

MICROCLIMATE MANIPULATION TO IMPROVE THE EFFICACY OF
ENTOMOPATHOGENS TARGETING PLUM CURCULIO LARVAE IN MICHIGAN
ORCHARDS

By

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ABSTRACT

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The goal of the research in my thesis was to determine whether the efficacy of augmentative releases of entomopathogenic fungi and nematodes for plum curculio control in apple and cherry orchards can be improved via microclimate manipulation and soil texture consideration. To meet this goal, the effect of soil moisture delivered through micro-sprinkler irrigation and the estimation of soil texture on entomopathogens was investigated in field efficacy experiments. Field trials indicated mixed levels of efficacy by entomopathogens against plum curculio, with only one application timing interval decreasing plum curculio emergence compared to untreated controls, and demonstrated a trend in which plum curculio emergence was greater in irrigated versus unirrigated treatments. In addition, plum curculio soil-dwelling life stage activity was studied in laboratory experiments to provide additional support for field study findings. Laboratory studies focused upon the influence of soil texture and moisture potential on plum curculio burrowing depth and pupation rates. At low moisture levels, plum curculio were not found in the pupal lifestage, while higher moisture treatments had significantly greater numbers of plum curculio found in the pupae. At low moisture levels, plum curculio depth was greatest in sandier soil treatments, while under high moisture treatments plum curculio were found at greater depths in clay-dominated soils. This research was aimed at providing producers with broader opportunities to adopt alternative pest management tactics to control plum curculio, including the implementation of entomopathogens as well as cultural and mechanical methods.

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CHAPTER 1

Literature Review

1.1. *Conotrachelus nenuphar*

1.1.1. Geographic Distribution

Conotrachelus nenuphar (Herbst) (Coleoptera: Curculionidae), the plum curculio, is a weevil native to North America. Populations are distributed east of the Rocky Mountains, with a northern boundary located at approximately 50° north latitude and southern boundary of 28° north latitude (Chapman 1938). A small population in Box Elder County in northern Utah, first detected in 1980, is the only known exception to this distribution (Alston et al. 2005).

1.1.2. Description and Life History

Adult plum curculio are predominantly dark brown and dark grey in color with distinguishing white and orange markings that distinguish it from other weevils. Plum curculio adult sex may be identified visually by examining the ventral surface for a groove located between the 2nd and 3rd pair of legs which is present in males and absent in females (Thomson 1932).

Adults overwinter in a variety of substrates including leaves, grass litter, and exposed soil, with some penetrating into loose soil to a maximum depth of 8.0 cm (Bobb 1949, Armstrong 1958, Smith and Flessel 1968, Lafleur et al. 1987). In Quebec apple orchards, a field study by Lafleur et al. (1987) also revealed that adults labeled with ⁶⁵Zn exhibited fall migratory patterns. When released within orchards, the majority of PC moved west-southwest towards

woodlots surrounding the orchards and when released within woodlots, the majority of adults migrated south.

Adult spring emergence has been correlated with temperature and host plant phenology (Quaintance and Jenne 1912, Whitcomb 1929, Cox 1951, Smith and Flessel 1968). Upon emergence, adults have diminished fat levels and thus females feed for ovary development and maturation (Smith and Salkeld 1964) with oogenesis being initiated in approximately 50.0% of females and generally completed by petal-fall (Smith and Salkeld 1964). Plum curculio feed on buds and leaves prior to the availability of developing fruit (Garman and Zappe 1929). The aggregation of adults, likely caused by the stridulation of both sexes (Mampe and Neunzig 1996), may occur at the outermost row of host trees within orchards (Lafleur and Hill 1987, Racette et al. 1991, Chouinard et al. 1993, 1994). In observational studies, male plum curculio have been observed to mate with as many as 3 females per day (Yonce and Jacklin 1978) or as few as 10.4 females per 30 days (Smith and Salkeld 1964).

Oviposition by females occurs on immature fruit and is described by Quaintance and Jenne (1912). Using their proboscis, females cut a crescent-shaped flap in the fruit skin and excavate a small chamber beneath in order to mitigate the probability of the immature stages being crushed by the internal pressure of the developing fruit. Eggs hatch within 3-12 d (Paradis 1956) and larva tunnel through the interior of the fruit, feeding on fruit tissue and developing through four instars which are classified by head capsule width ranges established by Garman and Zappe (1929) and Chapman (1938).

Fourth instar larva exit fruit and burrow into the ground to a depth of 1.0-8.0 cm where they pupate, forming a pupal chamber approximately twice the volume of the pupa (Quaintance and Jenne 1912, Chapman 1938). Adequate soil moisture is required for larval entrance into soil,

pupation, and adult emergence from soil, with frequent rainfall correlating with increased summer generation emergence (Chandler 1932). When introduced into dry soil, plum curculio larvae fail to survive (Armstrong 1958). In Connecticut, larvae were observed in soil for an average of 11.6 days post soil entrance, the pupal stage was observed to last 11.0 days, and adults remained in soil for an additional 9.8 days prior to emerging (Garman and Zappe 1929).

Adults of univoltine populations found in northern regions undergo obligate winter reproductive diapause. At the onset of spring emergence, oogenesis begins in females and is followed shortly by mating (Smith and Salkeld 1964). Geographic strain distribution described by Chapman (1938) places the boundary between uni- and bivoltine strains along the southern borders of Kansas, Missouri, Illinois, transecting through Kentucky and Tennessee, and extending north along the northwestern borders of North Carolina and Virginia. However, indications of biovoltine populations have been documented as far north as southern Delaware (Stearns et al. 1935) and West Virginia (Leskey and Wright 2004).

1.1.3. Hosts

Hosts of plum curculio are limited to the flowering plant family Rosaceae, with native hosts including serviceberry (*Amelacher* spp.), hawthorn (*Crataegus* spp.), chokecherry, pin cherry, and plum (*Prunus* spp.), and highbush blueberry (*Vaccinium* spp.) (Antonelli et al. 1992). Economically important exotic introduced hosts include apple (*Malus* spp.), peach, apricot, and nectarine (*Prunus* spp.), and pear (*Pyrus* spp.). Southern hosts are reported to be primarily restricted to peach (*Prunus persica*) and plum (*P. angustifolia* and *P. umbellate*), while host range in the Northeastern United States is more broad (Maier 1990, Jenkins et al. 2006a). A West Virginia field trial determined that plum curculio exhibit a greater preference for Japanese and

European plums, followed by: peach, sweet cherry, tart cherry, apricot, apple, and pear (Leskey and Wright 2007).

1.1.4. Pest Status and Economic Significance

Feeding and oviposition scarring by adult plum curculio and infestation by larvae are the two forms of damage caused by the insect, making it an economically important pest of pome and stone fruit. Oviposition and feeding scars on young fruit often result in malformed fully-grown fruit, rendering it undesirable and unsuitable for fresh market. Tart cherry production is further complicated due to a national zero-tolerance standard for infested fruit (USDA-AMS 1941) that can ultimately lead to the rejection of an entire fruit load, requiring the grower to pay for costs associated with the cleaning of processing lines and the disposal of the rejected load. In the mid-Atlantic and northern U.S. fruit growing regions, plum curculio feeding and oviposition most significantly affects apple and cherry production, whereas damage in the southern U.S. is most economically significant in peach production.

1.1.5. Management Tactics

Organophosphate insecticides have been relied upon over the past 50 years as the principal control tactic for plum curculio in tree fruit production. The Food Quality Protection Act (1996) ushered in a new era of pest management, amending the Federal Insecticide, Fungicide, and Rodenticide Act, standardizing the Environmental Protection Agency's management of pesticides, and legislating health-based standards for pesticide applications in food. As a result, the use of most organophosphate insecticides was terminated in 2012 and an emphasis was placed on the discovery and approval of reduced risk insecticides. Subsequent research efforts have been made to optimize plum curculio monitoring tools and strategies to

reduce the number of pesticide applications, including trap design and lure combinations (Coombs 2001, Leskey and Wright 2004). Additionally, novel chemistries including insect growth regulators, neonicotinoids, and oxadazines have been assessed for their efficacy and promise as alternative control tactics (Wise et al. 2006, Wise et al. 2007).

Prior the advent of reliable chemical insecticides, plum curculio control was achieved primarily through cultural and mechanical tactics (Racette et al. 1992) that are currently used infrequently. Areas surrounding orchards may be utilized by adults as overwintering sites and thus should be managed prior to the growing season. Overwintering habitat up to 300.0 m away from orchards should be modified to reduce plum curculio survival (Lafleur et al. 1987), which may be accomplished via the burning of overwintering habitat in winter or early spring (Stearns et al. 1935) and the removal of wild and neglected native hosts that provide food and oviposition sites (Maier 1990).

During the growing season, applications of kaolin clay-based particle films on young fruit have reduced plum curculio damage, as well as damage caused by other pests, and have demonstrated physiological benefits to trees resulting in improved fruit production (Thomas et al. 2004, Lalancette et al. 2005). However, frequent applications are required to maintain efficacious coverage during high precipitation periods, thus increasing labor and fuel costs for growers (Whalon Personal Communication). “Trap crops” or wild or unsprayed hosts, strategically planted along orchard borders to attract plum curculio could receive insecticide applications targeting locally elevated plum curculio populations (Prokopy et al. 2003, Leskey et al. 2008, Shapiro-Ilan et al. 2008). Historically, limb jarring and collection of adults has been promoted as a control tactic (Cook 1890), but low oviposition damage reduction (Chapman

1938) coupled with higher associated labor costs likely would make this tactic prohibitively expensive in most commercial orchards.

