REFINING TRUNK INJECTION STRATEGIES FOR CONTROL OF FOLIAR INSECT PESTS AND DISEASE IN MICHIGAN APPLE ORCHARDS

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ABSTRACT

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OF FOLIAR INSECT PESTS AND DISEASE IN
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In conventional apple orchards, insect pests are managed with insecticides delivered to the canopy using airblast sprayers, which provide good canopy coverage. However, spraying results in significant product loss: as little as 26% is estimated to reach the tree canopy due to spray drift and less than 0.1% of insecticide ends up reaching the target pest. The remainder is lost to the environment with potential to harm people or non-target organisms. Trunk injection is a discriminating pesticide delivery system which reduces insecticide inputs and environmental exposure by delivering chemicals directly to the vascular system. It is commonly used to deliver pesticides in ornamental and shade trees. Recent work with trunk injection in apple orchards has shown promise, but more research must be done to determine efficacy and safety in tree fruit crops. In the following studies, we injected emamectin benzoate, imidacloprid, dinotefuran, spinosad, chlorantraniliprole, and abamectin into apple trees to expand the list of insecticides compatible with trunk injection. Nectar and pollen were sampled from trees to compare the effects of injection timing on insecticide concentration in floral resources. In addition, two fundamental injection tool types were compared: drill-based and needle-based. To test compatibility of combined insect and disease management, an insecticide and a fungicide were injected simultaneously. Finally, low-volume injections were performed on nursery apple trees, which normally require high pesticide inputs and do not produce fruit for several years. Emamectin benzoate, chlorantraniliprole and abamectin resulted in moderate to high mortality

and reduced feeding in *Choristoneura rosaceana* bioassays using leaves sampled from trunk injected apple trees. Neonicotinoids reduced *Empoasca fabae* density in field evaluations, and also showed activity on *C. rosaceana* at higher concentrations. Spinosad was not well-transported within the apple tree vascular system. Numbers of *E. fabae* nymphs were lower on trees injected with imidacloprid using a drill-based tool compared with untreated trees in all years, despite a trend of initially higher foliar concentrations with the needle-based tool. This demonstrated that delivery method is an important factor in effective trunk injection based apple management. We found that when an insecticide and a fungicide are injected, they can interact dynamically within the vascular system of a tree. Injections of emamectin benzoate followed by phosphorous acid into the same set of injection ports resulted in higher mortality of *C. rosaceana* larvae and lower incidence of apple scab compared with untreated trees. This has important implications for expanding the utility of trunk injection for fruit tree management. Nursery tree injections were most effective when emamectin benzoate was injected into the trunk versus the taproot. A rate equivalent to 1/8 the rate used for mature tree injection reduced insect pests more than a 1/80 rate. The higher rate of emamectin benzoate was also persistent in the following year. Imidacloprid and emamectin benzoate were injected in the spring and fall, and nectar and pollen were sampled the following spring. Imidacloprid was not detected in nectar or pollen when injected in the previous spring. Conversely, emamectin benzoate was detected when injected in the previous spring, but was not detected in nectar or pollen when injected in the fall. This study expanded the list of insecticides compatible with trunk injection, demonstrated novel uses of trunk injection to reduce insect pests in apple trees, and introduced possible ways to mitigate accumulation of insecticides in nectar and pollen.

In girum imus nocte ecce et consumimur igni.

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KEY TO ABBREVIATIONS

a.i. – active ingredient

cv. – cultivar

DAT – days after treatment

df – degrees of freedom

ema – emamectin benzoate

phos – phosphorous acid

ppm – parts per million

ppb – parts per billion

RCBD – randomized complete block design

SAR – systemic acquired resistance

s.e. – standard error

UTC – untreated check

CHAPTER 1: LITERATURE REVIEW

Introduction

A major goal of integrated pest management is to optimize pest control and minimize pesticide use (Cross and Berrie 2009). However, reliance on broad spectrum insecticides continues, and large quantities of these materials are applied in agricultural systems worldwide (Devine and Furlong 2007). In apple tree (*Malus pumila* Mill.) (Mabberley et al. 2001) orchards, the most common pesticide application tools are tractor-pulled axial fan air-assist (airblast) sprayers. These sprayers provide good coverage but are susceptible to drift, and estimated to deliver 60-65% of material to apple tree canopies while a third is lost to the environment (Steiner 1969). In fact, coverage may be lower. McArtney and Obermiller (2008) found that only 26% - 38% of total spray volume reaches the canopy depending on wind speed, nozzle configuration, and row spacing. Using nozzles that produce larger droplets, matching water volume to sprayer type, and applying under ideal weather conditions can improve pesticide deposition and help reduce drift (Fox et al. 2008, Wise et al. 2010).

Though high canopy coverage may be achieved with airblast sprayers, far less material is actually needed to manage pests. It is estimated that only 0.1% of pesticides applied in the United States reach their target pests, while the remainder contaminates the environment (Pimentel and Levitan 1986, Pimentel 1995). This is damaging to the ecosystem and is a high cost to growers (Devine and Furlong 2007). Apple production is highly valuable in Michigan, and ranks third nationwide (NASS 2014). There is a need for pest management tools which minimize environmental impacts and reduce costs to growers by reducing pesticide inputs.

Trunk injection uses low volumes of pesticides in a discriminating delivery system,

which targets foliar pests and, minimizes pesticide losses and environmental impacts, and has low capital investment (VanWoerkom et al. 2014, Wise et al. 2014). Holes are made through the bark of trees in order to access the xylem, and formulated systemic chemicals are delivered to the vascular system. Uptake of chemicals can be passive and rely on transpiration, or pressurized to deliver high volumes (Wilson 1978). When performing trunk injections, the physiology of the tree must be taken into account, along with the physical properties of the chemicals to be injected. Whether pests are direct or indirect feeders, tree physiology, and pesticide chemistry will all determine if trunk injection is successful. This chapter will provide an overview of the woody plant vascular system, the factors influencing movement of introduced chemicals in plants, and trunk injection in apple trees.

Xylem and Phloem of Woody Plants

In vascular plants, water is transported from soil via the roots, to the leaves where it is used during photosynthesis along with carbon dioxide and sunlight (Pallardy 2007). Modern agricultural technologies have been able to take advantage of the vascular systems of plants through the use of systemic chemicals: fertilizers and pesticides which are distributed throughout the plant. The vascular system is composed of two major parts: xylem and phloem. As the plant grows, the apical meristems produce primary xylem and phloem. However, in woody plants most vascular tissue comes from secondary growth. The cambium produces secondary xylem and secondary phloem which increases the diameter of the stem (Pallardy 2007). Xylem primarily carries water from the roots to the leaves. Phloem carries nutrients from sources such as photosynthesizing leaves or roots as plants come out of dormancy, to sinks, which are storage organs, including roots, developing fruits, and seeds.

Xylem

Xylem tissues vary in structure and arrangement between angiosperms and gymnosperms. Angiosperms can be ring-porous, diffuse-porous, or semi-diffuse. In ring-porous species, the vessels grow larger in the beginning of the season, getting progressively smaller toward the end. Because of the larger vessel size, ring-porous species are more susceptible to embolism or cavitation and freezing damage. They must also rely on the outermost annual ring for sap flow. Diffuse-porous species, such as apple trees, have relatively uniform xylem vessels, and these species use multiple annual growth rings of xylem for sap flow (Anfodillo et al. 1993, Pallardy 2007).

In gymnosperms, xylem tissue is much simpler and consists of tracheid cells, ray parenchyma, and resin canals. Tracheids are vertically oriented, elongated, dead lignified cells with pits to connect them to adjacent tracheids. Overlapping tracheids form a continuous path for water to flow upward. Also present are rays, which include narrow rays and fusiform rays, which can surround resin canals. Angiosperms have both tracheids and vessel elements, as well as fibers and rays. The presence of vessel elements distinguishes hardwoods (with vessel elements) from softwoods (without vessel elements). The primary difference between vessel elements and tracheids is that they are much larger in diameter and have sieve plates in addition to pits, which connect cells end to end instead of to adjacent cells, though pits are still present (Pallardy 2007).

As xylem cells reach maturity, they die and become empty tracheids through which water travels. Instead of an active process, xylem works passively through a system of transpirational pull known as cohesion-tension theory (Dixon and Joly 1894). The entire cycle of moving water, solutes, and energy through the environment and plant is known as the soil-plant-atmosphere

continuum (Philip 1966). Water moves along a potential from highest to lowest, starting with high soil moisture, moving into the roots and through the xylem to the leaves, and ending with lower potential atmospheric humidity. Negative pressure is the tension or water potential gradient which enables the xylem to pull water up from the roots to the leaves (Norman and Anderson 2005). Most water is lost through the stomata during transpiration, and the evaporation creates a negative pressure which pulls water upward. Capillary action and the cohesion of water molecules maintain negative pressure created by evaporation at the leaf surface. Water flow in plants is affected by tension in soil and the humidity in the air. Sufficient dryness in the soil creates soil tension, which can impede root uptake. Dryness in the air will cause the plant to close its stomata to prevent excessive water loss. High temperatures will also cause plants to close their stomata (Pallardy 2007).

Root pressure also contributes to xylem movement, though to a lesser degree than transpiration. Because there is a higher concentration of solutes in the root tissue than in the surrounding soil, water enters roots across an osmotic gradient. This is typically described in terms of movement from high to low potential (Norman and Anderson 2005). As more water enters the roots it creates a positive water pressure. Thus lower potential from the leaves and higher potential from the roots drives xylem flow. At some times, such as in the early morning root pressure can be high enough to cause guttation, where droplets of sap are exuded on the leaves of plants. This occurs in non-woody plants such as grasses when soil moisture and humidity in the atmosphere are both high, and root pressure is increased due to a lower water potential than the surrounding soil (Sławiński and Sobczuk 2011).

Phloem

Phloem tissues in gymnosperms are made up of sieve cells. Angiosperms have much more derived tissues. These include sieve tube elements with sieve plates connecting them end to end, and companion cells to support them, as these phloem cells possess virtually no organelles of their own. Phloem translocation utilizes living cells, and is an active process. This means energy must be expended to drive it. In the leaf, xylem and phloem are paired together in the vein. As sugars are produced in the mesophyll by photosynthesis, they are actively pumped out to the phloem for transport. In general, chemicals actively moved into the phloem must be endogenous, i.e. naturally present in the plant, such as sugars and amino acids, for there to be a corresponding chemical pump (Denis and Delrot 1993, Sur and Stork 2003). A small amount of energy must be spent for active transport to take place. The phloem is first loaded with a high concentration of sugars. Water from adjacent xylem moves into the phloem via osmosis. This is known as the Pressure Flow Hypothesis (Münch 1930, Chaney 1978). Sugars then move along a gradient from sources to sinks. When they reach their destinations, sugars are often converted to starches for storage. This renders them insoluble in water and stops them from being further translocated. As sugars are converted to starch the osmotic pressure decreases, and water moves back into the xylem.

Properties Affecting Movement of Chemicals in Vascular Tissue

Successful systemic chemicals are highly soluble, stable within and biocompatible with the plant, effective against their targets, and highly mobile inside the plant (Chaney 1978). Rate of chemical movement is dependent primarily on xylem flow, which is determined by rate of transpiration (Aajoud et al. 2006). However, mobility of chemicals are also determined by their

individual chemistries. The Transpiration Stream Concentration Factor (TSCF) is a value introduced by Briggs et al. (1982) that defines the efficiency of translocation through xylem from roots to shoots by its concentration in the transpiration stream divided by its concentration in an external solution. Two properties are essential to understanding how a molecule will behave in a plant: its log octanol/water-partition coefficient (log K_{ow}) and its log acid dissociation constant (pK_a) (Sur and Stork 2003, Bonmatin et al. 2015). Log K_{ow} describes the molecule's solubility in water versus its solubility in organic solvents. A lower log K_{ow} means a molecule has a higher solubility in water and is more able to move across membranes. Systemic pesticides such as imidacloprid (log K_{ow} = 0.51) and dinotefuran (log K_{ow} = -0.549) are highly water soluble, therefore they are highly mobile in xylem (Sur and Stork 2003, EPA 2004). In apple trees for example, imidacloprid is distributed quickly and uniformly when trunk injected (Aćimović et al. 2014).

The pK_a describes how strong or weak an acid is; chemicals with a high pK_a are weaker acids. Phloem has a pH that is slightly basic (around 8) while xylem has a more acidic pH (around 5). The pH difference between xylem and phloem means chemicals that are strong acids (low pK_a) will tend to prefer entering phloem, while weak acids will not exhibit a preference (Sur and Stork 2003). Thus chemicals with a high pK_a will not prefer one over the other. Imidacloprid is non-ionized and thus is not restricted from moving between xylem and phloem, though it tends to be more concentrated in xylem because of higher water flow and because it is not actively transported into phloem (Sur and Stork 2003). The organic carbon-water partition coefficient (K_{oc}) describes how much the molecules adhere to the plant tissue while moving through the xylem. Movement will be impeded for chemicals with higher K_{oc} values.

Not all pesticides are exclusively xylem mobile. Spirotetramat, glyphosate, paraquat, and

some experimental insecticide-glucose conjugates such as IPGN, are reported to be phloem mobile (Hart et al. 1992, Denis and Delrot 1993, Nauen et al. 2008, Lei et al. 2014). Phloem mobile pesticides must be carrier-mediated in addition to meeting other chemical requirements, to overcome the much higher rate of xylem flow. Phloem mobile pesticides such as glyphosate and paraquat are relatively small and hydrophilic, and they also both possess endogenous compounds that allow carrier-mediated phloem loading. Glyphosate has a phosphonate group which is phloem loaded by a phosphate transporter (Denis and Delrot 1993). Paraquat can be transported by a polyamine carrier system in the phloem (Hart et al. 1992). IPGN is a relatively large hydrophobic pesticide, which might indicate that it would have difficulty moving into the phloem. However it has a glucose group attached to it, which is an endogenous compound that allows it to be actively loaded into the phloem (Lei et al. 2014). Therefore, when predicting how pesticides will move through a plant's vascular system, it is important to consider its relative size, its solubility, log K_{ow}, pK_a, and K_{oc} values, as well as any groups on the molecule that might be able to take advantage of carrier systems transporting endogenous chemicals into the phloem.

It is important to be aware of how injection chemicals and methods may post risks to pollinators due to presence in nectar and pollen. Some insecticides including emamectin benzoate may persist in trees for multiple years (Smitley, Doccola, et al. 2010, McCullough et al. 2011), though little data exists for concentrations of insecticides in nectar and pollen in the year following trunk injections (Smitley et al. 2016). In whole-flower samples of injected apple trees, concentrations of emamectin benzoate (0.0015 μg/g) and chlorantraniliprole (0.1117 μg/g) were recovered (VanWoerkom et al. 2014). No imidacloprid was recovered from whole-flower samples, however separate concentrations of trunk injected insecticides present in nectar and pollen are not yet known.

History and Current State of Trunk Injection

The earliest use of trunk injection is attributed to Leonardo da Vinci, who recommended trunk injections of arsenic to prevent people from stealing tree fruits (Wilson 1978, Perry et al. 1991). (Ethics aside, how da Vinci proposed to render fruit safe for later consumption is unclear). More recently in the early 20th century, trunk injected silvicides were in use to control unwanted trees and brush, and eradicate trees infected with Dutch elm disease (Wilson 1978). Trunk injection has recently been employed as a control method for the invasive beetle *Agrilus planipennis*, which infests targets ash trees (*Fraxinius* spp.) (Smitley, Doccola, et al. 2010, Doccola et al. 2011, McCullough et al. 2011, Tanis et al. 2012, Herms et al. 2014). This pest was introduced to Michigan in 2008 and has since become widely established in North America. Trunk injections of emamectin benzoate have been found effective at controlling *A. planipennis* for at least two years (Smitley, Doccola, et al. 2010). Injections resulted in minimal health impacts and trees were able to compartmentalize injection sites which prevented infection and allowed for wound closure (Doccola et al. 2011).

The method of injection can also be a critical factor, and several companies have developed trunk injection tools. Most tools rely on either a drill or needle for injection. Mauget capsules (JJ Mauget Company Inc, Arcadia, CA) also use drilled holes to inject with self-contained micro-infusion capsules. A feeder tube is inserted into the injection hole after drilling. The capsule is compressed, connected to the feeding tube, and hammered into the tree to activate. Product is drawn into the vascular system over time. Capsules are available in premeasured doses of insecticides, fertilizers, fungicides and antibiotics. ACECAP / MEDICAP / PHOSCAP (Creative Sales Inc., Freemont, NE) are dry formulated chemicals contained inside

gelatin capsules and reinforced inside a plastic housing. A hole is drilled and the entire unit is tapped into the trunk. The gelatin capsule dissolves and administers a premeasured dose of insecticide, fungicide, or fertilizer. The QUIK-jet (Arborjet, Woburn, MA) uses a hand-pumped syringe with a one-way valve leading to a pesticide tank. Four to eight holes are drilled into the tree trunk to a 2" depth, and a plastic plug is tapped into each hole. The plug has a silicone septum to prevent leaking. The syringe needle is inserted into the plugs and the insecticide dose is injected using the plunger. Over time the cambium layer heals over the hole and the plug. Other injection tools by Arborjet use various methods of pressure injection, which all use a similar plug inserted into a drilled hole for injection.

