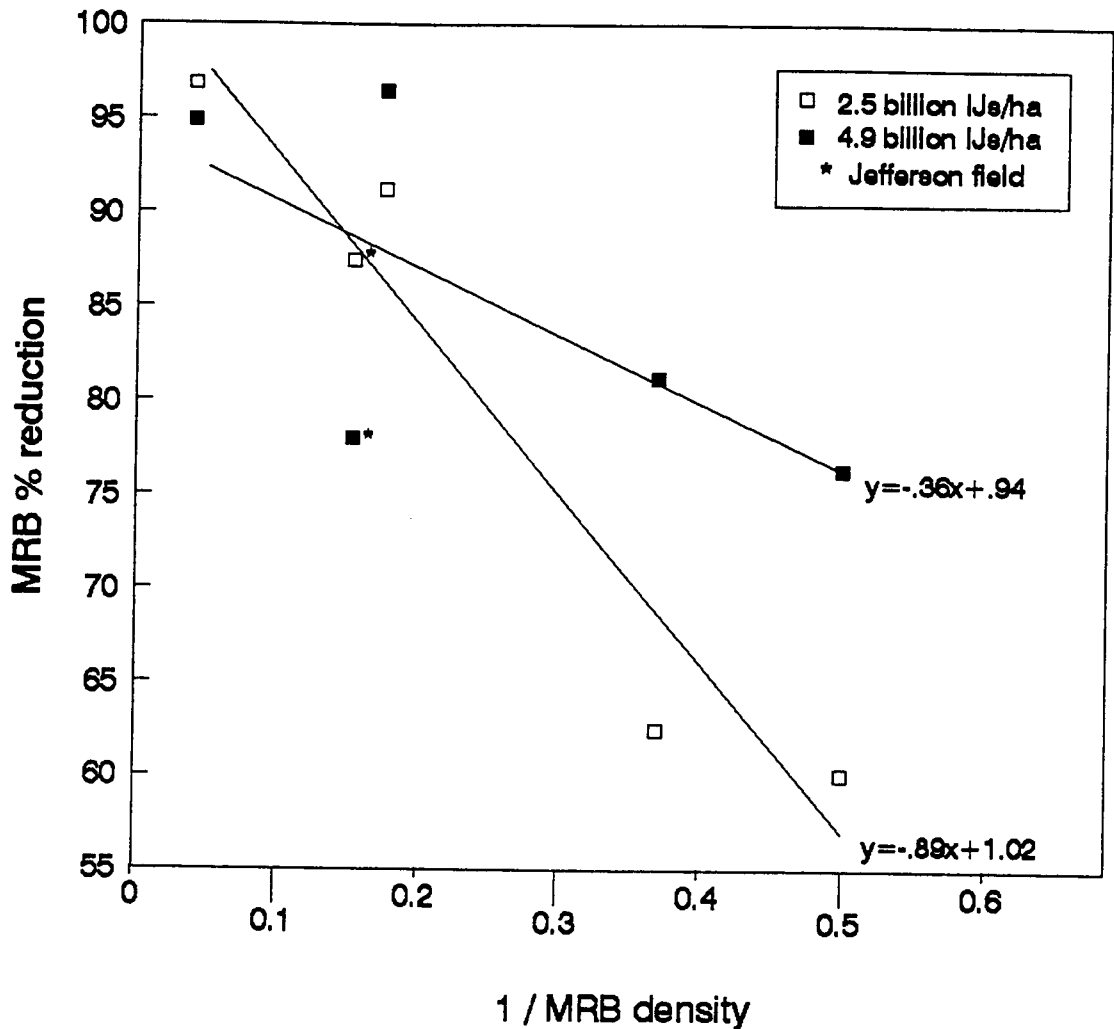


Figure 3.7 Relationship between mint root borer percent reduction and reciprocal mint root borer density for two application rates of *Steinernema carpocapsae*

in agreement with Poinar and Hom (1986) and Ishibashi and Kondo (1986) who observed a decrease to low levels over a 6-7 week period. Long-term nematode persistence also has been reported (Shanks and Agudelo-Silva, 1990; Klein and Georgis, 1992). Klein and Georgis (1992) observed 90% reduction of Japanese beetle, *Popillia japonica* Newman, larvae 290 days after nematode application; however, it is not clear what nematode level in the soil produces continued pest reduction. Fan and Hominick (1990) found that the *G. mellonella* bioassay recovers only 30-40% of entomopathogenic nematodes present in the

soil because not all IJs are infectious at the same time. Also, it is difficult to determine whether IJs survive over extended periods of time in the soil or whether nematode reproduction in dead hosts produces another generation of IJs.

To achieve good efficacy, nematodes must be applied prior to hibernacula formation due to the non-susceptibility of MRB prepupae to *S. carpocapsae*, even at extremely high concentrations. Non-susceptibility of the prepupal stage to nematodes should not be that surprising considering the MRB spends a major portion of its life cycle in the soil. Bedding and Akhurst (1975) suggest that soil insects may have evolved some protection from entomopathogenic nematodes. Moreover, lepidopterous pupae are variably susceptible to entomopathogenic nematodes with those pupating above ground more susceptible than those pupating in the soil or litter (Kaya and Hara, 1981). Timing becomes critical in achieving post-harvest MRB control not only because the nematode is ineffective against the prepupal stage, but because chlorpyrifos is probably ineffective as well (R. Williams, personal communication).

To complicate matters further, variability in development was observed between the two fields used in the 1992 large plot experiment. When the experiment was evaluated, the Corvallis field had 48.3% hibernacula formation while the Jefferson field only had 3.5% hibernacula formation. This phenomena was again observed in 1993 when a peppermint field in Jefferson had 17.9% hibernacula formation on September 8 while a field near Monroe had only 1.8% hibernacula formation on September 17. Additional studies are needed to determine what causes this variation in development. Soil texture and irrigation frequency may be possible factors that would influence soil temperature, hence insect development. Nevertheless, the consequence of variable development rates is that it narrows an already narrow treatment window, particularly for chlorpyrifos which cannot be applied pre-harvest. The nematodes, however, can be applied pre-harvest.

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**CHAPTER 4**  
**Control of Mint Root Borer in Peppermint with Pre-Harvest Applications of the Entomopathogenic Nematode, *Steinernema carpocapsae***

**ABSTRACT**

Field experiments were conducted to investigate the potential of pre-harvest applications of the entomopathogenic nematode, *Steinernema carpocapsae* (Weiser) strain All, to control the mint root borer (MRB), *Fumibotys fumalis* (Guenée). Pre-harvest applications ranging from 1.2 billion to 7.4 billion IJs/ha reduced MRB populations significantly compared to the control, but not always below the treatment threshold of 2-3 MRBs per hectare. Nematode persistence in soil decreased significantly from one day to 14 days post-application. Short nematode persistence combined with prolonged adult emergence and oviposition increases the risk of applying the nematodes prematurely under pre-harvest conditions.

**INTRODUCTION**

The mint root borer (MRB), *Fumibotys fumalis* (Guenée), is a serious pest of several commercially important mints (*Mentha piperata* L., *M. cardiaca* G. and *M. spicata* L.) grown primarily for their oil. The larvae tunnel into and feed within the underground stem or rhizome which weakens the stand and reduces yields (Pike et al., 1988; Berry and Fisher, 1993). Crop damage is especially severe at the end of the season when the larvae have reached their maximum size. Currently, chlorpyrifos (Lorsban) is the only chemical insecticide registered for use against the MRB. Treatment is recommended when infestations exceed 2-3 MRBs per 929 cm<sup>2</sup> soil sample (Berry and Fisher, 1993). Restricted to post-harvest use, chlorpyrifos targets late instars after damage has already

occurred. Consequently, chlorpyrifos is a preventative measure for the following year instead of a control measure for the current year.

The entomopathogenic nematode, *Steinernema carpocapsae* (Weiser) strain All (BioVector®), may provide control of MRB larvae before severe crop damage occurs. Unlike chlorpyrifos, the nematode is not restricted to post-harvest use; therefore, a pre-harvest nematode application, if effective, would minimize crop damage by targeting earlier instars. With the exception of neonate larvae (Kaya, 1985), early instars appear to be more susceptible than late instars (Kondo, 1987). Early instar susceptibility to entomopathogenic nematodes has been documented in other lepidopteran species (Kaya, 1985; Kondo, 1987; Turgeon and Finney-Crawley, 1991). Under laboratory conditions, *S. carpocapsae* was observed infecting early instar MRB (unpublished data); however, laboratory susceptibility does not necessarily translate into field susceptibility (Begley, 1990) and needs to be confirmed.

Premature application of nematodes pre-harvest is a concern given the prolonged egg laying and emergence of MRB adults. The MRB is univoltine with adult emergence beginning in late spring and continuing for a two month period (Berry, 1974). During this time, eggs are continuously deposited on the foliage, and the resulting larvae add to the existing population as they drop to the soil surface and tunnel into the rhizomes. Nematode persistence becomes an important issue because of the prolonged adult emergence. Nematode reproduction within the host which leads to the production of thousands of IJs is considered an advantage the nematode has over insecticides; however, it is not known to what degree this occurs and whether it will provide continuous control.

Post-harvest nematode persistence indicates that pre-harvest nematode persistence may not last beyond several weeks. Pre-harvest conditions, however, may be more favorable to nematode survival and persistence. Nematode survival is dependent on protection from UV radiation and favorable temperature and moisture (Kaya, 1990).

Because peppermint is frequently irrigated and has a dense canopy, favorable abiotic environmental conditions exist for nematode survival.

Our objective was to determine the pre-harvest potential of *S. carpocapsae* for MRB control. To compare the control efficacies of different nematode rates, field studies were conducted in 1991 and 1992. Small plot experiments were conducted both years; a large scale experiment was conducted in 1992 only. In addition, a nematode persistence study was conducted in 1992 to determine how long the nematode will persist in the soil.

## MATERIALS AND METHODS

### Efficacy Studies

The 1991 small plot experiment compared the following treatments: 0, 1.8, 3.7 and 7.4 billion infective juveniles (IJs) per hectare of *S. carpocapsae* strain All (biosys, Palo Alto, CA). In 1992, nematode rates of 0, 1.2, 2.5, 3.7 and 4.9 billion IJs/ha were compared. For both experiments, a randomized complete block design (RCBD) was used replicated five times in 14.9 m<sup>2</sup> and 22.3 m<sup>2</sup> plots in 1991 and 1992, respectively. Treatments were applied in 1991 on July 18 and in 1992 on July 14, using a CO<sub>2</sub> driven backpack sprayer delivering approximately 281 liters per hectare at 2.1 kg/cm<sup>2</sup> pressure. The plots were irrigated with approximately 0.3 cm of water to moisten the ground prior to nematode application. Treatments were then applied with the irrigation running, followed by approximately 2.5 cm of additional irrigation to move nematodes into the soil. The plots were evaluated 30 days post-application in 1991 and 14 days post-application in 1992. Six soil samples (464.5 cm<sup>2</sup> to a depth of six cm) were taken in each of the plots. The 1991 experiment was evaluated a second time 50 days post-treatment taking four soil samples (464.5 cm<sup>2</sup>) per plot. Data from each evaluation were analyzed separately. The soil was sifted through a screen (mesh size 0.6 cm) and inspected for larvae while

rhizomes were placed in Berlese funnels equipped with 75 watt bulbs to extract live larvae. Samples were extracted for four days or until the rhizomes were completely dry.

The 1992 large plot experiment compared three nematode rates: 0, 2.5 and 4.9 billion IJs per hectare. Using irrigated areas as experimental units, a RCBD was used with treatments replicated three times. One block was located in a commercial peppermint field near Eugene, OR (plot size 0.8 ha) while the remaining two blocks were located in a commercial peppermint field near Jefferson, OR (plot size 1.1 ha). Nematodes were applied in late afternoon or evening on July 21 and 22 in the Eugene field, and on July 27 and 28 in the Jefferson field. The plots was irrigated with approximately 0.3 cm of water prior to injecting the nematodes through the irrigation. The application was made over 30-60 minutes to ensure even distribution of nematodes and immediately followed with approximately 2.5 cm of irrigation. The experiment was evaluated twice and data from each evaluation were analyzed separately. The first sampling date was 18 days post-application; the second sampling date was approximately 30 days post-application. On each date, sixteen soil samples (464.5 cm<sup>2</sup> to a depth of six cm) were taken along each irrigation line. MRB numbers were determined using Berlese funnels and soil screens as described above.

