CONTROL OF PEAR PSYLLA IN PEARS AND BLACK STEM BORER IN APPLES WITH TRUNK INJECTION

By

Celeste Elizabeth Wheeler

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ABSTRACT

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The use of pesticides to produce fruit crops is essential to the economic success of farmers. Consumers have a high standard for acceptable fruit, and pesticides are key to the farmer's ability to produce marketable fruit.

Trunk injection should be considered as an alternative delivery method for pesticides. It delivers the pesticide directly into the tree's system without losing pesticide to drift and is contained within the tree. Trunk injection has been effectively used to control foliar pests in apple trees and wood boring pests such as the emerald ash borer. One injection can provide multiple seasons of control. In the current two studies, we use trunk injection to apply insecticides and evaluate their efficacy at controlling two very different orchard pests, the pear psylla in pear and the black stem borer in apple.

The objective of the first study is to compare the efficacy of abamectin and azadirachtin in the control of pear psylla in pear using two different application methods, airblast and trunk injection.

The objective of the second study is to evaluate the efficacy of two insecticides, emamectin benzoate and azadirachtin, and injection timing fall and spring, on their ability to control BSB in apple trees with simulated topworking and ethanol injection as an attractant.

Dedicated to Richard Randall Wheeler who first encouraged me to pursue my Master's degree.

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

History of Trunk Injection

The written history of plant injection begins with Ibn-al-Awam in 1158. He quoted from Hadj de Granade's methods to give perfumes, flavors, and medicinal qualities to fruits, and different colors to roses. A second ancient record of plant injection was in the 1400s where Leonardo da Vinci recorded methods to make fruit poisonous by injecting them with arsenic. He is the first to write about injecting into trunks of trees. His methods included boring a hole in the trunk of the tree, injecting the liquid with a syringe while the sap is rising, and plugging the hole tightly (Roach 1938,1939).

Another two early attempts, published anonymously, are as follows. The magazine Orchard and Garden published methods in 1602 on how to make sour fruits sweet and how to kill wood boring insects. JM Wilson's Rural Cyclopaedia in 1765 published methods on destroying insects on trees by injecting mercury. Both of these methods utilize injected materials that are insoluble, which raises questions as to their actual effectiveness (Roach 1938,1939).

More significant contributions to the body of knowledge stemmed from studies on the ascent of sap, transpiration stream, and root pressure. Various publications noted observations on the movement of dyes and the rate of movement of inorganic salts and metals and are summarized by Roach (1939). Roach mentions that, Magnol observed that dyes absorbed by cut stems move up the stem into the flowers and leaves, as well as notes from Meyer that submerging the stump of a cut tree into dye, the dye penetrates the roots.

Roach (1939), also notes McNab's work which showed that metals move through cut branches at different rates, and Pfitzer's observations that water travels more quickly than the dissolved lithium salt. Roach mentions studies by Sachs where he used dyes and inorganic salts

and noted that lithium salt travelled up the stem nearly as rapidly as the water. He also noted that substances that dyed the cell walls moved more slowly up the stems. Goppelsroeder also injected dyes and developed methods for selecting the most suitable dyes for injection experiments (Roach 1939).

Stoddard and Dimond (1949) have also summarized important contributions to trunk injection. For example they note that Shevyrev and Roth explored opposing views on air exposure while trunk injecting. Shevyrev thought the access of air to exposed tissues must be prevented, while Roth left the injection hole open for 36 hours before injecting and found this caused rapid absorption of the injection liquid (Stoddard and Dimond 1949).

From these experiments that explored the movement of injected materials in the plants, the next round of experiments of significance focused on the actual treatment of problems such as diseases, insects, plant nutrition, and many focused on the treatment of chlorosis in trees (Roach 1938,1939).

Roach notes several experiments where trees were injected with ferrous sulphate to treat chlorosis. He notes that Sachs observed that only the leaves on branches vertically above the injection port became green and healthy. Many others found the distribution of the treatment to be irregular, many of the branches not receiving treatment. Morkrezecki experimented with different concentrations of ferrous sulphate. He would allow the tree to uptake the solutions until no more was absorbed by the tree and found that after 4 days the treatment started having positive effects on the leaves, after 10 days the foliage was healthy, and after 3 weeks the foliage continued to be healthy (Roach 1938,1939).

Morkrezecki also used powdered iron pyrophosphate to treat the chlorotic trees. This and other inorganic nutrient solutions appeared to control scale insects on pears and apples. He

continued with similar methods to control bark beetles but was unsuccessful. He was the first to use a salicylic acid solution to control gummosis in apple, pear, and other trees (Stoddard and Dimond 1949).

In the early 1900's there was more research in the study of mineral nutrition in fruit trees, tree physiology and nutrition. Roach describes methods for plant injection including injection through leaves, shoots, branches, roots, and whole tree injection. These methods include detailed descriptions and diagrams of tools used for different types of injection. Roach also records his methods for diagnosing and controlling nutrient deficiency of fruit trees (Roach 1934).

In the late 1900's and early 2000's, there were various invasive insects that renewed interest in trunk injection (Berger and Laurent 2019).

Injection for control of invasive forest pests and pathogens

Emerald Ash Borer

Native to Asia, the emerald ash borer has killed millions of ash trees since it arrived in the US in the early 2000's (McCullough et al 2018). White and green ash trees in urban that are infested by the borer can be tricky to control. There are often laws and public resistance to the conventional sprays of pesticides to control the pest and save the tree, and in these instances, it makes sense to consider trunk injection as a treatment alternative (Grimalt et al 2011, Flower et al 2015).

Research has shown that trunk injection of systemic insecticides is successful in controlling the emerald ash borer and can have a lasting effect even 3 years post application (McCullough et al 2018). There has also been research into movement of compounds and the distribution throughout the trees (Mota-Sanchez et al 2009).

Asian Longhorn Beetle

Another invasive beetle to the United States is the Asian longhorned beetle. It was first discovered in the US in 1996 on its preferred host the maple (Ugine et al 2013). Trunk injected treatments of imidacloprid were researched and used extensively to control the beetle.

Hemlock woolly adelgid

Hemlock woolly adelgid, which attacks hemlock trees, is invasive to the United States. It feeds on fluids from the tree at the base of the needles. Imidacloprid is an effective tool to control sucking insects like the hemlock woolly adelgid, and the systemic activity is good as a trunk injected treatment as well (Eisenback et al 2014).

Dutch elm disease

Dutch elm disease is spread by a bark beetle that carries the vector for a fungus that causes a vascular wilt and is one of the most devastating diseases causing the loss of millions of elms in Europe and North America (Karnosky 1979). The spread of this disease inspired a surge of research in trunk injection a means of control (Perry et al 1991). Since the first incidence of Dutch Elm Disease in north America, it has wreaked environmental havoc. The Elm tree was once a dominant forest tree and commonly used in urban settings, but this is no longer the case (Karnosky 1979).

Injection research centered around injection techniques and fungicide injection challenges (Perry et al 1991, Haugen and Stennes 1999). Fungicides were used as a preventative method of control, and used post infection (Elliston and Walton 1979, Sherald and Gregory 1980, Lanier 1988). Macroinjection methods were refined to inject high volumes into large trees (Kondo 1978, Stennes and French 1987).

Trunk Injection as alternative to spraying in fruit crops

Most recently there has been an interest in using trunk injection in orchards as an alternative to foliar spraying of the canopy. Both insecticides and fungicides have been researched for effectiveness in orchards. In apples, the efficacy of fungicides and insecticides were tested (Acimovic et al. 2014, VanWoerkom et al. 2014).

Apple crops face a variety of insect pests, some of which are direct pests feeding on and burrowing into the fruit, while others are indirect pests that feed on the leaves and sap. Apple scab and fire blight require fungicides and antibiotic applications as well. As a result, a huge number of applications of fungicides and insecticides are required to produce a crop of apples. If injections could be used instead, it could reduce the negative effects of traditional sprays such as non-target exposure through drift and runoff and reduce the total amount of insecticide and fungicide used (Acimovic et al 2014, VanWoerkom et al 2014).

A broad spectrum of insecticides were injected and evaluated for efficacy against both direct and indirect pests, and were found most effective in controlling indirect pests, often with some degree of control into the second season after the injection (VanWoerkom et al 2014).

Imidacloprid, a widely used compound in insect pest control, was injected to discover its distribution in apple trees. Different numbers of injection ports were injected in an attempt to get a more uniform distribution in the crown of the tree and to more fully understand how the compound is transported in apple trees (Acimovic et al 2014). The use of four injection ports was enough to provide uniform distribution of the chemical in the crown of the trees. The diffuse porous xylem of apple trees moves injected imidacloprid in a counterclockwise spiral ascent (Acimovic et al 2014).

Apple scab is a fungus that infects apple trees in the spring and if unchecked can completely devastate a crop of apples. Fungicides injected to control apple scab were found to cause phytotoxicity with some control found in the second year with phosphites (VanWoerkom et al 2014). Through further investigation, differently timed injections resulted in better control. Repeated injections of phosphites were successful in scab control, and spring injected trees were protected from scab a second year. Phosphites provided the best scab control due to their ability to easily translocate and accumulate in the canopy (Acimovic et al 2016b).

Fire blight, a bacterial pathogen, can also have devastating effects on apple trees and has developed resistance to some antibiotics used in its control. Injecting antibiotics could reduce off target exposure to antibiotics to the environment. Early trunk injection to control fire blight showed positive results of control, however not all injected compounds successfully translocated throughout the tree (Acimovic et al 2015).

In grape vines, vine injection was used to control powdery mildew. The vines were injected with commonly used fungicides and were capable of reducing the intensity of the powdery mildew (Duker and Kubiak 2009).

Trunk injection has been used in avocado to control avocado thrips (Byrne et. al. 2014). Imidacloprid and dinotefuran both showed promise in their ability to control thrips in avocado, however concerns related to preharvest intervals and fruit residue are still to be investigated.

Also in avocado, emamectin benzoate was trunk injected to control shot hole borer, an invasive ambrosia beetle. In their experiments, emamectin benzoate was successfully injected and distributed throughout the tree, and effectively controls early shot borer infestations. This early control may reduce the need to treat an entire grove (Byrne et al. 2020).

In olive, peach, plum, orange, and pear trees, trunk injection was used to correct nutrient deficiencies. Iron chlorosis causes these trees to become unproductive. Ferrous sulfate was effective in alleviating chlorosis in both olive and peach. Like many other trunk injection experiments, these injections were effective in a second season as well (Fernández-Escobar et. al. 1993). Injections of ferrous sulfate were effective at correcting chlorosis in plum trees for two years (Yoshikawa and Stromberg 1982). Orange trees with chlorosis were improved temporarily by the injection of iron-salt solutions into the trees (Thomas and Haas 1928).

In pear trees, injections were compared to foliar applications. Anjou and Bartlett pear trees were either injected or sprayed with ferrous sulfate to correct chlorosis. Fall and spring injections were best at improving tree performance and increasing the level of iron and green color in the leaves and fruit. Injections lasted 4 years, while sprayed treatments only lasted for one season (Raese and Parish 1984).

Harries (1965), through bioassays, found that pear psylla were reduced on leaves from trunk injected trees. Two compounds, Bidrin® (3-hydroxy-Ar ,iV-dimethyl-c£.s-croton-arnide dimethyl phos- phate) and Bayer 47043 (O,O-dimethyl-S- (N,N'-dimethyl- malonicamide) thiophosphate) were injected into pear trees and psylla were exposed to the leaf disks (Harries 1965). In Iran injected pear trees with a mixture of azadirachtin and fertilizer lowered psylla eggs and nymphs (Sheikhigarjan et. al. 2016). Marcic et. al. (2015) demonstrated that sprays of azadirachtin and abamectin and caused high rates of mortality and reduction or termination of egg laying in both treatments. Burts (1985) demonstrated abamectin sprayed treatments reduced psylla populations just as well as the standard treatments. Arnaudov and Kutinkova (2009) showed that two applications of abamectin were sufficient for reducing psylla eggs and nymphs.

Trunk Injection for Systemic Acquired Resistance, SAR

Systemic acquired resistance (SAR), is a way for a plant to more quickly generate a defense response to a pathogen attack. These compounds can provide control for long periods of time and are effective against a broad range of pathogens including bacteria, viruses, and fungi. Injection of compounds that promote SAR to fire blight were also shown to provide control. Timing of the injection is just as important for the effects of the injections to have maximum fire blight control (Acimovic et al 2015).

In citrus trees, citrus greening (huanglongbing) is a disease that poses a huge risk for commercial growers, and it is thought that nearly all of Florida's citrus trees have been infected. Insecticide sprays to control the psyllid vectors has slowed the disease but is unable to stop it from spreading. Injections of SAR compounds and antibiotics has been shown to prolong the life of the trees and maintain crop yields (Hu et al 2018).

Biology of trees and trunk injection

Successful trunk injection depends on many biological factors. Tree species, water uptake, and xylem type are perhaps the most important biological factors in trunk injection.

Tree species vary in many ways that effects their ability to uptake and distribute injected compounds. Depending on the tree species, its size, xylem type, and water uptake will vary. Taller, larger trees such as maple or oak will take more time to translocate injected compounds to their canopies as compared to an apple or cherry tree (especially if grafted onto a dwarfing rootstock). As tree size increases, so does the number of resistance points for the injected compound, and overall trunk volume.

Different types of trees will have a different daily water uptake. This is driven by the rate of transpiration. Tall forest trees can uptake between 100-300 gallons of water per day

(Acimovic 2014) while apple trees can absorb 15-50 gallons of water per day. Smaller fruit trees are often grafted onto rootstocks that have a significant effect on their water uptake and are the reason for such a wide range of water consumption.

The lack of understanding of different physiological processes in trees is part of what slowed forward movement in the field of trunk injection. In the 1890s the cohesion-tension theory was developed by Dixon and Joly (Dixon and Joly 1894, 1895). The theory states that the water in trees is pulled upward through a tree via negative pressure in the air. This is a very important concept to understand when trunk injecting because it governs how easily the injected substance is taken up by the tree.

Water in the xylem of trees is under negative pressure. Boehm demonstrated this theory through an experiment where a transpiring branch was able to uptake mercury, and the cohesion tension theory came shortly after with experiments showing the leaves of a branch were able to transpire in a high-pressure environment (Dixon and Joly 1984, 1985). This theory was not readily accepted because of how difficult it was to imagine how the negative pressure can exist for such a long period of time (Zimmermann 1983).

Water movement patterns inside trees varies depending on the different types of xylem tissues in various tree species.

Hardwood trees such as ash, maple, and oak have xylem anatomy with vessels, tracheids, fibers and rays. There are two xylem types in hardwoods- diffuse porous and ring porous. Diffuse porous xylem has a more extensive network of vessels dispersed throughout the growth ring as compared to ring porous xylem. This promotes a more even distribution of the injected compound. This is due to the high density of vessels, increased vessel to vessel contact, higher

lateral pitting of vessel walls, larger pit size and higher density of inter-vessel pits (Acimovic 2014).

Softwood trees such as cedar, pine and spruce consist primarily of tracheids, rays and resin canals. The movement of injected compounds in softwood faces more resistance points. Conifers have resin canals in their xylem as a defense system. Very soon after injury, the wound becomes filled with resin. Resin can reduce the conductivity of xylem for injected compounds.

The sap in the phloem is slightly basic. When an injected solution is highly acidic, it will move first into the phloem (Sur and Stork 2003). As a plant comes out of dormancy, phloem carries nutrients from leaves or roots to sinks. The phloem, when it has a high concentration of sugars, receives water from the xylem through osmosis and the sugars then move along a gradient to the sinks. The sinks are anything that hold sugars such as seeds, fruit, or roots (Coslor 2017).

Water ascent in trees was shown, with the use of dyes, not to always move directly upward. Some tree species have predictable sap movement, while other tree species have variable unpredictable sap movement (Kozlowski & Winget 1963, Kozlowski et al 1967). The ascent of sap can be described as spiral right, spiral left, interlocked (zigzag), sectoral winding, and sectoral straight (Rudinsky and Vite 1959).