The Wedgle Direct-Inject System (ArborSystems, Omaha, NE) injects into the cambium layer instead of the xylem. A punch is used to make a ¼" hole, and a plastic septum is inserted. The injection tool has a handle on either side, a port for a proprietary premixed pesticide pouch, and a needle in the front. The needle is inserted into the trunk and the handles are squeezed multiple times to inject the correct dose. An adapter is included to allow non-ArborSystems pesticide bottles. The Blade for Infusion in TrEes (BITE, Bite Injection Systems, University of Padova, Italy) has a lenticular blade with vertical ports, designed to inject between wood fibers. A brass hammer is attached to the tool and is used to insert the blade into the trunk. A rubber washer compresses around the blade as it is hammered in, to prevent leaking. A disposable syringe with a standard Luer-type slip tip is loaded with the pesticide dose and inserted into the tool. Depressing the plunger injects the pesticide through the blade into the tree. In apple trees, blade type injection ports were found to heal faster than drilled and capped ports (Aćimović, Cregg, et al. 2016).

Trunk injections have been used to control indirect pests and diseases in fruit bearing

trees, including avocado (Byrne et al. 2012, 2014), date palm (Salem et al. 2014), and apple (Aćimović et al. 2014, 2015, VanWoerkom et al. 2014, Wise et al. 2014, Aćimović, VanWoerkom, et al. 2016). The avocado thrips *Scirtothrips perseae* was controlled with trunk injections of imidacloprid, dinotefuran, and acephate (Byrne et al. 2012). Timing avocado tree injections to coincide with mid and late leaf flush greatly improved uptake of the neonicotinoids (Byrne et al. 2014). Acephate residues were found in avocado fruit, though this was not the case for neonicotinoid residues. Several conventional and biorational insecticides were injected into date palm in lab and field settings, with Lannate showing good control of the red palm weevil (*Rhynchophorus ferrigineus*) and best recovery of palms from injury (Salem et al. 2014). Apple tree injections of imidacloprid, emamectin benzoate, and chlorantraniliprole control indirect pests like *Empoasca fabae* as well as indirect/direct pests such as *Choristoneura rosaceana* and *Grapholita molesta* which begin on leaves and switch to fruit later in development (VanWoerkom et al. 2014, Wise et al. 2014). The fungicides propiconazole, penthiopyad, cyprodinil+difenoconazole, and phosphorous acid were able to control apple scab, with phosphorous acid outperforming the others (VanWoerkom et al. 2014, Aćimović, VanWoerkom, et al. 2016). Fire blight was controlled using injections of phosphorous acid and acinbenzolar-*S*-methyl, both systemic acquired resistance pesticides, which activated key defense proteins in the apple trees. The antibiotic oxytetracycline was also able to control fire blight (Aćimović et al. 2015).

Pesticide Movement into Nectar

Because nectar is derived from phloem and not xylem, systemic insecticides that are not phloem loaded such as imidacloprid are less likely to be found in significant concentrations (De

la Barrera and Nobel 2004). Despite this, neonicotinoids and other xylem mobile pesticides may be found in phloem under certain conditions. They are able to move across biomembranes from xylem to phloem, and in fact can be present in sufficient quantities to control some phloem-feeding pests (Sur and Stork 2003, Bonmatin et al. 2015). Fipronil has also been found in the inflorescences of sunflowers (Aajoud et al. 2008). Therefore, pesticide movement in plants must be evaluated on a case by case basis.

Because there are multiple routes of exposure for honey bees and other pollinators, assessing risk can be difficult. Aside from pollen and nectar, guttation can be a major source of insecticide exposure because it is supplied by the xylem, whereas nectar and pollen are phloem-derived. Assessment of pollinator safety considers multiple forms of exposure, including contact, inhalation, and ingestion (Bonmatin et al. 2015). When trunk injecting fruit trees, risk of exposure through the pollen and nectar must be carefully considered.

Conclusion

Insecticides move through plant vascular systems according to specific chemistries. Knowing the chemical's properties such as solubility and pK_a will help predict how it will behave once introduced into a tree. In addition to predicting the systemicity of chemicals and to what organs they will be delivered, it is important to consider whether there will be potential for pollinator impacts. Xylem-mobile insecticides may not be actively loaded, but could passively cross into phloem in small amounts. In addition, insecticide residue may be present in guttation, though this is more of a concern in grasses and herbaceous plants and less so in larger woody plants. After identifying potential systemic chemicals, they should be evaluated on a case by case basis for movement and potential routes of exposure to the environment.

The following chapters address several key knowledge gaps in regards to trunk injection in apple trees. First, multiple formulated insecticides were tested in order to potentially expand the list of active ingredients effective for apple tree trunk injection. Nectar and pollen samples were collected separately to assess potential pollinator risks and effects due to injection date. The next chapter compared a drill based trunk injection tool with a needle based injection tool. Following that is a study which combined insecticide and fungicide trunk injections in trees. Finally, newly established non-bearing apple trees were injected with insecticides in a study which evaluated their performance for multiple seasons.

CHAPTER 2: EFFICACY OF TRUNK INJECTED INSECTICIDES IN MICHIGAN APPLE ORCHARDS

Abstract

Trunk injection is well established for delivering pesticides in ornamental and shade trees, but less research has been done to determine efficacy and safety in tree fruit crops. Apple trees were injected with the insecticides emamectin benzoate, imidacloprid, dinotefuran, spinosad, chlorantraniliprole, and abamectin. Additionally, emamectin benzoate and imidacloprid were injected in apple trees in the spring and fall. Nectar and pollen were sampled during bloom in the following spring to compare seasonal timing on insecticide accumulation to flower parts. Neonicotinoids reduced *Empoasca fabae* density in field evaluations. Emamectin benzoate, chlorantraniliprole and abamectin resulted in moderate to high mortality and reduced feeding in *Choristoneura rosaceana* bioassays using leaves sampled from trunk injected apple trees. Imidacloprid residues were below 25 ppm in fruit before harvest and within EPA tolerances. Spinosad was found not to be well transported within the apple tree vascular system. Imidacloprid was not detected in nectar or pollen when injected in the spring, and detected at 0.39 ppb when injected in the previous fall. Emamectin benzoate was not detected in nectar or pollen when injected in the fall, and detected at 7.36 ppb and 1.15 ppb in the nectar and pollen, respectively, when injected in the previous spring. By testing additional insecticides we have helped to refine the list of trunk injectable pesticides. We have also broadened our understanding of what chemical properties contribute to making a pesticide mobile in woody plants, and possible ways to mitigate accumulation of insecticides in nectar and pollen.

Introduction

Apple production is an important industry in Michigan, totaling $288,748,000 in 2014 revenue and ranking third nationwide (NASS 2014). A goal of apple integrated pest management is to reduce insecticide inputs while maintaining yield and quality. Typically, airblast sprayers are used in apple production systems to deliver pesticides in developed countries (Devine and Furlong 2007, McArtney and Obermiller 2008, VanWoerkom et al. 2014). However, studies show that much of the spray solution is lost to the environment, with only 29-56% of the product reaching the tree canopy and less than 0.4% contacting the target pest (Steiner 1969, Reichard et al. 1979, Pimentel and Levitan 1986, Zhu et al. 2006). In addition to the economic loss to growers, the non-target drift of pesticides can be environmentally damaging (Devine and Furlong 2007). Therefore other pesticide delivery systems need to be developed to reduce environmental exposure, improve accuracy, and reduce costs. Trunk injection is a discriminating delivery system that reduces pesticide losses and has low capital investment (Wise et al. 2014).

Trunk injection is performed by creating an opening through the bark of the tree to reach the xylem. Formulated pesticide is delivered to the site through a drilled hole or using a needle driven directly into the trunk. Pesticides can be delivered fully concentrated or diluted. The product is carried through the xylem and pulled up to the leaves by transpiration (Chaney 1978). In food crops, trunk injections have recently been tested in avocado (Byrne et al. 2014), date palm (Salem et al. 2014), and apple (Aćimović et al. 2014, 2015, VanWoerkom et al. 2014, Wise et al. 2014, Aćimović, VanWoerkom, et al. 2016). There are many injection tools on the market but fewer pesticides formulated specifically for injection. The most effective pesticides have high solubility which allows rapid distribution in the canopy (Campana 1978, Aćimović et al. 2014). Systemic insecticides include active ingredients such as imidacloprid and emamectin

benzoate. Recent studies on apple have shown that insecticide injections are most effective against indirect insect pests which feed on vegetative tissues, and direct pests that feed both on vegetative and fruit parts, including *Choristoneura rosaceana* (Harris 1841), *Dysaphis plantaginea* (Passerini 1860), *Empoasca fabae* (Harris 1841), and *Phyllonorycter blancardella* (Fabricius 1781) (VanWoerkom et al. 2014).

Safety to pollinators is a chief concern when using insecticides in apple production, as populations of European honey bee and native bees have been in decline for the last half-century (Pettis and Delaplane 2010). Systemic insecticides such as neonicotinoids have been shown in some cases to have lethal and sublethal impacts on bees (Wu et al. 2011, Williamson et al. 2013, Doublet et al. 2015). Recently the EPA released a pollinator risk assessment which set a residue No Observable Adverse Effect (NOAE) level for imidacloprid at 25 ppb (EPA 2016). Assessing the potential impacts of trunk injection on pollinators through nectar and pollen sources with respect to new EPA guidelines is critical in acquiring special registration for apple trees.

This research focused on expanding the spectrum of active ingredients for pest control via trunk injection in apple, by comparing emamectin benzoate, imidacloprid, spinosad, chlorantraniliprole, dinotefuran and abamectin. The injected products were compared with field evaluations of *E. fabae* and other pest species, bioassays with laboratory-reared *C. rosaceana*, and residue analyses of plant tissues. In addition, nectar and pollen samples were collected to assess the associated risks to pollinators.

Materials and Methods

Trunk Injections

Injections were performed in 2013 and 2014 into semi-dwarf Red Delicious apple trees

(17.8-20.2 cm diameter), with five replicate trees per treatment in a randomized complete block design at the Trevor Nichols Research Center (42°35'42.4"N, 86°09'22.0"W), Michigan State University, Fennville, MI. The first trial was injected on 30 May 2013; air temperature was 25.9°C, wind speed was 1.3 m/s, and relative humidity was 69.9% with zero precipitation. The second trial was injected on 30 May 2014; air temperature was 22.7°C, wind speed was 0.3 m/s, and relative humidity was 56.6% with zero precipitation. All products were diluted in 500 ml of water, and injected using the TREE I.V. system (Arborjet, Woburn MA). Four #4 Arborplugs® (Arborjet) per tree were tapped into a 3/8 inch (0.95 cm) hole drilled 5.1 cm deep, straight, and 30.5 cm above the ground. Injections were strategically placed under main branches. Treatments included emamectin benzoate (A16297A, 42.9 g a.i./L, Syngenta) injected at 0.93, 1.86, and 18.60 ml/tree, two treatments of imidacloprid (IMA-jet®, 5% a.i., Arborjet) injected at 8.00 and 16.0 ml/tree, spinosad (in 2013, Entrust® 80WP, 80% a.i., injected at 1 g/tree; in 2014, Entrust® SC, 22.5% a.i., injected at 3.33 ml/tree, Dow AgroSciences), dinotefuran (Venom 70SG®, 70% a.i., Valent USA Corporation) injected at 0.70 g/tree, and abamectin (AgriMek® .15EC, 2% a.i., Syngenta) injected at 2.4 ml/tree, plus an untreated control. In 2013, chlorantraniliprole (XCL-r8, 4% a.i., Arborjet) was injected at 10 ml/tree. Treatment rates were calculated based on labelled allowable a.i. per hectare per season divided by number of trees per hectare.

To test the effects of seasonal timing on residue uptake into nectar and pollen, injections of emamectin benzoate (TREE-äge, 4% a.i., Arborjet) at 1.86 ml/tree and imidacloprid (IMA-jet®, Arborjet) at 16.0 ml/tree were performed in the spring and fall of 2015. The first injections were on 4 June 2015; air temperature was 21.7°C, wind speed was 0.9 m/s, and relative humidity was 67.1% with zero precipitation. The second injections were on 19 October 2015; air temperature was 12.2°C, wind speed was 2.0 m/s, and relative humidity was 42.2% with zero

precipitation. Trees were injected using the QUIK-jet® (Arborjet) injection tool with no dilution of products. Samples of fruit buds and leaves were collected in the fall, and nectar, pollen, and leaves were collected in the fall and the following spring.

Field Evaluations and Bioassays

Crop protection was measured in-season by evaluating pest populations and associated damage. Numbers of *E. fabae* nymphs were counted approximately four weeks after treatment and injury due to *E. fabae* was counted approximately six weeks after treatment. Twenty young shoots were checked per tree and the numbers of *E. fabae* nymphs recorded. To evaluate injury, the number of *E. fabae* damaged leaves were counted. In 2013 the mean number of damaged leaves per shoot were reported. In 2014 the mean number of damaged shoots divided by the total number of leaves for twenty shoots per tree was reported. In 2013, at approximately four weeks after treatment, numbers of active *Aphis pomi* colonies were counted within a 2-minute period for each tree. At approximately five weeks after treatment, damage due to the direct fruit pest *Cydia pomonella* was evaluated by counting apples with frass-marked holes within a 2-minute period for each tree. In 2013, *P. blancardella* mines were counted at 83 days after treatment, in 100 spurs per tree.

In order to evaluate changes in insecticide activity over time, 2nd instar laboratory-reared *C. rosaceana* larvae were exposed to field-collected leaves at 1 and 8 weeks after injection, with additional bioassays conducted the following spring approximately one year later. In the 2013 one year after treatment bioassay, only the untreated, emamectin benzoate, and chlorantraniliprole treatments were included. Each experimental unit contained 8 leaf disks, cut from 2 leaves (high, low) on each side (N, S, E, W) of the tree. Moist filter paper (5.5 cm) was

pressed into a 5 cm wide polystyrene Petri dish and the leaf disks (2.4 cm) were placed into the dish. A cork borer was used to punch leaf disks and was rinsed in acetone between each treatment. Five neonate *C. rosaceana* larvae were placed on the leaf disks, then the dishes were sealed, labeled by treatment, and stored at a constant temperature and 16:8 light:darkness in a growth chamber. After one week the bioassays were taken apart and mortality and percent defoliation was evaluated. To measure defoliation, leaf discs were taped to sheets of paper and digitized with a flatbed scanner. Total number of pixels for each leaf was determined using ImageJ software and percent leaf area remaining was calculated based on a standardized reference leaf (Rasband 2017).

Leaf and Fruit Sampling

Plant samples were collected at one day after treatment, as well as one, two, four, eight, and 12 weeks after injection. All samples were taken from the first three replicates. Forty leaves were collected from each tree (approximately 20 g); ten leaves were collected from each quadrant of the canopy. Five fruit were taken from each tree, starting two weeks after treatment (approximately 40 g). Buds were sampled in 2015 by collecting 200 buds from each tree. Fruit, buds, and leaves were collected into 120 ml glass sample jars (Qorpak Bottle Beakers® with green PTFE lined cap, Berlin Packaging, Chicago IL), weighed, and stored in dichloromethane at 30ºC until processing. Samples were processed using the QuEChERS method (Anastassiades et al. 2003) and concentrations of active ingredients were quantified using HPLC.

Nectar and Pollen Sampling

In the spring of 2016, 400 apple blossoms were collected per tree at full bloom and the

nectar and pollen were sampled. Methods for sampling nectar and pollen were modified from Knäbe et al. (2014). Briefly: flower anthers were removed and dried under a heated fume hood. The remaining flower parts were placed into modified centrifuge tubes: a 1mL Eppendorf tube with a 2.5 cm square of 100 μm mesh filter secured in place with a second Eppendorf tube with its end removed. Nectar was extracted by centrifuging for 30 seconds; when approximately 1.5 – 2 ml of nectar was collected it was removed from the modified tubes, placed into 2 mL gas chromatography vials, and stored under refrigeration until ready for sample cleanup and analysis. Nectar was diluted with deionized water and run through C14 columns, then eluted with acetonitrile before residue analysis with HPLC. Pollen was extracted by placing the dried anthers in a stainless steel mesh rotary drum (Pollen Extractor, EURL PCompagnie, Avignon, France). Extracted pollen was vacuumed from the surfaces of the drum through a 1mL filtered pipet tip inserted into a vinyl tube connected to an electric vacuum pump. Pollen was flushed from the pipet tip into glass sample jars (120 ml jar with green PTFE lined cap, Qorpak Bottle Beakers®, Berlin Packaging, Chicago IL) with dichloromethane. Pollen was processed using the QuEChERS (Anastassiades et al. 2003) method and active ingredients were quantified using HPLC.