Historically, orchard sanitation was prescribed mid-growing season to remove any fallen infested fruit from orchards, thereby preventing plum curculio larvae from entering orchard soil and completing their lifecycle. Recommended methods for the removal of infested fruit included the integration of livestock into orchards, mechanical collection devices, or hand labor (Stedman 1904, Quaintance and Jenne 1912, Chapman 1938). Although early literature recommended the incorporation of poultry and other livestock in orchards to provide pest control via consumption of the insects (Cook 1890, Garman and Zappe 1929), contemporary research demonstrated that free-range poultry were unable to control plum curculio in Michigan orchards (Clark and Gage 1996). Recent research focused on the integration of hogs in organic apple production demonstrated a decrease in plum curculio damage via feeding on infested fruit, as well as control and increased economic benefit to growers from organic pork sales (Buehrer Personal Communication). Cultivating orchard soils in an attempt to disrupt soil-dwelling larval and pupal lifestages was recommended historically as another mechanical plum curculio control tactic which could be employed mid-growing season (Stedman 1904, Garman and Zappe 1929, Stearns et al. 1935).

1.2. Microbial Control Agents

1.2.1. Observations of Microbial Control Agents Infecting Plum Curculio

In addition to few natural enemies (Racette et al. 1992), including ants (Jenkins et al. 2006b), several entomopathogens have been observed as natural enemies of plum curculio. Early reports by Garman and Zappe (1929) included observations of larvae infected with “*Isaria* or

Sporotrichum spp” and adults exhibiting symptoms of green muscardine disease, and although unidentified, it is likely that the agents were *Beauveria bassiana* (Balsamo) Vuillimen and *Metharizium anisopliae* (Metchnikoff) Sorokin, respectively. More contemporary reports of natural fungal infestations in both lab and field studies identified the causal agent as *B. bassiana* (McGiffen and Meyer 1986, Lafleur et al. 1987). Although no historical reports of natural infections of entomopathogenic nematodes have been found in the literature, nematodes trapped from plum curculio-infested soils by highly susceptible species (waxworms, *Galleria mellonella* Linneaus) caused substantial mortality to plum curculio when applied in laboratory studies (Alston et al. 2005).

1.2.2. Entomopathogenic Fungi

Beauveria bassiana (Balsamo) Vuilemin is a filamentous facultative fungus that is the causal agent for the white muscardine disease of insects. This deuteromycete, hyphomycete fungi most commonly invades hosts through direct penetration after attachment of a germinating spore or conidium. Similar to some plant fungal pathogens, *B. bassiana* forms an appressorium and penetration peg in order to puncture the host cuticle. Upon entrance to the hosts’ haemocoel, the mycelium quickly spreads and develops blastospores, yeast-like hyphal bodies, and host death is a result of nutrient depletion, physical obstruction of blood circulation, toxic fungal metabolites, and infection of organs (Goettel and Inglis 1997). Upon host death and under the correct environmental conditions, hyphae bloom on cadavers and may be dispersed by water or wind to infect new hosts.

Insect host susceptibility to *B. bassiana* is influenced by fungal strain, environmental conditions, cuticular and epicuticular microorganisms, and host susceptibility, physiological

state, nutrition, and defense mechanisms (Goettel and Inglis 1997). The limited host range of entomopathogenic fungi may be due to distinct nutrient requirements for spore germination, such as hydrocarbons (Charnely 1984), although the majority of *B. bassiana* strains have a broad host range. Appressorium establishment occurs directly upon germination of a conidium on a flat cuticular surface and although most *B. bassiana* strains exhibit negligible specificity for infection site, hyphal growth may be directed away from deeply sclerotized regions (Pekrul and Grula 1979).

Several barriers may prevent cuticle penetration of a potential host by fungi. Barriers are either preformed or are induced upon recognition of the germinating spore, as elucidated by St. Leger (1993). Epi-cuticular preformed barriers include low relative humidity, low nutrient levels, competing microbial flora, surface structures, toxic cuticular lipids and phenols, electrostatic charges, and a hydrophobic barrier. Pro-cuticular preformed barriers include a dry and desiccated cuticle formed as the result of crystalline chitin and tanned proteins and protease inhibitors. Induced cuticular barriers include the attachment and activation of prophenoloxidase that results in the melanization of the spore.

B. bassiana has low internal reserves and thus initially relies on the host cuticle to provide nutrients required for penetration by the appressorium, which are made available by the production and secretion of cuticle-degrading enzymes of the fungus. A number of different cuticle-degrading enzymes produced by fungal entomopathogens correspond to the polymers by which the insect cuticle is constructed, including protein, chitin, and lipids (Charnely and St. Leger 1991). Host recognition mechanisms are keyed to nutrient levels available on appropriate host cuticles (St. Leger et al. 1992, St. Leger et al. 1995). Once the extra-cuticular nutrients are depleted, the pathogen forms structures for penetration. Using another species of

entomopathogenic fungi, *Metarhizium anisopliae*, Goettel et al. (1989) demonstrated that epicuticle penetration primarily involves enzymatic degradation, while procuticle penetration employs both enzymatic degradation and physical separation.

In a laboratory assay, infection of *Spodoptera exigua* by *Beauveria bassiana* elicited a notable reduction in counts of granulocytes, as well as an inhibition of their spread, post-infection. As *B. bassiana* spread throughout the host, the rate at which hemocytes were able to phagocytose invading fungal cells decreased, demonstrating the ability of the pathogen to elude and suppress cellular defense responses of hosts (Hung and Boucias 1992). Associated with the growth of new conidia and vegetative growth, *Beauveria bassiana* produces several low weight cyclic peptides exhibiting insecticidal and antibacterial activity including beauvericin, bassionolide, cyclosporins, enniatins, and oosporein (Roberts 1981, Vey et al. 2001, Weiser and Matha 1988). Both beauvericin and bassionolide are ionophorous secondary metabolites that dissolve into lipid bilayers and intensify cell membrane permeability to specific ions, with the resulting irregular ion transport disrupting the function of cells and organelles (Boucias and Pendland 1998). Oosporein reacts with proteins and amino acids, causing enzyme malfunction (Wilson 1971).

Beauveria bassiana and other species of entomopathogenic fungi are applied in inundative releases to soils and, less commonly, foliar surfaces. A number of different formulations for entomopathogenic fungi have been developed, including wettable powders, granules, water dispersible granules, suspension concentrates, oil miscible flowable concentrates, and oil dispersions (Faria and Wraight 2007). Alternative formulations of *B. bassiana* based on agriculturally-derived substrates, including maize (Nankina and Moore 2000) and rice (Nelson et al. 2004), have demonstrated effective levels of control against target weevil pests, although

US Environmental Protection Agency regulations prohibit the applications of mycoinsecticides in unapproved formulations.

1.2.3. Entomopathogenic Nematodes

Originally termed entomophagous, the nematode *Steinernema riobrave* Cabanillas, Poinar, and Raulston is microbivorous and not directly entomophagous (Kaya and Gaugler 1993). The nematode has a mutual association with the bacteria of the genus *Xenorhabdus* that is carried in the intestine of the nematode and resides in a quiescent stage (Boemare 2002). Once *S. riobrave* infective juveniles enter the haemocoel of insect hosts, the bacterium is released (Forst et al. 1997). Free-living infective juveniles enter into the haemocoel of a host by passing through the mouth, anus, or spiracles, and upon gaining entry, release the bacterium (Forst et al. 1997). After rapid growth and multiplication, infected hosts die of septicemia within 24-48 hours. Nematodes then feed on the bacteria within the host cadaver, giving rise to one to several generations until resources are depleted, and new infective juveniles leave the cadaver.

Insect immune systems employ a multi-faceted response to infection, including multiple cellular and humoral reactions. Immune system response to nematode infection has been reviewed by Dowds and Peters (2002). The instantaneous response by arthropod immune systems to nematode invasion is encapsulation, in which infective juveniles are trapped in either cellular or non-cellular capsules that are either solidified with a layer of melanin or consist of hardened melanin, respectively. Bacteria are phagocytized by cellular haemocytes and are sequestered into nodules. Humoral responses to injury and bacterial infection include the induced or constitutive antimicrobial peptides cecropins and lysozymes, although enzymes produced by the mutualistic bacteria may overcome such responses. Entomopathogenic nematodes may resist

encapsulation via three methods: evasion, in which surface lipids protect against recognition; tolerance, in which the insect haemocoel is overwhelmed by the number of infecting nematodes; and suppression in which nematode surface coat proteins reduce haemocyte concentrations. If the invading nematodes are able to release bacteria prior to encapsulation, they may be able to cause mortality without nematode reproduction occurring.

The pathogenicity of entomopathogenic nematodes in the families *Steinernematidae* and *Heterorhabditidae* and their mutualistic bacteria, *Xenorhabdus* and *Photorhabdus*, respectively, is not always straightforward. Certain entomopathogenic nematode species are pathogenic to insects when injected experimentally into insect haemocoel without their bacterial counterpart, while others are only pathogenic when injected combined (Bonifassi et al. 1999, Forst and Clark 2002). It is likely that the mutually associated bacteria provide nematodes with nutrition for the most effective development. Alternatively, some bacterial species mutually associated with entomopathogenic nematodes are pathogenic to insects when injected alone.

Entomopathogenic nematodes contain only one bacterial species, thus they are thought of as living in a state of “natural monoxeny”, referring to the state of microbiota in the intestine of the infective juvenile nematode and the state within the body of the infected insect (Bonifassi et al. 1999). To maintain this state of “natural monoxeny”, the bacterium associated with the nematodes produces a number of antimicrobial molecules including those to exclude any microbial competitors that may be present in the intestine of the infective juvenile or the haemocoel of the infected insect (Maxwell et al. 1994). The antimicrobial activity of symbiotic bacteria is reviewed by Webster et al. (2002), with small and large molecule antibiotics exhibiting efficacy against a wide variety of microorganisms including gram-positive and negative bacteria, fungi, and yeasts.

Entomopathogenic nematode infective juveniles may be stored in water for extended periods of time with proper refrigeration and aeration, although quality control issues and high costs prohibit this method, especially at a commercial scale. Grewal and Peters (2002) reviewed formulations designed to improve storage ability and reduce major factors influencing nematode quality, including high oxygen demand and susceptibility to microbial contamination. Formulations of entomopathogenic fungi include sponges, vermiculite, liquid concentrates, wettable powders, water-dispersible granules, and gels.