### **Nematode Persistence**

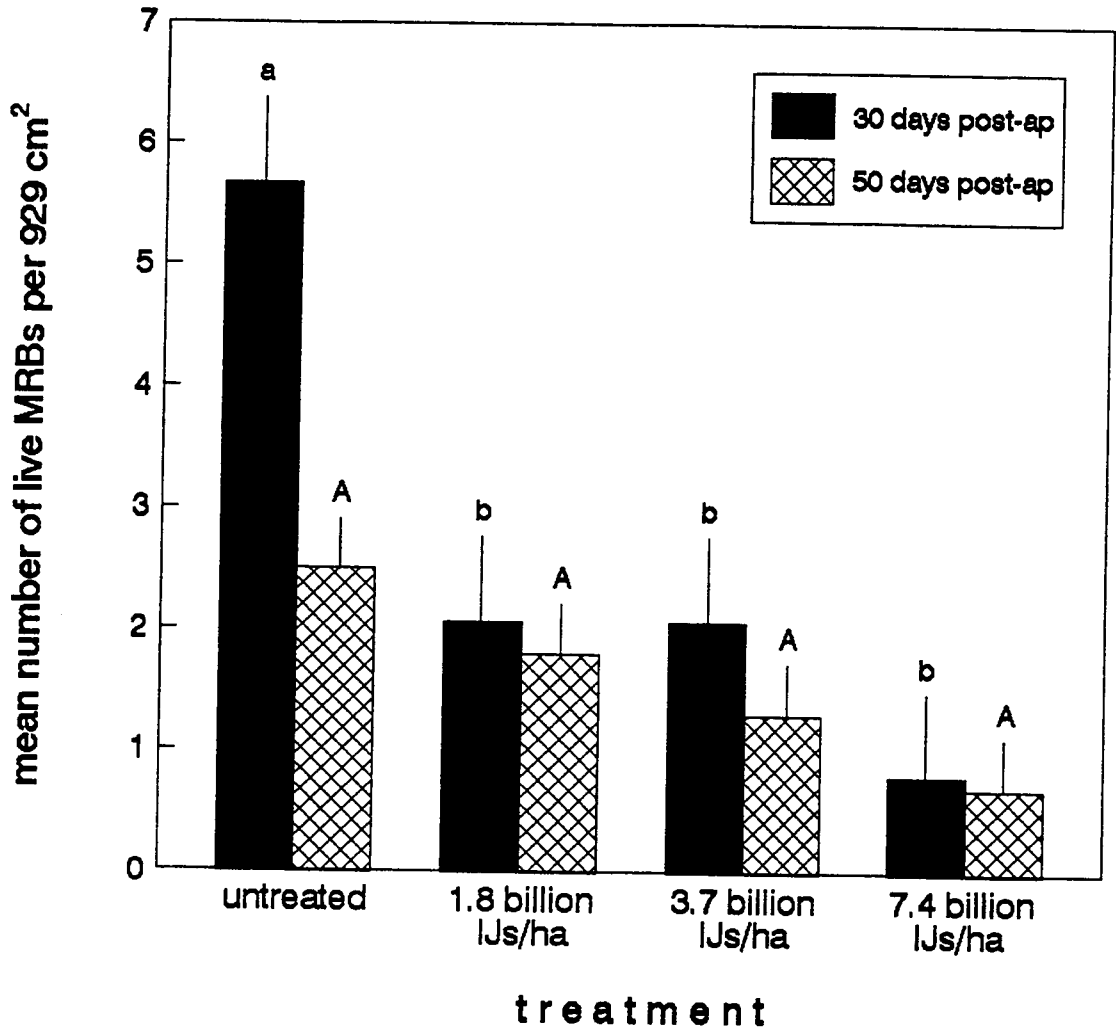
A nematode persistence study was conducted in the untreated and 4.9 billion IJs/ha treated areas of the 1992 large scale experiment. A modification of the methods described by Fan and Hominick (1991) was used to determine the relative number of IJs in the soil on four separate sampling dates (0, 1, 14, and 28 days post-application). On each sampling date, soil was collected at ten random sites along each irrigation line. Two 150 cm<sup>3</sup> samples to a depth of six cm were taken at each site and combined. The soil was then passed through a screen with 2.794 mm openings (W. S. Tyler Co., Mentor, OH) and

approximately 225 cm<sup>3</sup> of the screened soil was placed in a 237 ml plastic cup. The soil was moistened, if dry, and five late instar *Galleria mellonella* (L.) were placed on the soil surface. The containers were then covered and incubated in the dark at 20°C. After six days, the *G. mellonella* larvae were replaced with another five larvae, and again incubated for six days at 20°C. Dead larvae were dissected, and if nematodes were present, their numbers were recorded. Cadavers with characteristic signs of nematode infection (light brown coloration and disintegration of internal organs) were assigned a count of one even though nematodes were not found upon dissection. This method was used to obtain a conservative estimate for the samples collected 14 days post-treatment in the Eugene field because mortality data only was available for this block. A natural log transformation of the nematode counts stabilized the variance. Nematode recovery on two sampling dates (one day and seven days post-treatment) was analyzed using a t-test.

## RESULTS

### Efficacy Studies

In the 1991 small plot experiment, all three nematode rates significantly reduced MRB numbers from that of the control on the first evaluation (Figure 4.1). Percent reduction varied from 63.5% to 85.9%; however, only the highest rate of 7.4 billion IJs/ha reduced MRBs below the treatment threshold of 2-3 MRBs per 929 cm<sup>2</sup> soil sample. The second evaluation showed no significant differences in MRB numbers in any of the treatments including the control. This was due to a decrease in MRBs in the control probably as a result of cross contamination between plots. MRB levels in all nematode treatments on the second evaluation showed little change compared with the first evaluation. In the 1992 small plot experiment, all nematode treatments significantly reduced MRBs compared with the control (Figure 4.2). Percent reduction varied from

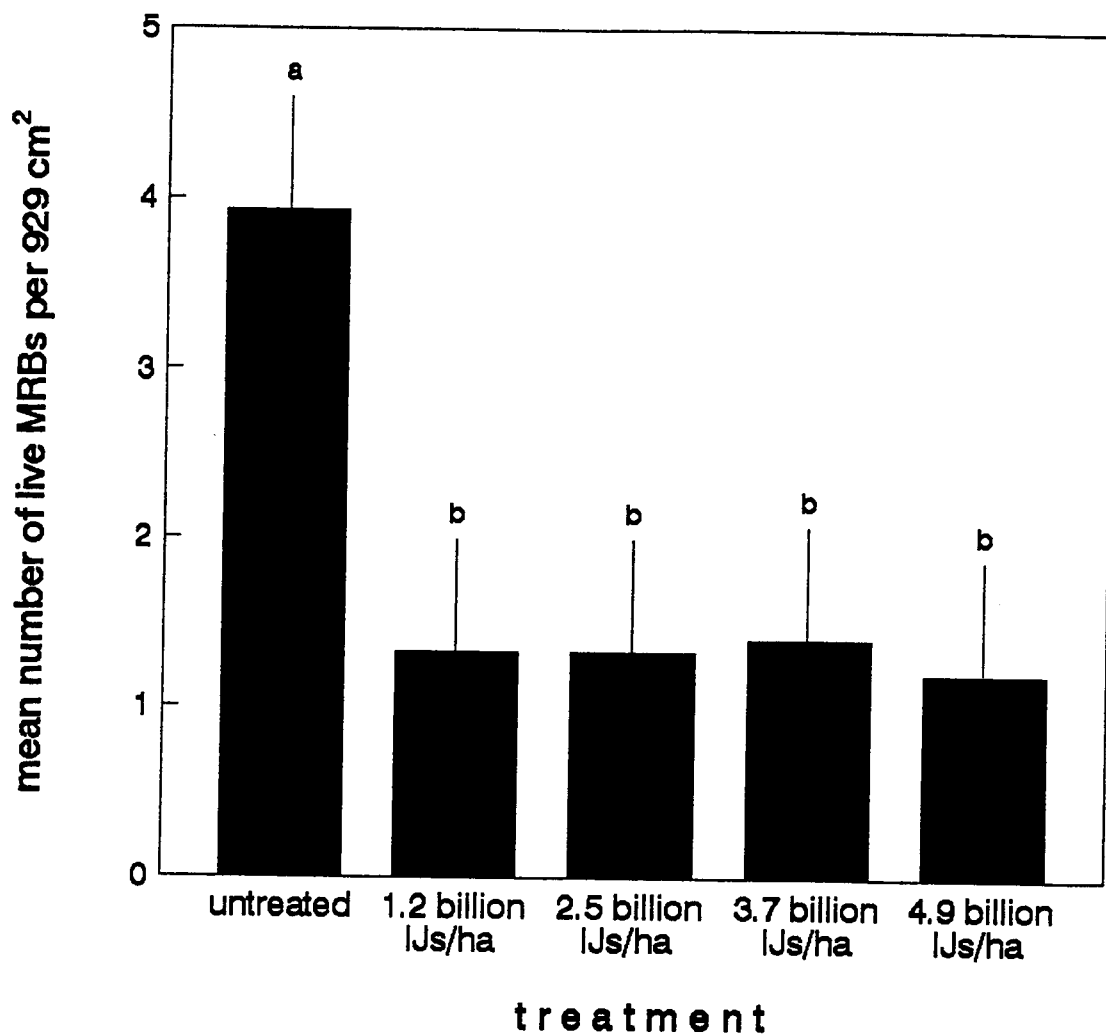


**Figure 4.1** Mean number of mint root borers recovered from 929 cm<sup>2</sup> soil samples in 1991 pre-harvest experiment (in small plots) conducted in a commercial peppermint field in Marion county, Oregon. Separation of means by Fisher's Protected LSD ( $p \leq 0.05$ ). On each sampling date, bars with different letters are significantly different.

64.4% to 69.5%. Significant differences were not detected among the nematode rates, all of which reduced MRBs below the treatment threshold.

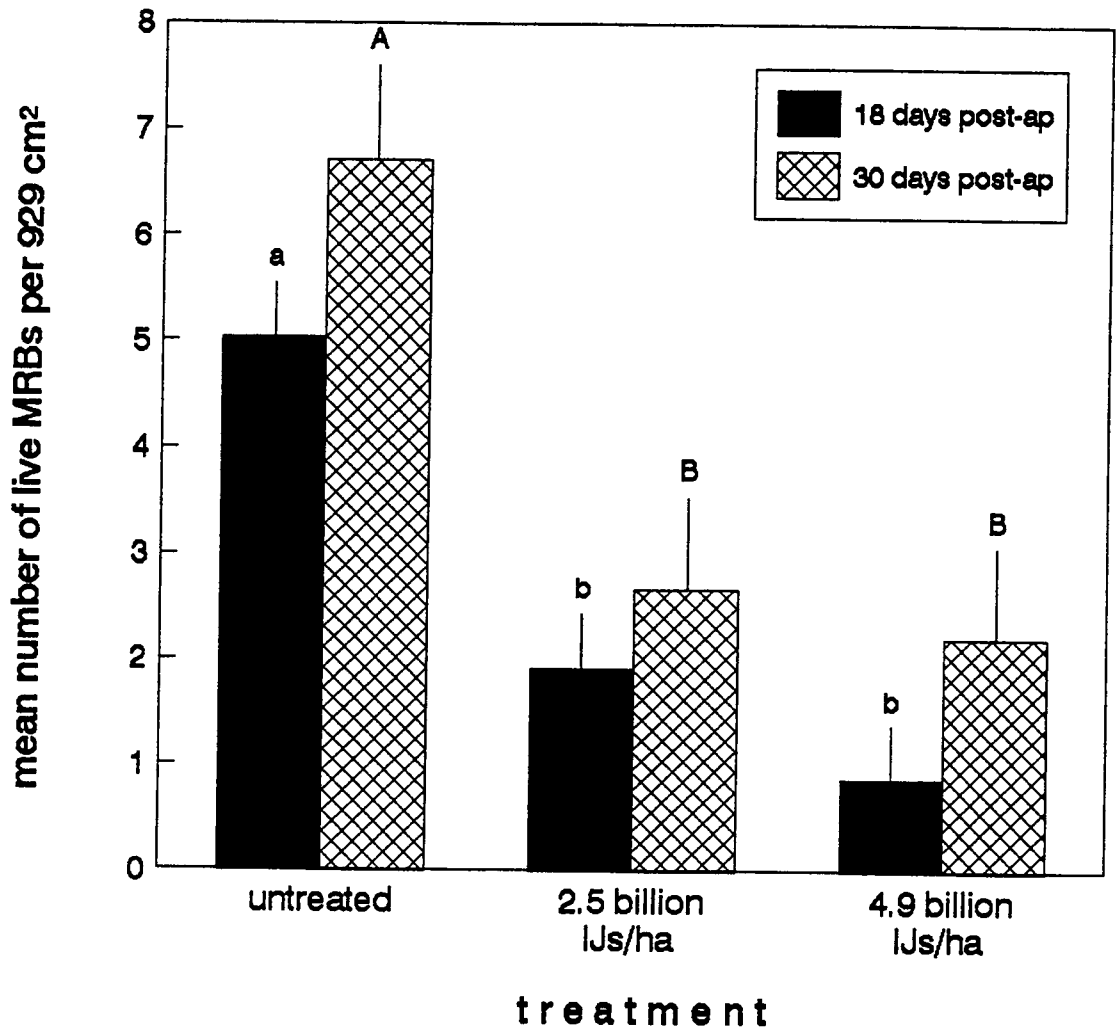
In the 1992 large plot experiment, significant differences were not detected between the 2.5 and 4.9 billion IJs/ha rates, but both nematode rates significantly reduced MRB numbers compared to the control on both sampling dates (Figure 4.3). On the first