Results

Field Evaluations

In 2013, the mean numbers of *E. fabae* nymphs were lowest in dinotefuran-treated trees treatments, followed by both rates of imidacloprid and abamectin ($F = 14.98$, $df = 10$, $P < 0.0001$) (Fig. 1). The highest mean numbers of *E. fabae* nymphs were found in the untreated, emamectin benzoate, spinosad, and chlorantraniliprole. Similarly in 2014, trees injected with

imidacloprid and dinotefuran had the lowest numbers of *E. fabae* out of all treatments. Mean numbers of nymphs were significantly lower than the untreated check in all treatments, with the exception of the lowest rate of emamectin benzoate ($F = 11.81$, $df = 8$, $P < 0.0001$). Response to emamectin benzoate was dose dependent, with 1.86 and 18.6 ml rates showing significantly lower numbers of *E. fabae* than the untreated check. Abamectin and spinosad performed similarly to emamectin benzoate.

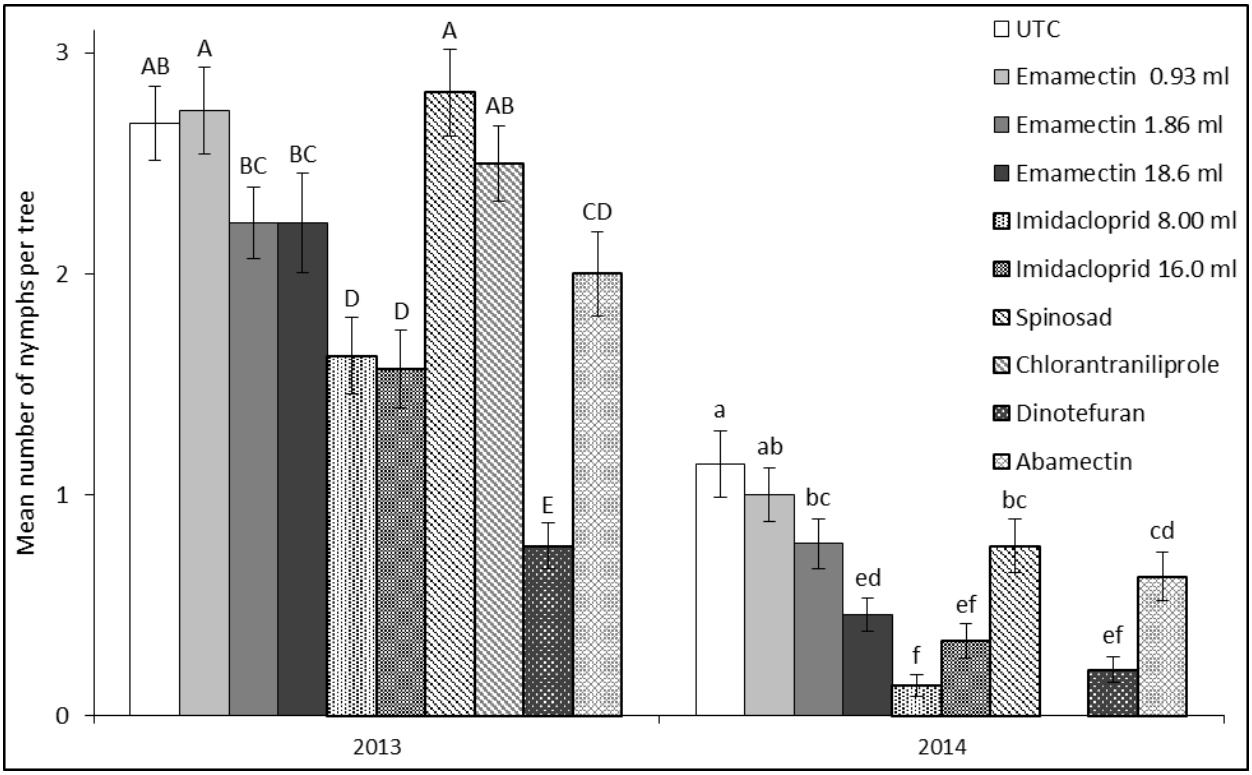

Figure 1. Mean numbers of *E. fabae* nymphs per tree. Twenty shoots were counted per tree. Field evaluations conducted 28 days after treatment in the 2013 trial, and 21 days after treatment in the 2014 trial. Chlorantraniliprole was dropped from the treatment list in 2014. Error bars are ± s.e. Means with the same letters are not significantly different (alpha = 0.05).

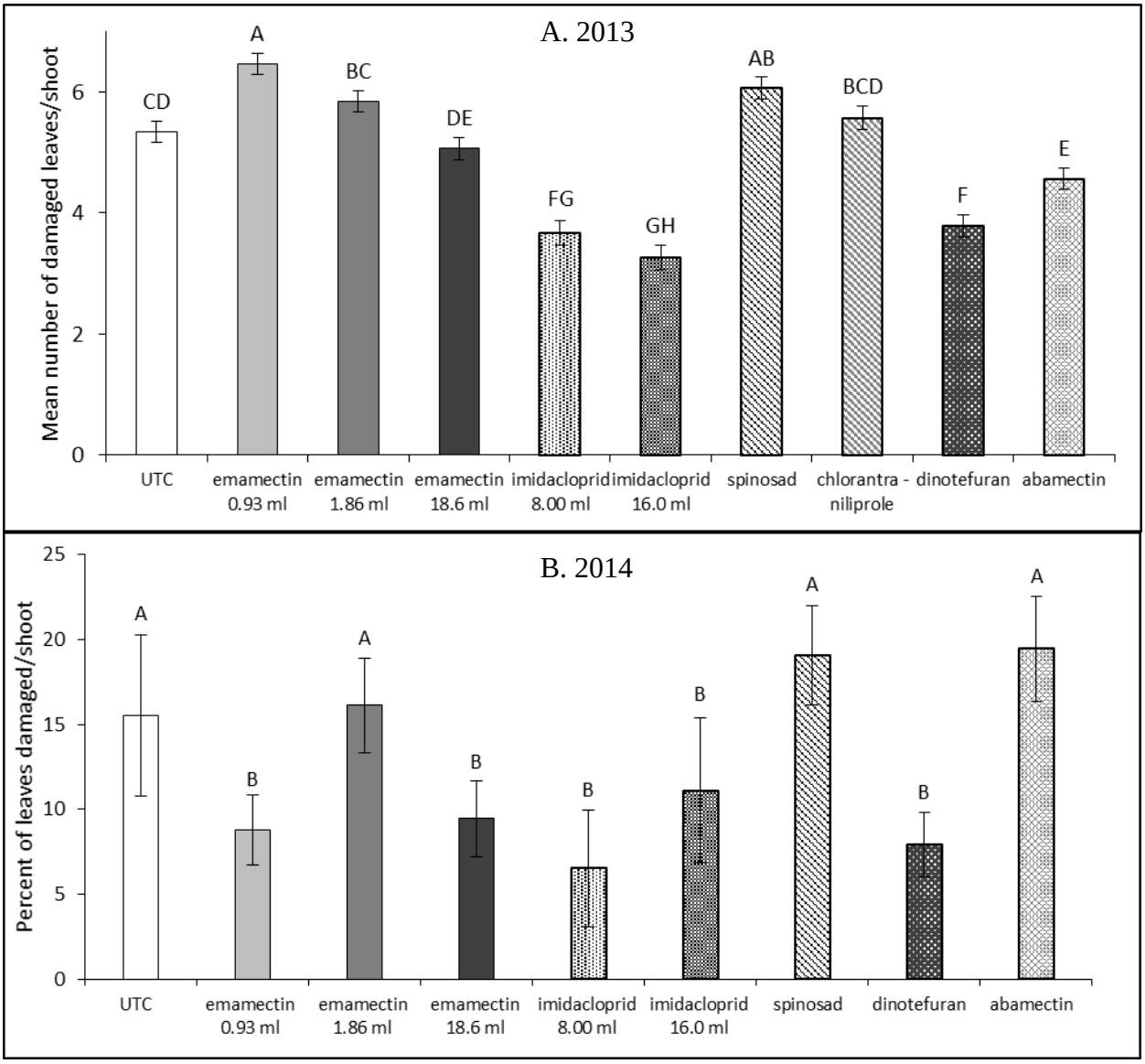

Figure 2. Damage due to *E. fabae* feeding. **A.** Field evaluation conducted 83 days after injection in the 2013 trial. Mean numbers of leaves damaged on 25 shoots per tree. **B.** Field evaluation conducted 46 days after treatment in the 2014 trial. Mean number of leaves damaged on 20 shoots per tree, divided by the total number of leaves per shoot. Error bars are ± s.e. Means with the same letters are not significantly different (alpha = 0.05).

Damage due to *E. fabae* was significantly lower in both the imidacloprid and dinotefuran-injected treatments than in untreated trees in 2013 (Fig. 2A). Spinosad, chlorantraniliprole, and

some emamectin benzoate treatments were not different than the untreated check ($F = 43.87$, $df = 10$, $P < 0.0001$). In 2014, all neonicotinoid treatments as well as the lowest and highest doses of emamectin benzoate outperformed the untreated check ($F = 11.34$, $df = 8$, $P < 0.0001$) and showed less signs of damage due to *E. fabae* (Fig. 2B).

A. pomi colonies in 2013 were higher on imidacloprid and dinotefuran-injected trees than other insecticide treatments and the untreated check, but not significantly so ($F = 2.08$, $df = 9$, $P = 0.0582$). In 2013 numbers of *P. blancardella* mines were numerically lower in trees injected with emamectin benzoate, chlorantraniliprole, and abamectin, but not statistically significant due to within-rep variability ($F = 1.99$, $df = 10$, $P = 0.0616$). Trees injected with the 8.00 ml rate of imidacloprid also had fewer leaf mines than the UTC. No significant differences were found for *C. pomonella* feeding damage in 2013 ($F = 0.32$, $df = 10$, $P = 0.9695$) or in 2014 ($F = 1.52$, $df = 8$, $P = 0.1890$).

Bioassays

In 2013 bioassays with *C. rosaceana*, all emamectin benzoate and abamectin treatments showed high levels of mortality, with the highest rate of emamectin benzoate reaching 100% at 7 days after treatment (Fig. 3A). Other treatments were not significantly different than the untreated check ($F = 12.34$, $df = 9$, $P < 0.0001$). At 56 days after treatment, mortality was significantly higher for chlorantraniliprole, abamectin, and all emamectin benzoate injections ($F = 8.36$, $df = 9$, $P < 0.001$). In the defoliation measurements, emamectin benzoate, chlorantraniliprole, and abamectin all had less leaf area missing compared with the untreated check at 7, 56, and 372 days after treatment. Imidacloprid, dinotefuran, and spinosad had defoliation levels comparable or higher than the untreated check (Fig. 4A).

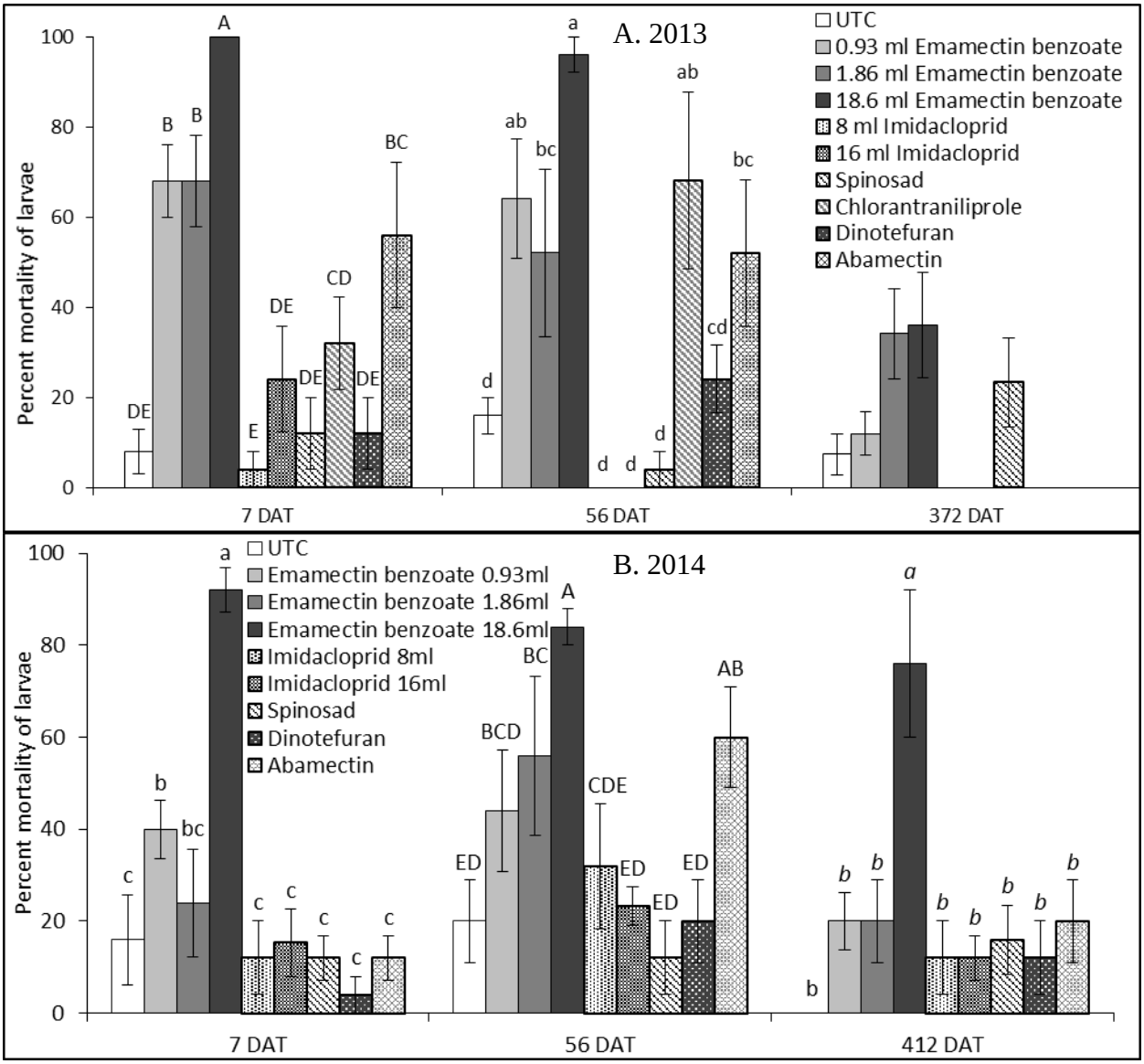

Figure 3. *C. rosaceana* larval mortality in bioassay arenas. **A.** Bioassays conducted 7, 56, and 372 days after 2013 injection date. Only emamectin benzoate and chlorantraniliprole were used in the bioassay conducted at 372 DAT, and no significant differences in mortality were found between treatments. **B.** Bioassays conducted 7, 56, and 412 days after 2014 injection date. Error bars are ± s.e. Means with the same letters are not significantly different (alpha = 0.05).