1.2.4. Abiotic Factors Limiting Entomopathogen Efficacy

The efficacy of entomopathogenic nematodes and fungi against soil-dwelling insect pests may be influenced by several abiotic factors including soil moisture. Pereault et al. (2009) concluded that water stress most likely had an impact on entomopathogen success against soil dwelling plum curculio larvae. When targeted with entomopathogenic nematodes, pecan weevil survival in laboratory assays decreased as moisture levels increased (Shapiro-Ilan et al. 2006). Laboratory experiments investigating the effect of water potential on *Beauveria bassiana* demonstrated *B. bassiana* conidia half-lives were longest at -15.0 bars (-1500.0 kPa) and decreased as the water potential either increased or decreased (Studdert and Kaya 1989). In a similar experiment, 25.0% saturation of soils with H₂O resulted in *B. bassiana* conidia with half-lives lasting up to 276 days (Lingg and Donaldson 1981).

In addition to water stress affecting entomopathogen success, Pereault et al. (2009) postulated that soil texture and composition also affected entomopathogen efficacy against plum curculio larvae. McCoy et al. (2002) observed that efficacy of nematodes against citrus weevil (*Diaprepes abbreviatus*) larvae improved in soils with higher proportions of sand. In laboratory

assays, *Steinernema carpocapsae* and *S. glaseri* exhibited higher survival rates in soils with increasing concentrations of sand, as well as higher levels of pathogenicity and persistence (Kung et al. 1990). Perault (2008) noted improved efficacy of *B. bassiana* against plum curculio larvae at sites with clay soils. Alternatively, clay coatings on *B. bassiana* have been reported help to reduce biodegradation of conidia (Fargues et al. 1983).

The UV B radiation component of natural sunlight is harmful to microorganisms, as it penetrates organisms and causes direct DNA damage (Tevini1993). Biological control agents and microbial pesticides including entomopathogenic nematodes and fungi are adversely affected by UV B exposure. Prolonged exposure to UV B radiation rapidly decreases viability and pathogenicity of entomopathogenic fungi and nematodes, making them ineffective for pest control (Gaugler and Boush 1978, Nickle and Shapiro 1994, Fargues et al. 1996, Huang and Feng 2009).

Temperature is an important environmental factor that may influence entomopathogen efficacy against target pests. Grewal et al. (1994) determined thermal niche breadths for infection, establishment, and reproduction of entomopathogenic nematodes in laboratory experiments with *Galleria mellonella*, and *S. riobrave* demonstrated the widest temperature range for infection, from (10.0-39.0°C). Superior virulence by *S. riobrave* against plum curculio larvae in laboratory experiments was observed under a range of temperatures in a laboratory assay (Shapiro-Ilan et al. 2011). Temperature was determined to significantly affect the in vitro germination and growth of several isolates of *B. bassiana*, with only 1 of 29 isolates demonstrating a thermal threshold above 43°C, while most demonstrated thermal tolerance to temperatures up to 25°C.

1.2.5. Experiments Targeting Plum Curculio Larvae with *Beauveria bassiana* and *Steinernema riobrave*

In a laboratory bioassay, a strain of *B. bassiana* isolated from South Carolina caused delayed mortality of 4th instar plum curculio larvae when compared to a strain of the entomopathogenic fungi *Metarhizium anisopliae* also isolated from South Carolina, although mortality rates at the end of the experiment were similar (Tedders et al. 1982). Laboratory immersion bioassays against last instar plum curculio demonstrated that *B. bassiana* GHA strain caused reduced mortality compared to the *M. anisopliae* F52 strain (Pereault 2008). When applied to larval-infested soil in the field, *B. bassiana* GHA strain did not significantly reduce the numbers of adults emerging in Georgia (Jenkins et al. 2006b). In a Michigan field efficacy study, *B. bassiana* GHA in granular rice and commercial oil formulations were effective in controlling plum curculio adult emergence when applied to Michigan apple and cherry orchard floors (Pereault et al. 2009).

Laboratory assays targeting final instar plum curculio larvae demonstrated that *S. riobrave*, as well as several other species of entomopathogenic nematodes, were efficacious (Shapiro-Ilan et al. 2002, Alston et al. 2005, Shapiro-Ilan et al. 2011). In a field study, *S. riobrave* 355 strain decreased adult emergence by up to 97.0% when applied to cages containing soil infested with final instar plum curculio in Georgia (Shapiro-Ilan et al. 2004). In a similar Michigan field study targeting soil-dwelling larvae, *S. riobrave* 355 strain reduced adult plum curculio emergence by up to 85.0% (Pereault et al. 2009). Soil-based applications of *S. riobrave* 355 strain targeting plum curculio larvae in an unmanaged plum thicket provided 100.0% control over two years, compared to less than 87.9% control caused by *S. riobrave* 3-8b strain (Shapiro-Ilan et al. 2008).

1.3. Thesis Research

The goal of the research in my thesis is to determine whether the efficacy of augmentative releases of entomopathogenic fungi and nematodes for plum curculio control in Michigan apple and cherry orchards can be improved via microclimate manipulation and soil texture consideration. In order to meet this goal, the effect of soil moisture delivered through micro sprinkler irrigation and the estimation of soil texture on entomopathogens was investigated in field efficacy experiments at three different orchards. Orchards were chosen based on the concentrations of sand, silt, and clay in the soil in order to determine the effect of soil type on entomopathogen efficacy against plum curculio larvae. In addition, plum curculio soil-dwelling life stage activity was studied in laboratory experiments to provide additional support for field study findings. Further studies focused upon the influence of soil texture and moisture potential on plum curculio pupation rates while depth of pupation was investigated using four soil substrates exhibiting a variety of textures as well soil moisture potentials. This research was aimed at providing producers with broader opportunities to adopt alternative pest management tactics to control plum curculio, including the implementation of entomopathogens as well as cultural and mechanical methods.

CHAPTER 2

Microsprinkler Irrigation for the Optimization of Field Efficacy of Entomopathogenic Fungi and Nematodes Targeting Last-Instar Plum Curculio (Coleoptera: Curculionidae) in Michigan Apple and Cherry Orchards

2.1. Introduction

The plum curculio, *Conotrachelus nenuphar*, (Herbst) (Coleoptera: Curculionidae) is a key pest of stone and pome fruit in central and eastern North America and an important factor in apple and cherry integrated pest management (Maier 1990). Adult plum curculio emerge from overwintering sites in the spring, feed, mate, and oviposit in fruit (Smith 1957). Larvae develop through four instars while feeding inside fruit. Fourth instar larvae exit from fruit, burrow into soil to a depth of 1.0-8.0 cm, and pupate (Quaintance and Jenne 1912). Adults emerge from pupation during the summer, cause late-season fruit damage and eventually overwinter, emerging the following year to continue infestations (Racette et al. 1992). Adults of univoltine Northern populations undergo obligate winter reproductive diapause, while adults of bivoltine Southern populations do not. Geographic strain distribution (Chapman 1938) places the boundary between uni- and bivoltine strains along a line formed by the southern border of Kansas, through Kentucky, and extending North along the northwestern borders of North Carolina and Virginia.

Organophosphate insecticides have been relied upon over the past 50 years as the principal control tactic for plum curculio in tree fruit production. The use of most organophosphate insecticides was terminated in apple (*Malus domestica*) and sweet and tart cherry (*Prunus avium*, *Prunus cerasus*) production in 2012 in compliance with the Food Quality

Protection Act (FQPA 1996). As a result, an emphasis was placed on the discovery and approval of reduced risk insecticides to provide control of plum curculio comparable to the recently cancelled insecticides. In response, research to optimize plum curculio monitoring strategies including trap design and lure combinations has reduced the number of pesticide applications (Coombs 2001, Leskey and Wright 2004). Along with monitoring strategies, novel chemistries including insect growth regulators, neonicotinoids, and oxadazines, have been assessed for their efficacy and promise as alternative control tactics (Wise et al. 2006, Wise et al. 2007). However, the dependence on insecticides raises concerns about the negative effects on non-target insects, especially natural enemies and other beneficials (Croft and Whalon 1982, Theiling and Croft 1988, Desneux et al. 2007), and the possibility of resistance development (Denholm and Rowland 1992, Nauen and Denholm 2005, Whalon et al. 2008). The utilization of microbial control agents, including entomopathogenic fungi and nematodes, orchard integrated pest management regimes has been suggested as a way to alleviate some of these concerns (Bathon 1996, Roy and Pell 2000, Lacey and Shapiro-Ilan 2008).

Pre- FQPA chemical insecticide sprays, including organophosphates, targeted only active adult plum curculio because of their visibility and ease of targeting, although some “curative” active against life stages within fruit has been documented (Wise et al. 2007). Historically, cultural and mechanical tactics were prescribed to control plum curculio immature life stages including orchard sanitation, livestock integration, and cultivation (Cook 1890, Stedman 1904, Garman and Zappe 1929, Racette et al. 1992). Augmentative applications of microbial control agents for the control of soil-dwelling life stages provides a strategy to control the pest at previously untargeted life stage, moving closer to the goal of a higher-level integrated pest

management and returning to the historical control model in which multiple life stages were targeted (Kogan 1988, Prokopy 1994).

In a laboratory bioassay, a strain of the entomopathogenic fungi *B. bassiana* isolated in South Carolina caused delayed mortality of 4th instar plum curculio larvae when compared to a strain of the entomopathogenic fungi *Metarhizium anisopliae* also isolated in South Carolina, although mortality rates at the end of the experiment were similar (Tedders et al. 1982). Laboratory immersion bioassays against last instar plum curculio demonstrated that *B. bassiana* GHA strain caused less mortality compared to the *M. anisopliae* F52 strain (Pereault 2008). When applied to larval-infested soil in the field, *B. bassiana* GHA strain did not significantly reduce the numbers of adults emerging in Georgia (Jenkins et al. 2006b). In a Michigan field efficacy study, *B. bassiana* GHA in granular rice and commercial oil formulations were effective in controlling plum curculio adult emergence when applied to apple and cherry orchard floors (Pereault et al. 2009). Laboratory assays targeting final instar plum curculio larvae demonstrated that the entomopathogenic nematode *Steinernema riobrave*, as well as several other species of entomopathogenic nematodes were efficacious (Shapiro-Ilan et al. 2002, Alston et al. 2005, Shapiro-Ilan et al. 2011). In a field study, *S. riobrave* 355 strain decreased adult emergence by up to 97% when applied to cages containing soil infested with final instar plum curculio in Georgia (Shapiro-Ilan et al. 2004). In a similar Michigan field study, *S. riobrave* 355 strain reduced adult plum curculio emergence by up to 85.0% (Pereault et al. 2009). Soil-based applications of *S. riobrave* 355 strain in an unmanaged plum thicket provided 100.0% control over two years, compared to less than 88.0% control caused by *S. riobrave* 3-8b strain (Shapiro-Ilan et al. 2008).