**Figure 4.2** Mean number of mint root borers recovered from 929 cm<sup>2</sup> soil samples in 1992 pre-harvest experiment (in small plots) conducted in a commercial peppermint field in Lane county, Oregon. Separation of means by Fisher's Protected LSD ( $p \leq 0.05$ ). Bars with different letters are significantly different.

evaluation, both nematode rates reduced MRB numbers below the treatment threshold. In contrast, neither rate reduced MRBs below the treatment threshold on the second evaluation. The increase in MRBs on the second evaluation was due to an increase in MRBs in the Jefferson field where two of the three replicates were located. The MRB



**Figure 4.3** Mean number of mint root borers recovered from 929 cm<sup>2</sup> soil samples in 1992 pre-harvest experiment (in large plots) conducted in commercial peppermint fields in the Willamette Valley, Oregon. Separation of means by Fisher's Protected LSD ( $p \leq 0.05$ ). On each sampling date, bars with different letters are significantly different.

population in this field was observed to be slower in development than the population in the Eugene field.

**Table 4.1** Recovery of nematodes from soil treated with 4.9 billion IJs/ha *Steinernema carpocapsae* using *Galleria mellonella* as a bioassay organism

	sampling date (days post-application)		
	1	14	28
mean number ( $\pm$ SE) of IJs in 225 cm <sup>3</sup> of soil	69.2 (7.9)	3.7 (1.2)	0.3 (0.1)

### Nematode Persistence

On the pre-application sampling date, most (280 out of 300) of the *G. mellonella* larvae used to bioassay the soil samples survived. Of those that died, there appeared to be mortality due to nematode infection in two larvae. One larva appeared to be infected with nematodes, but none was found inside the cadaver. The other larva was associated with what appeared to be infective juveniles; however, the cadaver had broken open and may have started to decompose. Thus, it is unclear whether the nematodes were entomopathogenic or saprophytic. A natural population of entomopathogenic nematodes in the fields used in this experiment, if present, was probably present in very low numbers. In the samples taken one day post-application, all *G. mellonella* larvae died with an average of 69.2 nematodes recovered in 225 cm<sup>3</sup> of soil (Table 4.1). Nematodes recovered in the *G. mellonella* bioassay decreased significantly ( $t=9.94$ ,  $p=0.005$ ) from one day to 14 days post-application. By 28 days post-application, very few nematodes were recovered.

## DISCUSSION

Pre-harvest nematode applications have the potential to effectively control the MRB. Pre-harvest nematode application rates ranging from 1.2 billion to 7.4 billion IJs/ha significantly reduced MRBs compared with the control. However, pre-harvest nematode applications did not necessarily reduce MRB levels below the treatment threshold. In the 1991 small plot experiment, the highest nematode rate (7.4 billion IJs/ha) reduced MRB numbers below the treatment threshold, but the lower rates did not. A second evaluation showed no increase in MRBs in the nematode treatments (apparently the nematode application in this experiment was properly timed), but there was a noticeable decrease in MRBs in the untreated plots. This may have been caused by lateral movement of the nematode between plots. IJs are capable of horizontal movement (Poinar and Hom, 1986), and phoresy on other soil dwelling organisms (Epsky et al., 1988) aid in their dispersal. Nematode contamination between plots is a common problem in field trials (K. Smith, personal communication). In the 1992 large plot experiment, MRBs were reduced below the treatment threshold in the first evaluation of the experiment, but not in the second evaluation. On the first evaluation, the 1.8 and 3.7 billion IJs/ha rates in the 1991 experiment did not reduce MRB numbers below the treatment threshold which suggest the nematodes were applied too early.

Nematode persistence decreased significantly after 14 days post-treatment, indicating nematode persistence cannot be relied upon to provide continuous control. It is not known what levels of nematode recovery in the *Galleria* bioassay translate into continuous control, but the 1992 small plot and large plot experiments illustrate the potential problems of applying a pre-harvest application too early. These results are expected if the treatments were applied too early. Continual increase in the larval population once nematode populations have declined will obstruct any trends seen previously in efficacy reduction and will result in lowering the percent reduction observed

initially. Unfortunately, time constraints did not allow a second set of samples to be taken in the small plot experiment; this may have further clarified what had occurred. The 1992 large plot experiment was sampled on two separate occasions and showed a decrease in control and higher MRB numbers over all treatments in the second set of samples. The Jefferson field, where two of the replicates were located and where MRB development was delayed, was where the MRB increase was observed. Variability in MRB development between fields not only complicates the timing of control measures post-harvest, but pre-harvest as well.

Higher application rates may be required when nematodes are applied pre-harvest. A possible explanation is that a certain proportion of the IJs are retained on the foliage. Nematode survival on foliage is poor due to UV radiation and dry conditions (Begley, 1990). Compared to the results of a post-harvest nematode persistence study, fewer nematodes were recovered in the pre-harvest soil samples on corresponding sampling dates. For example, in 225 cm<sup>3</sup> of soil taken one day post-application, there was an average of 123.6 nematodes recovered post-harvest while only 69.2 nematodes were recovered pre-harvest. Although these results support the hypothesis that the nematodes are lost on the foliage when applied pre-harvest, additional studies are necessary. Irrigation is important in moving the IJs into the soil profile (Shetlar et al., 1988; Zimmerman and Cranshaw, 1991); therefore, additional irrigation may be helpful in a pre-harvest nematode application to wash nematodes off the foliage. In this case, the presence of foliage, thought to create a more conducive environment, may actually hinder control efforts.

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## CHAPTER 5

### Evaluation of Mint Root Borer Populations in the Willamette Valley of Oregon

#### ABSTRACT

Experiments were conducted to evaluate different methods of determining larval density. Berlese funnel extraction and hand-sorting measure larval density directly. Berlese funnel extraction has advantages over hand-sorting in that it is less labor-intensive, more accurate, and useful in detecting early instars (provided soil is removed from the rhizomes prior to extraction). To be able to detect early instar MRBs with Berlese funnels allows field diagnosis to occur earlier in the season. In 1993, fields were correctly identified as infested or non-infested in mid-August. Efforts to correlate adult density with subsequent larval infestation were inconclusive using pheromone trap catches; however, a modified sweep search correctly ranked infestation levels in three fields monitored in 1994.

#### INTRODUCTION

The mint root borer, *Fumibotys fumalis* (Guenée), is a serious pest of peppermint (*Mentha piperata* L.) in the Willamette Valley of Oregon. Larvae feed within the underground stem or rhizome, hollowing out entire sections of the rhizome. This feeding damage weakens the stand, resulting in winter injury and yield loss (Berry and Fisher, 1993; Pike et al., 1988). Treatment is recommended when MRB numbers exceed 2-3 MRBs per 929 cm<sup>2</sup> (Berry and Fisher, 1993). Against the larval stage, chlorpyrifos (Lorsban) and the entomopathogenic nematode, *Steinernema carpocapsae* (Weiser) strain All (BioVector<sup>®</sup>), are currently the options available for control. Both can provide effective control; however, proper application timing is critical.



In order to properly time an application, infestations must first be diagnosed. In the past, larval infestation levels were typically assessed post-harvest after flaming for *Verticillium* wilt control. Sampling often did not start until after the irrigation was restored and regrowth was present in the field. Wilted plants are symptomatic of MRB feeding damage and are used as a diagnostic aid; however, waiting this long may not allow control measures to be properly timed. Variability in MRB development between fields as well as difficulty in controlling the MRB once it has entered the prepupal stage are important reasons to apply control measures earlier. Since fields cannot be treated without first being diagnosed, sampling also must begin earlier.

To determine larval density, Berry and Fisher (personal communication) recommend taking a soil sample (929 cm<sup>2</sup> in size to a depth of 5.1 to 7.6 cm) every hectare with a minimum of 25 samples taken per field. There are two methods available for processing the samples. Hand-sorting consists of manually checking the interior of the rhizomes for the presence of larvae. It is usually done in the field, requires little in terms of equipment, and allows fields to be diagnosed the same day they are sampled. Berlese funnel extraction is the other alternative. A Berlese funnel consists of a cylindrical container with a wire mesh bottom in which the sample is placed (Berry et al., 1981). A heat source, usually a light bulb, slowly dries the sample from above, driving soil organisms down an attached funnel and into a collection jar below. Although it may take several days to process the sample, Berlese funnels may reduce labor inputs because the insects move out of the sample on their own volition.

Berlese funnel extraction may be the preferred method for determining larval density; however, they may produce biased results if the insect is not very active, does not respond to desiccation or cannot free itself from the soil (Edwards, 1991). Pre-harvest application of a control measure requires earlier field diagnosis. Use of Berlese funnels to detect early instar MRBs may require some modifications since small larval size may limit mobility. It may be necessary to use only the plant portion of a sample for Berlese funnel

extraction (M. Morris, personal communication). This would require the removal of soil which would not only add time and labor to the extraction process, but also brings up the issue of whether a separate extraction must be done on the soil.

Evaluating fields later in the season not only jeopardizes the proper timing of a control measure, but if the MRB has already entered the prepupal stage, additional time and labor must be spent looking for hibernacula in order to properly assess the infestation. Hibernacula are teardrop-shaped earthen cells that are constructed by the last instar as it prepares to overwinter. Because they resemble clumps of soil, hibernacula are difficult to detect, even to the trained eye. It is important for fields to be sampled prior to hibernacula formation; however, if samples are collected too early, MRB density can be underestimated due to the prolonged adult emergence (Berry, 1974). Knowing how early sampling can begin and still obtain an accurate assessment of the infestation level is essential in properly timing control measures.

Monitoring the adult population with pheromone traps or sweep nets is relatively quick and simple, and may provide an alternative to the time-consuming and labor-intensive nature of collecting and processing soil samples. Berry and Fisher (1993) recommend sweep net samples to be taken during the growing season as a preliminary assessment of MRB numbers, but it is not known whether this method can be used as a predictive tool. The same is true for the MRB sex pheromone which is now commercially available. Compounds identified as (E,E)-11-tetradecadienyl acetate, (Z)-11-tetradecenyl acetate, (E)-11-tetradecenyl acetate, and (Z)-9-tetradecenyl acetate in a ratio of 100:18:8:4 was shown to be attractive to male MRB moths (Davis et al., 1991). Pheromone trap counts have been correlated to subsequent infestation for other lepidopterous pests (Shelton and Wyman, 1979; Tingle and Mitchell, 1981; Ramos et al., 1981; Faccioli et al., 1993; Qureshi et al., 1993); however, there also have been reports of non-correlation (Kehat et al., 1982; Hoffman et al., 1992). Assuming there is good

correlation between the adult MRB population and subsequent larval infestation, infestations would be diagnosed accurately and well in advance of treatment.

To accurately assess infestation levels, our objectives were to determine the best method for detecting MRB larvae and to examine the potential of predicting larval density from adult density. For MRB larvae, experiments were conducted to determine 1) accuracy of hand-sorting compared with Berlese funnel extraction, 2) how best to deal with the soil portion of a sample when using Berlese funnels, and 3) feasibility of early monitoring of larval populations. For MRB adults, preliminary studies were conducted investigating the potential of using pheromone trap counts and a modified sweep search as diagnostic tools to correlate adult numbers to larval infestation.

## **MATERIALS AND METHODS**

### **Hand-Sorting vs. Berlese Funnel Extraction**

In 1993, soil samples (929 cm<sup>2</sup> to a depth of six cm) were collected on two separate dates from a commercial peppermint field near Coburg, OR. Eight and twelve samples were collected on August 7 and August 20, respectively. Each sample was halved and placed in two separate plastic bags and transported back to the laboratory where the two halves were randomly assigned to either hand-sorting or Berlese funnel extraction. The soil was separated from the rhizomes, sifted with a screen (mesh size 0.6 cm) and examined for MRB larvae. Rhizomes for hand-sorting were halved lengthwise (larger rhizomes were quartered) using a razor blade and examined for the presence of larvae. Any lateral branching or budding of the rhizomes also was dissected, but root hairs were not. The rhizomes for Berlese funnel extraction were placed in Berlese funnels equipped with a 75 watt light bulb for 2-3 days. At the end of the extraction process, the contents

of the collection jar were examined for MRB larvae using a dissecting microscope. Data were analyzed with a paired t-test.