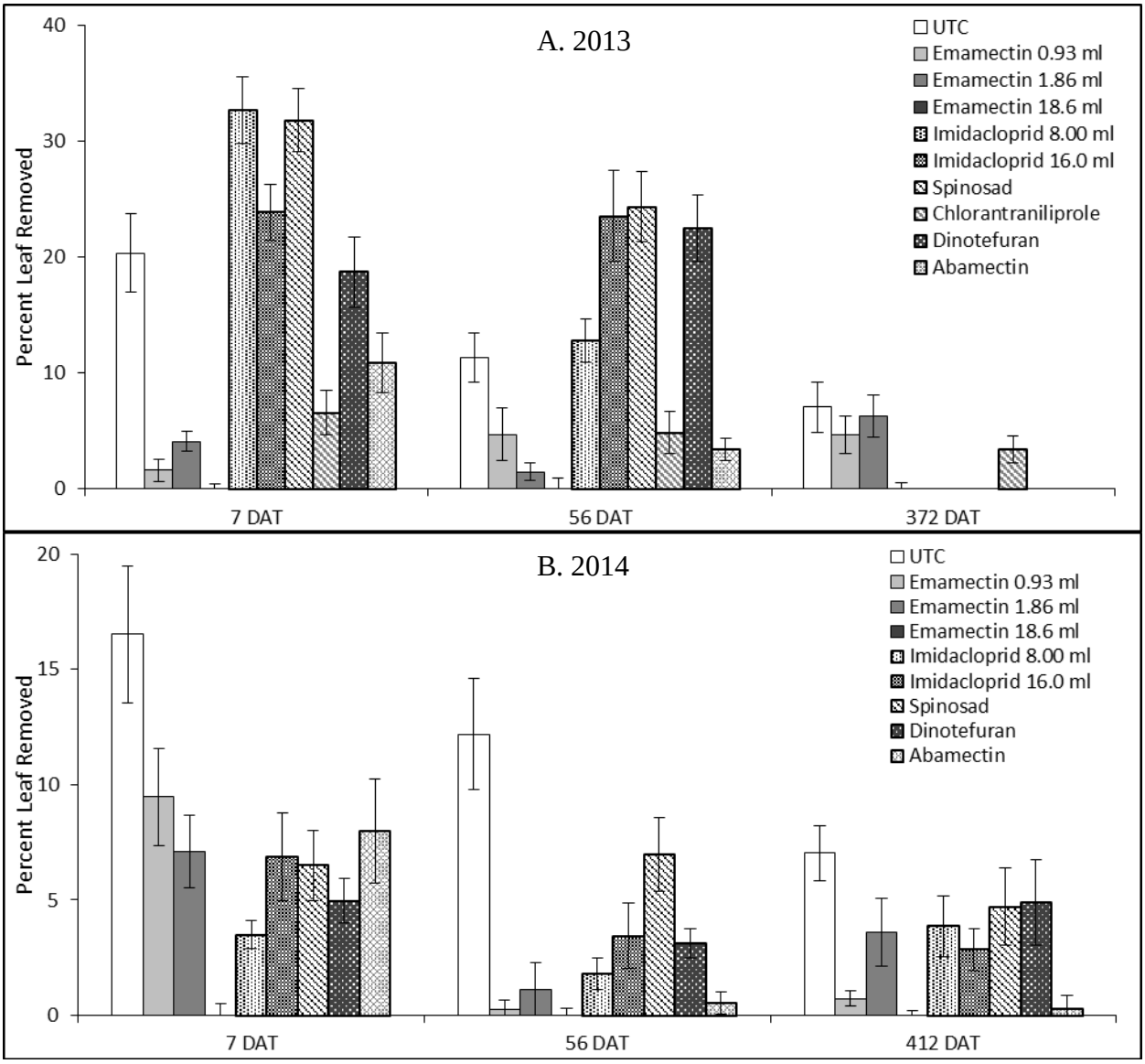

Figure 4. Larval feeding in 2013 and 2014 bioassays. **A.** Percent of leaf disc removed by *C. rosaceana* larvae at 7, 56, and 372 days after treatment in 2013 injections. Only emamectin benzoate and chlorantraniliprole were used in the bioassay conducted at 372 DAT. **B.** Percent of leaf disc removed by *C. rosaceana* larvae at 7, 56, and 412 days after treatment in 2014 injections. Percentages are based on total number of pixels compared to a reference of 18.6 ml emamectin benzoate, which experienced virtually no defoliation. Error bars are ± s.e.

In 2014, emamectin benzoate at the highest rate (18.6 ml) and lowest rate (0.93 ml) were

significantly higher than the untreated check in bioassays conducted at 7 days after treatment ($F = 14.65$, $df = 8$, $P < 0.0001$). Emamectin benzoate at the mid-rate (1.86 ml) was not significantly higher than the untreated check, and other treatments had lower mortality than the UTC (Fig. 3B). At 56 days after treatment, the highest two rates of emamectin benzoate as well as abamectin showed the highest percent mortality. Imidacloprid and dinotefuran treatments were not significantly different than the untreated check, and spinosad had low mortality ($F = 7.54$, $df = 8$, $P < 0.0001$). At all times in the 2014 defoliation measurements, leaf discs from emamectin benzoate injected trees showed dose-dependent effects with the 18.6 ml showing virtually no feeding. Imidacloprid, dinotefuran, and spinosad all experienced less defoliation than the untreated check treatment (Fig. 4B).

Leaf and Fruit Sampling

The highest dose of emamectin benzoate resulted in the highest concentration of active ingredient in leaves, and increased over time until the end of the study in 2013 (Fig. 5A). Concentrations increased more gradually in leaves for other treatments, reaching their peaks mid-season. Spinosad concentrations were very low and were only detectable once, at 56 days after treatment (0.002906 ppm) (Fig. 5C). In 2014, abamectin increased in concentration over the season, peaking at 23.121 ppm (Fig. 5F). Other treatments had a trend of peaking mid-season and tapering off by the end sample date. Between the two seasons there was approximately a 100-fold difference in leaf residue concentrations.

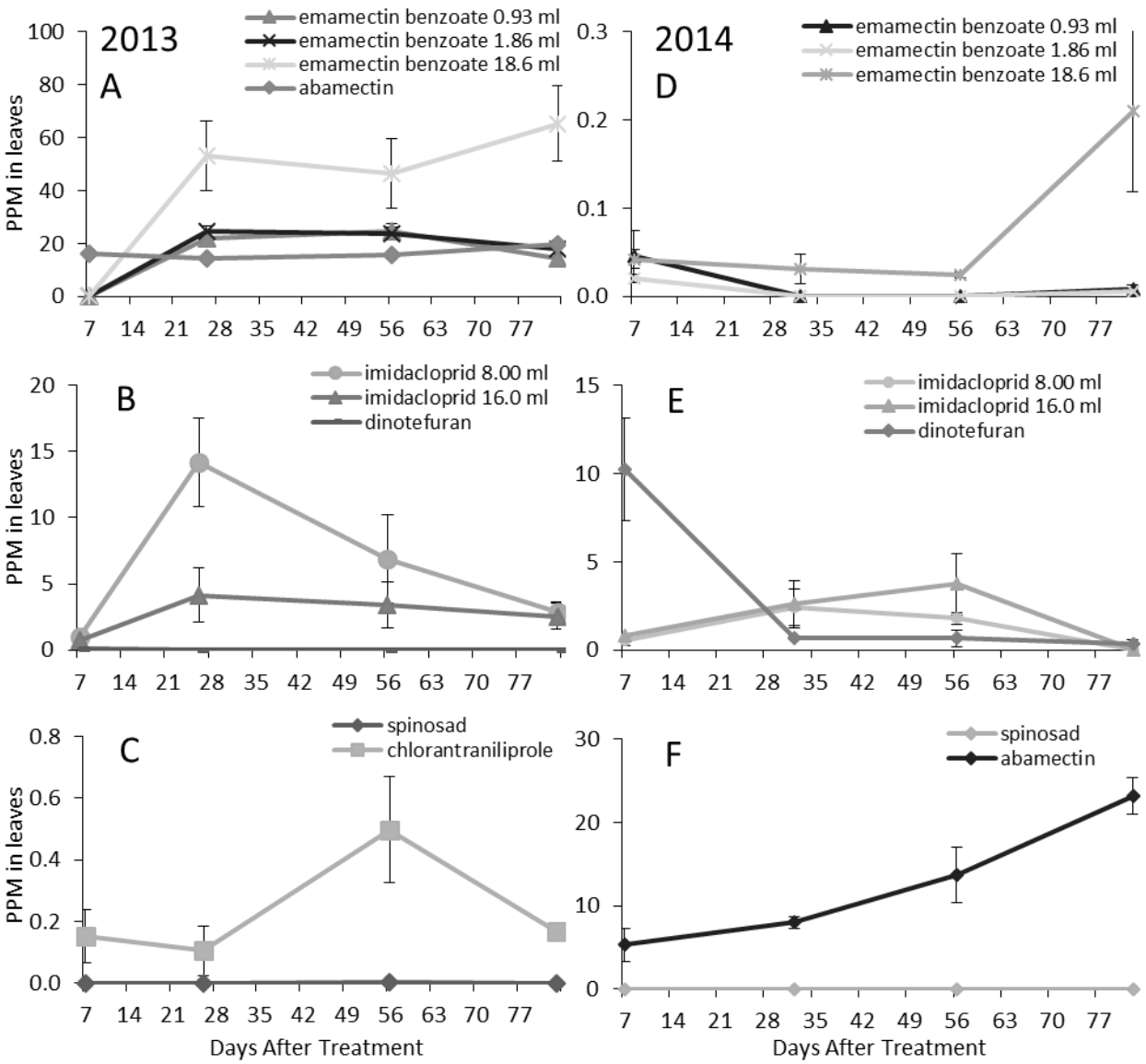

Figure 5. Concentrations of active ingredients in leaves throughout growing season, in 2013 (A, B, C) and 2014 (D, E, F). Error bars are ± s.e.

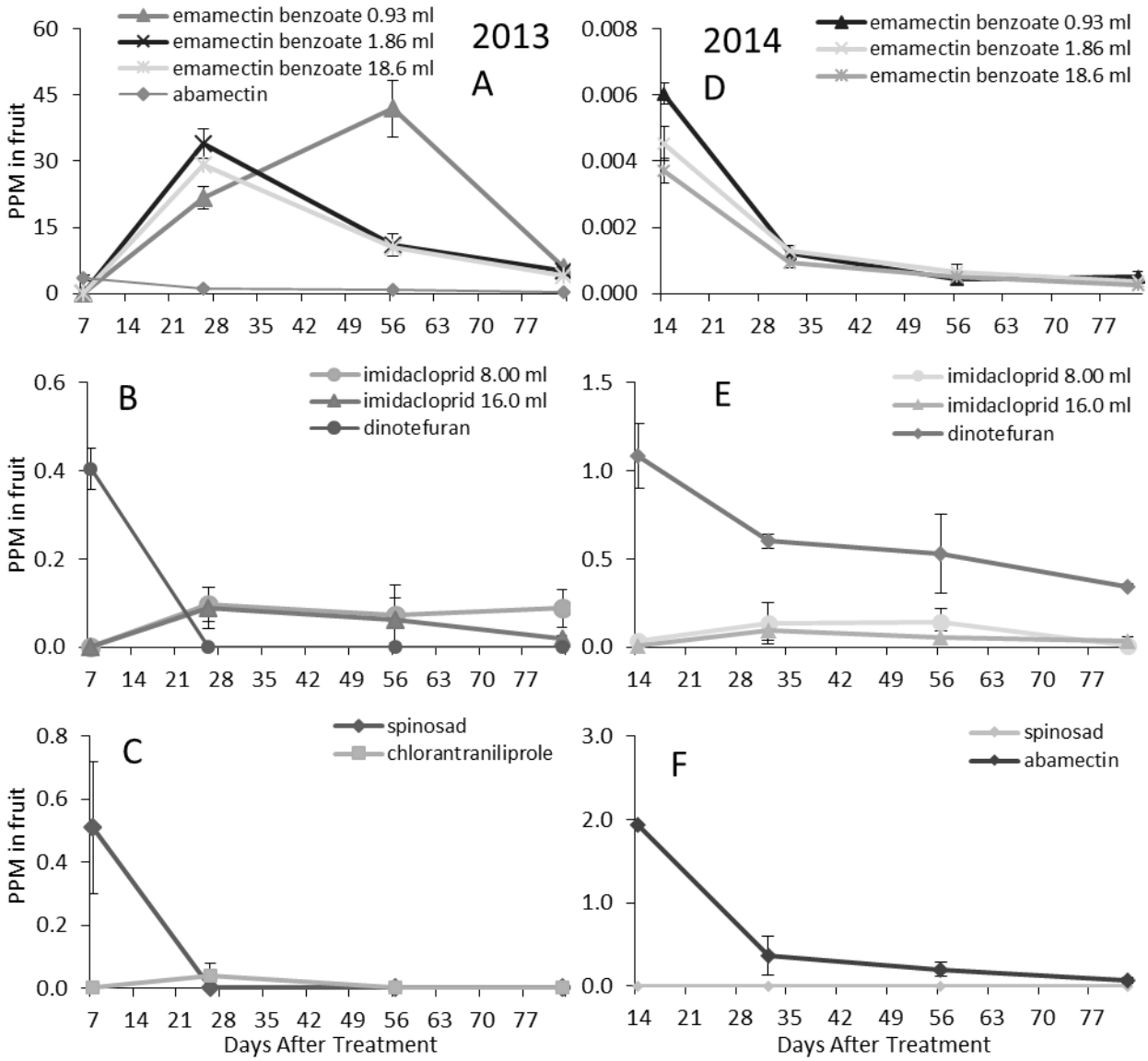

Figure 6. Concentrations of active ingredients in fruit throughout growing season, in 2013 (A, B, C) and 2014 (D, E, F). Error bars are ± s.e.

The residue concentrations in fruit were similar for emamectin benzoate treatments in 2013, though parent compound residues decreased in concentration after peaking mid-season at 26 and 56 days after treatment. By 83 days after treatment, the peak concentration of emamectin benzoate (injected at 0.93 ml per tree), was 6.044 ppm (Fig. 6A). Abamectin had a peak concentration at 7 days after treatment and was lower in the subsequent samples (Fig. 6C). All

other treatments had relatively low concentrations. In 2014, residue concentrations of dinotefuran and abamectin were highest at 14 days after treatment and decreased over the growing season (Figs. 6E, F). Imidacloprid treatments increased mid-season and tapered off. Emamectin benzoate had very low concentrations of residue detected, and were not detected in the last sample. Spinosad had a single detection in fruit at 0.0003 ppm for 32 days after treatment.

Nectar and Pollen Sampling

We did not detect emamectin benzoate in bud or leaf samples collected seven days after fall injections and 144 days after spring injections in 2015 (Fig. 7). In the spring, emamectin benzoate was detected in nectar (7.36 ppb ± 2.42 ppb), pollen (1.15 ppb ± 0.69 ppb), and in one leaf sample (0.0014 ppb) collected from trees injected in the spring of 2015.

Imidacloprid was in leaves for both fall and spring injections, as well as in buds for spring-injected imidacloprid (Fig. 7). Imidacloprid injected in the spring was only detected in leaves, at 0.06 ppb ± 0.03 ppb. For fall injections of emamectin benzoate, the active ingredient was only detected in the leaves at 21.63 ppb ± 9.70 ppb. Imidacloprid was detected in nectar at 0.39 ppb ± 0.05 ppb, and in leaves at 0.96 ppb ± 0.56 ppb.

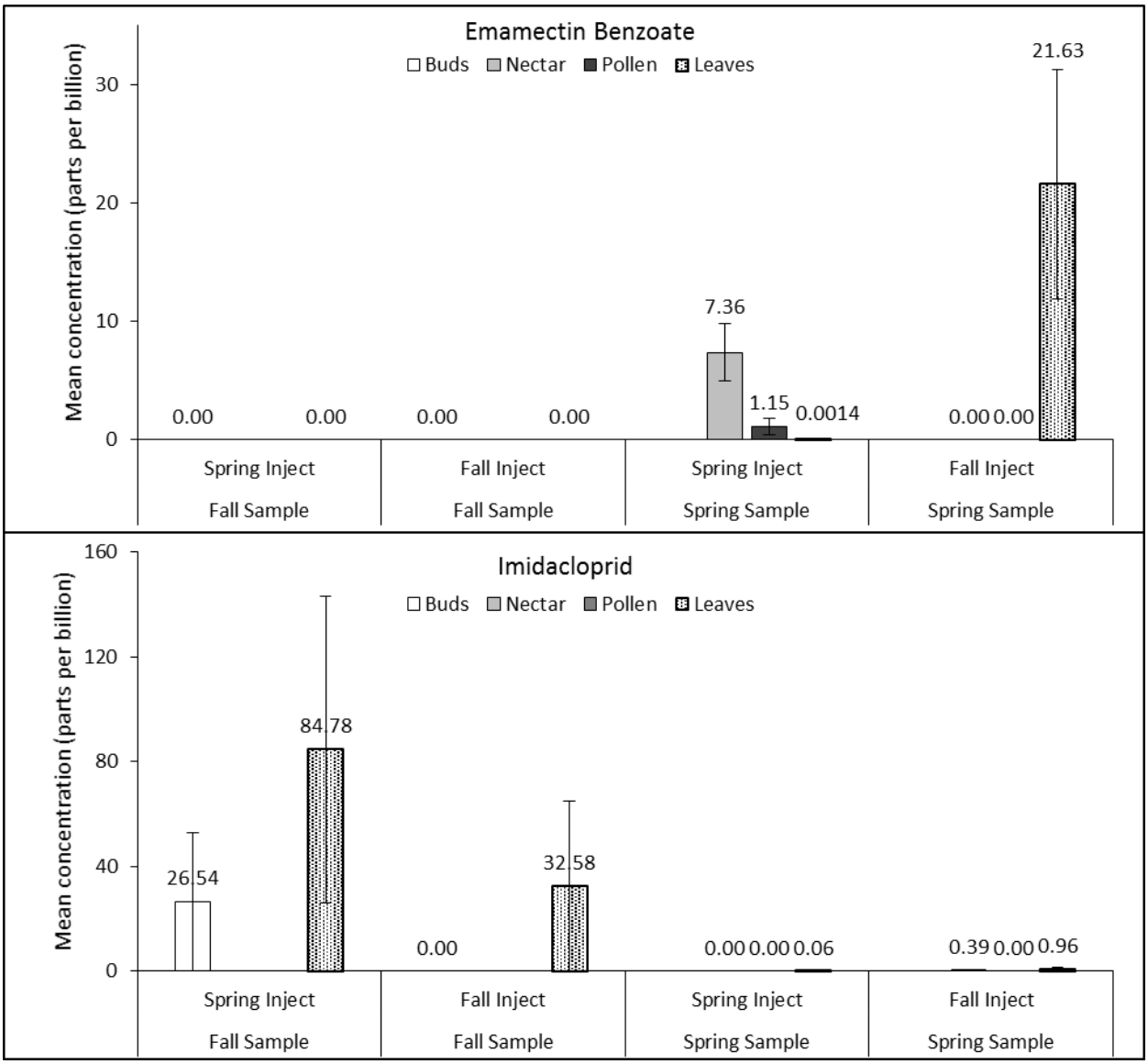

Figure 7. Mean concentration of emamectin benzoate and imidacloprid in buds, nectar, pollen, and leaves, for trunk injections performed in the spring (4 June) and fall (19 October) of 2015. Buds and leaves were sampled from both spring and fall injected trees at 7 days after the fall injections. Nectar, pollen, and leaves were sampled from both spring and fall injected trees after petal fall, 203 days after the fall injections. Error bars are ± s.e.