Depending on xylem type, there can be a sectorality in the distribution of an injected compound. Ash trees with ring porous xylem have a zigzag pattern of sap flow. This causes variable compound distribution in trunk injection. Diffuse porous xylem in hardwood trees has less sectorality. Apple and pear trees have diffuse porous xylem and show good distribution of injected compounds (Kozlowski et. al. 1997, Jackson 2003).

Trunk injection is physiologically different from animal injection. Tree tissues do not heal the same as animal tissues after wounding. In animals, a wound heals through a process in which injured tissues are repaired or replaced. In trees, the tissues are not repaired or replaced, they are compartmentalized (Shigo and Marx 1977, Shigo et al 1977, Shigo 1984, Shigo 1985). Trees have developed defensive systems for combating wounds. They confine the wound through protective systems.

Compartmentalization is a two-part process. First the tree reacts chemically by producing antimicrobial substances in order to slow the spread of invading microorganisms. Second, the cambium will respond to a wound by forming a thin layer of cells that are impervious to most bacteria and fungi that may infect the wound (Shigo 1984,). A tree has a maze of cellular walls that make it difficult for pathogens to spread, allowing the tree to live for hundreds of years.

The closure of wounds in trees occurs by the new generation of cells over the wound. This cannot be considered healing in a general sense because the cells are not repaired or replaced underneath the callus. The tree will close the wound on the outside with new growth, while the wound is compartmentalized under the new growth to contain pathogens (Shigo 1984).

The creation of an injection ports in trees creates a wound, and depending on the type of port, there is a difference in the level of injury to the tree. In apple trees, the two most common different types of injection technologies were compared to assess their severity and the trees rate of closure. Needle based technology was used to create a port that is unsealed after injection. Three different drill ports were created with two different size drill bits, some left unsealed and some closed with a plug. Ports with the smaller width were able to close more quickly than larger width ports. Larger drilled ports that plugged with a silicone plastic plug callused over the

slowest. In general, healing from trunk injection occurs in 2 seasons or less (Acimovic et al 2016).

There are advantages and disadvantages to each of these injection technologies. Drill based technologies create larger ports and removes the tree's tissue from the cavity before injection a treatment solution. The advantage to the larger wound is a greater surface area of xylem exposure, leading to quicker, more uniform distribution of the injected compound. The weather and environmental conditions are less of a concern in determining injection success in uptake especially when a pressurized injection system is used. When a plug is used to close the port, the tree is protected. If the port is left open, it can lead to sap leaking and potential fungal or bacterial infection (Acimovic et al 2016).

In needle-based technologies, there is no removal of trunk tissue, therefore the port closure process is faster. However, the port is much smaller with a much smaller surface area of the tree's xylem, leading to slower, less uniform distribution of the injected compound. There is more dependence on favorable weather and environmental conditions in determining injection success with needle-based technology (Acimovic et al 2016)

Injection timing is key to effectively controlling the targeted pest. The correct timing varies depending on the tree species. In one study, injection after harvest showed the most movement of the compound both up and down the trunk and into the branches. Before bud break, there was limited movement in the trunk and in the branches only at the end of the season, and injection at flowering the compound went to the branches and twigs (Clifford et al 1987).

In pears, fall injection just before leaf fall resulted in fast distribution of compounds into branches and leaves. Translocation continued the next season into green canopy growth. Winter

injection showed poor translocation, and spring injections showed considerable primary distribution, but delayed deposition in the developing leaves (Shabi et al 1974).

In recent years, there has been a lot of concern for the health of bees. In 2006, the CDC reported a phenomenon called colony collapse disorder (Cox-Foster et al 2007). The EPA has also set No Observable Adverse Effect Levels (NOAEL) for managing risks of pesticides on honeybees. The timing of injections in apple trees was shown to influence detectable residue levels in apple pollen and nectar (Coslor et al 2019a). Spring and fall injections of several insecticides were injected and pollen and nectar residues were analyzed. It was found that for emamectin benzoate and imidacloprid, it was possible to reduce the amount of residue found in nectar or pollen through injection timing (Coslor et al 2019a).

Injection of nursery apple trees before transplant was done to see if foliar pests could be controlled (Coslor et al 2019b). Emamectin benzoate was shown to translocate and distribute into the canopy of the nursery trees. The higher dose is likely to provide multiple years of protection.

Chemical factors that influence trunk injection

There are several factors that determine an injected chemical's success in translocating and distributing into the tree's canopy. These include water solubility, solution pH, carbon adsorption coefficient, molecular size, formulation, solution concentration, and environmental stability.

Two key components to predict how successful an injected solution will be are pH and water solubility. High water solubility is a key component for ease of uptake and translocation in trunk injection. The more soluble a compound is, the easier it will move through the membranes in the xylem of the tree.

A compound's pH is also a major factor in its ability to translocate through membranes. Acidic or neutral solutions translocate more readily, while basic solutions will adsorb to the xylem walls. This has been explained with a calculation called the Transpiration Stream Concentration Factor or TSCF (Briggs et al 1982).

TSCF is a can be described by a bell-shaped curve relating the solubility of the molecule to the solubility of the molecule to organic solvents or logKow, to the strength or weakness of the acidity of the compound pKa (Sur and Stork 2003, Briggs et al 1982). A lower logKow means the compound has a higher solubility in water, therefore an easier time moving through the xylem.

A solution's concentration in water and dose can affect the tree. Highly concentrated solutions will cause phytotoxicity, while excessively low concentrations will not be effective. The correct dose in agricultural production is essential to being in compliance with maximum dose levels set by the US EPA (Coslor et al 2018).

Injection of chemicals directly into the xylem of the tree bypasses many of the possible ways a chemical may bind or degrade when compared to soil application or spray application (Doccola et al 2012). Some compounds remain stable over time or have metabolites that continue to provide adequate protection to the plant.

The carbon adsorption coefficient (Koc) is a measurement of mobility of a solution in soil or plants (Doccola et al 2012, Doccola and Wild 2012). The higher the value, the more strongly it will be adsorbed onto the organic matter, and the less mobile it will be in the plant. The lower the Koc, the more mobile the chemical solution will be. Trunk injection will be more successful with a highly mobile solution, or one with a low Koc value.

Physically, chemical size or molecular weight will also be a factor in trunk injection. Molecules move through vessels and tracheids, and the diameter of these will determine whether these molecules can be transported through. If the physical size of the molecule is larger than the size that the vessel or tracheid allows, than little or none of the molecule will make it through (Doccola and Wild 2012). The smaller the molecule, or essentially the lower the molecular weight, the easier it will be transported.

Environmental factors that influence trunk injection

Factors in the environment that will influence trunk injection include water potential, vapor pressure deficit, relative humidity and temperature.

Water potential is the notion that water moves from an area of high concentration to an area of lower concentration to create an equilibrium. Water movement will occur this way in a plant moving from one area of the plant to another. The potential energy of water per unit volume at atmospheric pressure and ambient temperature is written with the symbol, ψ.

Water with less solutes dissolved will tend to move to areas with more solutes. The highest water potential is pure water. During injection, the water potential of sap is lowered, and the sap with the injected chemical is diluted with sap of a higher water potential. The water potential becomes more negative the further up the tree towards the leaves and into the air.

Vapor pressure deficit VPD is the difference between the potential amount of water vapor the air could hold at a certain temperature and the amount of water vapor that the air is currently holding at that temperature. Transpiration creates negative pressure in the water column due to the VPD.

Temperature has a large influence on VPD. At higher temperatures, air expands and can hold more water vapor. This increases the VPD and lowers the relative air humidity and allows

for more transpiration in the plant. However, when the air humidity is high, the VPD is low and transpiration is much lower in the plant (Zimmerman 1983).

Summary of ideal trunk injection conditions

Trunk injections are most successful when the weather is right for good translocation. A sunny day with medium to high temperatures and low relative air humidity are best. This will bring a high vapor pressure deficit and leaves will be in active transpiration. The plant will ideally be healthy with large leaf area.

Trunk injection with a larger diameter port will allow for faster, more even distribution of the compound. The injected compound will be a highly water-soluble compound of low molecular mass in a mildly acidic solution.

Simulating stress in Trees- Trunk injection of Attractants

Trees react to stress in order to survive. Tree stress can result from a variety of things including drought, flood, compacted soil, temperature extremes, mechanical injury, chemical injury, insect damage, and pathogens. Trees that are subjected to various stresses will prompt physiological responses in the tree.

Heat (Fire), or water related stress limits the trees ability to produce nonstructural carbohydrates that are needed to sustain hydraulic functions (Kelsey et al 2014). The tree will react with a physiological response. One of these responses is the synthesis of ethanol. This response allows the cells in the tree to survive for short time periods when under oxygen deprived stress.

Ethanol will dissipate as it accumulates in trees by way of diffusion, sapflow, and metabolism. It will move by diffusion from areas of high concentration to areas of low concentration. It moves unimpeded through cell membranes because it is nonionic and hydrogen

bods strongly to water. Ethanol is transported through sapflow and can move more rapidly than diffusion (Kelsey and Westlind 2017).

The ethanol is moved into the atmosphere or metabolized and converted to acetaldehyde that is released to the atmosphere (Kelsey et al 2016, MacDonald and Kimmerer 1993). Ethanol is released through the leaves and has been detected in the headspace of leaf samples (MacDonald et al 1989).

Ethanol accumulation in is linked with other insect and fungal problems for the tree. It stimulates the growth of a root feeding fungus in oak trees (Wargo and Montgomery 1983). Ethanol is a known lure for bark and ambrosia beetles including the black stem borer *Xylosandrus germanus* (Blandford).

Trunk injection of ethanol has been used as a means of attraction for boring beetles. This is done to normalize insect infestation when testing the efficacy of insecticide products on the trees (Kelsey and Joseph 2003, Reding et al 2013, Ranger et al 2016, Klingeman et al 2017, Wargo and Montgomery 1983).

Topworking

Grafting is the joining of different plant varieties to grow as a single plant. This process has been around for a long time. Evidence of grafting shows that it was practiced Europe, the Middle East and Asia by at least the 5th century BCE (Melnyk and Meyerowitz 2015).

Grafting is used often in fruit tree production. Cultivars of fruit trees will go in and out of popularity. As a cultivar becomes more popular with consumers, farmers will begin switching to that cultivar. They can plant a juvenile tree and wait several years for the tree to mature and begin flowering, or farmers can speed up the process through grafting. A healthy shoot (scion)

from the desired cultivar is grafted on scaffold branches of the existing tree. This process is commonly called topworking (Mudge et al. 2009).

Pear Psylla as a pest in pears

The biology of pear psylla:

Pear psylla, *Cacopsylla pyricola* (Förster) (Hemiptera: Psyllidae), is native to southern Europe and western Asia. Psylla are true bugs. They have piercing sucking mouthparts to feed on the sap of the pear tree. Psylla have three life stages, egg, nymph, and adult. The adults resemble small cicadas and are about 2-2.5mm in length. The wings are held roof-like over the abdomen and they can be green or dark brown in color depending on the season. They are seasonally dimorphic having a summerform or a winterform body that is determined by the photoperiod that the nymph experiences as it develops. In long summer days, the psylla will develop into the summerform, a smaller greenish colored adult. When the days become shorter, the adult becomes larger and darker (Campinera 2004).

Psylla overwinter in reproductive diapause which ends in mid to late winter. The post-diapause winterform adults begin laying eggs at the base of fruit and leaf buds, and then on new foliage as it begins to emerge. Each winterform female can produce upwards of 1,000 eggs. The summerform females produce about 400 eggs each. There can be 2-4 summerform generations depending on the climatic region (Campinera 2004).

There are five instars in which they feed on the phloem sap from the trees before becoming adults. The adults morph into an overwintering stage that is larger and darker. They will lay eggs onto the base of fruit buds in late winter (Nechols 1995).

Eggs are elongate and creamy white right after oviposition. They have pedicle that is partially inserted into the underside of the leaf, generally directly in the crux of the middle vein.

This is believed to tap into the water supply of the tree to sustain the egg. It will change to yellow just before hatching (Campinera 2004).

Psylla nymphs are the destructive phase of the insect and will damage the tree in several ways. First, they will feed on sap from the trees and excrete honeydew. This weakens the tree by killing leaf tissue. They inject of a toxin during feeding which causes leaves to yellow and sometimes fall, reducing tree vigor, and sometimes resulting in the loss of the tree (Pasqualini et al 2006).

Second, psylla can produce enough honeydew that it drips down onto leaves and fruit. The honeydew acts as a medium for a black sooty mold and this discoloration of the fruit substantially lowers its value. In cases where trees have been chronically heavily infested, they become stunted and can have a reduced fruit set the following years. In severe cases, the trees can lose their leaves and die (Howitt 1993).

And third, pear psylla is also a vector for *Candidatus Phytoplasma pyri*, a bacteria phytoplasma, that is associated with pear decline. Phytoplasmas are bacteria that inhabit the phloem of the pear tree and cause disease (Siemonsmeier et al 2019). This renders the tree unable to move nutrients to the roots (Seemüller et al, 2004). Most infected trees will have shorter shoots with small, pale green leaves that roll upward. In the fall, the leaves may turn orange-red and drop prematurely (Garcia-Chapa, 2003). In severe cases trees can collapse and die (Sabaté et al 2018).

Pear psylla distribution:

Pear psylla are non-native in the United states. It was first reported in North America in 1832 in Connecticut. It was likely brought to Massachusetts or Connecticut on pear stock from Europe (Chang et al 1975). It began to rapidly distribute east of the Mississippi river until it was

reported in Nelson, British Colombia. By 1939, it was reported in the Pacific Northwest in Spokane, Washington and by 1953 it had moved south to California. Today, it can be found in all pear orchards throughout the United States and Canada (Unruh et al. 1995).

Black stem borer as a pest in apples

Biology of Black Stem Borer:

Black stem borer (BSB), (Coleoptera: Curculionidae: Scolytinae) *Xylosandrus germanus* (Blandford), is nonnative and widely distributed ambrosia beetle in the United States. Xyleborini is one of the largest tribes of insects, and they attack hundreds of species of woody plants worldwide (Norris 1979). Unlike bark beetles, ambrosia beetles do not feed directly on the wood or plant tissue, they prefer to feed on symbiotic ambrosia fungi that they carry with them as they initiate galleries in their host tree.

The female transports spores of the ambrosia fungus, *Ambosiella gorsmanniae*, with her into the host tunnel. The BSB has independently evolved a specialized pouch-like organ called mycangium, which is used to transport actively growing and reproducing fungal propagules to newly established galleries (Skelton et. al. 2019, Hulcr and Cognato 2010). The fungus has also been observed acquiring the fungal crop from other ambrosia beetles (Hulcr and Cognato 2010). The mycangium is located between the prothorax and the mesothorax. The spores are transferred from the female to the tunnel during excavation. The ambrosia fungus makes up nearly 100% of the BSB diet, and the female will only initiate oviposition after the fungus is established within the gallery (Ranger et al 2016).

Female BSB are approximately 2 mm in length and 1 mm wide. They are black or dark brown and cylindrical with a shiny, compact body. The head is hidden under the pronotum. Key features to distinguish from other Xylosandrus include their lack of strial setae and shining

declivity (Rabaglia et. al. 2006). Their widely separated procoxae is also key to distinguishing them from other Xyleborini (Rabaglia et. al. 2006, Ranger et. al. 2016). Male BSB are much smaller than females and lighter brown in color. They are flightless and therefore rarely found outside the gallery (Ranger et. al. 2016).