### **The Role of Soil in Berlese Funnel Extraction**

Infested rhizomes were subjected to the following treatments in a completely randomized design (CRD) replicated six times: 1) Berlese funnel extraction of rhizomes in the absence of soil, 2) Berlese funnel extraction of rhizomes in the presence of soil, and 3) hand dissection. Peppermint rhizomes were cut into pieces approximately five cm long and were infested with six newly hatched MRB larvae. Larvae were obtained from eggs laid by laboratory-reared females and were placed on the rhizomes using a camel hair brush. The infested rhizomes were kept in petri dishes (two rhizomes per dish) containing wet cotton. Using two pieces of rhizome as the experimental unit, the experiment began two days after the rhizomes were infested. For the Berlese funnel treatments, a 60 watt light bulb was used to extract the larvae over a two day period. A layer of sandy loam soil (approximately two cm thick) was placed in the bottom of the Berlese funnels for the extractions in the presence of soil. Rhizomes were placed on top of the soil to force the larvae to move through the soil. Hand dissection, with the aid of a dissecting microscope, was done at the completion of the Berlese funnel extractions or four days after rhizomes were infested.

In a separate experiment, 14 samples (464.5 cm<sup>2</sup> to a depth of six cm) were collected on August 13, 1991 from a commercial peppermint field near Eugene, OR and stored overnight at room temperature in plastic bags. The following day, the rhizomes were separated from the soil for each of the samples and the two portions were placed in separate Berlese funnels. The soil and rhizomes were extracted using 75 watt light bulbs for two days, and the number of MRB larvae recovered from the rhizome and soil portions were recorded.

### **Early Monitoring of Larval Populations**

In 1993, eight fields were sampled for MRB larvae every two weeks from July 15 to August 15. A final post-harvest sample was taken in late August/early September. On each sampling date, eight soil samples (929 cm<sup>2</sup> to a depth of six cm) were taken in each of the fields which varied in size from 8-36 hectares. To keep the number of samples taken per unit area constant among all the fields, samples were taken from an area approximately eight hectares in size. Rhizomes were separated from the soil, placed in Berlese funnels equipped with 75 watt bulbs, and extracted for four days or until the rhizomes were completely dry. The soil was sifted and examined for the presence of larvae and hibernacula.

### **Correlation of Adult Density to Larval Infestation**

In 1993, Pherocon 1C traps baited with a MRB sex pheromone cap (Trece, Salinas, CA) were placed in eleven fields throughout the Willamette Valley. The traps, one per field, were hung from a shelf bracket attached to a broom handle (Cascade Handle Co., Eugene, OR) that was pushed into the ground approximately in the center of each field. A hose clamp connecting the shelf bracket to the broom handle allowed the trap height to be adjusted during the growing season so that the trap was positioned just above the foliage. The traps were monitored on a weekly basis from late May to harvest. Post-harvest soil samples (929 cm<sup>2</sup> to a depth of six cm) were collected from each field to determine the infestation level. One sample was taken every hectare and MRB larvae were extracted from the rhizome portion of the sample using Berlese funnels equipped with 75 watt light bulbs. The soil portion of the sample was sifted using a screen (mesh

size 0.6 cm) and inspected for larvae and hibernacula. Trap catch was represented either by highest trap catch or cumulative trap catch from June 11 to July 30.

In 1994, three commercial peppermint fields were sampled using pheromone traps and a modified sweep search for adults. Regular sweep net samples flushed out more moths than could be caught. To remedy this, the modified sweep search consisted of balancing on one leg while sweeping the foliage slowly from side to side with the other leg. The process was repeated along a transect. Each search was limited to five minutes, stopping the clock to capture each moth with a hand net as it emerged from the foliage. From mid-June to the end of July, three to six searches were conducted on a weekly basis. One person sampled the fields on June 17, June 24, and July 1 conducting three searches per field. Three people conducted two searches each for a total of six searches on the remaining sampling dates. The exceptions were on July 22 and 29 in the Jefferson and Corvallis fields, respectively. On these sampling dates, six (July 22) and four (July 29) searches were conducted by the same individual on the first three sampling dates. Captured moths were placed in plastic bags and taken back to the laboratory where they were counted and sexed. Some moths eluded capture and were noted. In each of the fields, three pheromone traps were placed approximately 75 m from each other in a triangular arrangement and checked weekly from late May to the end of July. After harvest, the larval infestation was determined by taking approximately one soil sample (929 cm<sup>2</sup> to a depth of six cm) every hectare. The samples were processed as described above.

## RESULTS

### **Hand-Sorting vs. Berlese Funnel Extraction**

On both sampling dates, more MRB larvae were recovered by Berlese funnel extraction than hand-sorting (Table 5.1). Significant differences between the two methods were detected on August 20 ( $p=0.001$ ) but not on August 7 ( $p=0.189$ ). The time invested in the two methods varied from approximately 15 minutes per sample for the Berlese funnel extraction to 30-45 minutes per sample for hand-sorting. Hand-sorting on August 7 took less time than on August 20 since fewer rhizomes are present earlier in the season.

### **The Role of Soil in Berlese Funnel Extraction**

There were significantly fewer early instar MRBs recovered in the Berlese funnel extractions with soil than without soil (Figure 5.1). Hand dissection had an intermediate extraction efficiency. For all treatments, larval recovery was quite low with recovery of less than half of the larvae used to infest the rhizomes. The 14 field collected samples yielded a total of 28 MRB larvae (Table 5.2). All but one were found in the rhizomes. Among the larvae recovered from the rhizomes was a first instar. The single larva found in the soil was large enough to be detected had the soil been sifted.

### **Early Monitoring of Larval Populations**

Four out of the eight fields did not have a treatable level while the remaining four fields had an infestation level above the treatment threshold of 2-3 MRBs per 929 cm<sup>2</sup> (Figure 5.2). In the infested fields, larval density increased over time. All fields were correctly diagnosed on the August 13 sampling occasion although heavily infested fields were correctly diagnosed earlier in the season.

**Table 5.1** Comparison of extraction efficiency between two sampling methods for mint root borer larvae

	mean number of MRB larvae ( $\pm$ SE) per 464.5 cm <sup>2</sup>	
	August 7	August 12
hand-sorting	4.75 (1.05)	16.75 (2.05)
Berlese funnel	6.62 (1.16)	26.08 (2.80)

**Table 5.2** Extraction of mint root borer larvae from rhizome and soil portions of soil samples taken on August 13, 1991 in a commercial peppermint field in Lane county, Oregon

<u>sample</u>	MRB numbers in 464.5 cm <sup>2</sup> of soil	
	<u>rhizome</u>	<u>soil</u>
1	2	0
2	7	0
3	1	0
4	2	0
5	0	0
6	1	0
7	0	0
8	0	1
9	1	0
10	4	0
11	0	0
12	0	0
13	5	0
14	4	0



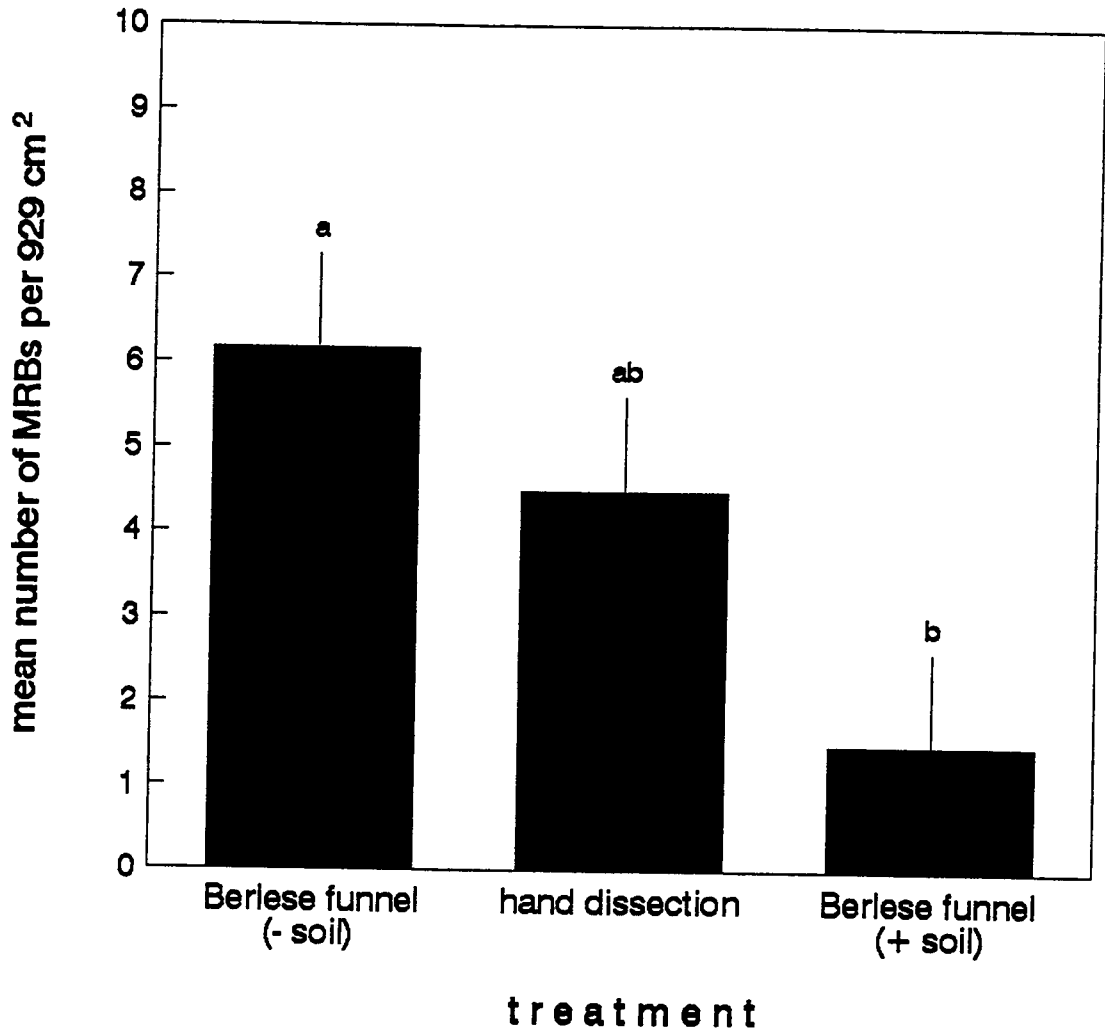
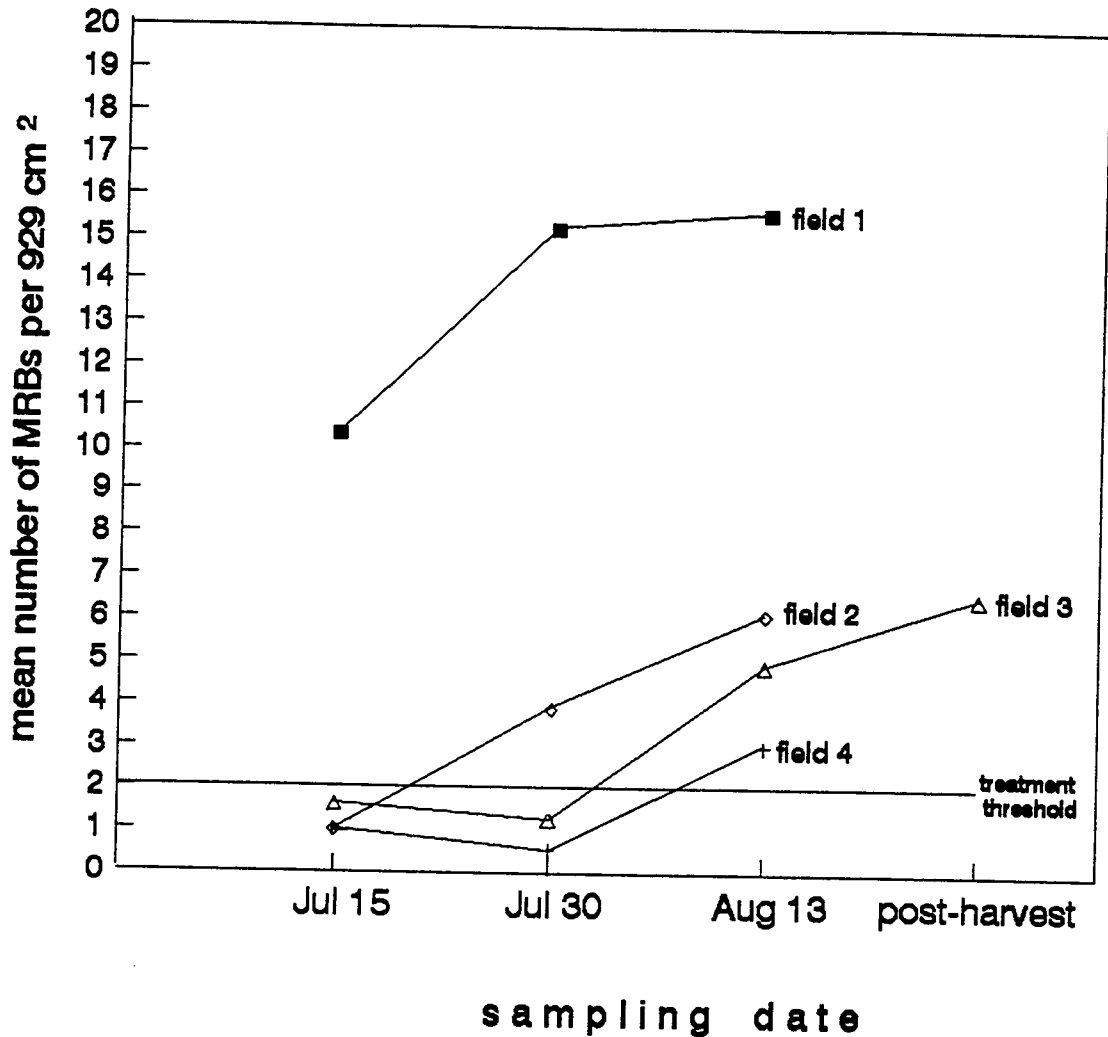