Discussion

This study provides evidence for an expanded list of compounds that are compatible with

trunk injection for apple pest management. In general, the neonicotinoids imidacloprid and dinotefuran reduced numbers of *E. fabae* and associated feeding damage in the field. In *C. rosaceana* bioassays, emamectin benzoate and abamectin resulted in the highest levels of mortality. Avermectins as well as chlorantraniliprole also reduced leaf feeding in *C. rosaceana* bioassays. Imidacloprid was not detected in nectar or pollen for injections performed in the previous spring. Emamectin benzoate was detected in nectar and pollen for injections performed in the previous spring, but not for injections performed in the previous fall.

Chlorantraniliprole showed lethality and reduced defoliation in the *C. rosaceana* bioassays. The two avermectins emamectin benzoate and abamectin showed comparable levels of efficacy on lepidopteran pests like *C. rosaceana* and *P. blancardella*. Emamectin benzoate was similarly toxic to *C. rosaceana* and *E. fabae* in previous apple trunk injection studies (VanWoerkom et al. 2014). Abamectin also showed activity on *E. fabae*, suppressing numbers and resulting in less shoot damage. High efficacy on homopterans from abamectin would not be expected when delivered by airblast sprayer. This suggests that trunk injection as a method of insecticide delivery can enhance the activity of a compound through ingestive exposure.

On *E. fabae*, the high and low rates of imidacloprid did not have a significant difference in effectiveness. The same was true for emamectin benzoate on *C. rosaceana*. The lowest rate and the "labeled field rate" of 1.86 ml/tree were about equally effective compared with untreated checks. This is an important consideration for management. Even at these doses, a small amount of insecticide delivered via trunk injection may be enough to be effective.

Colonies of *A. pomi* were higher on trees injected with imidacloprid and dinotefuran, which may be explained by presence of beneficial insect predators present on the untreated trees. Another possibility is that leaf damage due to *E. fabae* nymphs reduced the host quality of shoots

for the aphids, which led to reductions in their numbers. Spinosad was not effective at protecting against *E. fabae* or *C. rosaceana* in these trunk injection experiments. This is likely due to lack of translocation within trees. In both years spinosad did not translocate from the injection site to the leaves, based on residue data as well as insect observations. Unless a formulation can be produced that would improve mobility within the tree's vascular system, it is unlikely spinosad will be a viable candidate for trunk injection.

Nectar and Pollen

Imidacloprid concentrations were low in nectar and pollen. Imidacloprid was detected in the nectar and leaves when injected in the fall, but concentrations were lower in the spring of 2016 when injected the previous spring. Nectar and pollen are fed by the phloem and imidacloprid lacks endogenous chemical groups necessary to be actively loaded into the phloem (Denis and Delrot 1993, De la Barrera and Nobel 2004, Lei et al. 2014). This may explain why concentrations of imidacloprid were low in pollen, nectar, as well as in fruit, which are fed by the phloem for the majority of the growing period. However only parent compounds were analyzed in this study. The metabolite olefin-imidacloprid is also toxic, and was detected in nutmeat and walnut husk of black walnut (*Julans nigra*) following soil injections and trunk sprays of imidacloprid (Nix et al. 2013). Future studies of imidacloprid injection should also quantify toxic metabolites for a more complete picture of imidacloprid distribution.

When emamectin benzoate was injected in the spring, concentrations were found in nectar and pollen the following year but not in leaves. However, when injected in the fall after fruit buds had set, emamectin benzoate was detected only in the leaves. Emamectin benzoate did not accumulate in the buds regardless of whether injections were performed in the spring or fall.

Injecting during the spring allows emamectin benzoate to distribute throughout the woody plant tissue. Some of the compound may be accessible to the shoot and fruit buds the following year. But emamectin benzoate does not move via transpiration when injected in the fall, and can only access leaves when the transpiration stream is active again in the spring. This suggests that emamectin benzoate may be phloem mobile in apple trees. Bark beetles (Curculionidae: Scolytinae), have been controlled with injections of emamectin benzoate (Grosman et al. 2009, Grosman and Fettig 2010). Bark beetles feed primarily in the phloem and control using trunk injection requires phloem-mobile insecticides (Fettig et al. 2014). Emamectin benzoate and its metabolites are incorporated into natural plant products such as sugars as they break down (Allen et al. 1997). It may be that emamectin benzoate is metabolized at a faster rate than it can accumulate, as is reported in horse chestnut (*Aesculus hippocastanum*) (Burkhard et al. 2015). Future trunk injection studies should also quantify metabolites, especially considering that both the parent compound and metabolites are known to possess high contact toxicity to *Apis mellifera* (Chukwudebe et al. 1997, Anderson et al. 2009).

This research provides evidence for a broader suite of insecticides with potential usefulness for trunk injection in apples. Imidacloprid, chlorantraniliprole and emamectin benzoate had been previously demonstrated to be effective at controlling foliar apple pests. Dinotefuran, spinosad, and abamectin were previously untested active ingredients and some showed high levels of efficacy on tested pests. Continued testing will expand the pool of available trunk injected pesticides. This study has important implications for pollinator safety, as the EPA has recently set a threshold of 25 ppb for imidacloprid as part of its pollinator risk assessment (EPA 2016). It may be possible to mitigate transport of emamectin benzoate or imidacloprid residues to nectar and pollen through careful injection timing.

CHAPTER 3: TRUNK INJECTION TOOL COMPARISONS IN MICHIGAN APPLES USING EMAMECTIN BENZOATE AND IMIDACLOPRID

Abstract

Trunk injection is a pesticide delivery system with potential for application in tree fruit crop production. However, most of the injection tools available on the market were developed for use on ornamental and shade trees rather than on apple trees. Commercially available injection tools were compared by injecting systemic insecticides into apple trees. Two concurrent four-year studies using emamectin benzoate and imidacloprid, with QUIK-jet® (ArborJet Systems, Woburn, MA) and BITE Infusion (University of Padova, Italy) were conducted. The BITE delivered significantly higher peak foliar concentrations of both insecticides compared to the QUIK-jet in 2013 (imidacloprid: p-value = 0.0155; emamectin benzoate: p-value = 0.0021), and higher imidacloprid concentrations across all dates in 2014 (p-value = 0.0148). However, this trend was not consistent across years, and no significant differences in foliar concentrations between tools were found except for QUIK-jet which delivered a higher peak foliar concentration than BITE in 2016 (p-value = 0.0125). Numbers of *Empoasca fabae* nymphs were lower in the canopies of trees injected with imidacloprid using QUIK-jet compared with untreated trees in all years, despite more instances of higher foliar concentrations with BITE. This study demonstrated that delivery method is an important factor in effective trunk injection based apple management.

Introduction

A primary goal in pest management is to protect crops while improving precision of

control measures and reducing pesticide exposure to workers and consumers. Apples are a high value crop in the United States and worldwide. In Michigan, the apple industry is valued at $221M and ranks 7th in the US for fruit, tree nut and berry sales (NASS 2014). In order to protect apple orchards from insect pests, growers regularly apply insecticides using tractor pulled airblast sprayers. With this method, large volumes of insecticide must be sprayed in order to cover the canopy and effectively protect the trees. However a major drawback of sprayer technology is overspray which results in less than 0.1% overall insecticide reaching the target pest, potentially harming people and non-target organisms (Pimentel 1995). Currently, there is a need for application methods that reduce the amount of pesticide in the environment, and trunk injection provides a promising alternative. Trunk injection has advantages over airblast sprayers because it does not cause overspray, and it minimizes exposure to farm workers and non-target insects (VanWoerkom et al. 2014, Wise et al. 2014).

Trunk injection represents an alternative delivery system for pesticides in tree fruit productions systems (Wise et al. 2014). With trunk injection the treatment solution is injected into the xylem tissue, and the insecticide is translocated throughout the vascular tissue. This method is used to protect from insect pests in forests as well as ornamental and shade trees in urban environments (Wilson 1978). Injection of the insecticide emamectin benzoate has been used to mitigate the spread of the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Bupestridae) (Smitley, Rebek, et al. 2010, Tanis et al. 2012). More recently, there has been a renewed interest in trunk injection for pest control on fruit bearing trees (Byrne et al. 2012, 2014, Wise et al. 2014, Aćimović, Cregg, et al. 2016, Khalaf and Alrubeai 2016). In recent years a major foliar pest on apple has emerged: potato leafhopper, *Empoasca fabae* (Harris) (Homoptera: Cicadellidae), which feeds on phloem of young shoots. Systemic insecticides are

distributed through the vascular system to leaves, which makes trunk injection particularly capable at targeting foliar pests.

The scale of production and economic drivers of commercial apple production are different than the ornamental industry. Trunk injection technology has been developed for use in ornamentals and shade trees, where pests and diseases are treated at the individual tree level. Ornamentals do not have the same requirements for food safety as fruit trees. In addition, ornamentals do not get managed at the same scale as fruit trees and are often treated on a case-by-case basis in urban environments. Thus, there is a need for assessing the most effective tools for use in apples.

Trunk injection methods can be as important as the formulated products that are injected. Most tools are either drill-based and remove tissue to create an opening for product, or needle-based and pierce the trunk but do not remove tissue. Several companies have developed trunk injection tools for use in ornamental and shade trees. Arborjet (Woburn, MA) has developed the QUIK-jet, a hand-pumped syringe tool that injects through a self-sealing plug tapped into drilled hole. The company Bite Injection Systems (University of Padova, Italy) has developed a tool known as BITE (Blade for Infusion in TrEes). This tool has a lenticular blade with vertical ports, designed to inject between wood fibers.

We tested these two tools in a Michigan apple orchard during the years 2013-2016. Tools were compared using imidacloprid and emamectin benzoate, and injections were timed. We analyzed insecticide residue levels in leaves and measured the abundance of *E. fabae* nymphs on foliage.

Materials and Methods

Trunk Injection

Emamectin benzoate and imidacloprid were injected in separate concurrent trials during four field seasons (2013-2016), at the Michigan State University Trevor Nichols Research Center (TNRC) in Fennville, MI (latitude 42.5951° : longitude -86.1561°). Injections were performed on semi-dwarf 'Red Delicious' apple trees (17.78-20.32 cm diameter at breast height, DBH) with four replicate trees per treatment in randomized complete block designs. Injections were performed in late spring, shortly after petal fall. Two injection methods, QUIK-jet and BITE, were used to inject both imidacloprid and emamectin benzoate. For each tool, four equally spaced injection sites were placed around the trunk, under main scaffold branches where possible, 30.5 cm above the ground. QUIK-jet: Holes were drilled using a 9.525 mm brad-point bit to a 5.1 cm depth and a self-sealing Arborplug was tapped into each hole. The syringe needle was inserted into the plugs and the insecticide dose was selected by adjusting the plunger. BITE: A brass hammer (attached to the tool) is used to insert the blade into the trunk. A rubber washer compresses around the site which prevents leaking. The pesticide was drawn into a disposable Luer-type syringe is loaded with the pesticide dose and inserted into the tool.

Imidacloprid (ImaJet®, Arborjet) was injected at 16 ml/tree (equivalent to 0.8 g a.i./tree). On 14 June 2013, the air temperature was 20.0°C, wind speed was 0.8 m/s, and relative humidity was 66.6%, with zero precipitation. Total rainfall from the start date through the end of the study was 223.0 mm. The two injection methods were timed with a digital stopwatch by a second person on the 2013 injection date. Injections were also performed in 2014, 2015, and 2016, on trees in another apple orchard of the same variety. On 9 June 2014, the air temperature was 20.9°C, wind speed was 1.5 m/s, and relative humidity was 64.8% with zero precipitation. Total

rainfall from the start date through the end of the study was 233.9 mm. On 4 June 2015, the air temperature was 21.7°C, wind speed was 0.9 m/s, and relative humidity was 67.1%, with zero precipitation. Total rainfall from the start date through the end of the study was 257.1 mm. On 18 May 2016, the air temperature was 15.4°C, wind speed was 1.3 m/s, and relative humidity was 33.6% with zero precipitation. Total rainfall from the start date through the end of the study was 196.57 mm. The average rainfall for the region for 2013 – 2016 was 227.6 mm.

Concurrently on 14 June 2013, emamectin benzoate (A16297A, Syngenta AG, Switzerland) was injected at 1.86 ml/tree (equivalent to 0.08 g a.i./tree) using the QUIK-jet and the BITE. For injections in 2014, 2015, and 2016, TREE-Age® (emamectin benzoate, Arborjet Inc., Woburn, MA) was substituted for A16297A, at the same injection rate and equivalent a.i. In 2014, emamectin benzoate injections were performed three days later on 12 June rather than occurring on the same date as imidacloprid injections. For this date the air temperature was 22.7°C, wind speed was 0.3 m/s, and relative humidity was 56.6% with zero precipitation.

Residue Sampling

For all treatments, leaf samples were collected at one, two, four, eight, and 12 weeks after treatment. All samples were taken from the first three replicates for repeated measures analysis. Forty leaves were collected from each tree (approximately 20 g); ten leaves were collected from each cardinal direction. Leaves were collected into 120 ml glass sample jars (Qorpak Bottle Beakers® with green PTFE lined cap, Berlin Packaging, Chicago IL), weighed, and stored in dichloromethane at 30ºC until the samples were processed during the fall. Samples were processed using the QuEChERS (Anastassiades et al. 2003) method and concentrations of active ingredients were quantified using HPLC. Residue data were analyzed using PROC MIXED with

repeated measures in SAS® 9.4 (SAS 2013).

Field Evaluation

Crop protection was measured in-season with pest and associated damage evaluations. Numbers of *E. fabae* nymphs were counted two weeks after treatment. Twenty young shoots were checked per tree and the numbers of *E. fabae* nymphs were recorded. *E. fabae* nymph counts were analyzed with PROC GLM.

Results

Residue Analyses

Across the four years of injection trials, foliar concentrations of imidacloprid and emamectin benzoate followed distinctly different seasonal patterns. The effects of injection system on leaf concentration of both products were relatively small, and residues in leaves varied by injection system on only two out of five sample dates within each compound (Fig. 8).