Adult females overwinter in galleries and emerge sometime in March-May with 2-3 consecutive days of 21˚C already having mated with the overwintering brood siblings. Emergence varies across geographical region, occurring earlier in the south in North Carolina and Tennessee in March and into April in Ohio and Illinois (Webber and McPherson 1983, Oliver and Mannion 2001, Ranger et. al. 2010). In Michigan, emergence occurs in late April or early May (Haas et. al. 2016). The peak flight activity occurs late afternoon and into the night where females will fly low to the ground in search of a good host tree. The female will then initiate a gallery by burrowing into the tree, creating a hole about 1 mm in diameter (Ranger et al 2016).

Eggs are deposited at the far ends of the brood chamber where there is substantial fungal development. Development into an adult takes about 15 to 18 days during which time the BSB will feed on the ambrosia fungus. Overall BSB takes about 60 days from gallery initiation to adult emergence with 1-2 generations per year (Ranger et al 2016).

There is a large variation of eggs per gallery ranging from 1 to 54, where the sex ratios are highly biased towards females 10:1. Unfertilized BSB eggs result in male offspring, while fertilized eggs result in females. BSB will breed among siblings within the gallery, and occasionally males will leave their galleries to enter a neighboring gallery to mate (Ranger et al 2016). Overwintering galleries can consist of many different broods in higher numbers than

galleries during the season sometimes 100-200 BSB can found in a single overwintering brood (Ranger et al 2016).

BSB populations in one location can vary drastically from year to year, and populations will vary from region to region. Reding et. al. (2010) monitored BSB populations where annual cumulative captures ranged from 944-3617 BSB in Ohio, 5-68 BSB in Tennessee, and 49-1022 BSB in Virginia. Most activity declined in periods of cool, wet weather, and it is unknown whether BSB are inactive or traps are less attractive in those conditions (Reding et. al. 2010).

Black stem borer distribution:

BSB is an invasive species in the United States. It is native to Asia and was first reported in the US in 1932 in New York (Felt 1932). Because of their small size and inconspicuous nature, they are easily transported inside nursery trees and other wood import products and are often undetected in port inspections. They easily establish populations in new environments (Atkinson et al 1990).

BSB have become prevalent in the Midwest and Northeastern states and are present in 26 US states according to CABI (2019). They have wide host ranges that include deciduous trees (Reding et al 2013) and coniferous trees (Wood 1982, Paine and Lieutier, 2016). They are a serious pest to nursery trees including ornamental and landscape trees (Oliver and Mannion 2001, Agnello et al 2017).

BSB are not size-selective in regards to the size of tree or branch they attack. They can breed in both small and large trees so both nursery and established trees are at risk (Paine and Lieutier, 2016). They are also capable of attacking healthy trees as well as recently cut or stressed trees, making them even more of a formidable pest (Rabaglia et. al. 2006).

Most recently BSB have been found in orchards in the United States attacking trees and causing growers to become concerned. The BSB were reported attacking trees under stress especially those growing in waterlogged soils, suffering winter injury, or fire blight. They attack spindle trees and small diameter trees. Even more concerning, once in the orchard they will attack healthy trees as well (Lehnert 2015). BSB has been found in apple orchards in New York, Michigan, Ohio, North Carolina, Oregon, and Wisconsin (Hall et. al. 1982, Agnello et. al. 2014, Lehnert 2015, Haas et. al. 2016, Wilson et. al. 2014, Guedot, 2019).

The first reports of BSB infestations in apple orchards were in Ohio in 1982 (Hall et. al. 1982). New York commercial apple orchards were hit hard by BSB attacks in 2013. Some of the affected trees were also infected with fire blight as a secondary problem brought on by the original BSB attacks. The trees ooze at the gallery holes and create more opportunities for fire blight to infect the tree. Hundreds of apple trees were removed and destroyed (Agnello et. al. 2014).

In 2014 and 2015, BSB were discovered in Michigan in the southwest and near Grand Rapids in apple orchards (Haas et. al. 2016, Wilson et. al. 2014). In 2019, BSB were first discovered attacking apple trees in Wisconsin (Guedot, 2019).

In North Carolina, BSB were observed tunneling around the graft union of dwarfed apple trees. Rapid apple decline, a disease that causes midseason collapse of apple trees, originates on the graft union and is linked to the presence of BSB. The BSB does not cause RAD, however it is attracted to highly stressed trees. The most cases of RAD were in 2017 in North Carolina due to many stress factors including drought, winter damage, and spring freezes. This causes the tree to produce the ethanol that attracts BSB (Villani, 2017).

A good source of published information on the BSB has been compiled by Webber and McPherson in an annotated bibliography (Webber and McPherson 2017).

Trapping for Black Stem Borer:

Ethanol is a known attractant to BSB. A trap constructed of a 1-liter plastic bottle and an ethanol lure can be used. The inverted bottle has 4 large windows and an effective funnel at the capped end. This funneled end is filled with a small amount of antifreeze to act as a preserving agent. Ethanol lures will attract the BSB to the traps (Haas et. al. 2016).

Trap height and placement is important for accurate trap data collection. BSB flight patterns are quite low to the ground, and the traps set lower to the ground caught more BSB than higher traps (Webber and McPherson 1983, Reding et. al. 2010).

Webber and McPherson (1983) placed traps at various heights 7m down to 1m. They discovered that traps at 1m resulted in the most activity in their locations in North Carolina and Illinois. Reding et. al. (2010) placed ethanol bated traps at three different heights (3m, 1.7m, and 0.5m) and found that the 0.5m traps were most successful at all of the locations across three states (Ohio, Tennessee, and New Jersey).

BSB are attracted to stressed trees. Ethanol synthesis is a response trees have to drought stress but has no role in the tree's defense (Kelsey et. al. 2014). In fact, it has the opposite effect and acts as a kairomone that attracts many bark and ambrosia beetles (Joseph et al. 2001, Kelsey and Joseph 2001, 2003, Coyle et al. 2005, Gallego et al. 2008, Miller and Rabaglia 2009, Ranger et al. 2011, Kelsey et al. 2013).

Ethanol combined with (−)-α-pinene were commonly used lures to attract bark and ambrosia beetles (Gallego et al. 2008) in monitoring programs. It should be noted, however, these two compounds should not be used in combination when trapping for ambrosia. Miller and

Rabaglia (2009) showed how (−)-α-pinene has an interruptive effect on ambrosia beetles. It is necessary to have a separate trap for ambrosia beetles when monitoring a wide range of bark and ambrosia beetles (Miller and Rabaglia 2009). Verbenone is another kairomone for wood boring insects. This compound is shown to be a deterrent to BSB (VanDerLaan and Ginzel 2013).

CHAPTER 2: TRUNK INJECTION TO CONTROL PEAR PSYLLA IN PEARS

Abstract

Pesticide losses through drift, run-off, and leaching occur with every pesticide application made with airblast sprayers. Trunk injection as an alternative method of insecticide application has been explored in apple, avocado, cherry, and date trees, but no such work has been done in pear. The objective of this study is to compare the efficacy of abamectin and azadirachtin in the control of pear psylla using two different application methods, airblast and trunk injection. Trunk injections of abamectin and azadirachtin were compared to airblast applications of equal labeled rates on 33-year-old Bartlett Pear trees (*Pyrus communis* L., var "Bartlett"). The abamectin and azadirachtin trunk injected treatments performed equally or better than the airblast applied treatments in the control of the pear psylla. The trunk injected trees from the first season provided a moderate level of control into the second season, one year after the injections. Trunk injection of two insecticides effectively controls pear psylla as well as airblast application and does not pose the same risk of drift and non-target exposure as airblast application. There are still improvements to be made on pear trunk injection before it can become an alternative method for growers.

Introduction

Pear Psylla, *Cacopsylla pyricola* (Forster), is the number one insect pest to the pear industry. In fact, more than half of the money spent to control insect pests in commercial pear orchards are directed specifically at controlling pear psylla (Horton 1999). Psylla nymphs feed on sap from the trees and produce honeydew, which drips down onto leaves and fruit. A black sooty mold, also known as fruit russet, grows in the honeydew and the black color on the pears downgrades the fruit (Westigard et. al. 1981). In cases where trees have been chronically heavily infested, they become stunted, reduce fruit production, and lose their leaves (Howitt 1993). Pear

psylla also transmit a disease that causes pear decline, which renders the tree unable to move nutrients to the roots and can cause the death of the tree (Seemüller and Schneider, 2004).

Washington, Oregon, and California are the biggest US producers with over 90% of the pears produced coming from these three states (Ing 2000). Pear production in Michigan was once a much larger industry encompassing over 10,000 acres. Today pears are produced on less than 700 acres (Census of Agriculture 2019). Overcoming the challenges to pear production are important to face if the pear industry in Michigan will make a comeback.

Summer management of pear psylla is very difficult if the overwintered population is not controlled (Horton 1999). Historically, broad-spectrum synthetic pesticides have been liberally used to control pear psylla, but the pest has developed resistance (McMullen and Jong 1971, Whalon et al 2008). They have developed resistance to a wide range of insecticides including benzene hexachloride, chlorinated camphene, parathion, malathion, diazinon, carbaryl, dieldrin, and azinphosmethyl (McMullen 1971). With this widespread resistance there is a need for more integrated control of pests and the use of selective botanical insecticides that are safer to mammals and the environment.

Two insecticides with selective modes of action, azadirachtin and abamectin, have been shown to control psylla populations in pear (Marčić et al 2009, Civolani et al 2010). Azadirachtin is a neem-based botanical insecticide non-toxic to birds, mammals, bees, and plants (Bond et al 2012). Abamectin is an insecticide/miticide with low toxicity to mammals and is known to be xylem mobile (EPA 1986). Trunk injected abamectin has been shown to control leaf feeding insects in apple (Coslor et al 2019).

Airblast sprayers are commonly used to apply pesticides in conventional orchards, however, this method of pesticide applications has drawbacks. Pesticide losses through drift, run-off, leaching, and non-target exposure occur with each application. An estimated 45% of pesticides are lost to drift and sedimentation (Zhu et al., 2006, Steiner, 1969) posing risks of non-target exposure. Reducing these risks has become a priority in Europe and the United States (EU, 2009, US EPA, 2016). With the shift in governmental priorities towards environmental and worker protection, it is important to explore alternative pesticide application methods.

Trunk injection as an alternative pesticide application method does not pose the same non-target exposure risks as airblast application. The pesticide is delivered directly into the vascular system of the tree and is taken up through the xylem sap flow. Trunk injection is an effective application method in apple, avocado, and date trees (Acimovic et. al. 2014, Byrne et. al. 2012, Coslor et. al. 2018, Khalaf et. al. 2016) but no such research has been done in pear.

The objective of this study is to compare the efficacy of abamectin and azadirachtin in the control of pear psylla using two different application methods, airblast and trunk injection.

Methods and Materials

Field Plots and Treatment Compounds

This experiment targeted natural populations of pear psylla at the MSU Trevor Nichols Research Center in Fennville, MI, USA (latitude 42.5951°: longitude -86.1561°). The two insecticide formulations used were Agri-Mek™ (Syngenta AG. Grensboro, NC), and Azasol™ (Arborjet Inc. Woburn, MA). Treatments were made on 33 year old Bartlett Pear trees (*Pyrus communis* L., var "Bartlett") with single tree replicates and four replicate trees per treatment in a randomized complete block design.

Rates of compounds were based on labeled rates for use in pears. Trunk injections were applied with the equivalent amount of active ingredient per tree based on orchard tree spacing (Table 1.).

Table 1. Treatment rates for pear trunk injection and airblast application comparison at Trevor Nichols Research Center, Fennville, MI in 2017 and 2018.

Treatment/Application Method	Trade Name	Active Ingredient	Application Rate	Active Ingredient per Tree
Untreated Control	-	-	-	-
Abamectin/ Trunk Injection	Agri-Mek .15EC	abamectin	2.40 ml/tree	0.04 g
Abamectin/ Airblast	Agri-Mek 0.7 SC	abamectin	0.29 l/ha	0.04 g
Azadirachtin/ Trunk Injection	Azasol 6%	azadirachtin	4.0 g/tree	0.24 g
Azadirachtin/ Airblast	Azasol 6%	azadirachtin	2.45 kg/ha	0.24 g

Application Method

Trunk Injection

Treatment injection applications were made 7 days after petal fall (23 May 2017). Injections were preformed using an Arborjet Tree IV with 4 portals equally spaced along the circumference of each trunk. The injection equipment included; the Arborjet Tree IV™ kit (Tree IV, #4 arbor plugs, and plug tapper), hammer, cordless drill, and a 0.95 centimeter wood drill bit. First, four holes were drilled into the apple trunk approximately 5 centimeters deep, 90 degrees horizontal from the trunk and 30 centimeters above the ground spaced as equally as possible while strategically placing under the main scaffold branches of the tree to distribute maximum compound volume throughout the canopy. Next, the plugs were tapped into place deep enough so that the outside rim of the plug was just below the bark. At this stage the tree is ready for injection.

Before each injection, the Tree IV was cleaned with a sanitizing solution (Arborjet Cleanjet™, Arborjet, Inc, Woodburn, MA) solution and water to rinse out residues. The insecticide was measured out and diluted into distilled water so that the final volume was 500 ml. The compound was then poured into the Arborjet injector holding tank. The needles were inserted into the plugs and the compound was injected via hand operated pressurized pump into the tree.

Foliar Application

Foliar applications were preformed using an FMC 1029 airblast sprayer in 935 Liters of water per Hectare (100 GPA). First applications were made May 23, 2017 and May 30, 2018. A second application of the foliar treatments was made on June 23, 2017 and July 17, 2018 after psylla nymphs reached an action threshold of 1 nymph per 3 leaves (Horton 1999). Individual trees were airblasted by using the nozzles on one side of the airblast sprayer only to apply the treatment on one side of the tree at a time. Foliage on both sides of the tree were thoroughly covered by the output of the sprayer.

Field Evaluations

To evaluate the pear psylla, field evaluations were conducted every two weeks. Samples of 50 leaves were taken (randomly throughout the entire tree- high, low, shielded, exposed and all four quadrant areas) for each replication. Pear psylla eggs and nymphs were counted using a stereomicroscope. In 2017, PP nymph and egg evaluations were made on 31 May, 12 June, 19 June, 28 June, 14 July, and 21 July. In 2018, PP nymph and egg evaluations were made on 18 June, 16 July, 25 July, and 8 August. Field evaluations were performed in 2017 and 2018 seasons. All the field evaluations on the 2017 injection trees continued into the 2018 season with field evaluation dates occurring June through mid-August.

At the end of the season, on 17 August 2017 a two minute count for sooty mold occurred. Each tree was surveyed for two minutes and leaves with sooty mold were counted. This evaluation is used to quantify the negative impact of psylla honeydew on leaves at the end of the season.

Residue Sample Collection and Preparation

Pear leaf and fruit samples were taken for residue analysis. Leaf samples were taken on 1, 7,14, 28, 56, and 84 days after treatment (DAT). Fruit samples were taken on 7 and 84 days after treatment. For the leaf samples- 40 leaves were randomly sampled from each replication (approximately 25 grams). For the fruit samples, 5 pears were randomly sampled from each replicate tree. Each pear was diced into half inch squares and homogenized in a bowl, and 25 grams was taken from this sample. Pears taken at 7 DAT were not as large as the 84 DAT fruit, therefore after dicing and homogenizing the 7 DAT samples, most of the homogenized sample was used to make 25 grams. Samples were weighed and held in 50 mL of dichloromethane (DCM) until processing. Samples were mixed with 4 grams magnesium sulfate and 1 g sodium chloride and allowed to sit for 48 hours in a 4.44°C walk in cooler. The DCM was then filtered through a funnel lined with filter paper and 10 grams of sodium sulfate to remove water and allowed to evaporate under a hood 4-12 hours. We added 2 mL acetonitrile to the evaporated jars and swirled for 90 seconds to ensure maximum uptake of the dried pesticide residue. The acetonitrile solution was then analyzed on a HPLC utilizing a previously reported method (Bayer 1998; Wise et al. 2006).