The efficacy of microbial control agents, including entomopathogenic fungi and nematodes, against soil-dwelling plum curculio may be influenced by abiotic factors, especially

soil moisture. Pereault et al. (2009) concluded that moisture stress most likely had an impact on microbial control agent efficacy against soil dwelling plum curculio larvae. When targeted with entomopathogenic nematodes, pecan weevil (*Curculio caryae*) survival in laboratory assays decreased as moisture levels increased (Shapiro-Ilan et al. 2006). Laboratory experiments investigating the influence of soil matric potential on *Beauveria bassiana* indicated *B. bassiana* conidia half-lives were longest at -15.0 bars (-1500.0 kPa) and decreased as the water potential either increased or decreased (Studdert and Kaya 1989). In another experiment, soils saturated with water at 25.0% produced *B. bassiana* conidia with half-lives lasting up to 276 days (Lingg and Donaldson 1981).

Research is needed to understand the influence of soil moisture on entomopathogen efficacy against soil dwelling plum curculio. Thus, the objective of this study was to determine the effect of micro sprinkler irrigation on the efficacy of two microbial control agents, *Steinernema riobrave* and *Beauveria bassiana*, against last-instar plum curculio larvae, scheduled precisely against emerged last-instar or against last instars as they emerged over time.

2.2. Materials and Methods

2.2.1. Insect Material and Rearing

Adult plum curculio were collected in April, May, and June 2010 from apple and cherry orchards in Benzie, Leelanau, and Manistee Counties, Michigan, using circle traps (Mulder et al. 1997) and black corrugated plastic “Whalon-modified Tedders Traps” (1.6 by 0.8 m) with a 5.0 cm wide strip of high-contrast reflective tape along the edges of the traps (Great Lakes IPM, Vestaburg, MI). Traps were baited with the attractive plant volatiles plum essence and benzaldehyde (Coombs 2001, Leskey and Wright 2004, Leskey et al. 2005). Thinned apples

were harvested from Trevor Nichols Research Center (Fennville, Michigan) in June 2010, treated with two fungicides (Captan 80 WDG and Benlate 50W) and a plant growth regulator (Diphenylamine), and stored at 5.0° C.

Plum curculio larvae were reared from field-collected adults held in cages for 5 days with thinned apples for oviposition. Apples made available for oviposition were rinsed to remove any fungicide residues and held in the laboratory on metal mesh racks over aluminum trays lined with moistened paper towels. Trays were checked daily for larval emergence and newly emerged larvae were held in groups of 10 in 90.0 mm petri dishes at 25.0° C in the dark. The dishes were lined with two pieces of Whatman filter paper moistened with 1.5 ml sterile water and sealed with Parafilm. Larvae used in bioassays were added to the soil surface of arenas within 24 h of emergence.

2.2.2. Microbial Control Agents

Commercialized strains of *Beauveria bassiana* (GHA strain Mycotrol-O liquid formulation; Laverlam International, Butte, MT) and *Steinernema riobrave* (355 strain in a petroleum-based gel formulation; Becker Underwood, Ames, IA) were used in this study. *B. bassiana* was applied at a rate of 1×10^{14} conidia/hectare and *S. riobrave* was applied at a rate of 4×10^9 infective juveniles/hectare in 50.0 ml aqueous suspensions. Controls consisted of H₂O. Treatments were poured evenly onto the soil surface of each assay arena.

2.2.3. Assay Arenas

Assay arenas were similar to those used by Pereault et al. (2009). Arenas were constructed of round (8.0 cm tall by 9.0 cm diameter) plastic deli containers (Sweetheart Cup Company Inc., Owings Mill, MD). A 4.0 cm diameter hole was removed from the bottom of the containers and fitted with mesh screen to allow for water drainage. Tops of the containers were fitted with conical boll weevil trap tops (USDA Boll Weevil Eradication Foundation, Abilene, TX) that were modified to fit tightly in the pot's upper rim to contain emerging plum curculio adults. Arenas were installed under the canopy of trees within 0.5 m of the trunk by removing soil cores with a 10.0 cm diameter golf hole cutter (Bayco Golf Inc., Winnipeg, MB, Canada). Soil from the golf hole cutter was transferred into arenas, making sure that the soil column was not modified, and filled arenas were placed back into the holes so that pot rims were level with the soil surface.

2.2.4. Microsprinkler Irrigation

SuperNetTM SR model micro sprinklers (Netafim USA, Fresno, CA) were utilized and were fitted with model # 30 (Brown) nozzles with a nozzle size of 0.1 cm. Micro sprinklers were supplied with water at a rate of approximately 2.5 bar via on-farm water sources, resulting in a nominal flow rate of 0.5 Liters per minute over a wetted diameter of 5.5 m. Micro sprinklers were used to irrigate treatment plots for 1 h at four intervals: -1, 0, 1, and 3 d from pathogen application, applying 31.0 Liters of water per irrigation interval.

2.2.5. Orchards

This field trial was performed in two apple orchards and one cherry orchard. The first apple orchard was located in Charlotte, Eaton County, Michigan (41.637242° N, -84.797974 W)

and the second was located in Flushing, Genesee County, Michigan (43.027788° N, -89.913381° W). The cherry orchard was located in Honor, Benzie County, Michigan (44.726217° N, -86.036725° W). At each orchard site, eight 15.0 cm deep soil samples were taken randomly from beneath tree drip-lines. Soil samples from each site were homogenized and 0.5 L sub-samples were placed into plastic bags and submitted to the Michigan State University Diagnostic Service Laboratory for nematode community structure analysis.

2.2.6. Experimental Design

The study was established at three orchards under two tree rows in each orchard in a split-plot design. Irrigation treatments (irrigated vs. unirrigated) were assigned to either row as the whole plot factor. Split plot factors were assigned randomly and included three pathogen treatments (*B. bassiana*, *S. riobrave*, and H₂O control) and timing of plum curculio larvae addition. Plum curculio larvae were added -5, 0, or 10 d from pathogen application. Each pathogen by timing treatment combination was replicated 12 times per row. Emerging adults from individual pots were counted and recorded, beginning 21 d post treatment and ending 60 d post treatment.

2.2.7. Statistical Analysis

Data were analyzed using SAS Version 9.3 (SAS Institute Inc., 2011) with $\alpha=0.05$. Adult emergence data from the three orchard blocks were combined and analyzed using proc Glimmix. Mean separations were conducted with Tukey's Honest Significant Difference.

2.3. Results

There was a significant effect of the three way interaction between irrigation, microbial control agent, and agents timing on the emergence of adult plum curculio ($F_{4, 402}=13.1$, $P<0.0001$) Adult emergence was significantly reduced by both *S. riobrave* (2.6 ± 0.3) and *B. bassiana* (2.8 ± 0.4) in treatments receiving micro sprinkler irrigation at the 10 d timing interval, compared to untreated controls (5.2 ± 0.4). All other entomopathogen by timing by irrigation combinations were not significantly reduced compared to corresponding untreated controls. Additionally, a trend was observed in which the mean number of adults emerging from treatments receiving micro sprinkler irrigation (3.6 ± 0.2) was greater compared treatments not receiving micro sprinkler irrigation (2.6 ± 0.1).

Table 2.1. Soil Type, pH, Soil Organic Matter, and Soil Composition.

Site	Soil Type	Soil pH	Organic Matter (%)	Soil Composition (%)		
				Sand	Silt	Clay
1	Sandy Loam	7.3	3.5	55.4	33.2	11.4
2	Loam	6.8	2.9	50.4	36.8	12.8
3	Sand	7.1	2.1	96.1	3.7	0.2

Table 2.2. Nematode Community Structure.

Site	Soil Herbivores						Tylenchus	Aphelencus	Dorylamis	Mononahs	Bacterial Feeders	Mycorrhizal Fungi	Oligochaetes
	Lesion	Dagger	Ring	Stunt	Pin	Spiral							
1	0	5	0	0	1	0	60	10	10	15	65	10	0
2	0	15	122	4	3	10	30	20	15	0	175	80	0
3	0	8	30	0	0	0	25	10	45	10	185	25	0

Table 2.3. Mean (\pm SEM) number of adult plum curculio emerged from microbial control agent-treated soil in pots. Ten larvae were added to each pot either -5, 0, or 10 d from microbial control agent applications. Values with an asterisk are significantly lower from the control within the same irrigation by larval addition timing (Tukey's HSD, $\alpha=0.05$).