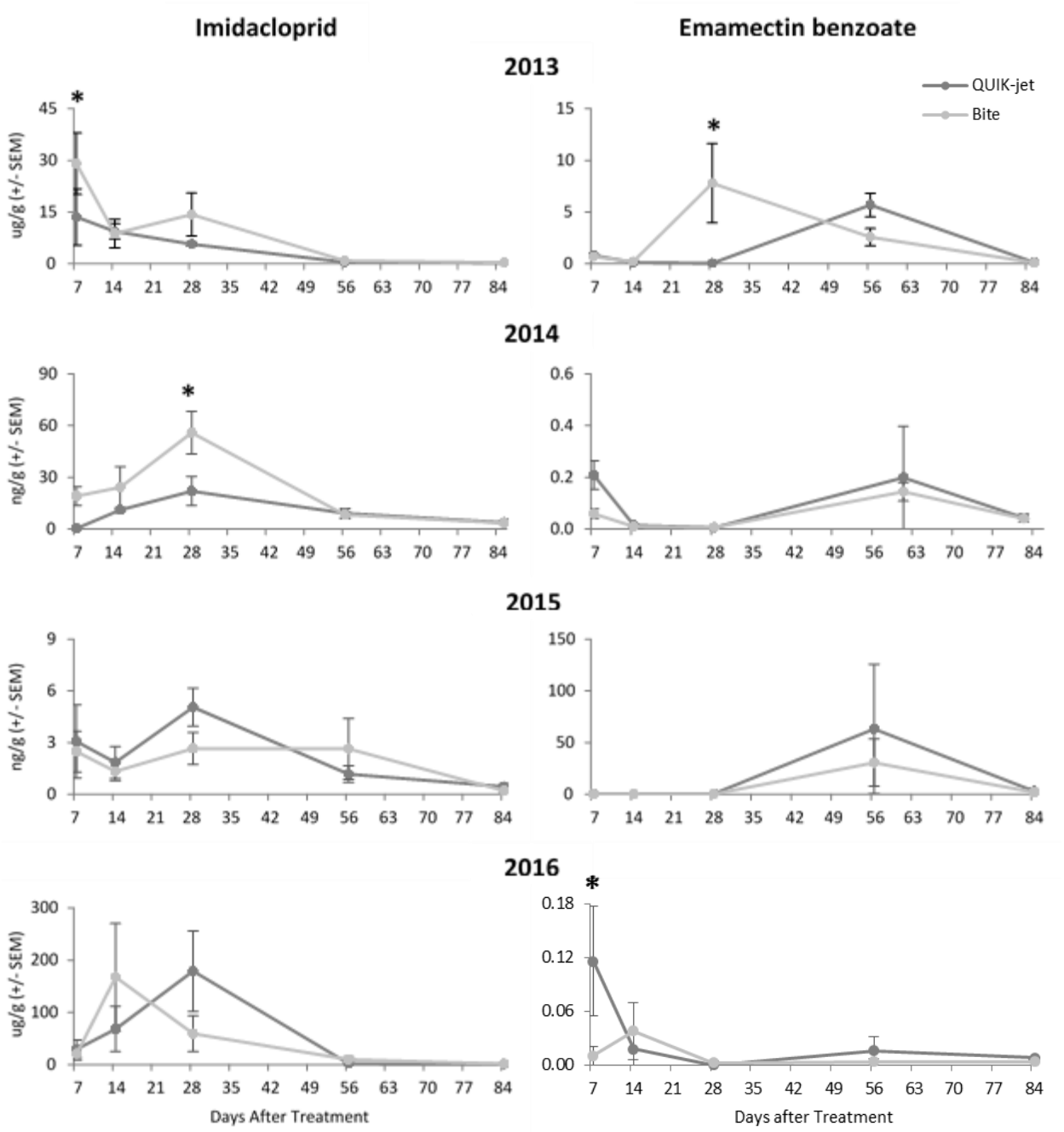

Figure 8. Mean insecticide residues recovered from leaves sampled at 7, 14, 28, 56, and 84 days after treatment (DAT) for injections performed in 2013-2016. Asterisks indicate significance within sample dates (alpha = 0.05). Overall treatment effect for imidacloprid was significant in 2014. Both treatments were significantly higher at 28 DAT than other dates. Error bars are ± s.e.

In 2013, the overall effect of injection method on foliar imidacloprid concentrations was not significant (F-value = 3.32, df = 1, p-value = 0.1575) (Fig. 8). Effect of day was statistically significant; concentrations were higher on 7 days after treatment (DAT) than on 56 or 85 DAT (F-value = 5.87, df = 4, p-value = 0.0155). Injection method by day interactions were not significant (F-value = 1.08, df = 4, p-value = 0.4239). When sliced for simple differences, the BITE delivered higher concentrations to the canopy than QUIK-jet at 7 DAT (t-value = -2.35 df = 14.14, adjusted p-value = 0.0459). No other treatment by day slices were found to be significant (14 DAT: t-value = 0.09, df = 14.14, adj. p-value = 0.9282; 28 DAT: t-value = -1.31, df = 14.14, adj. p-value = 0.2262, 56 DAT: t-value = -0.06, df = 14.14, adj. p-value = 0.9537; 85 DAT: t-value = -0.01, df = 14.14, adj. p-value = 0.9925).

For 2014 imidacloprid injections, BITE delivered the highest concentration overall to the canopy (F-value = 10.07, df = 1, p-value = 0.0148). Concentrations in the canopy were significantly higher at 28 DAT than on other dates (F-value = 8.77, df = 4, p-value = 0.0025). The interaction of injection tool with day was not significant (F-value = 2.54, df = 4, p-value = 0.1050). In 2015 imidacloprid injections, there were no significant effects for injection method (F-value = 0.47, df = 1, p-value = 0.5178), for day (F-value = 2.60, df = 4, p-value = 0.5602), or for interaction between these levels (F-value = 0.78, df = 4, p-value = 0.5602).

For imidacloprid injections in 2016, there were no significant effects for injection method (F-value = 0.03, df = 1, p-value = 0.8717). Concentrations were significantly higher at 14 and 28 DAT for both treatments (F-value = 3.34, df = 4, p-value = 0.0386). There was no significant interaction between injection tool and day (F-value = 1.37, df = 4, p-value = 0.2926).

The overall effect of injection method on emamectin benzoate foliar concentrations in 2013 was not significant (F-value = 1.12, df = 1, p-value = 0.3400) (Fig. 8). Effect of day was

statistically significant, and there were higher concentrations of emamectin benzoate recovered from leaves at 28 DAT than other sample dates (F-value = 4.60, df = 4, p-value = 0.0262). There was also a significant injection method by day interaction (F-value = 5.46, df = 4, p-value = 0.0159). When sliced for simple differences, BITE delivered significantly higher emamectin benzoate to the canopy at 28 DAT (t-value = -4.24, df = 11.36, adjusted p-value = 0.0021). No other treatment by day slices were found to be significant (7 DAT: t-value = 0.07, df = 11.36, adj. p-value = 0.9424; 14 DAT: t-value = -0.04, df = 11.36, adj. p-value = 0.9722, 56 DAT: t-value = 1.70, df = 11.36, adj. p-value = 0.1225; 85 DAT: t-value = 0.02, df = 11.36, adj. p-value = 0.9874).

In 2014 emamectin benzoate injections, there were no significant effects for injection method (F-value = 1.07, df = 1, p-value = 0.3417), for day (F-value = 2.18, df = 4, p-value = 0.1657), or for interaction (F-value = 0.42, df = 4, p-value = 0.7891). No slices for simple effects of injection tool by day were significant (7 DAT: t-value = 1.58, df = 10.81, adj. p-value = 0.1552; 14 DAT: t-value = 0.05, df = 10.81, adj. p-value = 0.9620; 28 DAT: t-value = -0.01, df = 10.81, adj. p-value = 0.9891; 61 DAT: t-value = 0.58, df = 10.81, adj. p-value = 0.5787; 82 DAT: t-value = 0.03, df = 10.81, adj. p-value = 0.9756).

For emamectin benzoate injections in 2015, no significant effects were found for injection method (F-value = 0.30, df = 1, p-value = 0.6074), day (F-value = 1.81, df = 4, p-value = 0.2070), or for interaction (F-value = 0.22, df = 4, p-value = 0.9212). In addition, no slices for significant simple effects of injection tool by day were found (7 DAT: t-value = 0.02, df = 12.63, adj. p-value = 0.9857; 14 DAT: t-value = 0.01, df = 12.63, adj. p-value = 0.9956; 28 DAT: t-value = 0.01, df = 12.63, adj. p-value = 0.9918; 56 DAT: t-value = 1.09, df = 12.63, adj. p-value = 0.3032; 84 DAT: t-value = 0.03, df = 12.63, adj. p-value = 0.9755).

No significant effects were found for 2016 emamectin benzoate injection methods (F-value = 1.58, df = 1, p-value = 0.3624), day (F-value = 1.57, df = 4, p-value = 0.2515), or for interaction between these levels (F-value = 2.19, df = 4, p-value = 0.1388). When sliced for simple differences, QUIK-jet delivered significant higher concentrations to the canopy at day 7 (t-value = 3.00, df = 6.935, adj. p-value = 0.0125). No other treatment by day slices were found to be significant at an alpha of 0.05 (14 DAT: t-value = -0.58, df = 6.935, adj. p-value = 0.5745; 28 DAT: t-value = -0.07, df = 6.935, adj. p-value = 0.9482; 56 DAT: t-value = 0.35; df = 6.935, adj. p-value = 0.7322; 85 DAT: t-value = 0.13, df = 6.935, adj. p-value = 0.8967).

Field Evaluation

The impact on *E. fabae* numbers varied between inject tool within each insecticide treatment and year. Imidacloprid injections performed with the QUIK-jet reduced numbers of *E. fabae* nymphs compared to the UTC in all four years (Fig. 9A). In contrast, nymphs were significantly lower than the UTC for trees receiving BITE injections in 2013 and 2014. Trees injected with emamectin benzoate had significantly lower numbers of *E. fabae* nymphs than UTC trees in 2013 after injecting with BITE, but not in other years (Fig. 9B). Similarly, emamectin benzoate injections using QUIK-jet had significantly lower numbers of *E. fabae* nymphs than UTC trees only in 2015.

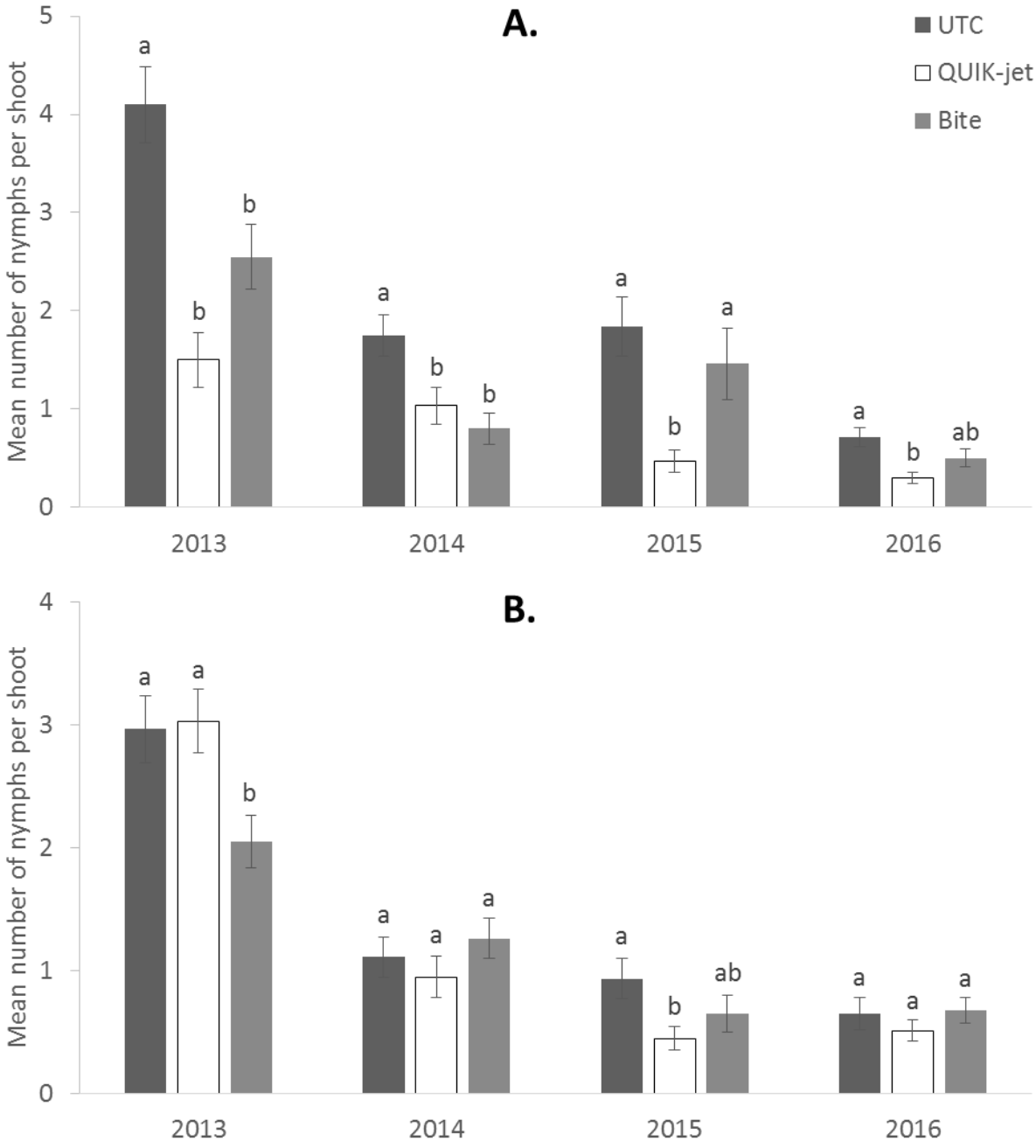

Figure 9. Mean numbers of *E. fabae* nymphs found on shoots of untreated and injected trees, for **A.** imidacloprid, and **B.** emamectin benzoate injections. Means with the same letter are not significantly different within year (alpha = 0.05). Error bars are ± s.e.

In 2013, *E. fabae* nymph counts were significantly higher on imidacloprid-injected trees

using either QUIK-jet or BITE, compared with UTC trees (F-value = 5.01, df = 2, p-value < 0.0076) (Fig. 9A). No significant differences were found between QUIK-jet and BITE. Similarly in 2014, nymph counts were significantly lower in trees treated with either of the two injection tools compared with untreated trees (F-value = 6.82, df = 2, p-value = 0.0014). In 2015, trees that were injected with QUIK-jet had significantly fewer *E. fabae* nymphs than untreated trees or those treated with BITE (F-value = 6.22, df = 2, p-value = 0.0023). Nymph counts on trees injected with QUIK-jet in 2016 were significantly lower than on untreated trees (F-value = 5.94, df = 2, p-value = 0.0031). There were no significant differences between BITE and QUIK-jet, nor were there significant differences between BITE and the untreated trees.

There were significantly fewer *E. fabae* nymphs during 2013 when emamectin benzoate was injected with BITE compared with QUIK-jet or UTC trees (F-value = 7.23, df = 2, p-value = 0.0010) (Fig. 9B). There were no significant differences between tools or the untreated trees for emamectin benzoate injections in 2014 (F-value = 0.95, df = 2, p-value = 0.3880). In 2015, trees injected with emamectin benzoate using QUIK-jet had significantly lower numbers of nymphs than the untreated trees, while not being different than trees injected using BITE (F-value = 3.11, df = 2, p-value = 0.0471). Numbers of nymphs were not significantly different from untreated trees for any injection treatments in 2016 (F-value = 0.64, df = 2, p-value = 0.5289).

Time Series Data

Injection using the BITE required approximately twice as much time as the QUIK-jet (F-value = 47.43, df = 1, p-value = 0.0063) (Fig. 10). The need to tap and remove the BITE tool by hand was the main factor which added to injection time compared with the QUIK-jet, which used a battery powered electric drill.

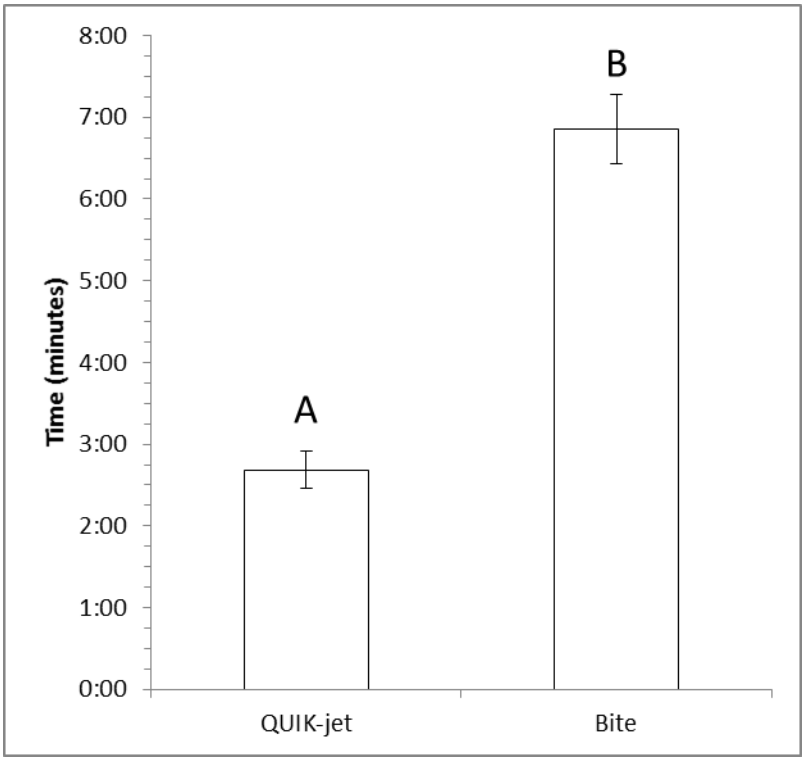

Figure 10. Time required to inject an apple tree at four positions around its trunk, using QUIK-jet or BITE. Means with the same letter are not significantly different (alpha = 0.05). Error bars are ± s.e.

Discussion

In this study we investigated how injection tools and insecticide products can interact to produce a range of delivery results within apple trees. Under realistic field and user conditions, both imidacloprid and emamectin benzoate were taken up more rapidly when injected with the BITE in 2013 and for imidacloprid in 2014, compared to the QUIK-jet. However, this trend was not consistent across all years. QUIK-jet delivered equivalent amounts of products to leaves in other years, notably in 2016 when under comparatively drought-like conditions it delivered emamectin benzoate to leaves earlier and in higher concentrations compared to BITE. Numbers of *E. fabae* nymphs were consistently lower on imidacloprid-injected trees than on untreated

trees when injecting with QUIK-jet, whereas nymphs were lower on trees injected with BITE in only two years. Injections with emamectin benzoate were less consistent across years and between tools, which indicates that at the rate used emamectin benzoate is not likely to be effective for *E. fabae* control. Results indicate that although residue analyses in the initial year suggested that BITE would yield better results, imidacloprid proved most effective when injected with QUIK-jet. This variation between canopy delivery and efficacy on *E. fabae* demonstrates that route of entry is another important aspect of trunk injection. Injection tools can play as important a role in pest management for apple production as pest phenology and insecticide selection.