Figure 5.1 Recovery of early instar mint root borers with Berlese funnel extraction (in the presence and absence of soil) and hand dissection

### Correlation of Adult Density to Larval Infestation

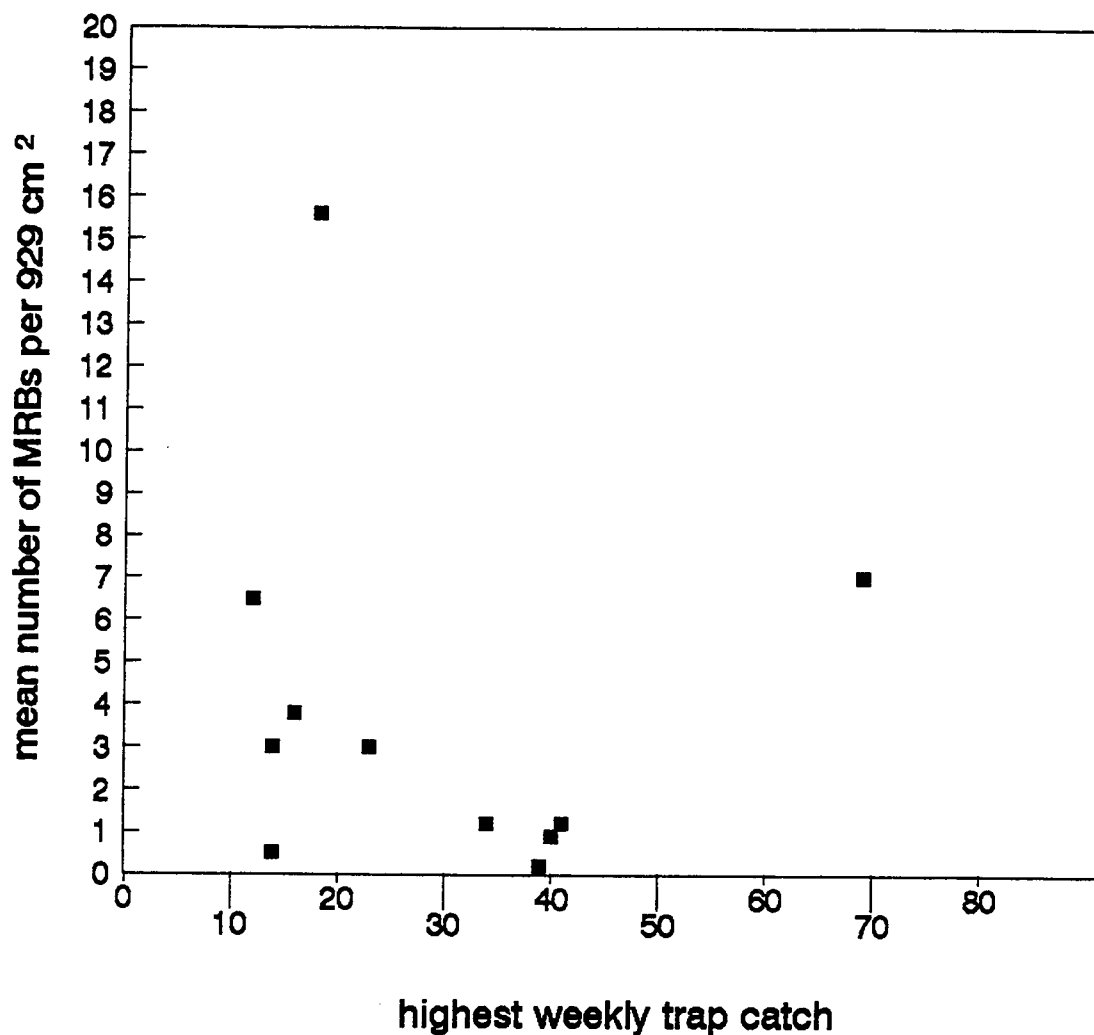
In 1993, the correlation between highest trap count (Figure 5.3) and cumulative trap count (Figure 5.4) to larval infestation were inconclusive. Field observations confirmed the erratic nature of trap catches. In several instances, trap catches remained low despite a large increase in the number of moths in the field. In 1994, pheromone trap catches were highly variable among traps in the same field and over time (Table 5.3).



**Figure 5.2** Populations of mint root borer larvae in four infested Willamette Valley peppermint fields in 1993

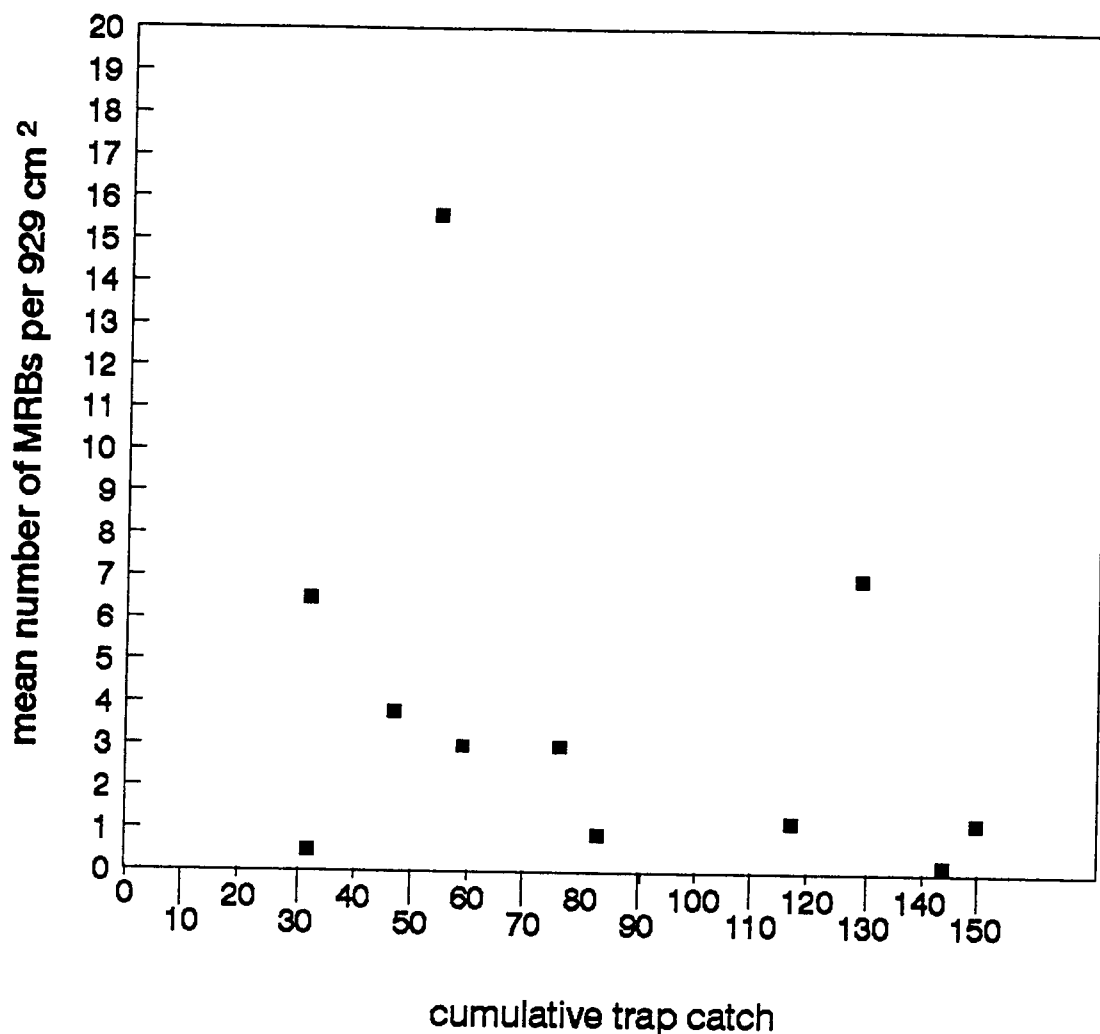
The modified sweep search correctly predicted the order of relative infestation levels in the three fields from June 24 to July 15 (Table 5.4) as did the cumulative counts over the season. Moth capture by the modified sweep search displayed a gradual increase followed by a decrease in the Corvallis field (Figure 5.5) while a more erratic pattern was observed in the Coburg and Jefferson fields (Figures 5.6 and 5.7).

The male to female ratio of the captured moths varied over time (Table 5.5); however, only in the Corvallis field did this ratio exhibit a clear pattern. The percentage of



**Figure 5.3** Relationship between highest weekly pheromone trap catch of mint root borer moths and subsequent larval infestation in eleven Willamette Valley peppermint fields monitored in 1993

females exhibited a similar pattern starting at a low level, increasing to a maximum and decreasing at the end of the season. Preliminary samples taken on June 9 in the Corvallis field captured twenty moths captured, all of which were males.