Pathogen	Timing	Irrigation	Mean Adult PC Emergence (\pm SEM)
<i>B. bassiana</i>	-5 d	Y	4.5 (± 0.4)
Control (H ₂ O)	-5 d	Y	2.3 (± 0.5)
<i>S. riobrave</i>	-5 d	Y	5.3 (± 0.5)
<i>B. bassiana</i>	-5 d	N	1.8 (± 0.4)
Control (H ₂ O)	-5 d	N	2.7 (± 0.4)
<i>S. riobrave</i>	-5 d	N	2.1 (± 0.3)
<i>B. bassiana</i>	0 d	Y	3.8 (± 0.5)
Control (H ₂ O)	0 d	Y	2.3 (± 0.5)
<i>S. riobrave</i>	0 d	Y	3.9 (± 0.5)
<i>B. bassiana</i>	0 d	N	3.6 (± 0.5)
Control (H ₂ O)	0 d	N	4.4 (± 0.4)
<i>S. riobrave</i>	0 d	N	3.6 (± 0.4)
<i>B. bassiana</i>	+10 d	Y	2.8 (± 0.4)
Control (H ₂ O)	+10 d	Y	5.2 (± 0.4)
<i>S. riobrave</i>	+10 d	Y	2.6 (± 0.3)*
<i>B. bassiana</i>	+10 d	N	1.5 (± 0.2)*
Control (H ₂ O)	+10 d	N	1.6 (± 0.3)
<i>S. riobrave</i>	+10 d	N	2.2 (± 0.4)

2.4. Discussion

Entomopathogenic fungi and nematodes have been demonstrated as potential control tactics for plum curculio in several studies, although overall their potential for widespread adoption may be limited. In laboratory assays, a number of microbial control agents, including *Steinernema riobrave* and *Beauveria bassiana*, have been demonstrated to cause significant mortality against plum curculio larvae (Shapiro-Ilan et al. 2002, Alston et al. 2005, Pereault 2008). Field studies have indicated more variability in the efficacy of such microbial control agents targeting soil dwelling larvae, with *S. riobrave* capable of causing 40.0-80.0% mortality and *B. bassiana* causing 48.0-77.0% mortality, although mortality rates varied from year to year

(Shapiro-Ilan et al. 2004, Shapiro-Ilan et al. 2008, Pereault et al. 2009). The lab-to-field, site-to-site, and year-to-year variability of augmentative microbial control applications are characteristic of such studies (McCoy et al. 2000). Thus, the goal of this research was to determine how the efficacy of two microbial control agents, *S. riobrave* and *B. bassiana* against plum curculio, are affected by micro sprinkler irrigation and improved, precise microbial control agent application timing, and soil texture. The results indicate that the application timing of the two microbial control agents, as well as micro sprinkler irrigation, have a significant effect on their efficacy against plum curculio larvae (Table 2.3).

Previous research in Michigan orchards indicated that soil moisture likely was a factor limiting the efficacy of several microbial control agents targeting plum curculio in soil (Pereault et al. 2009). Similarly, laboratory assays have indicated soil moisture affects microbial control agent efficacy. When targeted with entomopathogenic nematodes, pecan weevil survival decreased as moisture levels increased (Shapiro-Ilan et al. 2006), while optimal water potentials significantly lengthened *B. bassiana* conidia half lives, which decreased as the water potential increased or decreased (Studdert and Kaya 1989). To mitigate soil moisture issues, micro sprinkler irrigation systems have been used to successfully lengthen the larval suppression of *Diaprepes abbreviatus* by entomopathogenic nematodes (McCoy et al. 2000, McCoy et al. 2002). In this study, micro sprinkler irrigation improved the efficacy of *S. riobrave* and *B. bassiana* against plum curculio, significantly reducing the mean number of adult plum curculio emerging from 10 d irrigated treatments when compared to the corresponding control (Table 2.3).

Beauveria bassiana and *Steinernema riobrave* only demonstrated significant control of plum curculio when larvae were introduced to experimental plots 10 d post application of the

two microbial control agents, but not when the plum curculio were introduced 5 d prior to or on the day of the application of the microbial control agents. Although the original intent of this experiment was not to determine any differences in plum curculio life stage susceptibility to microbial control agents, the results of this indicate that there may be a difference. Historical reports of plum curculio biology indicate that plum curculio may commence pupation as soon as 5 d post introduction to soil or 9-16 d post soil introduction (Quaintance and Jenne 1912, Armstrong 1958). Thus, when plum curculio were introduced into soil at 10 d post microbial control agent application, microbial agents were more likely to come into contact with the insects in the larval stage. When insects were introduced into soil at 5 d before and the day of the application of the microbial control agents, there was an increased chance that the plum curculio had developed to the pupal stage by the time of contact with the microbial control agents, a life stage that may be less susceptible to such microorganisms. Differences in life stage susceptibility to entomopathogenic fungi and nematodes have been documented in a number of different pest species (Daniel and Wyss 2009, Shanina et al. 2009, Angel-Sahagún et al. 2005), and thus future research should focus on determining any difference in susceptibility of plum curculio larvae and pupae to *B. bassiana* and *S. riobrave*.

Early reports on plum curculio biology noted the importance of soil moisture to the survival and development of the insect from immature to mature stages. Quaintance and Jenne (1912) reported that when introduced to “normally moist” soil in an experiment, 90.0% of plum curculio emerged as adults. Similarly, Chapman (1938) noted, “The increase in infestation in periods of rainfall which has been noted by others is apparently due to the fact that it facilitates emergence.” The results from this experiment indicated that when experiment pots received micro sprinkler irrigation, regardless of timing and pathogen treatments, they exhibited a trend in

which adult emergence was greater compared to unirrigated treatments (Table 2.3). Future studies should investigate this interaction to determine if micro sprinkler irrigation is actually detrimental to plum curculio control as it has the potential to reduce the natural mortality caused by low soil moisture conditions.

The experimental methods used in this study created conditions that may be unrealistic in orchard settings. Plastic pots were used as three-dimensional experimental arenas for efficient evaluations in the field. This allowed little interaction with the rest of the orchard soil and thus may have created isolated soil columns with levels of soil moisture higher or lower than the surrounding orchard. Future studies should incorporate experimental arenas that increase soil interface interactions. Additionally, as larvae were introduced to the experimental arenas at a rate of 10 larvae per 77.0 cm^2 , these levels of infestation likely are much higher than what would occur in orchards with moderate to extreme levels of plum curculio infestation. Unnaturally increased levels of plum curculio density in soil may have caused further infections and reduction in adult emergence due to the cycling of both microbial control agents, through subsequent generations occurs on host cadavers.

Although this research was performed at three different orchard sites with soils consisting of varying levels of sand, silt, and clay, a statistically sound comparison of the effects of soil texture on the efficacy of the microbial control agents was not possible due to the experimental design. Previous field studies in Michigan orchards indicated that soil texture likely impacts the efficacy of microbial control agents against plum curculio larvae, with *S. riobrave* performing better in sandier soils and *B. bassiana* performing better in soils with increased clay concentrations (Pereault et al. 2009). Similar effects of soil texture on entomopathogenic fungi and nematodes have been observed (Fargues et al. 1983, McCoy et al. 2002) and consequently

future research investigating the control of plum curculio with microbial control agents should examine soil texture effects on efficacy.

Thus far, microbial control agents, including entomopathogenic fungi and nematodes, have demonstrated variable success in controlling the plum curculio in field studies. This study indicated that while micro sprinkler irrigation may improve the efficacy of two microbial control agents, *S. riobrave* and *B. bassiana* against plum curculio larvae, a great deal of more information regarding their biology and ecology is required if they are to be successfully and economically employed as control tactics. Before recommendations can be made to growers and the widespread adoption of microbial control agents for plum curculio can occur, a clearer understanding of the impact of increasing soil moisture through micro sprinkler irrigation on plum curculio and microbial control agent biology is necessary.

The loss of reliable, conventional pesticide control tactics will necessitate the continued development of novel and efficacious plum curculio control measures. Data produced from this study should serve to increase the body of knowledge pertaining to the biology of entomopathogenic fungi and nematodes, and plum curculio, with the end goal of developing effective alternative control tactics.

CHAPTER 3

Influence of Soil Texture and Moisture Potential on Plum Curculio (Coleoptera: Curculionidae) Depth and Pupation

3.1. Introduction

The plum curculio, *Conotrachelus nenuphar*, (Herbst) (Coleoptera: Curculionidae) is an economically important pest of pome and stone fruit in central and eastern North America (Maier 1990). Scars caused by adult feeding and oviposition and larval infestation make fruit unfitting for fresh market and are thus devalued via processing. Adult plum curculio emerge from overwintering in the spring, feed, mate, and oviposit in immature fruit (Smith 1957). Larvae then develop through four instars while feeding inside fruit prior to dropping out of the fruit and pupating in soil. Adults emerge from pupation mid- to late summer, cause late-season damage and overwinter in the fall, emerging the following year to continue infestations (Racette et al. 1992).

Tree fruit producers have relied on organophosphate insecticides for over 50 years as the principal management tactic for plum curculio. With the passage of the Food Quality Protection Act (FQPA 1996), the use of azinphos-methyl was terminated in 2012 and an emphasis has been placed on the discovery and approval of reduced risk insecticides. As a result, plum curculio research efforts post-FQPA have focused on optimizing plum curculio monitoring techniques as well as the assessment of novel chemistries for control (Coombs 2001, Leskey and Wright 2004, Wise et al. 2006, Wise et al. 2007). These efforts focused on control of the adult life stage of the pest with insecticide applications are contrary to higher-level integrated pest management

programs in which numerous pests at different life stages should be targeted with a range of tactics (Kogan 1988, Prokopy 1994).

Historically, cultural and mechanical tactics were prescribed to control plum curculio immature life stages including orchard sanitation, livestock integration, and cultivation (Cook 1890, Stedman 1904, Garman and Zappe 1929, Racette et al. 1992). Integration of hog grazing in organic apple orchards one example of recently developed interest in historical control methods, which demonstrated effective reduction in plum curculio damage (Grieshop Personal Communication). Similarly, preliminary studies of strip cultivation for weed management in orchards have shown the potential to disrupt plum curculio in abscised fruit and soil (Grieshop Personal Communication). Advancements in formulation and application technology of microbial control agents, especially entomopathogenic fungi and nematodes, have demonstrated efficacy against soil-dwelling plum curculio larvae and are thus novel control options with the potential for on- farm adoption (Shapiro-Ilan et al. 2002, Alston et al. 2005, Pereault et al. 2009).