BITE uses a lenticular blade-shaped needle with vertical ports and does not remove xylem tissue. Piercing between trunk fibers may initially allow faster access to the xylem tissue versus drill-based injection, explaining initially higher concentrations. Higher foliar insecticide concentrations in trees injected with BITE for 2013 are in line with earlier findings with fungicide injections (Aćimović 2014), though this was not the case for 2015 and 2016 for imidacloprid or 2014-2016 for emamectin benzoate.

There were differences in *E. fabae* nymph counts between injection methods, which were most evident in the imidacloprid injections. Generally, peak foliar concentrations did not predict reductions of nymphs in the field. Nymphs were counted at approximately 14 DAT each year; however peak levels of imidacloprid in the canopy often occurred later in the season, notably in 2014. Concentrations of imidacloprid in foliage were nearly equivalent between both tools at 14 DAT in 2013 and both tools significantly reduced numbers of nymphs at this date, but this was not consistent across years. There were significantly higher foliar concentrations of imidacloprid in trees injected using BITE throughout 2014, but numbers of nymphs were significantly lower

than the UTC for both tools. In 2015 nymphs on trees injected using BITE were not significantly different than UTC trees though foliar concentrations were not significantly different. This suggests that even though peak residues may be higher with the use of BITE, QUIK-jet delivers imidacloprid to the foliage in a way that better reduces numbers of *E. fabae.* Aćimović et al. (2016) concluded that fast solution uptake and high xylem exposure were key advantages to drill-based technologies.

The residue profile of emamectin benzoate over time was different than imidacloprid. The two insecticides were not combined into the same experimental designs, so there were not statistical analyses performed comparing them in this study. Generally, concentrations peaked later in the season in 2013, 2014, and 2015. This may be due in part to emamectin benzoate being a larger molecule and unable to travel as quickly through the xylem as imidacloprid, a highly soluble compound. Overall rainfall was also low during 2014 and 2016 compared with the other seasons. In particular, there was a period from 2 July through 23 July 2016 during which there was very little precipitation. These dry periods would have limited transpiration and may have affected insecticide uptake. Another contributing factor is the reservoir effect, which causes emamectin benzoate to accumulate in parenchyma tissue and provides long, multi-year residual activity compared with other systemic insecticides (Jansson et al. 1997, Smitley, Doccola, et al. 2010).

Time was a significant factor, with QUIK-jet taking an average of 2 min. 41 sec. and BITE requiring nearly 7 min. Another important consideration when selecting a tool to use for pest management is damage to the tree. In comparisons between drill and needle-type injection tools, Aćimović et al. (2016) found that smaller holes similar to those made by the BITE heal faster than larger holes such as those needed for the QUIK-jet, although all methods heal within

2 years.

Conclusion

Trunk injection tools offer a variety of delivery methods which affect efficacy of the product, similar to the way different sprayers provide different methods of delivery. Evaluating commercially available trunk injection tools is crucial to developing this method for use in apple orchards. These tools work in concert with pesticides to determine the method's overall effectiveness. This study showed that higher peak foliar concentrations of insecticide earlier in the growing season do not necessarily translate to greater pest control. Drill-based injection may be the best choice for apple production when targeting *E. fabae*. This study is an important step toward developing recommendations and facilitating adoption by growers. Future research should focus on comparing additional injection tools and other low-volume methods such as solid-set sprayer delivery and drip-line chemigation. It is also critical that exposure risk to pollinators via nectar and pollen be carefully evaluated.

CHAPTER 4: COMBINING TRUNK INJECTIONS OF SYSTEMIC INSECTICIDE AND FUNGICIDE IN APPLE TO CONTROL FOLIAR PESTS AND APPLE SCAB

Abstract

Trunk injections reduce pesticide inputs and environmental exposure in trees, and recent work has addressed apple production systems. Insecticides and fungicides have been demonstrated for apple tree injection, however combinations of the two have not yet been tested. Trunk injections of the systemic insecticide emamectin benzoate and systemic acquired resistance (SAR) fungicide phosphorous acid were performed on mature apple trees to combine management strategies for foliar insect pests and apple scab (*Venturia inaequalis*). Injections of emamectin benzoate followed by phosphorous acid into the same set of injection ports resulted in higher mortality of *Choristoneura rosaceana* larvae and lower incidence of apple scab compared with untreated trees. Scab incidence on trees in which phosphorous acid was injected into the same set of ports before emamectin benzoate were not different from untreated trees early in the growing season. Injections of emamectin benzoate and phosphorous acid into the same holes in either order showed higher mortality and reduced larval feeding in *C. rosaceana* bioassays compared with products injected into separate holes. This study demonstrates that two pesticides can interact dynamically within the vascular system of a tree, which has important implications for expanding the utility of trunk injection for fruit tree management.

Introduction

Novel pesticides have been developed rapidly throughout the last half-century. However, the means of delivering pesticides in tree fruit systems have remained relatively unchanged

(Wise 2016). Trunk injection is an alternative delivery method which precisely delivers pesticides, improving upon less discriminating methods such as airblast sprayers (Wise et al. 2014). When pesticides are sprayed using conventional sprayers, as little as 0.1% reaches insect pests, and more pesticide is sprayed than necessary (Pimentel and Levitan 1986).

Insecticides including emamectin benzoate and imidacloprid have shown high efficacy in multiple systems. Emamectin benzoate is effective at controlling emerald ash borer (*Agrilus planipennis*) for two or more years in *Fraxinius* spp. (Smitley, Doccola, et al. 2010). Studies of trunk injection in apple trees have shown that trunk injection is effective at controlling foliar pests such as obliquebanded leafroller (*Choristoneura rosaceana*) (VanWoerkom et al. 2014). Trunk injection has been demonstrated in apple cropping systems to control apple scab (*Venturia inaequalis*) using a variety of fungicides, including phosphorous acid (Percival and Boyle 2005, Aćimović, VanWoerkom, et al. 2016). However, no trunk injection studies to date have investigated treatment combinations to address multiple pests or diseases.

Adoption of trunk injection as a management strategy for apple crops relies on comprehensively addressing the system—not simply demonstrating control of individual insect pests and diseases but integrating management strategies and improving efficiency for growers. Combining treatments of fungicides and insecticides would be important in order to minimize the number of ports needed for injection. More damage is incurred with each additional port, which also increases the risk of infection or bark splitting (Aćimović et al. 2016). Ideally, all pesticides would be injected into a single set of ports in an apple management program designed to target multiple pests and diseases. Little has been done to demonstrate insecticides and fungicides combined for complex systems with multiple pests such as those found in apple orchards. It is common for pesticides to be tank mixed, though manufacturers recommend

mixing on a case-by-case basis and offer limited insight into expected outcomes. For example, the product label for PHOSPHO-jet (phosphorous acid) suggests that it is compatible with most agricultural products, but notes that it is acidic and should be tested first for safety and compatibility (Arborjet Inc. 2012). Little is known about mixing emamectin benzoate with phosphorous acid, and even less is known about how different pesticides will interact when injected into trees.

The objective of this study was to determine the compatibility of the insecticide emamectin benzoate and the systemic acquired resistance (SAR) inducing fungicide phosphorous acid when applied by trunk injection. We hypothesized that injecting an insecticide and a fungicide into separate holes would show better efficacy for both emamectin and phosphorous acid. We also hypothesized that injecting emamectin benzoate first would avoid potential degradation due to phosphorous acid.

Materials and Methods

Experiments were initiated in 2014 and repeated in 2015. In both years, semi dwarf apple trees, *Malus pumila* Mill. cv. 'Red Delicious', were used in an orchard at the Michigan State University Trevor Nichols Research Center in Fennville, MI. All trees were approximately 15.24 cm in diameter at 30.48 cm above the ground. Maintenance sprays of mancozeb at 2.52 kg a.i. / hectare (Dithane 75DF, Dow AgroSciences, Indianapolis, IN) were performed on a 7-10 day cycle prior to the injection dates, to prevent early establishment of apple scab before petal fall stage in apples. Trees were assigned treatments in a randomized complete block design with 5 replications, and injections were performed after petal fall on 30 May 2014 and 29 May 2015. Five untreated check trees (UTC) were not injected but received maintenance sprays prior to

injection. Holes were drilled with a 9.5 mm diameter brad point bit to an approximately 5.08 cm depth, with four holes arranged in cardinal directions. After drilling, holes were sealed with #4 Arborplugs (Arborjet, Woburn, MA). For treatments using separate holes for injection, a second set of holes were drilled between cardinal points about 5.08 cm above the first.

Injections were performed using the Tree I.V. system (Arborjet, Woburn, MA) with pesticides diluted in 500 ml water. Treatments consisted of emamectin benzoate (Tree-Age, ArborJet) at 1.86 ml/tree (0.08 g a.i.), and phosphorous acid (Phospho-jet, Arborjet) at 30.96 ml/tree (12.43 g a.i.) based on the labeled rate according to tree diameter. Injections were performed through the same four injection holes or a separate set of four hole, with either emamectin benzoate or phosphorous acid first (Table 1). For expediency, treatments 2 and 4 were injected with emamectin benzoate first, followed by phosphorous acid injections into all treatments, and finally a second round of emamectin benzoate injections for treatments 3 and 5. Approximately 1 to 3 hours lapsed between each round of injections.

Table 1. Injection treatment combinations.

1	UTC			
2	same hole		Ema 1st	Phos 2nd
3	same hole		Phos 1st	Ema 2nd
4	different hole		Ema 1st	Phos 2nd
5	different hole		Phos 1st	Ema 2nd

Ema (Tree-Age)	1.86	ml/tree	0.08	g AI/tree
Phos (Phospho-jet)	30.96	ml/tree	12.43	g AI/tree

Apple Scab Evaluations

Apple shoots were evaluated for scab by selecting 20 shoots, approximately 5 shoots per quadrant. Numbers of leaves with scab lesions were recorded along with the total numbers of leaves per shoot. Fruit were evaluated for scab by randomly counting 100 fruit per tree.

Evaluations were conducted on 25 June, 30 July, and 19 August 2014; 27, 62, and 83 days after treatment (DAT) respectively. Follow up evaluations of shoots and spurs were conducted one year later on 27 May (362 DAT), and shoots only on 10 July 2015 (406 DAT). In 2015, shoots were evaluated on 10 July (42 DAT); shoots and fruit were evaluated on 28 June 2016 (396 DAT).

Insect Bioassays

Leaves were sampled from trees injected in 2014 on 26 June and 18 August 2014 (27 and 80 DAT), and from 2015 trees on 15 June, 29 June, 10 July, and 29 July 2015 (17, 31, 41, and 61 DAT). Eight leaves were sampled, two from each quadrant of the crown, then punched with a 15 mm cork borer rinsed with acetone between treatments and placed in 5.5 cm polystyrene Petri dishes lined with moist filter paper. Five laboratory-reared *Choristoneura rosaceana* larvae (neonate, first or second instar) were placed in each Petri dish and kept in an incubation chamber with 16:8 day:night light cycle. After 7 days, dishes were opened and evaluated for mortality. Leaf discs were cleaned and imaged with a flatbed scanner at 600 dpi. Defoliation of leaf discs was quantified by number of pixels using ImageJ (Rasband 2017). In 2015, bioassays conducted on 10 July and 29 July were evaluated at 5 days for mortality, and 7 days for defoliation.

Data Analyses

Data were analyzed with one-way ANOVA using the GLM procedure in SAS 9.4 (SAS 2013). Data were arcsine transformed when necessary to meet assumptions of homoscedasticity. Fisher's LSD was used to construct pairwise *t*-tests for means separations ($\alpha = 0.05$).

Results

Apple Scab Evaluations

In 2014, shoot scab was lower than the untreated check (UTC) for most treatments, with some exceptions (Fig. 11). At 27 DAT (25 June 2014), treatment combination 3, 'same hole, phos 1st ema 2nd', had the highest scab infection on shoots out of all treatments, and was not different from the UTC ($F = 5.79$, $df = 4$, $p = 0.0001$). Treatment combination 4, 'different hole, ema 1st phos 2nd' was lower but not different than the UTC. Treatments 2 and 5, 'same hole, ema 1st phos 2nd' and 'different hole, phos 1st ema 2nd' respectively, were significantly lower than the UTC and not significantly different from each other or treatment 4. At 62 DAT (30 July), UTC had the highest scab incidence, followed by treatment 3 ($F = 16.44$, $df = 4$, $p < 0.0001$). Treatments 5, 4, and 2 had the lowest scab infestations and were not significantly different from one another. On 83 DAT, shoot scab incidence was highest for the UTC. Treatment 2 was lower but not different from the UTC or from treatments 3, 4, or 5 ($F = 2.34$, $df = 4$, $p = 0.0543$).

At 362 DAT, all treatments reduced the incidence of scab relative to the control ($F = 10.65$, $df = 4$, $p < 0.0001$) but scab ratings were similar among treatments. Scab incidence on shoots was much higher at 406 DAT (10 July 2015). Treatment 3 (63.94% ± 1.52% s.e.), treatment 4 (63.76% ± 1.45% s.e.), and treatment 5 (67.96% ± 2.05% s.e.) were not different from the untreated check (66.59% ± 1.47% s.e.) ($F = 3.70$, $df = 4$, $p = 0.0055$). Treatment 2 was lower (59.68% ± 1.79% s.e.) than the UTC or treatment 5, although not different than treatments 3 or 4. Fruit scab incidence showed few differences between treatments in 2014. At 27, and 62 DAT, all treated fruit had significantly lower scab incidence than the UTC ($F = 3.8$, $df = 4$, $p = 0.0233$; $F = 4.39$, $df = 4$, $p = 0.0138$). At 83 DAT, treatments 2, 3, and 4 had less scab than the UTC; treatment five was not different from either the UTC or the other treatments ($F = 3.61$, $df =$

4, p = 0.0279).

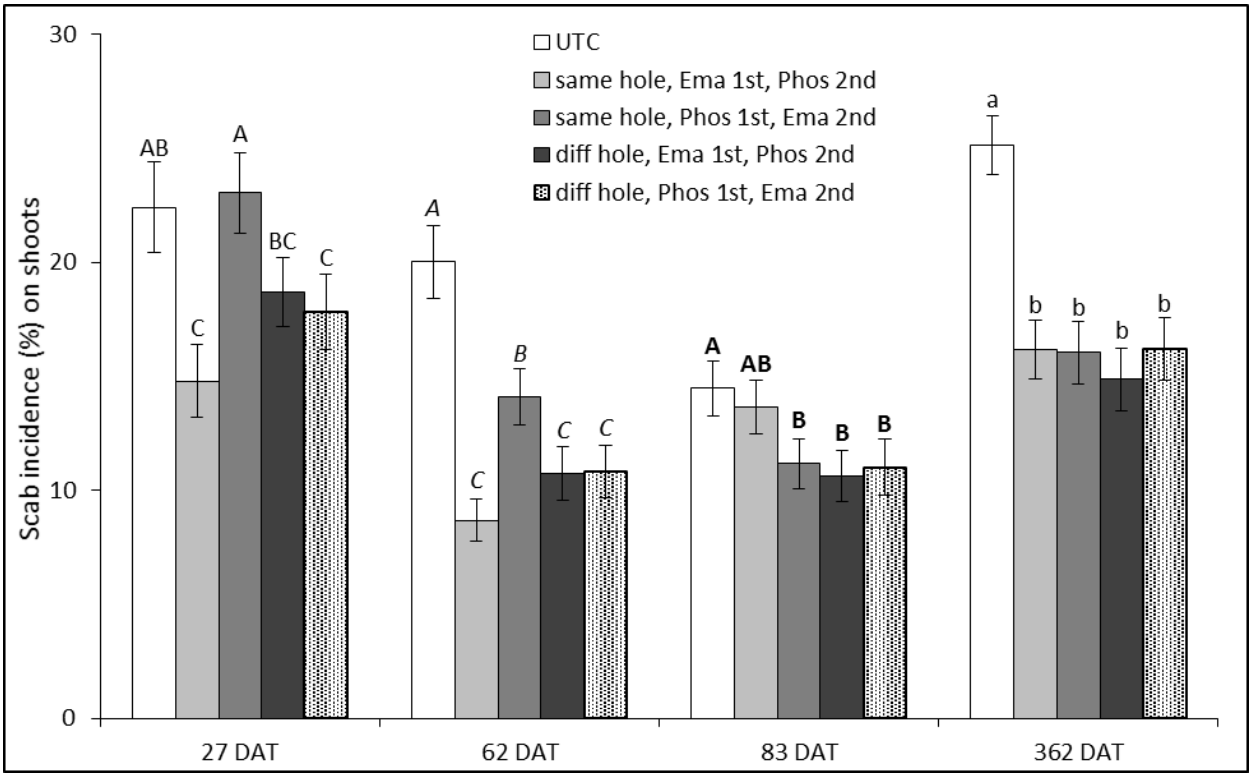

Figure 11. Mean percent scab infection on shoots recorded in 2014, at 27 DAT, 62 DAT, 83 DAT, and 362 DAT. Means within sampling dates with the same letter are not significantly different ($\alpha = 0.05$). Error bars are ± s.e.