**Figure 5.4** Relationship between cumulative trap catch of mint root borer moths from June 11 to July 30 and subsequent larval infestation in eleven Willamette Valley fields monitored in 1993

## DISCUSSION

Berlese funnels appear to be a more accurate method for sampling MRB larvae. The August 20 comparison between hand-sorting and Berlese funnel extraction showed Berlese funnels to recover significantly more MRB larvae. The August 7 comparison

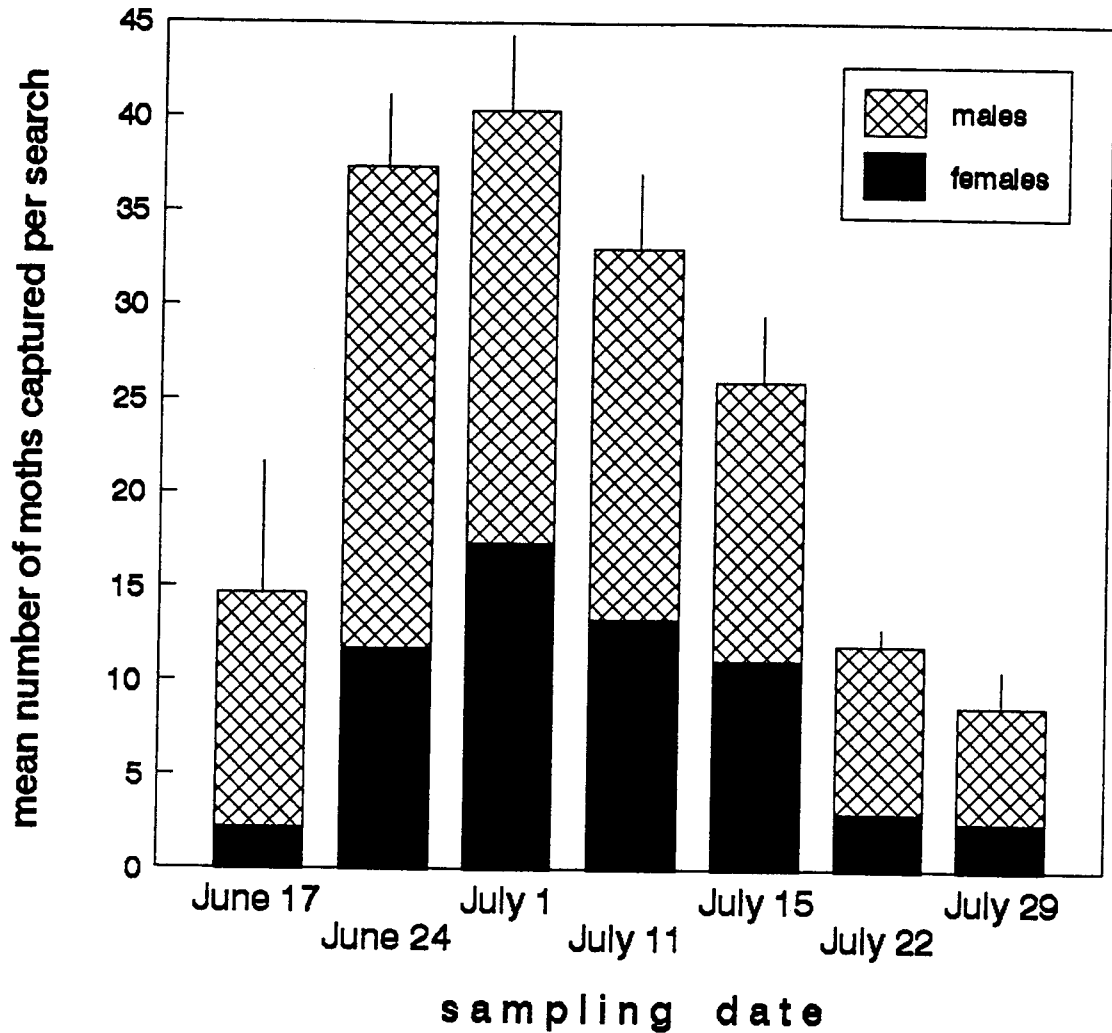
**Table 5.3** Mean number of mint root borer moths caught per pheromone trap in three Willamette Valley peppermint fields in 1994

		number of moths per trap								
		-----field-----								
date	trap	Corvallis			Coburg			Jefferson		
		1	2	3	1	2	3	1	2	3
May	26	2	1	0	0	1	1	0	0	1
June	2	1	2	0	1	4	0	0	0	1
	9	7	2	0	1	1	1	7	0	3
	17	2	2	1	3	8	3	3	1	2
	24	21	7	5	13	15	8	17	12	12
July	1	11	10	3	15	26	7	18	11	21
	8	6	5	3	8	21	7	7	6	6
	15	8	3	6	4	8	2	7	4	11
	22	14	6	8	9	4	5	5	20	8
	29	7	5	1	1	3	1	4	2	5
	total	79	43	27	55	91	35	68	56	70

**Table 5.4** Mean number of mint root borer moths caught per five minute modified sweep search in three Willamette Valley peppermint fields in 1994

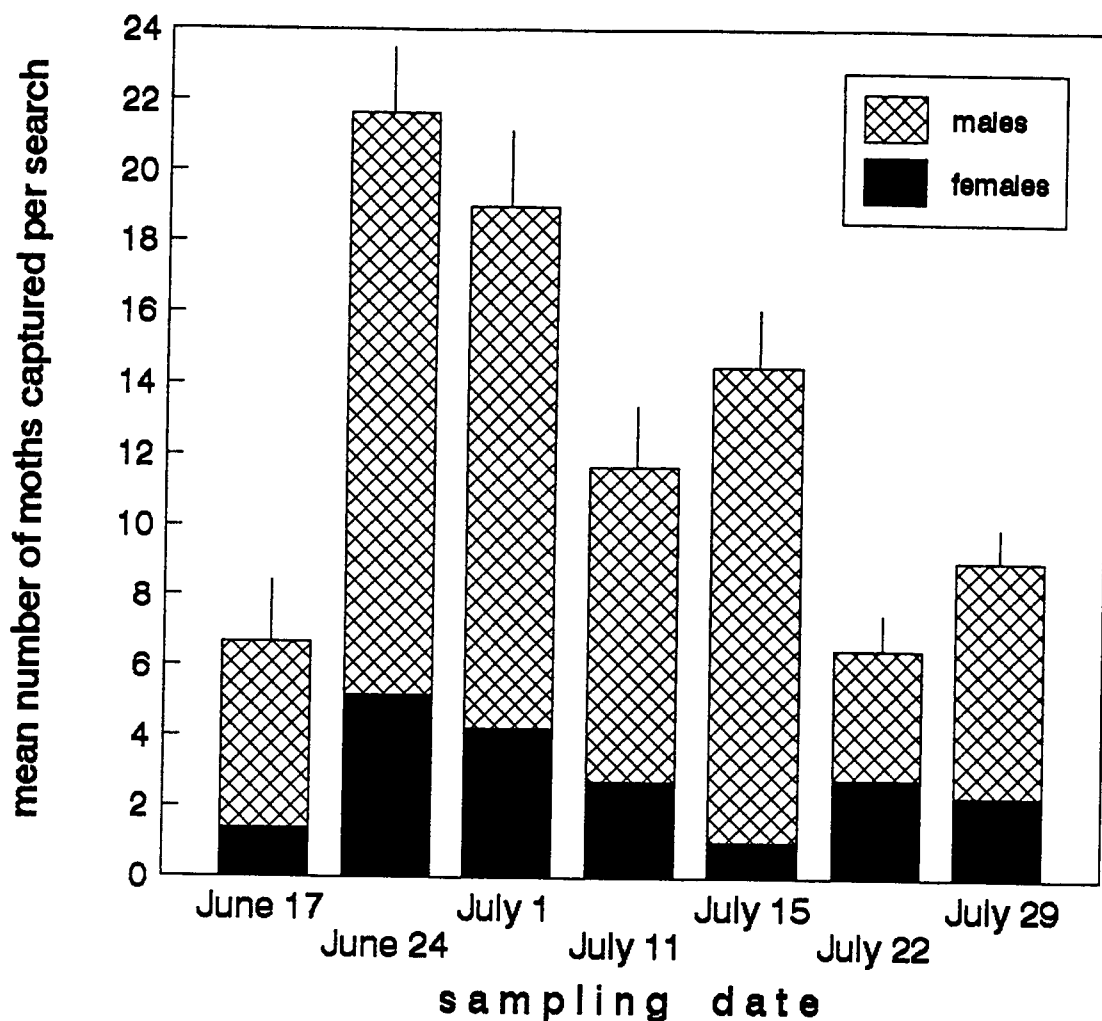
		mean number ( $\pm$ SE) of moths caught by field		
date		Corvallis	Coburg	Jefferson
June	17	14.7 (7.4)	6.7 (1.8)	7.0 (1.2)
	24*	37.3 (2.2)	21.7 (1.9)	8.3 (3.2)
July	1*	40.3 (4.2)	19.0 (2.1)	14.7 (2.9)
	11*	20.2 (4.2)	9.2 (2.5)	6.0 (0.9)
	15*	20.2 (3.6)	10.3 (1.5)	4.8 (0.7)
	22	10.2 (0.9)	6.3 (1.4)	6.3 (0.9)
	29	8.8 (1.9)	9.5 (0.8)	0.5 (0.3)
	total	151.7	82.7	47.6
infestation level (MRBs per 929 cm <sup>2</sup> )		12.3	7.5	3.8

\* correct ordering of infestation level according to moth capture



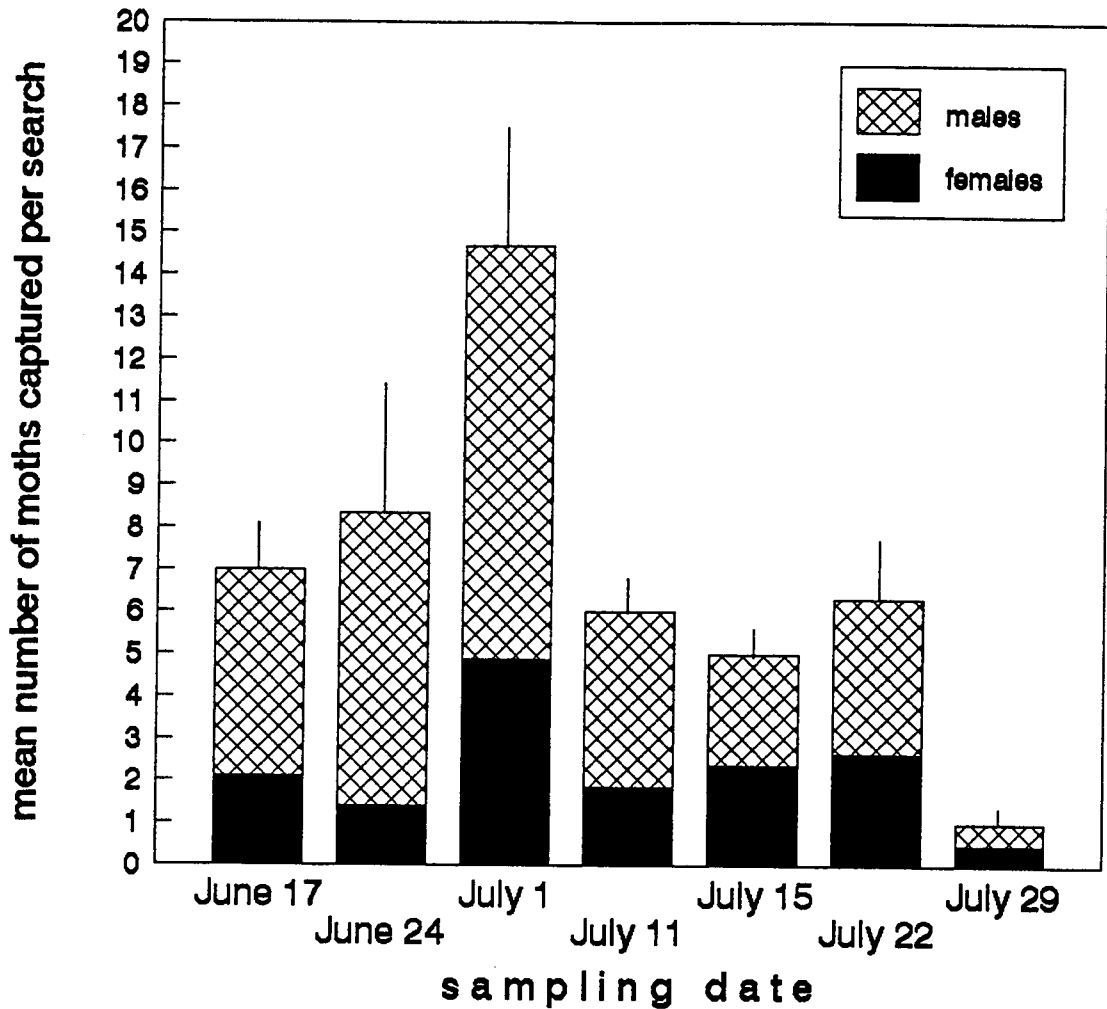
**Figure 5.5** Mean number of mint root borer moths captured in 1994 in a commercial peppermint field near Corvallis, Oregon using a five minute modified sweep search

failed to detect significant differences between the two methods even though higher larval numbers were collected with the Berlese funnel extraction. In terms of labor input, hand-sorting took 2-3 times longer than Berlese funnel extraction. Although samples must be left on the Berlese funnel for several days until they are completely dry, the extraction process itself does not require an input of labor.



**Figure 5.6** Mean number of mint root borer moths captured in 1994 in a commercial peppermint field near Coburg, Oregon using a five minute modified sweep search

Perhaps where Berlese funnels are most valuable is in detecting earlier instars. Smaller larvae would be difficult to detect by the hand-sorting method, leading to an underestimation of larval density. Berlese funnels are able to extract early instar MRBs, but the removal of soil is essential. Because the presence of soil appears to hinder the movement of early instar MRBs, the soil should be removed as much as possible prior to placing the rhizomes into Berlese funnels. A separate Berlese funnel extraction on the soil



**Figure 5.7** Mean number of mint root borer moths captured in 1994 in a commercial peppermint field near Jefferson, Oregon using a five minute modified sweep search

portion of the sample does not appear to be necessary since most larvae occur in the rhizomes; however, sifting the soil and conducting a visual examination is useful for detecting larger larvae in the soil. The presence of soil may not affect late instars as it does early instars, but because the prolonged MRB adult emergence results in a wide range in larval sizes at any given time, soil removal is recommended.