The integration of hogs into organic apple production provides control of plum curculio in two manners: first, hogs consume abscised fruit containing larvae, and second, hogs rooting the ground while foraging for food. Younger hogs (under 45.0 kg) introduced into orchards rooted into soil 10.0-15.0 cm in depth, on average, while larger hogs rooted deeper (Buehrer Personal Communication). Cultivation of orchard soils to disrupt plum curculio holds potential as a control tactic as there are a wide variety of powered and unpowered implements including discs, rotary cultivators, spaders, and tillers, with differing abilities to cultivate to different soil depths (Grieshop Personal Communication). While entomopathogenic fungi and nematodes have been shown to control plum curculio, the vertical distribution of both microbial control agents in soil varies, can impact efficacy against pests, and may be influenced by soil texture, moisture,

and orchard management (Storey et al. 1988, Storey et al. 1989, Koppenhöfer et al. 1995, Duncan and McCoy 1996, Gouge et al. 2000).

Alternative plum curculio control tactics focused on targeting immature, soil-dwelling life stages currently rely on historical data and observations for information about the pest's behavior and biology in soil. Quaintance and Jenne (1912) reported that the depth of the pupal cell is 1.0-8.0 cm with a majority of pupae located within 5.0 cm of the soil surface. In the same account, authors reported that in "normally moist" soil, 90.0% of plum curculio adults emerged, compared to 31.0% emergence in treatments in which soil was allowed to dry out, and 0.0% emergence in dry soil. Chapman (1938) described plum curculio pupation to occur at a depth of about 2.5 cm and noted, "The increase in infestation in periods of rainfall which has been noted by others is apparently due to the fact that it facilitates emergence." Armstrong (1958) stated that plum curculio larvae pupate in soil at depths of 1.3 to 5.0 cm and that 0.0% of adults emerged when larvae were introduced into dry soil, compared to 82.0% emergence in wet soil to which water was added 45 d post larval introduction, and 19.0% emergence in wet soil to which water was withheld for 60 d post larval introduction.

In more contemporary literature, precise measurements of soil moisture and texture have provided a thorough understanding of the influence of abiotic factors influencing certain insect biology and behavior in soil. In a laboratory bioassay, it was determined that optimal soil moisture is important for the development of *Diaprepes abbreviates* (Linnaeus) larvae into pupae, with fewer larvae surviving at high (80.0%) and low (20.0-40.0%) soil moisture contents (Lapointe and Shapiro 1999). In a similar laboratory study it was determined that extremely wet (0.0%) or dry (100.0%) soils significantly hindered *C. nasturtii* (Kieffer) emergence, regardless of soil type (Chen and Shelton 2007). Dimou et al. (2003) determined that both soil texture and

soil moisture significantly impacted the pupation depth of *Bactrocera oleae* (Rossi), while MacDonald and Ellis (1990) found that *Diabrotica virgifera virgifera* (LeConte) larvae moved farthest in soils with intermediate levels of moisture compared to wet or dry soils.

While early reports of plum curculio biology and behavior in soil provide basic information, methods were rarely standardized, if described at all, and soil texture and moisture were not accurately measured (Quaintance and Jenne 1912, Chapman 1938). Greater understanding of abiotic factors that influence the behavior of soil dwelling insects has been utilized to improve the management of insects using multiple tactics including cultural measures, host plant resistance, and biological and chemical control agents (Villani and Wright 1990). Thus, the overall goal of my research was to improve the understanding of plum curculio biology in soil. Two abiotic factors, soil texture and soil moisture, were specifically studied to determine their influence on the pupation rate and soil depth of plum curculio. Plum curculio were studied under controlled environmental conditions in laboratory assays utilizing four different soil substrates (three typical of fruit growing regions in Michigan) and three levels of soil moisture potential.

3.2. Materials and Methods

3.2.1. Insect Materials and Rearing

Freshly emerged (<12 h) final instar plum curculio larvae were collected from a colony maintained by the Pesticide Alternatives Laboratory, Michigan State University, East Lansing, MI. Northern strain adult plum curculio and larvae from infested apples (*Malus domestica*) and tart cherries (*Prunus cerasus*) were used to reinitiate the colony on a yearly basis. Infested fruit and adults were collected from orchards in Benzie, Ionia, Leelanau, and Manistee Counties,

Michigan. Adults were trapped using black corrugated plastic “Whalon-modified Tedders Traps” (1.6 by 0.8 m) with a 0.5 cm wide strip of high-contrast reflective tape along the edges of the traps (Great Lakes IPM, Vestaburg, MI), and circle traps (Mulder et al. 1997). Circle traps and pyramid traps were baited with two attractive plant volatile lures, plum essence and stabilized benzaldehyde (Coombs 2001, Leskey and Wright 2004, Leskey et al. 2005).

Plum curculio larvae were reared from thinned apples held in cages with adults for oviposition. Thinned apples were harvested from Washington State Tree Fruit Research & Extension Center (Wenatchee, Washington) in June 2012, treated with two fungicides (Captan 80 WDG and Benlate 50W) and a plant growth regulator (Diphenylamine), and stored at 5.0° C. Apples made available for oviposition were rinsed to remove any fungicide. To break the obligate diapause period of the Northern strain plum curculio and induce oviposition, adults were provided thinned apples treated with pyriproxyfen (Esteem[®] 35 WP, 750.0 mg/ L water) according Hoffmann et al. (2007). After exposure to adult plum curculio for 5 d, apples were transferred to metal mesh racks over aluminum trays lined with moistened paper towel and held in the laboratory. Trays were checked daily for larval emergence and newly emerged larvae were used within 12 h.

3.2.2. Soils

Soil substrates were collected from an apple orchard in Flushing, Genesee County, Michigan (43.027788° N, -89.913381° W) and a cherry orchard in Honor, Benzie County, Michigan (44.726217° N, -86.036725° W). Soils were collected from the top 15.0 cm located beneath the drip lines of orchard trees and returned to the Pesticide Alternatives Laboratory at Michigan State University for processing. In the laboratory, soils were air-dried and large pieces

of vegetation and debris were removed by hand, followed by passing through a number 18 sieve to remove large particles. To eradicate antagonists, soils were sterilized via autoclaving and held for 14 d to allow any toxic byproduct components produced from the autoclaved soil to dissipate (Kaya and Koppenhofer, 1996, Kaya and Stock 1997). Soils were then baked to remove any extra soil moisture. In addition to field-collected soils, play sand (Quikrete International Inc., Atlanta, GA) also was used and processed with the same methods used for the field-collected soils.

Soils and sand were submitted the Michigan State University Soil and Plant Nutrient Lab for analysis of texture, percent organic matter, and pH (Bouyoucos 1951, Watson and Brown 1998). The apple orchard soil (Soil #4, collected from Flushing, MI) was categorized as clay loam, while the cherry orchard soil (Soil #2, collected from Honor, MI) was categorized as sand, and a 50-50 mixture of the two soils (Soil #3) was categorized as sandy loam. The contractor's sand (Soil #1) was categorized as sand. Soil matric potential, a more biologically meaningful measure of soil moisture (Kaya and Stock 1997), was determined according to methods by Hamblin (1981). Water was added to produce moisture potentials of -10^1 , -10^2 , and -10^5 kPa.

3.2.3. Experimental Design

Experimental arenas were constructed with 50 ml polystyrene centrifuge tubes (11.3 cm tall by 3.0 cm diameter) (Denville Scientific Inc., Mutechen, NJ). The soils were adjusted to three standardized soil moisture potentials (-10^1 , -10^2 , and -10^5 kPa) by incorporating sterile, de-ionized (SDI) H₂O. Tubes were filled with soil to a depth of 10.2 cm, tapped vigorously to compact the soil, and filled again to a depth of 10.2 cm. A single plum curculio larva was

immediately added to the substrate-filled centrifuge tubes. If a plum curculio larva had not entered the soil after 1 h it was replaced with another newly emerged final instar. Centrifuge tubes were held on Styrofoam racks (Denville Scientific, Inc., Mutechen, NJ) and were incubated at 25.0° C in complete darkness for 10 d. After 10 days the vials were carefully excavated and the life stage of the plum curculio (larvae or pupae) and the depth of the deepest part of the plum curculio were recorded. Life stage data were assigned a binary value, either 1=pupae or 0=larvae. There were 4 tubes per treatment and the entire study was repeated on four dates. Arena tubes were arranged in a randomized complete block design and were blocked by date.

3.2.4. Statistical Analysis

All data was analyzed using R Studio Version 0.97.449 (R Core Team 2013) with $\alpha=0.05$. Plum curculio depth data was box cox transformed ($\lambda=0.4592547$) to meet the assumptions of normality (Box and Cox 1964). Analysis of depth data was performed using a linear mixed effects model, with the full model including block as a random variable, and soil texture and soil matric potential and their interactions as fixed effects. Contrasts between treatment means were developed within soil and matric potential treatments, and were utilized in mean separations conducted with Fisher's Least Significant Difference Test. Analysis of life stage data was performed with a generalized linear model, with the full model including soil texture, soil matric potential, and their interactions as fixed effects. Means separations were conducted with Tukey's Honest Significant Difference.

3.3. Results

3.3.1. Plum Curculio Depth

The mean depth of all experimental treatments pooled was 31.5 mm. The shallowest depth at which a plum curculio was retrieved was 0 mm (insects were found on the surface of soil) and the deepest depth was 99.0 mm. Analysis of variance of the plum curculio depth data showed significant effects of soil texture, soil matric potential, and the soil texture by soil matric potential interaction ($F_{3, 177}=4.7079$, $P<0.05$; $F_{2, 177}=123.3974$, $P<0.0001$; $F_{6, 177}=22.5159$, $P<0.0001$ respectively). Significant mean separations occurred between texture treatments and soil matric potential treatments (Table 3.2). In the low treatment, at a soil matric potential of -10^5 kPa, plum curculio depth was significantly greater in clay loam soils (26.4 ± 3.5 mm) compared to sand (10.6 ± 3.5 mm) and field sand (0.5 ± 0.4 mm) treatments. In the high level of moisture treatment, at -10^1 kPa, mean plum curculio depth was significantly greater in sand (60.3 ± 6.1 mm) than sandy loam (30.4 ± 4.5 mm) and clay loam (33.0 ± 4.4 mm) soils. In treatments containing field sand, plum curculio depth was significantly greater in treatments with a high moisture level at -10^1 kPa (18.9 ± 2.6 mm), versus a low moisture level - 10^5 kPa (0.5 ± 0.4 mm). In treatments containing clay loam, no significant difference was detected across moisture levels from -10^1 kPa (18.9 ± 2.6 mm) to -10^5 kPa (26.4 ± 3.5 mm).