Residue Sample Analysis

The residue levels were quantified using a waters 2695 separator module HPLC equipped with a Waters MicroMass ZQ mass spectrometer detector (Waters, Milford, MA), and a C18 reversed phase column (50 by 3.0mm bore, 3.5 um particle size (Waters, Milford, MA).

The mobile phase, solvent A, was water with 0.1% formic acid, and solvent B was acetonitrile with 0.1% formic acid, and was initially held at 80% solvent A and 20% solvent B and followed by a gradient shown in Table 2. Column temperature was 40ºC.

Monitored ions for abamectin were 158.3, and 886.7 m/z (Da). The HPLC level of quantification was 0.0023 mg kg -1 of a.i., and level of detection was 0.001 mg kg -1. By using above described extraction method, mean parent compound recovery from four pear leaf samples (each 100 g) treated only with standard imidacloprid solution (0.046 mg kg -1), then agitated and left to dry, was 73% (level of detection 0.009 mg kg -1). The results have not been corrected for abamectin recovery.

Monitored ions for azadirachtin were 685.4, and 703.4 m/z (Da). The HPLC level of quantification was 0.015 mg kg -1 of a. i., and level of detection was 0.005 mg kg -1. By using above described extraction method, mean parent compound recovery from four pear leaf samples (each 100 g) treated only with standard imidacloprid solution (0.046 mg kg -1), then agitated and left to dry, was 73% (level of detection 0.009 mg kg -1). The results have not been corrected for azadirachtin recovery.

Statistical Analysis

The psylla field evaluation data was analyzed using a repeated measures analysis as a two-way RCBD using PROC MIXED procedure in SAS 9.4 (SAS Institute, Cary, NC, 2013). The following statistical model was fitted to the data:

$$PearPsylla_{ijk} = \mu + Block_j + Trt_i + \varepsilon_{1ij} + Time_k + (Trt_i * Time_k) + \varepsilon_{2ijk}$$

$$i=1,2,3,4,5 \qquad j=1,2,3,4 \qquad k=1,2,3,4,5,6$$

The degrees of freedom for the model components were equal to 1 for the grand mean (μ); 4-1=3 for $Block_j$; 5-1=4 for Trt_i; 3*4=12 for the residual ε_{1ij}; 6-1=5 for $Time_k$; 4*5=20 for (Trt_i*$Time_k$); and 120-(1+3+4+12+5+20)=75 for the residual ε_{2ijk}.

The normality assumption was assessed by checking normal probability plots and histograms of residuals. The equal variance assumption was assessed by checking plots of residual v. predicted values, side by side box plots, and Levene's test.

The data indicates that as time goes on, the variability in the variances increases therefore making it necessary to fit a variance-covariance structure with unequal variances. When treatments were significant, all pairwise comparisons among the treatment means were analyzed. PROC GLIMMIX was run to generate a plot of pear psylla least-squares means for treatment by evaluation day sliced by treatment and adjusted for Tukey-Kramer honestly significant difference ($p \leq 0.05$).

Results

Field Evaluations

2017 Season

The overall treatment effect for mean psylla eggs was significant (F=16.01, Num df=4, Den df=23.4, $P < 0.0001$). Differences of treatment by evaluation day LS means sliced by

treatment indicated a total of 5 significant differences between treatments (Figure 1A and Figure 2A).

The overall treatment effect for mean psylla nymphs was significant (F=39.46, Num df=4, Den df=22, $P < 0.0001$). Differences of treatment by evaluation day LS means sliced by treatment indicated a total of 11 significant differences between treatments (Figure 1B and Figure 2B).

Abamectin injected treatment significantly reduced the number of eggs as compared to the untreated treatment for the evaluation dates 6-12-2017 (P=0.0119) and 6-19-2017 (P=0.0003). Abamectin airblast treatment reduced the number of psylla eggs as compared to the untreated check on evaluation date 6-12-2017 (P=0.0022) (Figure 1A).

Abamectin injected treatment reduced the number of nymphs as compared to the untreated treatment for the evaluation dates 6-12-2017 (P=0.0112) and 6-19-2017 (P<0.0001), and 6-28-2017 (P=0.0098). Abamectin airblast treatment reduced the number of psylla nymphs as compared to the untreated check on evaluation dates 6-19-2017 (P<0.0001), and 6-28-2017 (P<0.0032) (Figure 1B).

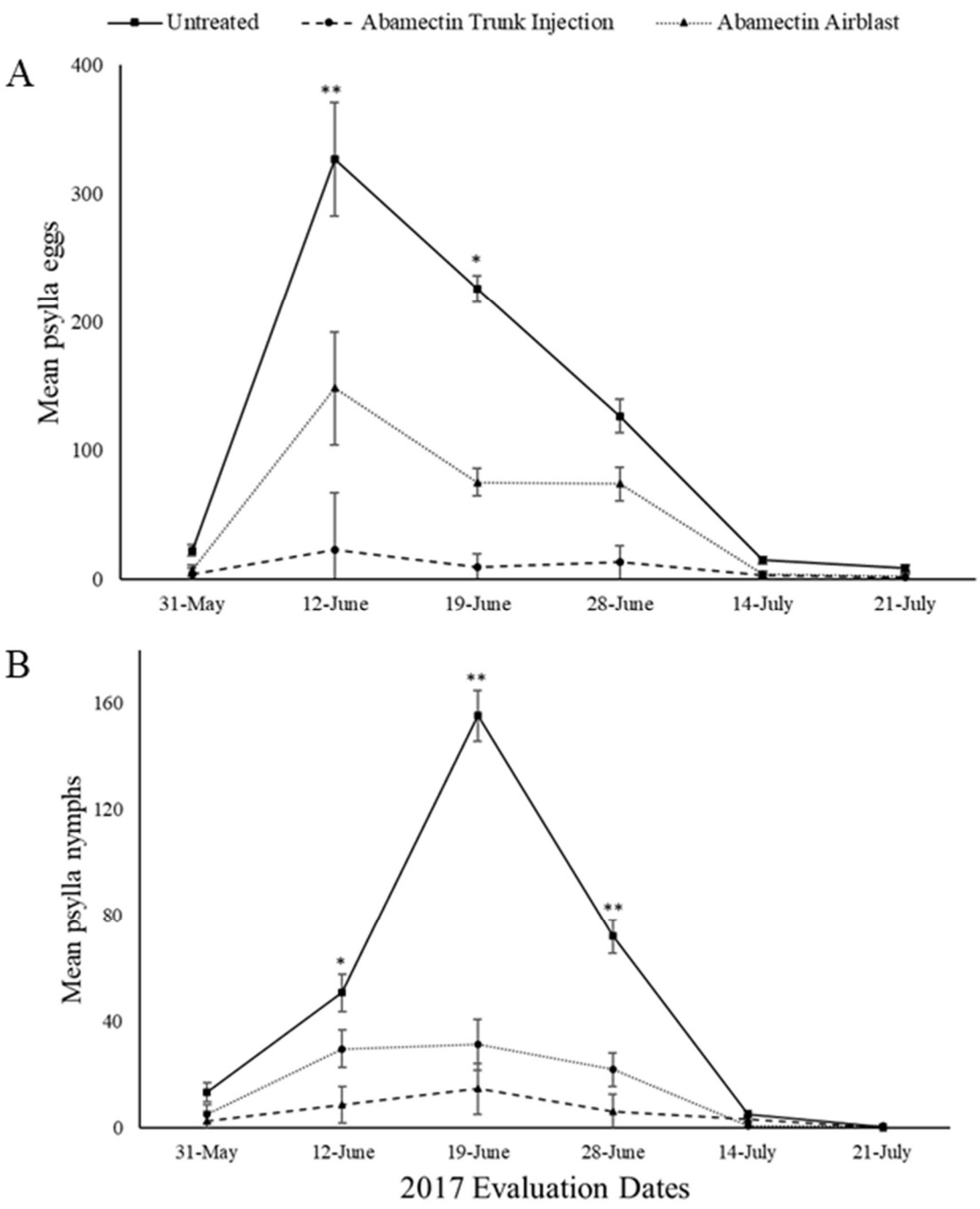

Figure 1. Mean number of pear psylla eggs (A) and nymphs (B) per 50 leaves in 2017 evaluations for the untreated and abamectin treatments. Values with * above them represent a significant difference ($\alpha \leq 0.05$) between the untreated and the trunk injected treatment only. Values with ** above them represent a significant difference ($\alpha \leq 0.05$) between the untreated and both injection and airblast treatments.

Azadirachtin injected and azadirachtin airblast treatments reduced the number of eggs as compared to the untreated on evaluation date 6-19-2017 (azadirachtin injection P=0.003, azadirachtin airblast P=0.0012) (Figure 2A).

Azadirachtin injected treatments reduced the number of nymphs as compared to the untreated on evaluation dates 6-12-2017 (P=0.0313), 6-19-2017 (P<0.0001), and 6-28-2017 (P=0.0074). Azadirachtin airblast treatments reduced the number of nymphs as compared to the untreated on evaluation dates 6-12-2017 (P<0.0385), 6-19-2017 (P<0.0001), and 6-28-2017 (P=0.0066) (Figure 2B).

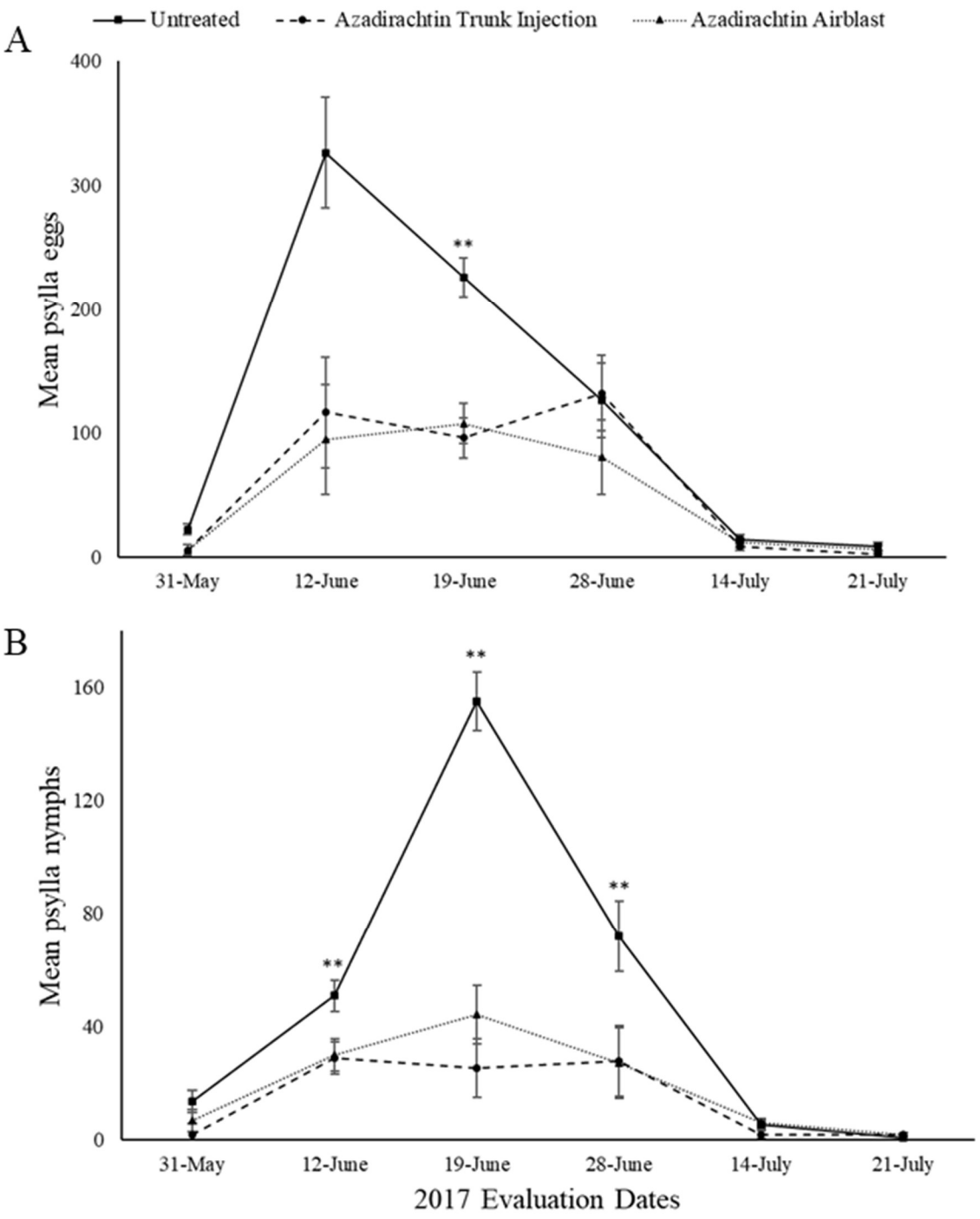

Figure 2. Mean number of pear psylla eggs (A) and nymphs (B) per 50 leaves in 2017 evaluations for the untreated and azadirachtin treatments. Values with ** above them represent a significant difference ($\alpha \leq 0.05$) between the untreated and both injection and airblast treatments.

The sooty mold evaluation that was conducted at the end of the 2017 season showed fewer leaves were infected on trees treated with either trunk injected treatments or airblast applied treatments compared to untreated check trees (F=17.92, NumDF=4, DenDF=12, P<0.0001). All treatments averaged less than 16 leaves with sooty mold as compared to the untreated check with 71.5 leaves with sooty mold (Table 2.).

Table 2. Mean leaves with black sooty mold per treatment after conducting a 2 minute visual count on 17-Aug 2017. Means followed by same letter do not significantly differ (P<0.0001, $\alpha \leq 0.05$, Tukey-Kramer honestly significant difference).

Treatment/ Application Method	Mean leaves with black sooty mold
Untreated	71.5 a
Abamectin/Trunk Injection	15.8 b
Abamectin/Airblast	10.3 b
Azadirachtin/Trunk Injection	8.5 b
Azadirachtin/Airblast	14 b

2018 Season

The pear trees that were injected in 2017 were evaluated in 2018 along with airblast applicated treatments applied in 2018. The overall treatment effect for mean psylla eggs was significant for abamectin (*F*=6.57, Num df=2, Den df=12.7, *P* =0.0109) (Figure 3A). Differences of treatment by evaluation day LS means sliced by treatment indicated a total of 2 significant differences between treatments.

Abamectin treatments reduced psylla nymphs was significant for abamectin (*F*=13.8, Num df=2, Den df=11.2, *P* =0.002) (Figure 3B). Differences of treatment by evaluation day LS means sliced by treatment indicated a total of 2 significant differences between treatments.

Abamectin trunk injection and airblast treatments reduced treatments reduced the number of eggs on 6-18-18 (P<0.05) relative to the untreated control but there was no effect of

treatment on the number of eggs the rest of the season.0308). (Figure3A). Without treatment, the number of nymphs peaked in mid-summer (7/25) but populations were reduced (P<0.0001) were controlled by both insecticide treatments at that time (Fig. 3B).