**Table 5.5** Percentage of female mint root borer moths collected during five minute modified sweep searches in three Willamette Valley peppermint fields in 1994

<b>date</b>	<b>percent females by field</b>		
	<b>Corvallis</b>	<b>Coburg</b>	<b>Jefferson</b>
June 17	15.2	21.1	30.0
24	31.5	24.0	16.7
July 1	43.1	22.2	33.3
11	40.4	23.5	30.8
15	42.9	7.1	47.4
22	25.5	43.3	41.9
29	29.6	26.2	50.0

The experiment comparing hand-sorting with Berlese funnel extraction for detecting early instars had a high percentage of missing larvae. For example, hand-sorting recovered 27 out of 72 larvae, leaving 45 larvae unaccounted. Because dead larvae and feeding damage are visible under a dissecting microscope, it is unlikely that the 45 missing larvae would go unnoticed. The most likely explanation is that prior to starting the experiment, the larvae escaped from the petri dishes containing the infested rhizomes. Hand-sorting was performed four days after rhizome infestation while the Berlese funnel extractions were performed after only two days. As a result, more larvae may have escaped from the rhizomes assigned to hand-sorting. Although more larvae may have been recovered by hand-sorting if all treatments had been initiated on the same day, hand-sorting is not a practical alternative to Berlese funnels for processing field samples.

MRB sampling can begin earlier, especially in heavily infested fields. In 1993, eight fields were correctly diagnosed on August 13. At that time, many fields had yet to be harvested, but fields can be sampled pre-harvest if necessary. Also, post-harvest samples can be collected while the hay is still in windrows instead of waiting for the irrigation to be restored. Wilted plants present in the fall regrowth would be more useful

as an indicator of control efficacy rather than as a diagnostic tool. Although the infestation level can be underestimated if samples are collected too early, the risk can be reduced by sampling heavily infested fields first. Heavily infested fields are most in need of early diagnosis and treatment to minimize damage to the crop. Knowing which fields are heavily infested prior to sampling would be ideal. Fields that are likely to be heavily infested are those with an infestation the previous year, but either the field was not treated or the treatment was not effective. Any field with a large number of moths earlier in the season are candidates for early sampling.

The five minute modified sweep search may not only serve as an indicator of which fields should be sampled first, but also may be useful in estimating the infestation level. Unfortunately, only three fields were sampled in 1994 and all fields were infested; however, the results are encouraging in that the total moth catch in each of the fields is approximately proportional to the infestation levels. Monitoring MRB adults using the modified sweep search is probably subject to several sources of variability. For example, differences were observed among samplers. Also, variability in time (daily as well as weekly) may be a factor influencing the number of moths caught. Additional studies are needed to determine whether moth catch can precisely predict larval infestation levels.

Although using the MRB sex pheromone to monitor the adult population would provide a simple and quick method of diagnosing fields, further studies are needed to determine what causes variable trap catches. Inconclusive results from the 1993 season may be explained by the use of only one trap per field; however, trap catches appear to be variable within the same field as seen in 1994. Possible explanations for this variability include wind direction, trap density, and the presence of competing females (Wall and Perry, 1980; Knight and Croft, 1987; Elkington and Cardé, 1988). Also, it should be noted that pheromone trap catches are an indirect measure of subsequent larval infestation because the traps attract male instead of female moths.

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**CHAPTER 6**  
**Control of Mint Root Borer in Peppermint with Multiple Applications of the**  
**Entomopathogenic Nematode, *Steinernema carpocapsae***

**ABSTRACT**

Field experiments were conducted to compare multiple nematode applications using lower rates (three applications of 1.2 billion IJs/ha, two applications of 1.2 billion IJs/ha, two applications of 1.8 billion IJs/ha, or two applications of 2.5 billion IJs/ha) with a single application using a higher rate (3.7 billion IJs/ha or 4.9 billion IJs/ha). All multiple application treatments significantly reduced MRBs compared with the control, significant differences were not detected between single and multiple applications. Multiple nematode applications offer an alternative to precisely timing a single application and may be particularly useful in heavily infested fields and in fields where a delay in harvest or in post-harvest irrigation is anticipated.

**INTRODUCTION**

The entomopathogenic nematode, *Steinernema carpocapsae* (Weiser) strain All (BioVector®), is capable of controlling the mint root borer (MRB), *Fumibotys fumalis* (Guenée), both pre- and post-harvest. Since pre-harvest nematode applications target earlier instars, crop damage is minimized; however, fields may be treated too early. Despite being univoltine, the MRB has a prolonged adult emergence which spreads oviposition over a two month period (Berry, 1974). This, in combination with the short persistence of *S. carpocapsae* in the soil, makes timing an important factor in reducing MRB populations. To benefit from a pre-harvest application, the nematode should be

applied as early as possible to minimize crop damage, yet late enough to ensure control of larvae resulting from later ovipositing activity.

Just as a pre-harvest application can be applied too early, a post-harvest application can be applied too late. Prior to entering the prepupal stage, late instar MRBs construct a silk-lined earthen cell called a hibernaculum in which to overwinter (Berry and Fisher, 1993; Pike et al., 1988). Once it enters the prepupal stage, the MRB is no longer susceptible to the nematode (see Chapter 3); therefore, fields must be treated before hibernacula form. Since nematode applications must be accompanied by irrigation to move the nematode into the soil and to ensure nematode survival (Shetlar et al., 1988; Zimmerman and Cranshaw, 1991), the lack of irrigation immediately before and after harvest may affect proper timing of a post-harvest application. Also interfering with post-harvest timing is the time-consuming nature of diagnosing and treating fields. Processing soil samples either by hand-sorting or with Berlese funnels is a slow, labor-intensive process. If an infestation is found, growers typically need up to a week to treat a field, depending on how long it takes to cover a field with irrigation.

The discovery of variability in MRB development between fields has created further difficulties in properly timing a nematode application. For example, soil samples taken from two fields in mid-September 1992 revealed 48.3% hibernacula formation in one field but only 3.5% hibernacula formation in the other field. At the same point in time, while it is essentially too late to treat one field, another field may still be treatable. Variability in MRB development only serves to narrow an already narrow treatment window. Faster developing populations run a higher risk of having too many hibernacula formed by the time larvae can be treated post-harvest; slower developing populations run a higher risk of having a pre-harvest treatment applied too early.

Extensive sampling is the key to properly timing a control measure. Given the time-consuming nature of MRB sampling, there may be some merit to treating more than once with a lower nematode rate instead of a single application at a higher rate. Results

from a pre-harvest small plot experiment conducted in 1992 showed that a rate as low as 1.2 billion IJs/ha significantly reduced MRB numbers compared to an untreated control. Although the percent reduction (64.4%) was not sufficient in itself to provide satisfactory control, multiple applications may be more effective. Initiating multiple nematode applications with a pre-harvest treatment not only contributes to minimizing crop damage, but also provides assurances that the control measure will be properly timed. Moreover, in the event that control is obtained with the first application, subsequent applications would not be necessary and would translate into savings for the grower. To investigate the potential of multiple nematode applications using lower rates, a small plot experiment was conducted in 1993 and a large plot experiment was conducted in 1994.

## MATERIALS AND METHODS

In the small plot experiment, a randomized complete block design (RCBD) replicated five times was used to compare the following treatments: 1) untreated, 2) 3 applications of 1.2 billion IJs/ha on July 24, August 5 and August 28, 3) 2 applications of 2.5 billion IJs/ha on August 5 and August 28, and 4) 1 application of 4.9 billion IJs/ha on August 5. The applications on July 24 and August 5 were pre-harvest; the application on August 28 was post-harvest. The experiment was conducted in small plots (29.7 m<sup>2</sup>) with 0.6 m borders surrounding each plot. The plots were irrigated with approximately 0.3 cm of water to moisten the ground prior to application. Infective juveniles of *S. carpocapsae* (biosys, Palo Alto, CA) were then applied with a CO<sub>2</sub> driven backpack sprayer (168 liters per hectare at 2.1 kg/cm<sup>2</sup> pressure) and immediately followed with approximately 2.5 cm of irrigation. The experiment was evaluated on September 11-13 by taking eight soil samples (929 cm<sup>2</sup> to a depth of six cm). Rhizomes were separated from the soil and placed in Berlese funnels equipped with 75 watt bulbs to extract live larvae. The extraction process lasted four days or until rhizomes were completely dry. The soil was

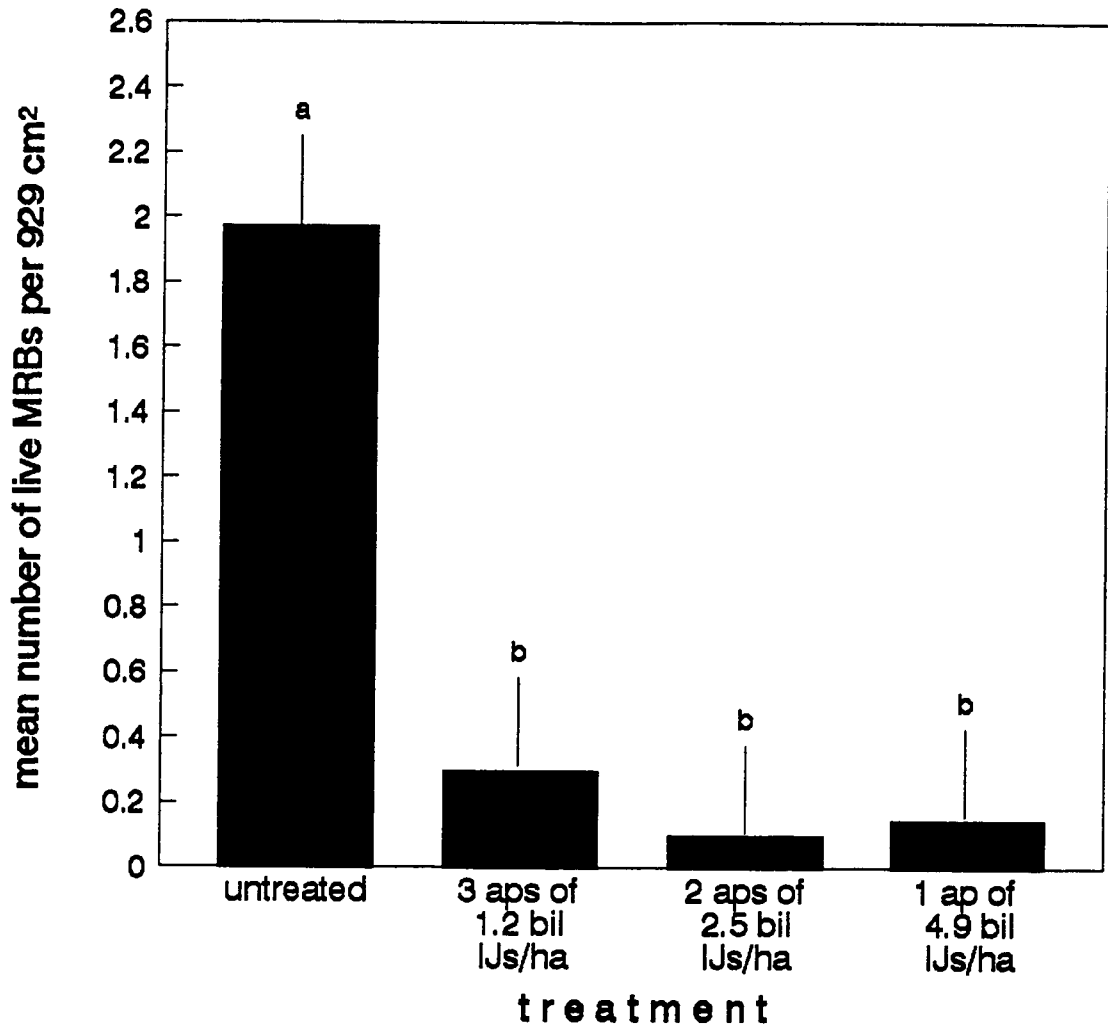
sifted using a screen (mesh size 0.6 cm) and a visual search was made for larvae and hibernacula.