3.3.2. Plum Curculio Life Stage

Soil texture and matric potential had a significant effect on the number of plum curculio found in the pupal life stage, (χ^2 (3, 188)=13.85, $P<0.001$, χ^2 (2, 186)=104.965, $P<0.0001$, respectively). Significant mean separations occurred between texture treatments and soil matric potential treatments (Table 3.2). Across both sand and clay loam treatments, the percent of plum

curculio in the pupal stage was significantly greater in higher moisture treatments at -10^1 kPa (sand: 93.8 ± 6.3 ; clay loam 68.8 ± 12.0) than lower moisture treatments at -10^5 kPa (sand: 0.0 ± 0.0 ; clay loam: 0.0 ± 0.0). Across treatments with higher moisture levels, the percent of pupal stage plum curculio was higher in sand (-10^1 kPa sand: 93.8 ± 6.3) than in clay loam (-10^1 kPa clay loam: 68.8 ± 12.0). In low moisture treatments, at -10^5 kPa, no plum curculio pupae were found (sand: 0.0 ± 0.0 ; field sand: 0.0 ± 0.0 ; sandy loam: 0.0 ± 0.0 ; clay loam: 0.0 ± 0.0).

Table 3.1. Soil Type, pH, Percent Soil Organic Matter, and Percent Soil Composition.

Soil#	Soil Type	Soil pH	Organic Matter (%)	Soil Composition (%)		
				Sand	Silt	Clay
1	Sand	8.9	0.1	97.4	0.5	2.1
2	Field Sand	6.8	1.4	90.6	5.0	4.4
3	Sandy Loam	6.6	2.5	70.6	22.0	7.4
4	Clay Loam	6.0	3.3	31.3	40.0	28.7

Table 3.2. Plum curculio mean depth and mean percent in pupal life stage. Mean depth values followed by upper case letters indicates mean separation by soil texture, values followed by lower case letters indicates mean separation by soil matric potential (Fisher's LSD, $\alpha=0.05$). Mean percent of PC in pupal life stage followed by upper case letters indicates mean separation by soil texture, values followed by lower case letters indicates mean separation by soil matric potential (Tukey's HSD, $\alpha=0.05$).

Soil Matric Potential (kPa)	Soil Texture	Mean Depth (mm) (\pm SEM)	Mean Percent of PC in Pupal Life Stage (\pm SEM)
-10^5	Sand	10.6 (3.5)Ab	0.0 (0.0)Ba
-10^2	Sand	60.3 (6.1)Aa	93.8 (6.3)Ba
-10^1	Sand	60.9 (4.3)BCa	93.8 (6.3)Aa
-10^5	Field Sand	0.5 (0.4)Ab	0.0 (0.0)Bb
-10^2	Field Sand	51.8 (5.8)Aba	56.3 (12.8)Bb
-10^1	Field Sand	59.8 (6.2)Ca	62.5 (12.5)Ab

Table 3.2. (cont'd)

Soil Matric Potential (kPa)	Soil Texture	Mean Depth (mm) (\pm SEM)	Mean Percent of PC in Pupal Life Stage (\pm SEM)
-10^5	Sandy Loam	14.9 (3.2)Ab	0.0 (0.0)Bb
-10^2	Sandy Loam	30.4 (4.5)Ba	31.3 (12.0)Bb
-10^1	Sandy Loam	40.6 (5.1)ABa	56.3 (12.8)Ab
-10^5	Clay Loam	26.4 (3.5)Ba	0.0 (0.0)Bb
-10^2	Clay Loam	33.0 (4.4)Ba	25.0 (11.2)Bb
-10^1	Clay Loam	18.9 (2.6)Aa	68.8 (12.0)Ab

3.4. Discussion

Plum curculio pupate in soils at depths ranging from 1-8 cm (Quaintance and Jenne 1912, Chapman 1938, Armstrong 1958), though these studies provided little information regarding soil texture and moisture. Additionally, historical studies and observations of plum curculio biology indicated that adequate levels of soil moisture coincided with increased rates of adult emergence from soil (Chandler 1932, Armstrong 1958). The results of this study indicate that both soil texture and soil moisture significantly affect the depth of plum curculio in soils, as well as the percent of plum curculio initiating pupation (Table 3.2).

At the lowest soil moisture level, -10^5 kPa, plum curculio were found at significantly shallower depths in sandy soils (sand and field sand, Table 3.1), than in clay loam soils (Table 3.2). During the experiment, small aggregates (2.0-3.0 mm in diameter) were observed to form while preparing the clay loam soil -10^5 kPa treatment, resulting in larger pore space or “cracks” in the soil that likely allowed for increased plum curculio movement, similar to phenomena observed in studies with clover root weevil, *Sitona lepidus* (Pacchioli and Hower 2004) and the olive fruit fly, *Bactrocera oleae* (Dimou et al. 2003) in fine, moist soils. Dry soils containing

increased levels of sand are typically more abrasive to insect cuticles compared to moist soils (Collis-George 1959), and thus may have prevented plum curculio larvae from penetrating into the soils, similar to observations of abrasive soils having detrimental effects on the western corn rootworm, *Diabrotica virgifera virgifera* in laboratory studies (MacDonald and Ellis 1990).

When soils of higher sand concentrations were prepared at greater moisture levels (10^1 kPa, 10^2 kPa), mean plum curculio depth was significantly greater compared to the corresponding clay loam soil (Table 3.2). At higher levels of moisture, the shear strength of soil is reduced (Collis-George 1959), thus requiring less effort on behalf of the plum curculio to penetrate and move through the sandier soils. However, as soil moisture levels increase, the amount of pore space available for insect movement within soils decreases (Villani and Wright 1990), likely impeding movement within the clay loam soil with its smaller particle and pore sizes than those of sandier soils. Similarly, in soils composed primarily of clay particles, burrowing depth of the walnut husk fly, *Rhagoletis completa*, was reduced because of small pore space (Boyce 1934). Alternatively, much larger aggregates were formed in clay loam sands (5.0-10.0 mm in diameter), resulting in more homogenous soil columns with less cracks or pore spaces that might obstruct larval movement. In higher moisture level treatments of sandier soils, the size of cracks and volume of space between aggregates increased, allowing for improved movement of plum curculio larvae through the soil column, again similar to observations of clover root weevil (Paccholi and Hower 2004) and the olive fruit fly (Dimou et al. 2001).

Across all soils at the lowest moisture level, -10^5 kPa, no plum curculio were found in the pupal life stage at the end of the experiment, 10 d post larval introduction (Table 3.2). Across all soils at high soil moisture levels (-10^1 kPa, -10^2 kPa), the percent of plum curculio in the pupal

life stage was significantly improved, ranging from 25.0-93.0% (Table 3.2). Similarly, pupation of the oriental fruit fly, *Bactrocera dorsalis*, the swede midge, *Contarinia nasturtii*, and the Southern corn rootworm, *Diabrotica undecimpunctata howardi*, was found to be most influenced significantly by soil moisture, with minimal pupation occurring at low soil moisture levels and increased pupation occurring at higher soil moisture levels (Brust and House 1990, Hou et al. 2006, Chen and Shelton 2007). Interestingly, at higher levels of soil moisture (-10^1 kPa, -10^2 kPa), the percent of plum curculio in the pupal lifestage was significantly higher in sand treatments compared to other soils (Table 3.2). Minimal evidence and discussion of the effect of soil texture on the pupation of insects is present in the literature and thus warrants further investigation.

Previous reports of plum curculio phenology in soil are inconsistent, with pupation commencing as soon as 5 d post introduction to soil (Armstrong 1958) or 9-16 d (Quaintance and Jenne 1912) post soil introduction. When plum curculio were excavated from experimental arenas in this study at 10 d post soil-introduction, the percentage of pupae found ranged from 0.0% in soils with low soil matric potentials to 93.8% in soils with matric potentials improved for pupation, indicating that soil moisture influences pupation rates. Future studies should focus on developing a phenology model of plum curculio life stages in soils with differing levels of moisture, based on degree-day accumulation. Such data, as part of a complete plum curculio phenology model, would provide information beneficial to predicting plum curculio life stage within soil which may be important to ascertain differing levels of susceptibility to microbial control agents such as entomopathogenic fungi and nematodes (Daniel and Wyss 2009, Shanina et al. 2009, Angel-Sahagún et al. 2005).

With the results of this study indicating that increased levels of soil moisture are necessary for plum curculio pupation, this relationship may help to explain the biogeography of the pest in North America. As reported by Chapman (1938), the plum curculio is distributed east of the Rocky Mountains, with a northern boundary located at approximately 50° north latitude and southern boundary of 28° north latitude. Soils near the Rocky Mountains in the Western border of the plum curculio distribution are generally more arid, while soils south of 28° north latitude are generally arid as well. One exception to this distribution, reported by Alston et al. (2005), is in Box Elder County, UT, where plum curculio were found in backyard and hobbyist orchards which may have had soils with increased levels of moisture due to irrigation.

The conclusions of this study are limited in their application to the improvement of plum curculio biological and behavioral models primarily because the results in a laboratory setting as opposed to a field study. A number of factors were unaccounted for study and should be addressed in future studies to improve understanding of the immature plum curculio life stages within soil. Soils utilized in this study were homogenized prior to experimental use, and not representative of aggregates and particles that naturally form in field settings and may influence larval insect behavior and movement through soil (Villani and Wright 1990). All vegetation was removed from the soils after their collection, resulting in an artificial soil column that lacked any ground cover and the accompanying root structure that is often characteristic of orchard understories. Akotsen-Mensah et al. (2012) determined that orchard weed management can influence plum curculio adult emergence from soil, thus future studies should attempt to incorporate naturally occurring plant material into experimental arenas to ascertain their effect, if any, on plum curculio depth and pupation.