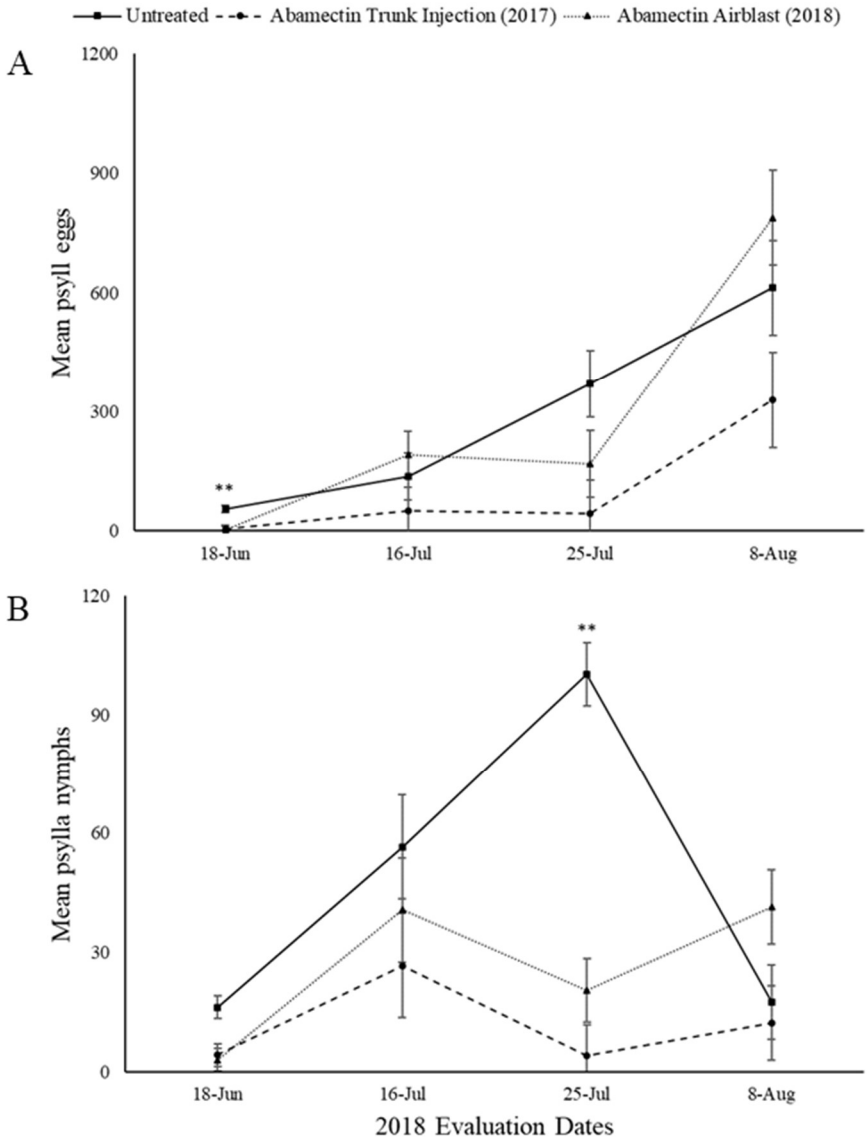

Figure 3. Mean number of pear psylla eggs (A) and nymphs (B) per 50 leaves in 2018 evaluations for the untreated and abamectin treatments. Values with ** above them represent a significant difference ($\alpha \leq 0.05$) between the untreated and both injection and airblast treatments.

The overall treatment effect for mean psylla eggs not significant for azadirachtin (F=0.11, Num df=2, Den df=9.01, P =0.8972) (Figure 4A).

The overall treatment effect for mean psylla nymphs not significant for azadirachtin (F=3.12, Num df=2, Den df9.71, P =0.0897) (Figure 4B).

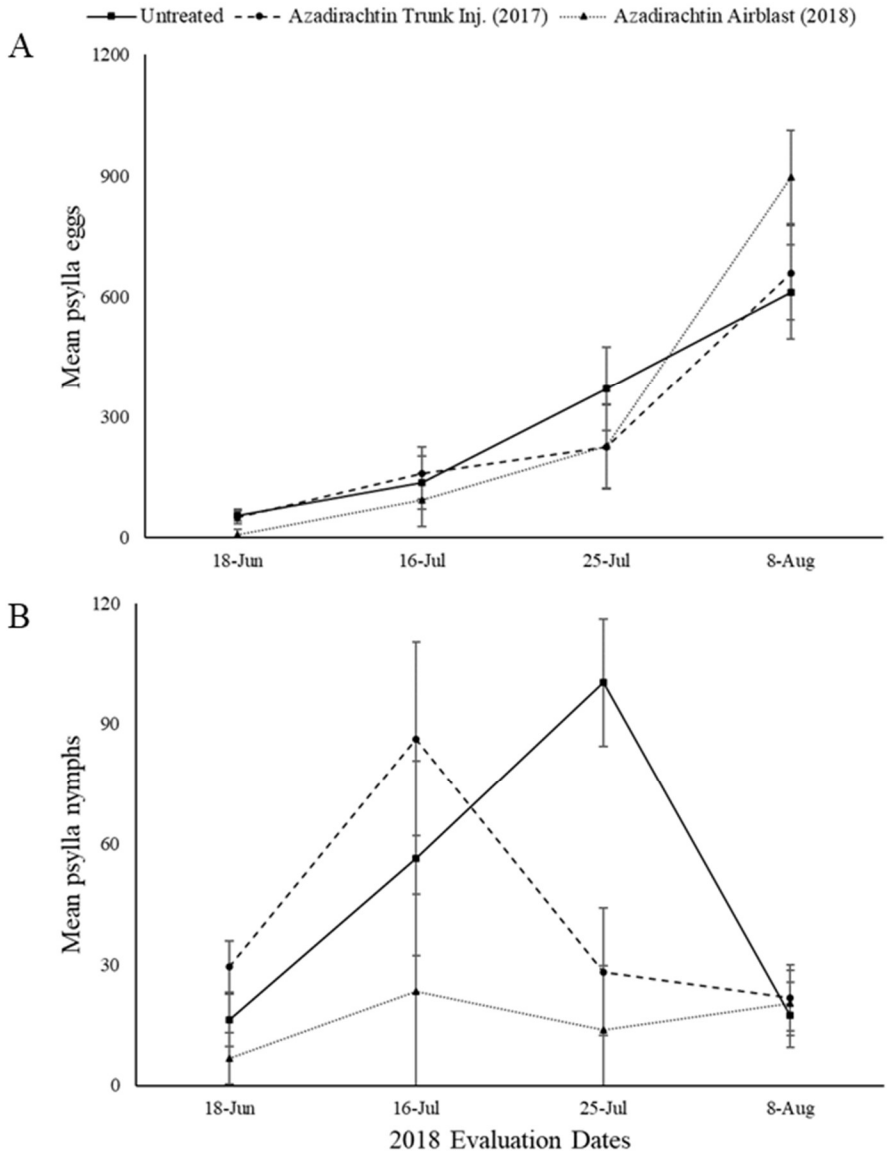

Figure 4. Mean number of pear psylla eggs (A) and nymphs (B) per 50 leaves in 2017 evaluations for the untreated and azadirachtin treatments. There was no significant difference found between any of the treatments ($\alpha \leq 0.05$).

Residue Profiling

Overall, detections of residue were quite low. Fruit samples had no residue detected for abamectin treatments (Table 3), and only one sample had residues above the level of detection for azadirachtin (Table 4). No residues were detected beyond 7 days after treatment, and all residues were well below the MRL for fruit (abamectin MRL=0.02 ppm, azadirachtin is exempt from the tolerance requirement) (US EPA 2020).

Leaf sample residue for abamectin were low with all residue detections below 0.25ppm, and most detections below 0.1ppm (Figure 5A). Leaf sample residue for azadirachtin were also low and steadily decreased throughout the season (Figure 5B). All azadirachtin residues were below 30ppm, and no residue was detected beyond 28 days after treatment.

Table 3. Mean abamectin residue on fruit taken 7 days and 84 days after the first treatment (DAT). No residue was detected (nd) above the level of detection (0.001 ppm).

Treatment/ Application Method	7 DAT	84 DAT
Untreated	nd	nd
Abamectin/Trunk Injection	nd	nd
Abamectin/Airblast	nd	nd

Table 4. Mean azadirachtin residue on fruit taken 7 days and 84 days after the first treatment (DAT). Residue (ppm) reported above the level of detection (0.005 ppm), or below the level of detection (nd).

Treatment/ Application Method	7 DAT	84 DAT
Untreated	nd	nd
Azadirachtin/Trunk Injection	1.507	nd
Azadirachtin/Airblast	nd	nd

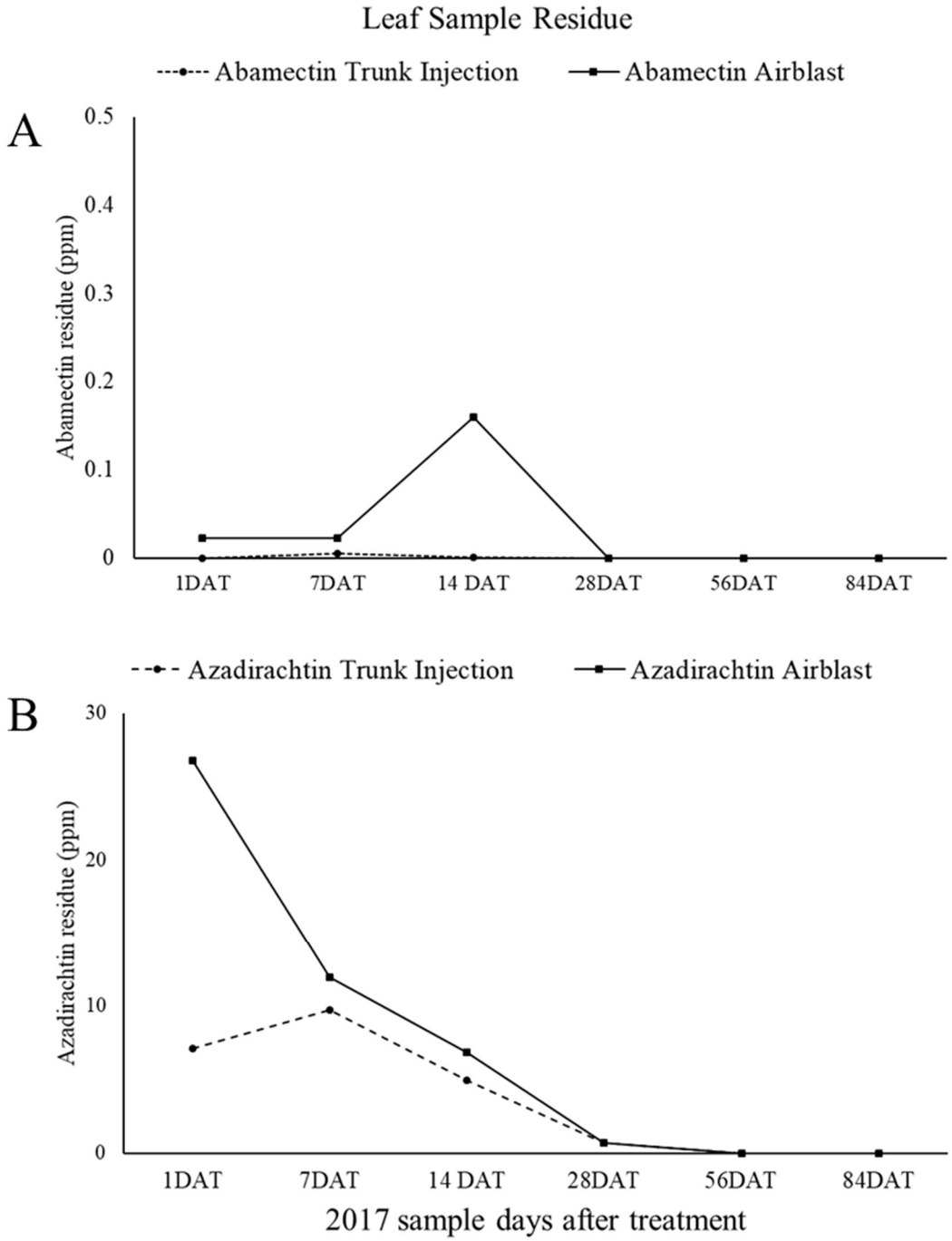

Figure 5. Mean residue from leaf samples taken in 2017 of abamectin trunk injection and airblast treatments (A) and azadirachtin trunk injection and airblast treatments (B). Samples were taken 1,7,14,28,56, AND 84 days after treatment (DAT). Residue is presented as ppm (ug/ml) above the level of detection (LOD) (abamectin LOD=0.001ppm) (azadirachtin LOD=0.005ppm). No residue was detected after 28 DAT for both abamectin and azadirachtin.

Discussion

This study contributes new information on trunk injection in pear trees to control pear psylla. A single injection of abamectin or azadirachtin provided two seasons control of pear psylla. Most importantly, one trunk injected application results in the same or better control than 4 foliar sprays throughout two seasons.

Abamectin is a neurotoxin, permanently opening the glutamate-gated chloride channels, and inhibiting the nerve and muscle cells to communicate. When ingested, the psylla are affected by uncoordinated movement, paralysis, starvation, and ultimately death (Lasota 2002). The neurotoxicity effects the nymph stage most, stopping life before reproduction occurs and our data reflect this. Egg numbers stayed quite low never ramping up to a peak in egg production, and always staying at a low level flat line. Nymph numbers were consistently below threshold (0.3 nymphs/leaf) for the abamectin trunk injected treatments. While airblast treatments were not statistically different from the trunk injected treatments, they were consistently above the nymph threshold that farmers use to act with another insecticide application. If a second application can be avoided, time and money can be saved and less pesticide introduced into the environment.

Abamectin rapidly degrades when exposed to light (Bai et al 2016, Lacosta 2002). Abamectin is formulated for foliar application (Agri-Mek), horticultural oils are recommended to add to foliar sprays in order to move the product to the foliage. Foliar application of abamectin with horticulture oil is generally expected to control pear psylla for half the season. Trunk injected abamectin provides season long control and may degrade slower when contained in the xylem of the tree. Perhaps this is why the injected treatment was superior in its ability to control pear psylla. The rapid degradation could also explain why there were no residues detected in the leaf analysis for airblast abamectin treatments after the seven days.

Azadirachtin is an anti-feedant, repellant, and insect growth regulator (Karnavar 1987, Subrahmanyam 1990, US EPA 1993). Azadirachtin affects the morphogenesis, ovarian development, fecundity, egg viability and molting of psylla through the endocrine system (Karnavar, 1987). The injection data reflected the IGR effect with significantly lower nymphs starting just a week after injection. The anti-feedant and repellant properties of azadirachtin likely played a role in lowering psylla numbers as well.

Leaf residue for azadirachtin was detected through four weeks after treatment for trunk injection, but only through two weeks after initial treatment for airblast application. Azadirachtin has systemic activity, which may be why the trunk injected treatments had detectible residues two weeks longer than airblast application. The tree can store azadirachtin in the leaves as a metabolite without changing its biological effect (Pavela et al. 2013; Cevenini & Minelli 2010).

Azadirachtin breaks down quickly under ultraviolet light (Barnby et. al. 1989, Dureja and Johnson 2000). The biological activity is significantly reduced around 200 hours of exposure to UV light (Barnby et. al. 1989). The anti-feedant potency is rapidly decreased with exposure to sunlight (Stokes and Redfern 1982). The half-life of azadirachtin was found to be less than an hour (Dureja and Johnson 2000). Sun exposure degradation may explain why the residues for the airblast application were detected for a much shorter period of time, and why airblast applicated azadirachtin were not effective for as long of a period of time as trunk injected azadirachtin.

This is the first modern study in the United States demonstrating the potential for using trunk injection in pear trees. Our study showed that injected insecticides achieved a high level of control over psylla for two seasons. Likewise, this has been documented in apple trees for similar phloem feeding insects such as potato leafhopper and rosy apple aphid (VanWoerkom et al.,

2014; Wise et al., 2014, Coslor et. al. 2019a, 2019b). Residues in pear fruit were extremely low and often zero, well below the MRL allowed by the US EPA, similar to other studies in apple (Coslor et. al. 2019a).

In conclusion, our study shows that trunk injection of insecticides to control pear psylla in pear trees has many promising aspects for future pear production. First, one injection provides a high level of control of pear psylla for two seasons using 75% less insecticide than with airblast application. Second, the insecticide is delivered directly to the feeding psylla through the sap of the tree, therefore it is not lost to drift, runoff, or subjected to photodegradation. This saves farmers money on insecticides and saves the environment unnecessary non-target exposure. And third, pesticide residue for abamectin and azadirachtin were low in the fruit, at zero or well below MRL. This reduces exposure and risk to humans who will consume the fruit. With governmental policies shifting towards more responsible pesticide use, this study shows that trunk injection should be strongly considered as a good alternative to airblast application.