A large plot experiment was conducted using plots approximately 0.6 ha in size. A randomized complete block design (RCBD) replicated four times was used. The following treatments were compared: 1) untreated control, 2) 2 applications of 1.2 billion IJs/ha on July 26-29 and August 15-20, 3) 2 applications of 1.8 billion IJs/ha on July 26-29 and August 15-20, and 4) 1 application of 3.7 billion IJs/ha on August 15-20. Applications on July 26-29 were pre-harvest; applications on August 15-20 were post-harvest. On the date of application, the ground was moistened with approximately 0.6 cm of water prior to applying the nematodes. Nematodes were injected through the irrigation over a 30-60 minute period to ensure even distribution and immediately followed with approximately 5.7 cm of additional irrigation. The first evaluation was made on August 11 and 15, sampling two blocks on each date by taking eight samples (464.5 cm<sup>2</sup> to a depth of six cm) along each irrigation line. Because the single application of 3.7 billion IJs/ha had yet to be applied, MRB numbers were determined for the control and the pre-harvest applications of the multiple application treatments only. The second and final evaluation was made on August 25 for the control plots, and September 1 for the nematode treatments. The control was sampled first to allow the grower to treat those areas as soon as possible. Along each irrigation line, ten soil samples (464.5 cm<sup>2</sup>) were taken, and MRB numbers were determined as described above for the small plot experiment. Data from each evaluation were analyzed separately.

## RESULTS

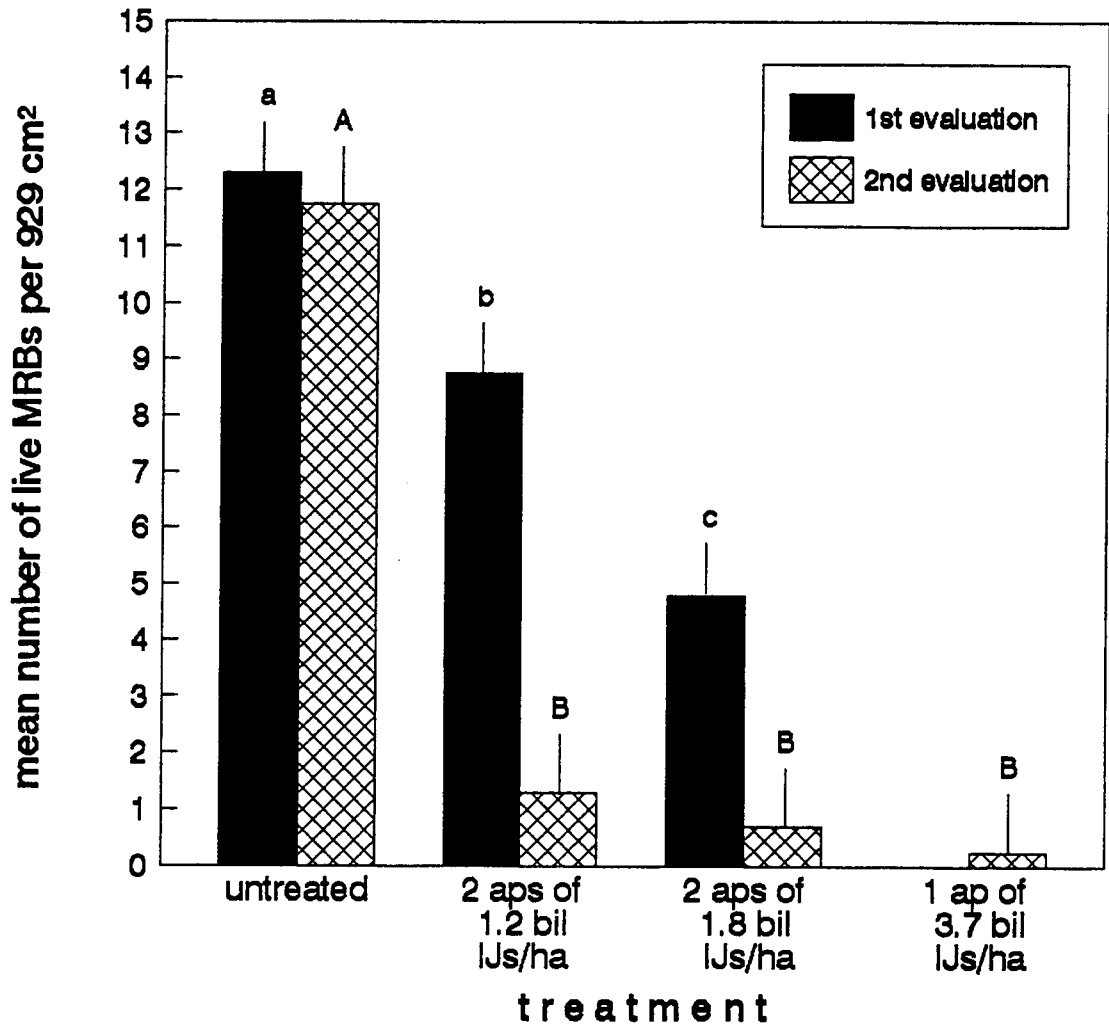
In the small plot experiment, significant differences were not detected among any of the nematode treatments, and all nematode treatments significantly reduced MRB numbers compared with the control (Figure 6.1). Total percent reduction compared to the





**Figure 6.1** Mean number of mint root borers recovered from 929 cm<sup>2</sup> soil samples in 1993 multiple application experiment (in small plots) conducted in a commercial peppermint field in Benton county, Oregon. Separation of means by Fisher's Protected LSD ( $p \leq 0.05$ ). Bars with different letters are significantly different.

control ranged from 84.8% for the treatment consisting of three applications of 1.2 billion IJs/ha to 94.9% for the treatment consisting of two applications of 2.5 billion IJs/ha. The single pre-harvest application of 4.9 billion IJs/ha, resulted in 92.4% reduction. Because the field used in this experiment had a relatively low infestation of 2.0 MRBs per 929 cm<sup>2</sup>,



**Figure 6.2** Mean number of mint root borers recovered from 929 cm<sup>2</sup> soil samples in 1994 multiple application experiment (in large plots) conducted in a commercial peppermint field in Benton county, Oregon. Separation of means by Fisher's Protected LSD ( $p \leq 0.05$ ). On each sampling date, bars with different letters are significantly different.

all nematode treatments significantly reduced MRB levels below the treatment threshold of 2-3 MRBs per 929 cm<sup>2</sup>.

In the large scale experiment, there were significant differences detected among all treatments on the first evaluation (Figure 6.2). An average of 12.3 MRBs per 929 cm<sup>2</sup> found in the control was reduced 28.9% with the pre-harvest application of 1.2 billion

IJs/ha. Better control was achieved with the higher rate of 1.8 billion IJs/ha, resulting in 60.9% reduction in MRBs. Neither rate, however, was successful at lowering the MRB population below the treatment threshold of 2-3 MRBs per 929 cm<sup>2</sup>. On the second evaluation, all nematode treatments significantly reduced MRB numbers compared with the control (Figure 6.2). In addition, all nematode treatments significantly reduced MRB numbers below the treatment threshold. The multiple application using 1.2 billion IJs/ha resulted in a total percent reduction of 88.9% while the multiple application using 1.8 billion IJs/ha resulted in total percent reduction of 94.3%. Total percent reduction for the single application of 3.7 billion IJs/ha was 97.9%. Significant differences were not detected among any of the nematode treatments on the final evaluation.

## DISCUSSION

In addition to either pre-harvest or post-harvest applications, multiple applications of *S. carpocapsae* are yet another option for MRB control. All multiple application treatments in both the small and large plot experiments reduced MRBs to a level comparable to a single application using a higher rate. Keeping in mind, however, that a significant increase in MRB control was not observed and that implementation would require additional labor inputs, multiple nematode applications are not a substitute for a properly timed single application. Only in certain situations should multiple applications be considered.

Multiple nematode applications should be considered if a field is scheduled to be harvested late or a delay is anticipated in restoring the irrigation after harvest. In the past, MRB control typically occurred in September. Taking into consideration the non-susceptibility of the prepupal stage to *S. carpocapsae* and the variability in MRB development between fields, inconsistent MRB control may be attributed in part to too many hibernacula in the field before treatment. Higher efficacy may result if control

measures targeting the larval stage are initiated sooner; however, attempts to treat fields earlier will increase the probability that harvest will conflict with the application. In such cases, multiple applications will ensure proper timing.

Heavily infested fields are also candidates for multiple nematode applications. The greater the infestation, the more critical is the need to initiate control pre-harvest due to the threat of severe crop damage. A single pre-harvest application, however, carries some risk since the consequences of an improperly timed treatment are magnified in heavily infested fields. Without a high percent reduction, enough MRBs would survive to result in an infestation the following year. Studies on MRB population dynamics are in order to determine the population density below which a field would not require treatment the following year.

Multiple applications starting with a pre-harvest treatment provides assurances that proper timing will occur with minimal crop damage. In heavily infested fields, it may be more important to initiate control earlier in heavily infested fields than to suffer severe crop damage. A crop damage index was not conducted for the large plot experiment; however, the 60.9% reduction in MRB numbers obtained with the pre-harvest application of 1.8 billion IJs/ha may have prevented substantial reduction in yield. Another potential advantage of using multiple nematode applications is the possibility of obtaining economic control with the first application. In the large plot experiment, none of the split application treatments provided adequate control with the first application, but further studies are needed to evaluate different nematode rate combinations. For example, a higher pre-harvest rate may be desirable to further minimize crop damage at an early stage, followed by a lower post-harvest rate, if necessary. This combination may yield better control than multiple applications using the same rate.

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## SUMMARY

Control of the mint root borer (MRB), *Fumibotys fumalis* (Guenée), appears to be heavily dependent on properly timing a control measure. In a laboratory experiment, MRB prepupae were not susceptible to the entomopathogenic nematode, *Steinernema carpocapsae* (Weiser) strain All, even at the highest nematode concentration of 10,000 infective juveniles (IJs) per insect. Because the chemical insecticide chlorpyrifos is probably also ineffective against the prepupal stage, fields need to be treated prior to prepupal formation in the late summer/early fall.

Unlike chlorpyrifos which is restricted to post-harvest use, *S. carpocapsae* can be applied pre-harvest. Pre-harvest nematode applications have the advantage of reducing crop damage; however, they also need to be properly timed. Nematode persistence in the soil appears to be short-lived, decreasing significantly from one day to 14 days post-application. Short nematode persistence combined with prolonged adult emergence and oviposition increases the risk of applying the nematodes prematurely if applied pre-harvest. The discovery of variability in MRB development between fields further narrows the treatment window, increasing the risk of applying a pre-harvest application too early and a post-harvest application too late.

Mint production also can interfere with application timing. Both the nematode and chlorpyrifos must be applied with irrigation. Consequently, fields cannot be treated immediately before harvest and irrigation may be delayed after harvest. Moreover, field evaluation is a time-consuming and labor-intensive process. Peppermint fields in the Willamette Valley are typically sampled after harvest as late as mid- to late September. If an infestation is found, the field must be treated. Growers need up to a week to treat a field, depending on how long it takes to move the irrigation across the field.

Experiments were conducted evaluating different methods and techniques to determine larval density so fields can be diagnosed quickly and accurately. Berlese funnel

extraction was shown to have advantages over hand-sorting because it is less labor-intensive, more accurate, and useful in detecting early instars (provided soil is removed from the rhizomes prior to the extraction process). The ability to detect early instar MRBs with Berlese funnels allows field diagnosis to occur earlier in the season, particularly for heavily infested fields. In 1993, fields were correctly identified as infested or non-infested in mid-August. Efforts to correlate adult density with subsequent larval infestation were inconclusive using pheromone trap catches; however, a modified sweep search correctly ranked infestation levels in three fields monitored in 1994.

Field experiments were conducted both pre- and post-harvest to investigate the potential of *S. carpocapsae* for MRB control. Pre-harvest nematode application rates ranging from 1.2 billion to 7.4 billion IJs/ha reduced MRB populations significantly compared to the control, but not always below the treatment threshold of 2-3 MRBs per 929 cm<sup>2</sup>. Retention of nematodes on the foliage may have occurred under pre-harvest conditions to affect efficacy. Post-harvest nematode application rates equal to or above 2.5 billion IJs/ha were as effective as chlorpyrifos applied at 2.24 kg active ingredient (a.i.) per hectare.

Multiple nematode applications using lower rates offer an alternative to precisely timing a single application. Multiple applications (three applications of 1.2 billion IJs/ha, two applications of 1.2 billion IJs/ha, two applications of 1.8 billion IJs/ha, or two applications of 2.5 billion IJs/ha) performed as well as single applications (3.7 billion IJs/ha or 4.9 billion IJs/ha). Multiple nematode applications may have their greatest utility in treating heavily infested fields. Since the first application can be initiated pre-harvest, crop damage can be minimized. Subsequent applications provide additional MRB reduction which is important in heavily infested fields because even survival of a small proportion of the MRB population is likely to result in an infestation the following year. Multiple nematode applications also should be considered in fields where a delay in harvest or post-harvest irrigation is anticipated.

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