The results of this experiment should help to improve the understanding of plum curculio biology and behavior in soil during immature life stages. Current pest management tactics are based largely on targeting adult plum curculio with insecticidal sprays, but changes in legislation have reduced the number of effective insecticide options, requiring growers to pursue alternative management techniques (FQPA 1996). Higher-level integrated pest management programs optimally rely on multiple management tactics, including cultural techniques, to control pests (Kogan 1988, Prokopy 1994). Based on the results of this research, growers and pest managers should reduce irrigation inputs in orchards during and after final instar plum curculio drop out from fruit to reduce movement and pupation within soil to increase natural mortality. By sequestering the immature stages of the pest on or near the soil surface, the mortality caused by natural enemies such as ants, and augmentative applications of microbial control agents, such as entomopathogenic fungi and nematodes, likely would be greatly improved (Shapiro-Ilan et al. 2002, Alston et al. 2005, Jenkins et al. 2006b, Pereault et al. 2009).

With this study's information on immature plum curculio biology and behavior below ground, it is now feasible to design improved management tactics that can target these stages in the pest's life. However, it is unlikely that such novel pest management tactics alone will contribute to control of the pest at levels below economic significance and thus should be considered integral parts of a higher-level pest management program developed in response to changes in pesticide legislation.

CHAPTER 4

Conclusions and Future Research

The plum curculio remains a primary pest of tree fruit production in North America with a host range limited to the flowering plant family Rosaceae, including economically important native and exotic host species (Maier 1990, Antonelli et al. 1992, Jenkins et al. 2006a). Feeding and oviposition scarring by adult plum curculio and infestation by larvae are the two forms of damage caused by the insect, making it an economically important pest of pome and stone fruit. Over the last 50 years, organophosphate insecticides have been the principal control tactic for plum curculio in tree fruit production. The Food Quality Protection Act (1996) ushered in a new era of pest management by terminating the use of most organophosphate insecticides in tree fruit production and placing an emphasis on the discovery and optimization of reduced risk insecticides and alternative control tactics.

Alternative control tactics, including the application of microbial control agents to target soil-dwelling plum curculio larvae, have been variable in efficacy against plum curculio (Shapiro-Ilan et al. 2004, Shapiro-Ilan et al. 2008, Pereault et al. 2009). Additionally, interest in cultural and mechanical tactics historically prescribed for plum curculio control, including orchard sanitation, livestock integration, and cultivation has recently developed (Cook 1890, Stedman 1904, Garman and Zappe 1929, Racette et al. 1992). To develop such novel and historical control tactics into reliable and efficacious components of tree fruit integrated pest management programs, an improved understanding of the biology and ecology of plum curculio, and the microbial control agents, *Steinernema riobrave* and *Beauveria bassiana*, is necessary. Thus, the goal of this thesis research was to improve the understanding of the effects of soil

moisture on the efficacy of two microbial control agents targeting plum curculio larvae in soil. Additionally, the effects of two abiotic factors, soil texture, and soil moisture, were specifically studied to determine their influence on the pupation rate and depth of plum curculio in soil. The conclusions of the experiments performed and presented in this thesis were consistent with these goals.

In field trials, micro sprinkler irrigation did improve the efficacy of *S. riobrave* and *B. bassiana* against plum curculio, significantly reducing the mean number of adult plum curculio emerging from 10 d irrigated treatments when compared to the corresponding control (Chapter 2, Table 2.3). Previous research in Michigan orchards indicated that soil moisture was likely a factor limiting the efficacy of several microbial control agents targeting plum curculio in soil (Pereault et al. 2009), and thus micro sprinkler irrigation was employed to increase soil moisture, as it has demonstrated lengthened periods of larval suppression of *Diaprepes abbreviatus* by entomopathogenic nematodes in citrus production (McCoy et al. 2000, McCoy et al. 2002). However, a trend was noted in which adult plum curculio emergence was greater in irrigated versus unirrigated treatments, regardless of microbial control agent treatments (Chapter 2, Table 2.3). Early reports on plum curculio biology noted the importance of soil moisture to the survival and development of the insect from immature to mature stages, with increased levels of soil moisture resulting in greater adult plum curculio emergence from soil (Quaintance and Jenne 1912, Chapman 1938). Laboratory studies were then developed to assess the impact of soil moisture on immature plum curculio biology and behavior.

In laboratory studies, at low moisture levels, -10^5 kPa, no plum curculio were found in the pupal life stage at the end of the microcosm experiments, 10 d post larval introduction into experimental assay arenas (Chapter 3, Table 3.2). Across soil treatments with high soil moisture

levels (-10^1 kPa, -10^2 kPa), the percent of plum curculio in the pupal life stage was significantly improved, ranging from 25.0-93.0% pupation (Table 3.2). Similar patterns of soil moisture influencing development have been observed in other insect species, including the oriental fruit fly, *Bactrocera dorsalis*, the swede midge, *Contarinia nasturtii*, and the Southern corn rootworm, *Diabrotica undecimpunctata howardi* (Brut and House 1990, Hou et al. 2006, Chen and Shelton 2007).

Both soil moisture and soil texture had a significant effect on the depth at which plum curculio were found in laboratory experiments. At the lowest level of moisture, -10^5 kPa, plum curculio were found at significantly shallower depths in soil treatments with higher concentrations of sand (Chapter 3, Table 3.1), as opposed to the clay loam treatment that had a greater depth (Chapter 3, Table 3.2). However, when soils of higher sand concentrations were prepared at greater moisture levels (-10^1 kPa, -10^2 kPa), mean plum curculio depth was significantly greater compared to the corresponding clay loam treatment (Chapter 3, Table 3.2). This variation in plum curculio depth likely is caused by aggregates formation and the development of larger pore space or “cracks” in soil, allowing for increased movement. Similar phenomena have been observed in studies with clover root weevil, *Sitona lepidus* (Pacchioli and Hower 2004) and the olive fruit fly, *Bactrocera oleae* (Dimou et al. 2001).

As the results of both chapters indicate that soil moisture has an impact on plum curculio development in soil, this relationship may help to explain the biogeography of the pest in North America. As reported by Chapman (1938), the plum curculio is distributed east of the Rocky Mountains, with a northern boundary located at approximately 50° north latitude and southern boundary of 28° north latitude. The relationship between soil moisture levels and plum curculio

development may serve as a factor to explain the biogeography of the arthropod in North America, as soils near the Rocky Mountains in the western boarder of the plum curculio distribution are generally more arid, while soils south of 28° north latitude are generally arid as well.

Literature reporting immature plum curculio phenology below ground is inconsistent, with pupation occuring as quickly as 5 d post introduction to soil (Armstrong 1958) or 9-16 d post soil introduction (Quaintenance and Jenne 1912). At 10 d post introduction to soil in arenass, the percent of pupae found ranged from 0.0% in soils with low soil matric potentials to 93.8% in soils with matric potentials, indicating that soil moisture may have an influence on pupation rates. Future research should focus on developing a phenology model of plum curculio pupation in soils with differing levels of moisture, based on degree-day accumulation. Such data, as part of a total phenology model, would provide information that would predict plum curculio life stage within soil, which may be essential as arthropods in different life stages may have differing levels of susceptibility to microbial control agents (Daniel and Wyss 2009, Shanina et al. 2009, Angel-Sahagún et al. 2005).

Observations and trends generated from this thesis should help to improve the understanding of plum curculio biology and behavior in soil during immature life stages and the impact of micro sprinkler irrigation on microbial control agent efficacy against plum curculio. Current pest management tactics are based largely on targeting adult plum curculio with insecticidal sprays, but changes in legislation have reduced the number of effective insecticide options, requiring growers to pursue management techniques alternative to the conventional standards (FQPA 1996). Higher-level tree fruit integrated pest management programs optimally

rely on multiple management tactics to control pests (Kogan 1988, Prokopy 1994), including cultural techniques.

Thus far, microbial control agents, including entomopathogenic fungi and nematodes, have demonstrated variable success in controlling plum curculio in field studies. Results indicate that while micro sprinkler irrigation may improve the efficacy of two microbial control agents, *S. riobrave* and *B. bassiana*, against plum curculio larvae, a great deal of more information regarding their biology and ecology is required if these agents are to be successfully and economically used as control tactics. Based on the laboratory research results, growers and pest managers should reduce irrigation inputs in orchards during and after final instar plum curculio drop out to reduce plum curculio movement and pupation within soil, thus increasing natural mortality. Before any final recommendations can be made to growers regarding the application of microbial control agents, a clearer understanding of the impact of increasing soil moisture through micro sprinkler irrigation on plum curculio and microbial control agent biology is necessary to determine whether or not such a pest management strategy is logical and justifiable.

The loss of reliable, conventional pesticide control tactics will continue to necessitate the development of novel and efficacious plum curculio control measures. The information this thesis has generated regarding immature plum curculio biology and behavior below ground, and the impact of soil moisture on microbial control agent efficacy, increases that body of knowledge. Such knowledge should help facilitate in the design of experiments to elucidate further crucial information and develop improved, ecologically sound plum curculio management tactics. However, it is unlikely that such novel pest management tactics alone will contribute to control of the pest at levels below economic significance and thus should be

considered integral parts of higher-level pest management programs developed in response to changes in pesticide legislation.

APPENDIX

Record of Deposition of Voucher Specimens

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2013-11

Author and Title of thesis:

Peter N. Nelson

Microclimate manipulation to improve the efficacy of entomopathogens targeting plum curculio larvae in Michigan orchards

Museum(s) where deposited:

Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Table A.1. List of voucher specimens.

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Curculionidae	<i>Conotrachelus nenuphar</i>	adult	30	pinned
Curculionidae	<i>Conotrachelus nenuphar</i>	larvae	30	75% ETOH
Rhabditidae	<i>Steinernema riobrave</i>	juvenile	18	images on CD
Rhabditidae	<i>Steinernema riobrave</i>	adult	3	images on CD

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