Trunk injection works well in reducing pear psylla in pear, however, the injection process is not yet time efficient enough to be economically feasible for most commercial farms. Future research should address ways to improve the economics of trunk injection for pear production.

CHAPTER 3: TRUNK INJECTION-BLACK STEM BORER IN APPLES

Abstract

The black stem borer (BSB), (Coleoptera: Curculionidae: Scolytinae) *Xylosandrus germanus* (Blandford), is an ambrosia beetle that has recently been found attacking seemingly healthy orchard trees in the United States. BSB are attracted to ethanol produced by stressed trees, which can be the case when topworking (grafting a new cultivar onto established trees). The objective of this study is to evaluate the efficacy of two insecticides, emamectin benzoate and azadirachtin, and injection timing fall and spring, on their ability to control BSB in apple trees with simulated topworking and ethanol injection as an attractant. To induce BSB colonization, trees were injected with ethanol using a previously reported method (Reding et. al. 2013). Our study shows evidence that both emamectin benzoate and azadirachtin injections can reduce BSB infestations. Timing of the injection influences the outcome in terms of protecting apple trees from BSB, with spring injected azadirachtin being more effective than fall injections. Emamectin benzoate likely affects BSB adults directly by reducing successful attacks/entries, while and azadirachtin appears to reduce BSB attacks and limits gallery success.

Introduction

Black stem borer (BSB), (Coleoptera: Curculionidae: Scolytinae) *Xylosandrus germanus* (Blandford), is an ambrosia beetle that burrows into woody tissue of seemingly healthy trees. BSB do not feed on wood, but prefer a diet of their symbiotic ambrosia fungus they transport into the galleries of their host tree. They are an invasive species in the United States and were first reported in the US in 1932 in New York (Felt 1932). BSB can easily adapt to new environments and establish populations in a range of woody plants (Atkinson et al 1990).

Female BSB are small, black or dark brown, and cylindrical. Their body is shiny and compact, approximately 2 mm in length and 1 mm wide with widely separated procoxae (Rabaglia et. al. 2006, Ranger et. al. 2016). Male BSB are much smaller and lighter brown than females. Males are flightless and therefore rarely found outside the gallery (Ranger et. al. 2016).

Adult females overwinter in galleries and emerge sometime between March and May with 2-3 consecutive days of 21˚C, already having mated with the overwintering brood siblings. In Michigan, emergence occurs in late April or early May (Haas et. al. 2016). Females will then initiate a gallery by burrowing into the tree and creating a hole about 1 mm in diameter (Ranger et al 2016). Overall BSB takes about 60 days from gallery initiation to adult emergence with 1-2 generations per year (Ranger et al 2016).

BSB populations in one location can vary drastically from year to year, and populations vary from region to region. Reding et. al. (2010) monitored BSB adult populations where annual cumulative captures ranged from 944-3617 BSB per site in Ohio, 5-68 BSB per site in Tennessee, and 49-1022 BSB per site in Virginia. Most activity declined in periods of cool, wet weather, and it is unknown whether BSB are inactive or traps are less attractive in those conditions (Reding et. al. 2010).

BSB have become prevalent in the Midwest and Northeastern states and are present in 26 US states according to CABI (2019). Most recently, BSB have been found in orchards in the United States. In 2014 and 2015, BSB were discovered in southwest Michigan and near Grand Rapids in apple orchards (Haas et. al. 2016, Wilson et. al. 2014).

Topworking in apple orchards could lead to potential attacks by BSB. Topworking is a process where an existing established rootstock are used to speed the process of changing to a more popular cultivar. This can reduce the time it takes for the orchard to begin producing marketable fruit and, with the right choice of cultivar and rootstock, can be very successful (Blazek et. al 2002). In topworking, most of the apple tree scaffold limbs are cut off, leaving a nursing branch. Next, 2-4 shoots from the new cultivar are grafted on top of the remaining stump of the central leader limb. This process wounds the tree and causes the tree to synthesize ethanol, which is known to be an attractant to BSB (Moeck 1970). The risk of BSB attacking newly topworked apple trees could deter growers from practicing topworking. Because of the rise in concern for orchard trees, it has become important to research methods of control for this pest.

Pyrethroids applied as trunk sprays in nursery settings have been used with success at suppressing BSB attacks, however applications must be closely timed with BSB attacks to be effective (Frank and Sadof 2011, Reding et. al 2013, Agnello et. al. 2015). Organophosphates like chlorpyrifos were also used in apple trials against BSB with inconsistent and marginal success at preventing new BSB infestations (Agnello et. al. 2015). Bio-repellents applied as trunk were inconsistently successful at reducing BSB attacks (Reding et. al. 2013). Fungicides have also been used to deter the growth of the ambrosia fungus and thus eliminate the BSB through its food source. Fungicides were tested in the lab with success at inhibiting the growth of the ambrosia fungus (Erper et. al. 2018). However, fungicides did not prevent attacks in the field

(Brown 2018). Results from efficacy testing trunk sprays have been variable and inconstant at suppressing BSB attacks, and none have completely prevented attacks.

Trunk injection of insecticides may be a good option to consider controlling BSB. Many pesticide products injected into the xylem of the apple trees readily diffuse into the woody tissues of the tree into which BSB bore (Mota-Sanchez et al. 2009). Although they do not feed directly on the woody tissue of the tree, their bodies and mouthparts come into close contact with treated wood. They have a high potential to ingest the insecticide compounds during their grooming processes. When creating and living in galleries, BSB are in contact with the treated wood constantly and perhaps rub against the walls exposing the ambrosia fungal spores to the insecticide. The BSB may ingest the ambrosia fungus with small amounts of injected insecticide. The tunnels and galleries are moist environments with the sap flow of the tree bringing a constant supply of sap laden with insecticide and could ultimately be a toxic environment for BSB.

Injections of azadirachtin have been used in efficacy trials to manage bark boring beetles and wood boring beetles. Azadirachtin injections to control emerald ash borer in ash trees was very successful against larvae. All larvae failed to complete development, however it was not effective in controlling adults (Audley et. al. 2016). These data suggest that azadirachtin may be a good option in the control of BSB larvae in apple trees.

Byrne et. al. (2020) used trunk injection of emamectin benzoate to control two different types of ambrosia beetles, the polyphagous shot hole borer and the Kuroshio shot hole borer in avocado trees. Researchers have had much success with trunk injection treatments in avocado trees ranging from nutrient correction to plant protection injections (Whiley et. al. 1991, Masikane et. al. 2020, Byrne et. al. 2012, 2014, 2020). The shot hole borers are ambrosia beetles

and are similar in anatomy and behavior to BSB. These experiments and bioassays showed trunk injection of emamectin benzoate as a potential method of control, and worth investigating in apple trees (Byrne et. al. 2020).

Injection timing may also factor into successful control of BSB attacks. When insecticides are injected in the fall, the product stays near the injection site and then slowly diffuses in the spring after breaking dormancy (Coslor 2018). Spring injections will readily diffuse with sap flow.

Inconsistent BSB infestations present a challenge to insecticide efficacy trials. Ethanol injection may induce BSB attacks on experimental trees. Reding et. al. (2013) injected potted trees with ethanol to see if this would homogenize BSB attacks. Overall, ethanol injected trees experienced more attacks than untreated trees, and attacks declined 8 days after ethanol injection (Reding et. al. 2013).

The objective of this study is to evaluate the efficacy of two insecticides, emamectin benzoate and azadirachtin, and injection timing fall and spring, on their ability to control BSB in apple trees with simulated topworking and ethanol injection as an attractant.

Methods and Materials

Field Plots and Treatment Compounds

This experiment targeted natural populations of black stem borers at the MSU Trevor Nichols Research Center in Fennville, MI, USA (latitude 42.5951°: longitude -86.1561°). Treatments were made on 26 year old apple trees (*Malus domestica* Borkhausen, var. "Johnafree") with single tree replicates and four replicate trees per treatment set in a randomized complete block design on trees adjacent to a natural wooded area.

Insecticide injections were preformed using an Arborjet Tree IV ™ (Arborjet Inc., Woburn, MA) with 4 ports equally spaced along the circumference of each trunk. The injection

equipment included; the Arborjet Tree IV™ kit (Tree IV, #4 arbor plugs, and plug tapper), hammer, cordless drill, and a 0.95 centimeter wood drill bit.

For each injection, four holes were drilled into trees trunks 5 centimeters deep, 90 degrees horizontal from the trunk and 30 centimeters above the ground spaced as evenly as possible while strategically placing under the main scaffold branches of the tree. Next, the plugs were tapped into place deep enough so that the outside rim of the plug was just below the bark.

Before each injection for every tree, the Tree IV was sanitized with the Arborjet Cleanjet™ (Arborjet Inc., Woburn, MA) solution and water to rinse out residues. The insecticides were measured and diluted with distilled water so that the final volume was 500 ml. The treatment solution was then poured into the Arborjet injector holding tank. The needles were inserted into the plugs and the solution was injected via hand operated pressurized pump into the tree.

Compounds injected include azadirachtin Azasol™ (Arborjet Inc. Woburn, MA), and TREE-äge™ (Arborjet Inc. Woburn, MA). Rates of compounds were based on maximum labeled rates for apples and applied with the equivalent amount of active ingredient on a per tree basis (Table 5 and Table 6).

Table 5. Treatment rates for apple trunk injection fall and spring comparison at Trevor Nichols Research Center, Fennville, MI Fall 2017 and Spring 2018. Applications were made on 19 Oct 2017 and 22 May 2018.

Treatment/Application Timing	Trade Name	Active Ingredient	Application Rate	Active Ingredient per Tree
Untreated Control	-	-	-	-
Emamectin benzoate/ Fall 2017	TreeAge	emamectin benzoate	1.86 ml/tree	0.08 g/tree
Azadirachtin/ Fall 2017	TreeAge	azadirachtin	4.00 g/tree	0.24 g/tree
Emamectin benzoate/ Spring 2018	Azasol	emamectin benzoate	1.86 ml/tree	0.08 g/tree
Azadirachtin/ Spring 2018	Azasol	azadirachtin	4.00 g/tree	0.24 g/tree

Table 6. Treatment rates for apple trunk injection fall and spring comparison at Trevor Nichols Research Center, Fennville, MI Fall 2018 and Spring 2019. Applications were made on 10 October 2018 and 30 May 2019.

Treatment/Application Timing	Trade Name	Active Ingredient	Application Rate	Active Ingredient per Tree
Untreated Control	-	-	-	-
Emamectin benzoate/ Fall 2018	TreeAge	emamectin benzoate	1.86 ml/tree	0.08 g/tree
Azadirachtin/ Fall 2018	TreeAge	azadirachtin	4.00 g/tree	0.24 g/tree
Emamectin benzoate/ Spring 2019	Azasol	emamectin benzoate	1.86 ml/tree	0.08 g/tree
Azadirachtin/ Spring 2019	Azasol	azadirachtin	4.00 g/tree	0.24 g/tree

Fall treatments were timed prior to leaf senescence. Spring treatment applications targeted two things, first the rise in natural BSB population, and second normal timing for topworking. Normal timing for topworking is in April or May when the bark slips freely. Bark slippage is when the bark can be separated, or peeled back, easily from the wood with little damage. This stage indicates that the vascular cambium of the tree is actively growing.

One set of trees was used in the 2017/2018 study, and a different set of trees was used in the 2018/2019 study. Different trees were used for fall and spring injection treatments. Fall 2017 and Spring 2018 treatment insecticide applications were made at 19 Oct and 22 May. Fall 2018 and Spring 2019 treatment insecticide applications were made at 10 Oct and 30 May.

On the day BSB numbers correlated well with bark slippage the trees were cut to simulate topworking. On the same day in the afternoon the spring treated trees were injected with insecticides. Then on the following day, all trees were injected with an ethanol solution to attract BSB.

Black Stem Borer Monitoring

Local BSB adult monitoring was done throughout the 2018 and 2019 growing seasons. Traps were constructed of one liter plastic bottles inverted with four large windows cut out of the

body of the bottle. Standard release ethanol lures (AgBio Inc. Westminster, CO) were clipped to the inside of each trap, and 25mL of antifreeze was poured in the bottom funnel of the trap as a preservative. Traps were placed approximately 0.5 meters above the ground just inside the wooded area adjacent to the plots in early spring (17 Apr 2018 and 7 May 2019). Traps were monitored weekly during peak season, and the number of adults captured was recorded.

Top-working Simulation

Spring treatments were initiated when the BSB trap catch was greater than 10 adults. At this point the tops of trees were cut to simulate top-working procedures (Hertz 1979). All branches except one were removed from the trees in all treatments (22 May 2018 and 30 May 2019).

Ethanol Injection

Ethanol injections were made a day after spring insecticide injections (23 May 2018 and 31 May 2019) to increase attraction of BSB adults, according to Reding et al (2013). Ethanol was injected a second time when BSB attacks plateaued approximately 10-20 days after the first ethanol injection (1 Jun 2018 and 18 Jun 2019).

Ethanol injections were preformed using an Arborjet Tree IV with 2 portals placed on opposite sides as low as possible to the ground and staggered below the insecticide injection ports to reduce potential interactions between compounds.. Injected compounds move quickly up the xylem in the tree but rely on diffusion to move latterly (Acimovic et. al. 2014). Coslor et. al. (2019), observed less interaction of injected products with separate injection holes due to the limited lateral movement of the product, and the compartmentalization of the xylem pathways above injection ports. The same injection equipment from the insecticide injections was used to perform the ethanol injections.

The same process was used in the ethanol injections, the only difference was that two holes were drilled into the apple trunk instead of four. The holes were placed opposite sides of the tree and staggered below the injection ports so that each ethanol injection port was not directly below any injection port.

Field Evaluations

Weekly BSB evaluations were made for each tree. The trunk of each tree was inspected for evidence of BSB attacks. This was done by performing a 5 minute visual analysis of each trunk to record BSB entry holes. Holes that were found were circled with permanent marker as an indication for the next evaluation.

BSB Tube Evaluations

To evaluate the fate of BSB entries, we placed plastic centrifuge tubes over the holes to capture BSB emerging from holes (Figure 6.). Three to five holes were randomly selected on each tree to be sampled for BSB gallery success fifty days after the first BSB attacks occurred. Due to varying number of assessable BSB holes, we were unable to place an equal number of tubes on each tree. Plastic centrifuge tubes were coated with Tanglefoot™ (The Scotts Company LLC, Marysville, OH) on the inside and punctured with 3 small holes to allow for airflow. They were then glued to the tree over holes with super glue (Gorilla Glue®, The Gorilla Glue Company, Cincinnati, OH). Gaps between the tube and the bark where BSB might escape were covered with expanding glue (Gorilla Glue®, The Gorilla Glue Company, Cincinnati, OH). The tubes were removed and assessed for BSB two weeks after they were installed.

Figure 6. centrifuge tubes glued over BSB entry holes to capture BSB as they emerge.

Residue Sample Collection and Preparation

Apple leaf and trunk core samples were taken for all treatment plots 7 days after spring application timing for residue analysis. For the leaf samples, 40 leaves were randomly sampled from each replication (approximately 25 grams). For the upper and lower trunk core samples, a wood borer (Haglöf 25 cm Case Hardened Steel Bit, Haglöf Inc., Madison, MS) was used to extract a wood sample 10 centimeters above or below each insecticide injection port (approximately 5 grams).

Samples were weighed and held in 50 mL of dichloromethane (DCM) until processing. Samples were mixed with 4 g magnesium sulfate and 1 g sodium chloride and allowed to sit for 48 hours in a 4°C walk-in cooler.

For leaf samples, the DCM was then filtered through a funnel lined with filter paper and 10 grams of sodium sulfate to remove water and allowed to evaporate under a hood 4-12 hours.

For wood core samples, the samples were sonicated inside the jars before replicating the filtering and evaporation process done with the leaf samples.

Next, we added 2 mL acetonitrile to the evaporated jars and swirled for 90 seconds to ensure maximum uptake of the dried pesticide residue. The acetonitrile solution was then analyzed on a HPLC utilizing a previously reported method (Bayer 1998; Wise et al. 2006).

Residue Sample Analysis

The residue levels were quantified using a waters 2695 separator module HPLC equipped with a Waters MicroMass ZQ mass spectrometer detector (Waters, Milford, MA), and a C18 reversed phase column (50 by 3.0mm bore, 3.5 um particle size (Waters, Milford, MA).

The mobile phase, solvent A, was water with 0.1% formic acid, and solvent B was acetonitrile with 0.1% formic acid, and was initially held at 80% solvent A and 20% solvent B and followed by a gradient to 20% Solvent A and 80% Solvent B and returned to initial conditions at the end of the run. Column temperature was 40ºC.

Monitored ions for emamectin benzoate were 886.6, and 158.2 m/z (Da). The HPLC level of quantification was 0.149 mg kg -1 of a.i., and level of detection was 0.045 mg kg -1. By using above described extraction method, mean parent compound recovery from four apple leaf samples (each 100 g) treated only with standard imidacloprid solution (0.046 mg kg -1), then agitated and left to dry, was 73% (level of detection 0.009 mg kg -1). The results have not been corrected emamectin benzoate recovery.

Monitored ions for azadirachtin were 685.4, and 703.4 m/z (Da). The HPLC level of quantification was 0.186 mg kg -1 of a.i., and level of detection was 0.056 mg kg -1. By using above described extraction method, mean parent compound recovery from four apple leaf samples (each 100 g) treated only with standard imidacloprid solution (0.046 mg kg -1), then

agitated and left to dry, was 73% (level of detection 0.009 mg kg -1). The results have not been corrected for azadirachtin recovery.

Statistical Analysis

Field Data Analysis

The BSB evaluation data were analyzed with a repeated measures analysis as a two-way RCBD using PROC MIXED procedure in SAS 9.4 (SAS Institute, Cary, NC, 2013). The following statistical model was fitted to the data:

$$BSB_{ijk} = \mu + Block_j + Trt_i + \varepsilon_{1ij} + Time_k + (Trt_i * Time_k) + \varepsilon_{2ijk}$$

$$i=1,2,3,4,5 \qquad j=1,2,3,4 \qquad k=1,2,3,4,5,6$$

The degrees of freedom for the model components were equal to 1 for the grand mean (μ); 4-1=3 for $Block_j$; 5-1=4 for Trt_i; 3*4=12 for the residual ε_{1ij}; 2-1=1 for $Time_k$; 4*1=4 for (Trt_i*$Time_k$); and 120-(1+3+4+12+1+4)=95 for the residual ε_{2ijk}.

The normality assumption was assessed by checking the normal probability plot and histogram of the residuals. Normality was corrected by square root transforming the data. The equal variance assumption was assessed by checking the plot of residual v. predicted values, side by side box plot, and Levene's test. It was necessary to fit a variance-covariance structure with unequal variances.

When treatments were significant, all pairwise comparisons among the treatment means were analyzed. PROC GLIMMIX was run to generate BSB least-squares means for treatment by evaluation day sliced by treatment and adjusted for Tukey-Kramer honestly significant difference ($p \leq 0.05$).

BSB Tube Data Analysis

The BSB tube data were analyzed as unbalanced subsamples using PROC MIXED procedure in SAS 9.4 (SAS Institute, Cary, NC, 2013). The error terms for testing treatment effect were adjusted to a composite mean square to reflect the unbalance.

Residue Analysis

The residue data was analyzed using a two sided t-test comparing residue from spring samples to residue from fall samples. The normality assumption was assessed using PROC UNIVARIATE procedures in SAS 9.4 (SAS Institute, Cary, NC, 2013) after data was square root transformed. T-tests were performed using PROC TTEST.

Results

Field Evaluations

The overall treatment effect for mean BSB in 2018 was significant (F=3.61, Num df=4, Den df=15, P = 0.0298). Differences of treatment by evaluation day LS means sliced by treatment indicated a total of 3 treatment means were significantly different than the untreated check following the first application of ethanol (Figure 7A).

The fall injection of emamectin benzoate significantly reduced the number of BSB attacks after the first ethanol injection in 2018. Fall injected emamectin benzoate had significantly lower BSB attacks than the untreated (P=0.0215), as well as spring injected emamectin benzoate (P=0.0214). Azadirachtin significantly reduced the number of BSB attacks in the spring treatment (P=0.0119). Azadirachtin injected in the fall had lower mean BSB attacks when compared to the untreated, however it was not significant. After the second ethanol injection there were no treatments that were significantly different from the untreated (Figure 7B).

The overall treatment effect for mean BSB in 2019 was not significant (F=0.33, Num df=4, Den df=15, P = 0.8511) (Figure 8).

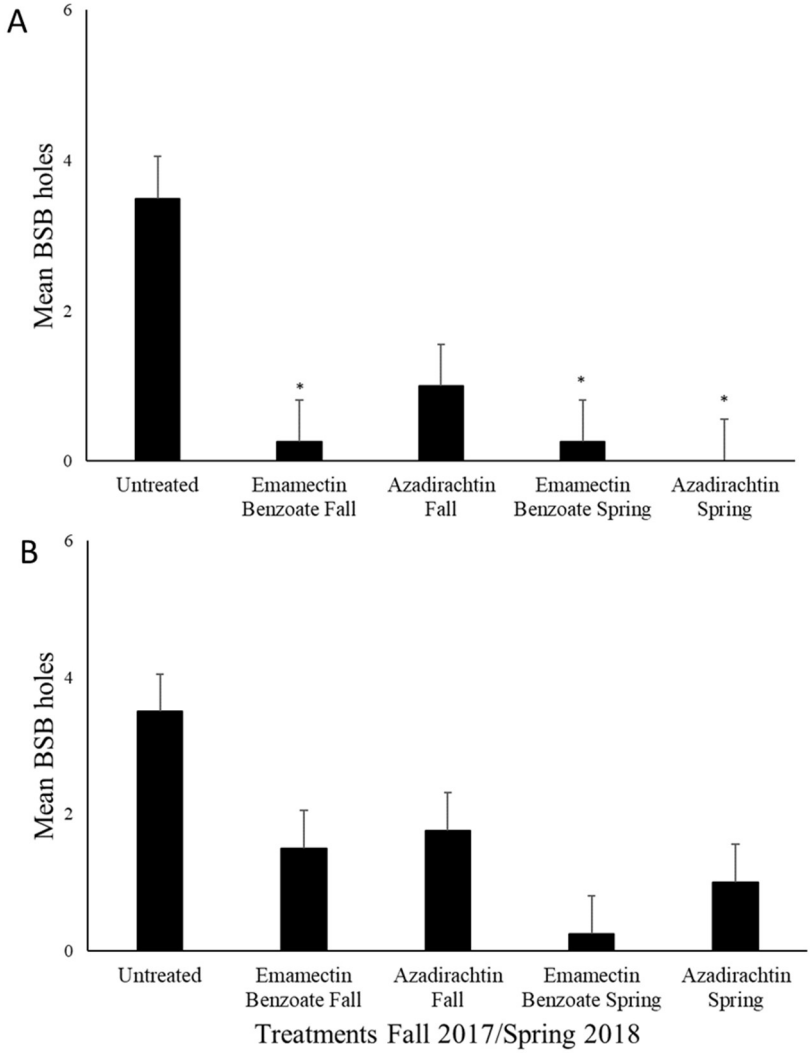

Figure 7. Mean number of BSB entry holes per treatment for the Fall 2017/Spring 2018 treatment evaluations after the first ethanol injection 23-May (A) and after the second ethanol injection 23-Jun (B). Values with * above them represent a significant difference ($\alpha \leq 0.05$) between the untreated and the treatment for evaluation date.

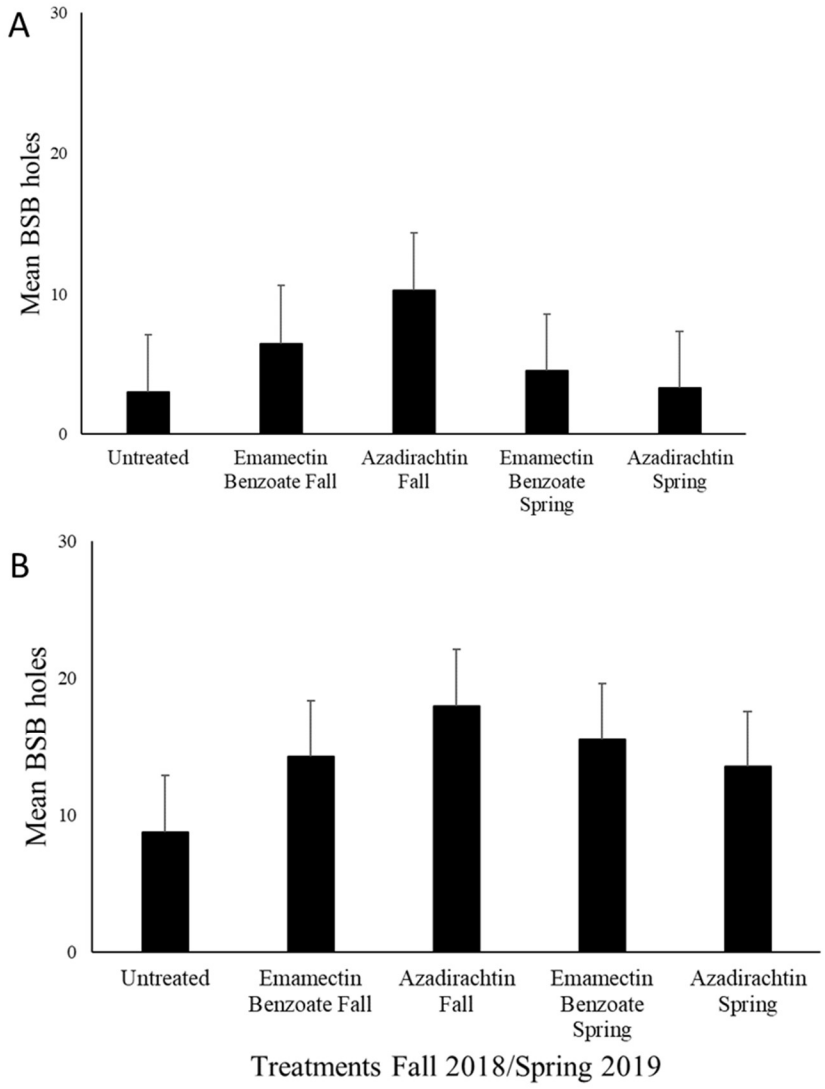

Figure 8. Mean number of BSB entry holes per treatment for the Fall 2018/Spring 2019 treatment evaluations after the first ethanol injection 31-May (A), and after the second ethanol injection 18-Jun (B).

Ethanol Traps

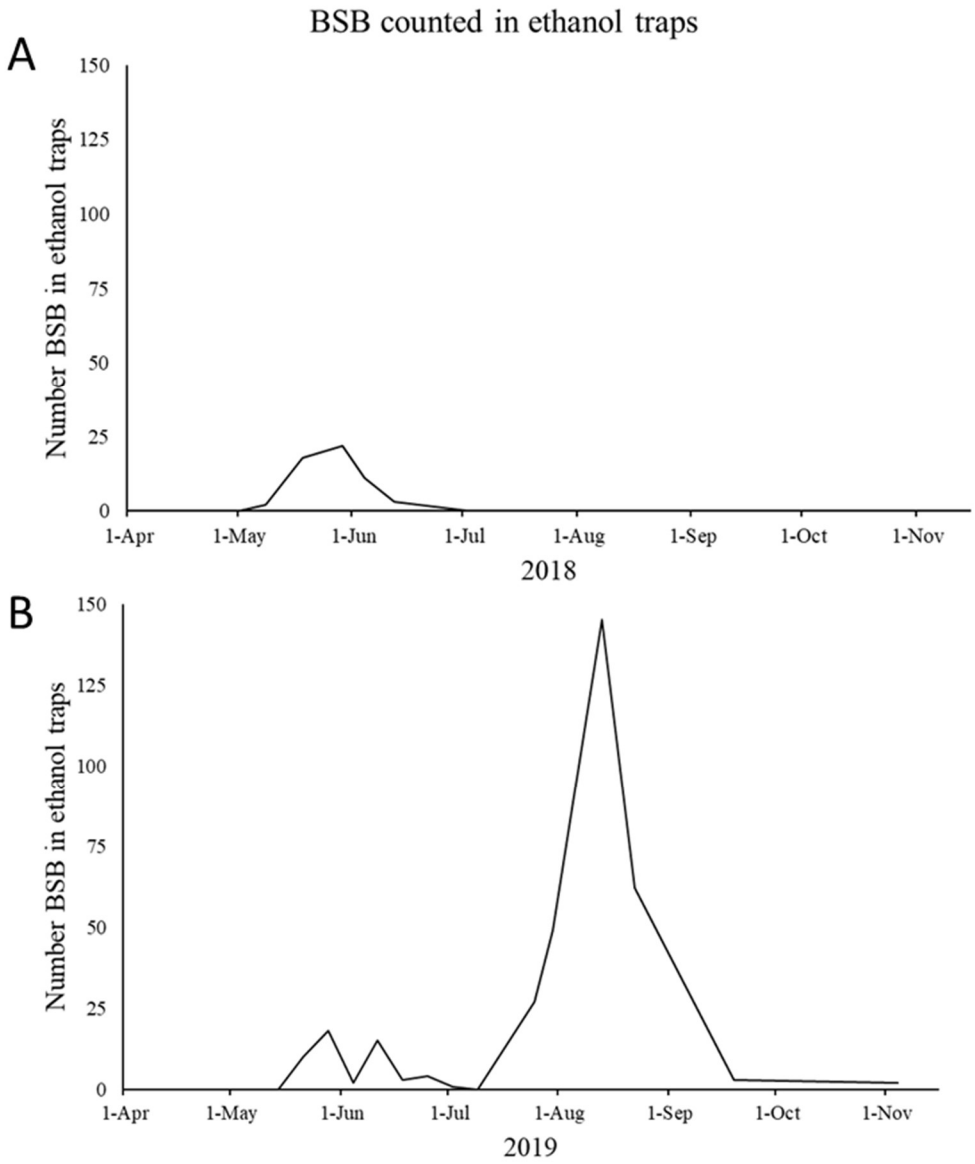

Figure 9. Total number of BSB in ethanol traps in the 2018 (A) and 2019 (B) seasons.

Local BSB adult populations were monitored during 2018 and 2019 using ethanol traps. The number of BSB counted in 2018 (Figure 9A), was comparable to 2019 (Figure 9B) early season (mid-May to mid-June). However, in mid-August there was a large emergence of BSB that was not seen in 2018 trap data, with upwards of 130 BSB caught in both traps.

BSB Tube Evaluations

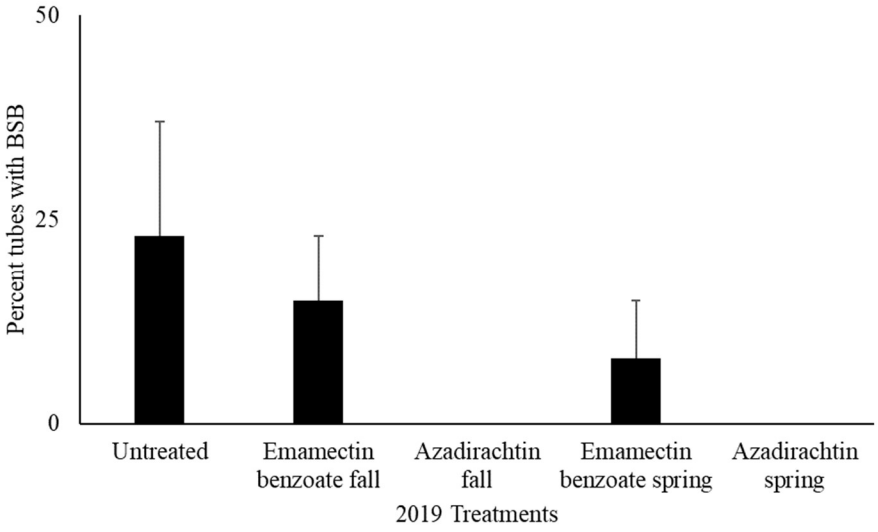

Figure 10. Mean percent tubes-covered galleries with emergent BSB.

The overall treatment effect for the subsample tubes was not significant (Num DF=4, Den DF=8.17, F=1.08, P=0.4281). The untreated check had 23% of tubes with BSB. Emamectin benzoate fall and spring treatments had 15% and 8% of tubes with BSB respectively. Azadirachtin treatments had no tubes with BSB (Figure 10).

Residue Samples

Overall in 2018, emamectin benzoate had no significant differences between fall and spring treatments (t(22)=-1.72, p=0.1000). Leaf samples taken 224 DAT and 7 DAT for fall treatments and spring treatments in 2018 had no detectable residue. Overall in 2019, emamectin benzoate had no significant differences between residue levels in fall and spring treatments (t(19.018)=-0.94, p=0.3596)(Figure 11).

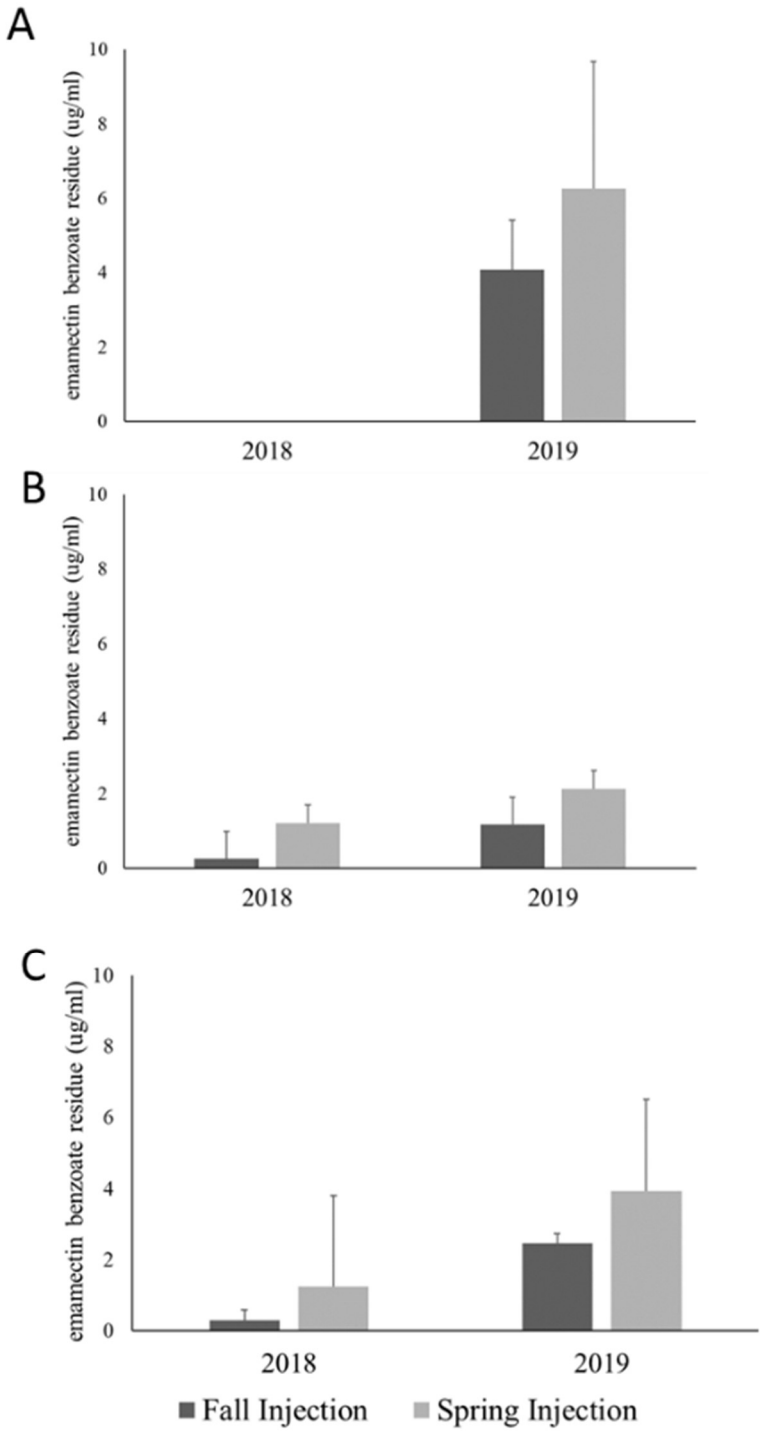

Figure 11. Residue levels for emamectin benzoate treatments. Mean residue levels for leaf samples (A), upper trunk core samples (B), and lower trunk core samples (C). Residue is presented as ppm (ug/ml) above the level of detection (LOD)(emamectin benzoate LOD_{leaves}=0.045ppm, LOD_{wood}=0.045ppm). No emamectin benzoate residue was detected in 2018 leaf samples. Fall Injection residue samples were taken 224 and 232 days after treatment for 2018 and 2019 respectively. Spring injection residue samples were taken 7 days after treatment in 2018 and 2019.

Overall in 2018, azadirachtin had higher residue levels detected in spring injection treatment samples than in fall injection treatment samples $t(17.425)=-6.60$, $p<0$. Leaf, upper core, and lower core all had significantly higher residue detected in spring injection samples than fall injected samples (Leaves: $t(5.6168)=-5.68$, $p=0.0016$, Upper Core: $t(6)=-7.43$, $p=0.0003$, and Lower Core: $t(5.4734)=-3.87$, $p=0.0098$) (Figure 12).

Overall in 2019, azadirachtin had higher residue levels detected in spring injection treatment samples than in fall injection treatment samples ($t(20.576)=-3.00$, $p=0.0069$). Leaf samples had higher residue in spring injection samples than fall injection samples $t(4.966)=-4.04$, $p=0.0100$. Upper and lower core samples had no significant differences in residue between spring and fall treatments (upper core: $t(5.8223)=-1.18$, $p=0.2840$, lower core:$t(6)=-1.25$, $p=0.2571$) (Figure 12).

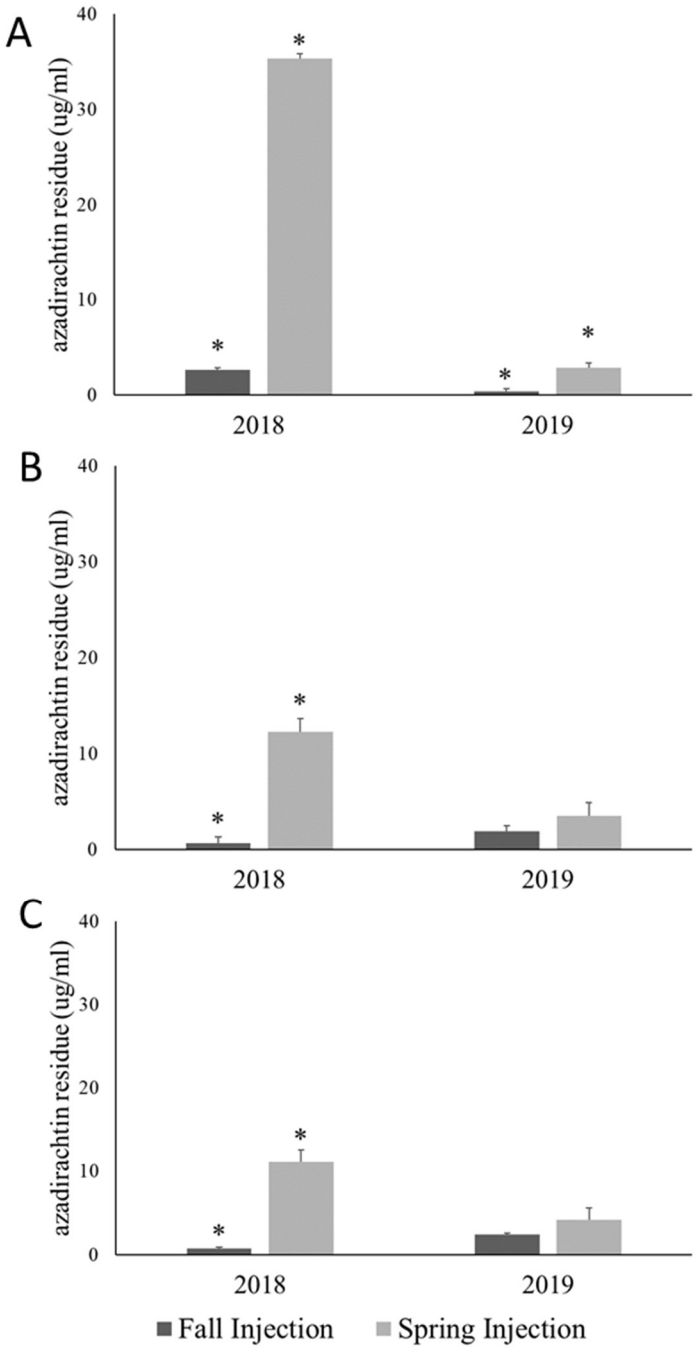

Figure 12. Residue levels for azadirachtin treatments. Mean residue levels for leaf samples (A), upper trunk core samples (B), and lower trunk core samples (C). Residue is presented as ppm (ug/ml) above the level of detection (LOD)(azadirachtin LOD_{leaves}=0.056ppm, LOD_{wood}=0.056ppm). Fall Injection residue samples were taken 224 and 232 days after treatment for 2018 and 2019 respectively. Spring injection residue samples were taken 7 days after treatment in 2018 and 2019. Bars with * represent a significant difference between the fall treatment residue and spring treatment residue samples for that corresponding year.

Discussion

This study contributes new information on the potential for using trunk injection to control of BSB in apple trees. The 2018 study shows evidence that both emamectin benzoate and azadirachtin injections can reduce BSB infestations. Our study suggests that the timing of the injection influences the outcome in terms of protecting apple trees from BSB, with spring injected azadirachtin being more effective than fall injections (Figure 6A). This study suggests that emamectin benzoate likely affects adults directly by reducing successful attacks/entries, while and azadirachtin appears to reduce BSB attacks and limits gallery success (Figure 8).

In the 2018 season, emamectin benzoate reduced the number of BSB attacks most effectively after the first application of ethanol. Azadirachtin also reduced the number of BSB attacks in the spring treatments after the first application of ethanol. This shows that both compounds have the potential for good control of BSB attacks when attacks are within 7 days of injection. After the second injection of ethanol, there were no significant differences in BSB attacks between the treatments and the untreated. It is worth noting that the untreated check did not experience further BSB attacks after the second ethanol treatment, while fall treatments and spring azadirachtin treatment did experience a rise in BSB attacks. There was no residue data collected after the second ethanol injection, thus it is not clear if concentrations of the two compounds diminished rapidly after 7 days. We did not conduct a tube evaluation in 2018 and it would have been interesting to see if the attacks after the 2nd ethanol injection resulted in any successful galleries.

These results were not repeated in the 2019 season, likely because of the unusually cold and wet spring. (include a brief figure or table to summarize weather data (GDD accumulation, mean summer temp/rainfall) This delayed the adult emergence and resulted in high levels of

BSB attacks late in the season and resulted in limiting the effectiveness of the injected compounds (Figure 6B). Reding et. al. (2010) had similar observations and noted that most BSB activity declined in periods of cool, wet weather, although it is unknown whether BSB are inactive or traps are less effective in those conditions.

Our focus was on reducing BSB attacks, but it is also important to investigate whether initiated galleries were successful. We found no successful galleries in azadirachtin injected trees and lower numbers of success in emamectin benzoate injected treatments as compared to the untreated check in our tube evaluations. While these data had no statistical significance, the numerical data is compelling and warrants further investigation.

Azadirachtin is an anti-feedant, repellant, and insect growth regulator (Karnavar 1987, Subrahmanyam 1990, US EPA 1993). Azadirachtin affects the morphogenesis, ovarian development, fecundity, egg viability and molting of BSB through the endocrine system (Karnavar, 1987). It also has fungicidal properties (Schmutterer 1990, Dubey and Kumar 2003). Neem oil was observed to significantly reduce germination in an entomopathogenic fungus (Aguda et. al 1986). It is likely a combination of these properties that impacts the BSB in azadirachtin injected treatments.

Emamectin benzoate belongs to avermectin group of insecticides. These compounds affect the nervous system in arthropods. They inhibit the neurotransmitters by permanently opening glutamate gated channels and allowing an influx of chloride ions into nerve cells, rendering the cells non-functional. Ultimately, invertebrates are paralyzed irreversibly and stop feeding. Avermectins are taken up by arthropods via contact and ingestion, although ingestion is the primary route (Ishaaya and Degheele 1998). It is also xylem mobile, making it a good candidate for trunk injection.

Emamectin benzoate has been used successfully through trunk injection to control bark beetles in previous work. Fettig et. al. (2014) conducted a study controlling mountain pine beetle in lodgepole pine. Their data indicated that injections of emamectin benzoate applied in late summer or early fall will provide adequate levels of tree protection the following summer. McCullough et. al. (2011) used emamectin benzoate injections with great success to control emerald ash borer. Emerald ash borer density on trees treated with emamectin benzoate were less than 1% of control trees. They noted similar findings that injection treatments were equally effective a year later suggesting 2 years of effectiveness for one injection. It was also reported that emamectin benzoate induces mortality in adult beetles with little exposure and at low concentrations. Coslor et. al (2019) injected apple trees with emamectin benzoate, along with phosphorous acid, and resulted in significant leafroller control. Coslor et. al (2018), found that timing also effected availability of emamectin benzoate in leaves, nectar and pollen. Spring injections allowed for movement via transpiration, where fall injections did not until the following spring.

Emamectin benzoate is also metabolized and incorporated into plant products and might be metabolized at a faster rate than it can accumulate. (Burkhard et al. 2015). (Allen et al. 1997).

In conclusion, trunk injection of the two insecticides emamectin benzoate and azadirachtin have potential for controlling BSB in apple trees. Fall and spring injections of emamectin benzoate preformed equally well in the 2018 season after the first ethanol injection. Spring injected azadirachtin treatments were more effective in the 2018 season after the first ethanol injection than fall injected azadirachtin treatments. In the second year, attempts were made to assess the level of success of the initiated gallery holes. Interestingly, azadirachtin treatments had no successful galleries and emamectin treatments had numerically fewer

successful galleries than the untreated trees. Given that topworking of apple orchards is well-planned and high investment practice, the necessary economic investment of injecting trees to protect against BSB may be justified. Effort should be made in further studies to assess not only the number of BSB attacks, but also the success rate of the galleries. Further studies could also be done to determine if it is growth regulator or fungicidal properties that ultimately cause galleries to be unsuccessful.

APPENDICES

APPENDIX 1: 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: 2020-07

Author and Title of thesis:

CONTROL OF PEAR PSYLLA IN PEARS AND BLACK STEM BORER IN APPLES WITH TRUNK INJECTION

By:

Celeste Elizabeth Wheeler

Museum(s) where deposited:

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

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Psyllidae	*Cacopsylla pyricola*	Nymph	10	Alcohol
Psyllidae	*Cacopsylla pyricola*	Adult	10male,10female	Pinned/Pointed
Curculionidae	*Xylosandrus germanus*	Adult	10male	Pinned/Pointed

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