On 10 July 2015 (43 DAT), scab incidence was lower than the UTC on all treated trees (33.61% ± 2.16) and no differences between treatments were found (treatment 2: 25.45% ± 1.71; treatment 3: 21.8% ± 1.84; treatment 4: 22.89% ± 1.74; treatment 5: 26.13% ± 1.98) ($F = 6.24$, $df = 4$, $p < 0.0001$) (Fig. 12). On 28 June 2016 (396 DAT), treatment 4 had the highest scab incidence (27.37% ± 2.61) on shoots ($F = 7.74$, $df = 4$, $p < 0.0001$). No differences were found between the UTC (17.79% ± 1.89) and treatment 3 (21.78% ± 1.99), treatment 2 (18.1% ± 1.86), or treatment 5 (13.57% ± 1.87). However, treatment 3 had significantly higher scab incidence than treatment 5. No significant differences were found for fruit scab incidence at 396 DAT

(overall mean 38.64% ± 2.44) (F = 0.15, df = 4, p = 0.9614).

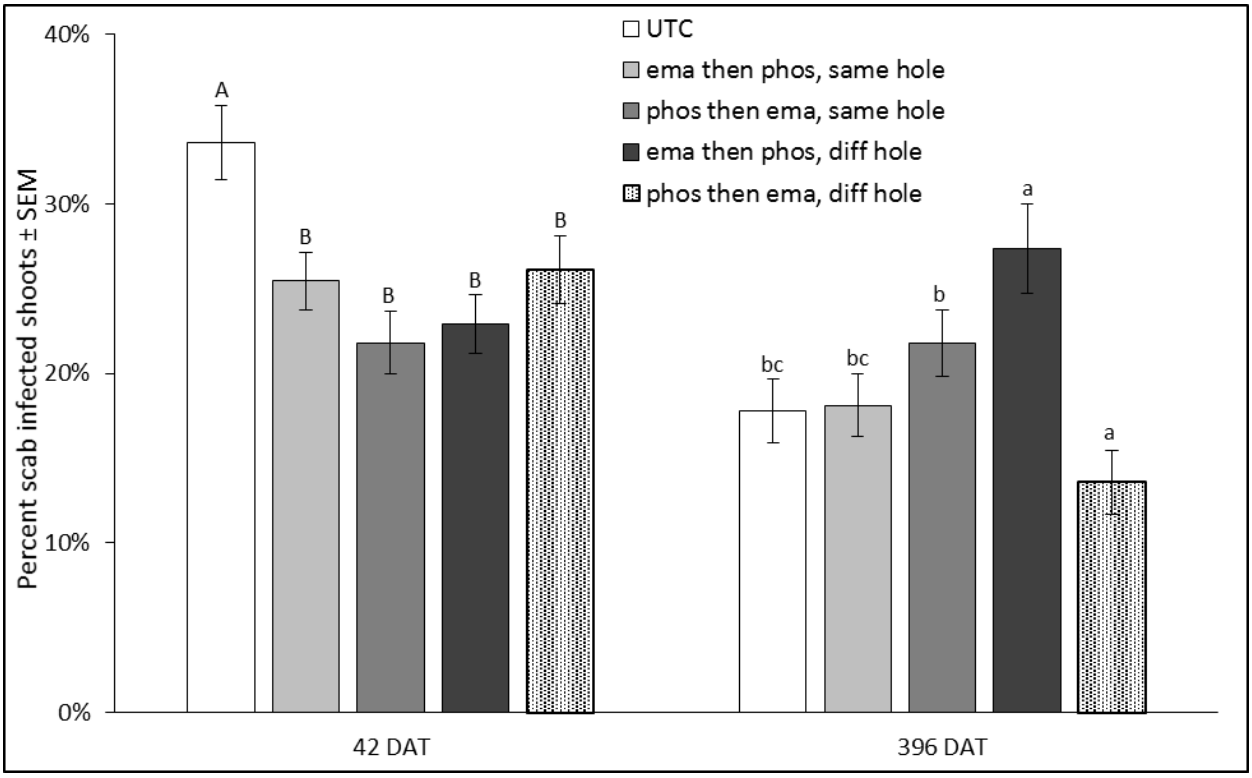

Figure 12. Mean percent scab infection on shoots recorded in 2015, at 42 and 396 DAT. Means with the same letter within dates are not significantly different (α = 0.05). Error bars are ± s.e.

Insect Bioassays

On 26 June 2014 (27 DAT) *C. rosaceana* larva mortality was highest on treatments 2 and 5 (F = 2.71, df = 4, p = 0.0673). Mortality of larvae exposed to leaf discs from treatments 3 and 4 did not differ from mortality of the untreated control (Fig. 13). Mortality data for bioassays had unequal variances for treatments, suggesting a higher chance of Type 1 error. On 18 August 2014 (80 DAT), all treatments were higher than the UTC and not different from one another (F = 5.98, df = 4, p = 0.0039).

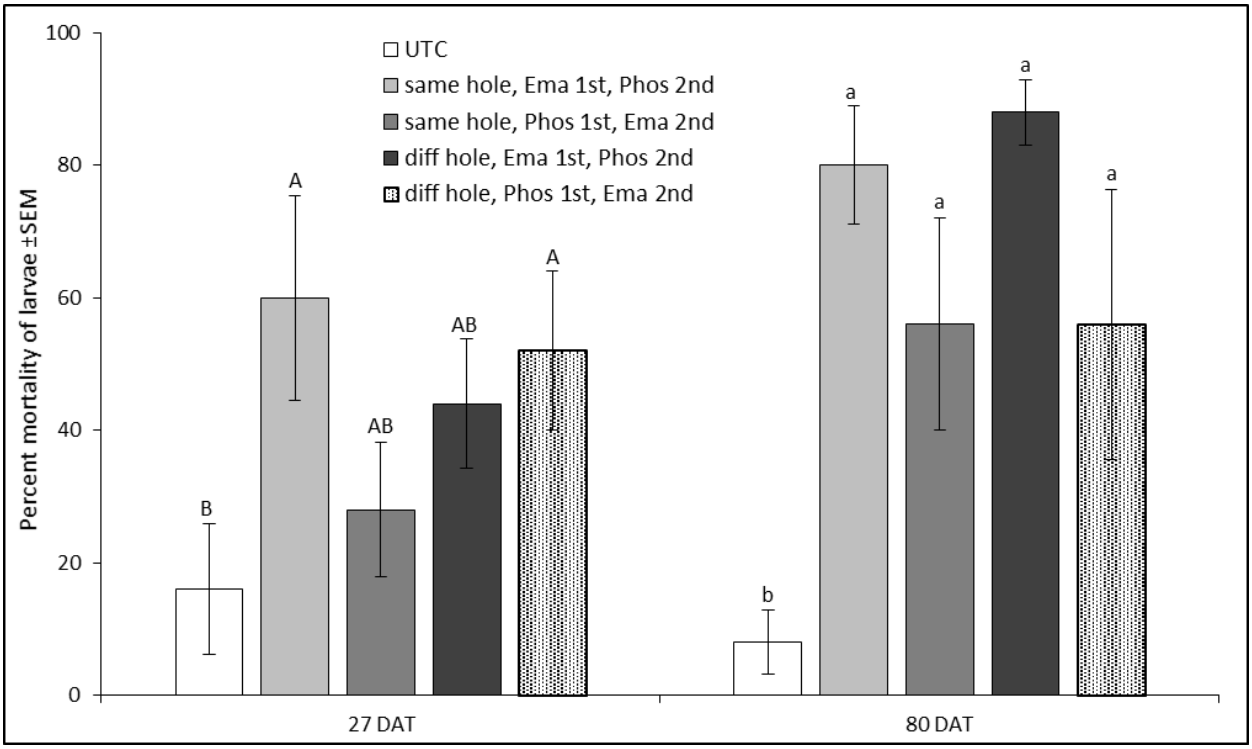

Figure 13. Mortality of *C. rosaceana* larvae exposed to treated leaves in 2014, at 27 DAT and 80 DAT. Means with the same letter within dates are not significantly different. Error bars are ± s.e.

At 27 DAT, treatments did not affect larval feeding ($F = 0.4$, $df = 4$, $p = 0.8053$). For the bioassay at 80 DAT, all treatments reduced larval feeding compared to the UTC ($F = 14.78$, $df = 4$, $p < 0.0001$). Treatment 4 had the greatest amount of leaf disc remaining followed by treatment 5, which was not different from the other injection treatments. Treatments 2 and 3 had more feeding than treatment 4 but less than the UTC (Fig. 14).

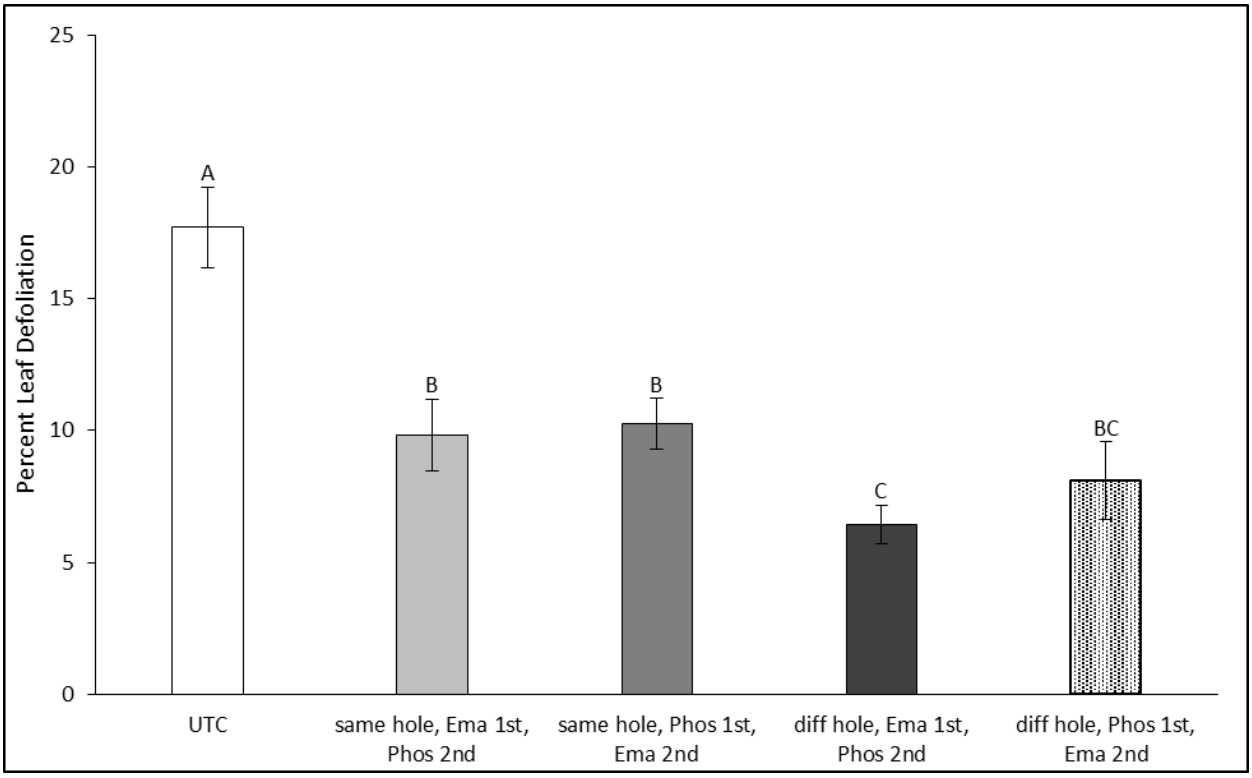

Figure 14. Percent leaf defoliation after the *C. rosaceana* bioassay conducted in 2014 at 80 DAT. Percentages based on an unfed reference leaf. Means with the same letter are not significantly different (alpha = 0.05). Error bars are ± s.e.

For bioassays conducted at 17 and 31 DAT, mortality of *C. rosaceana* larvae was high and did not differ among treatments (17 DAT: F = 0.17, df = 4, p = 0.6029; 31 DAT: F = 0.75, df = 4, p = 0.5741). To reduce mortality among UTC larvae, bioassays were shortened to 5 days followed by leaf disc evaluations at 7 days. No significant differences were found at 41 DAT (F = 1.53, df = 4, p = 0.2418). Again at 61 DAT, no significant differences were found in mortality between treatments (F = 1.13, df = 4, p = 0.3792).

There were no differences in larval feeding between any treatments or the UTC at 17 DAT (F = 1.21, df = 4, p = 0.3096). On 31 DAT, treatment 5 reduced larval feeding relative to the UTC (F = 4.45, df = 4, p = 0.0018). Larval feeding on other treated leaves was no different

than the UTC at 31 DAT. At 41 DAT, feeding on the UTC leaves was greater than all treatments, which had no differences from one another ($F = 8.98$, $df = 4$, $p < 0.0001$) (Fig. 15). On 61 DAT, treatments 2, 3, and 4 had less removed leaf area than the UTC ($F = 5.60$, $df = 4$, $p = 0.0003$). Treatment 4 was not different than treatment 5, and defoliation for treatment 5 leaves was not different than the UTC.

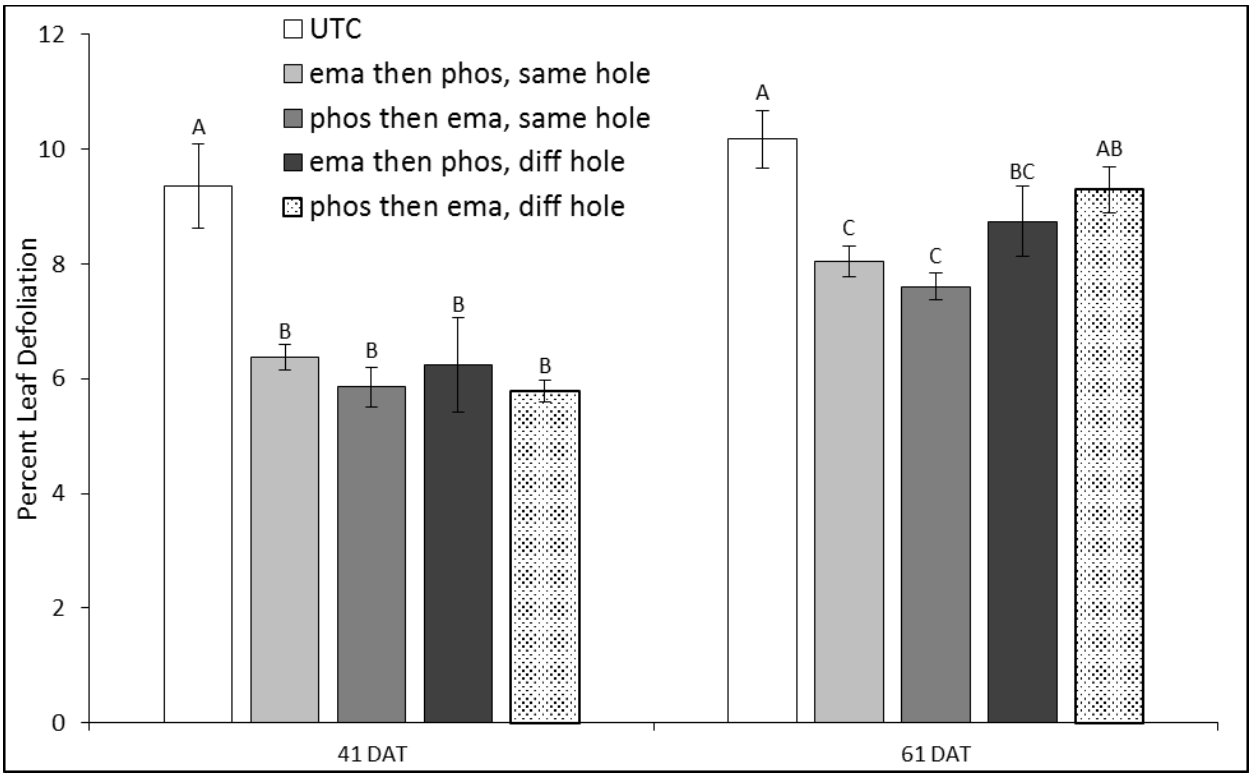

Figure 15. Percent leaf defoliation after the *C. rosaceana* bioassays conducted in 2015 at 41 DAT and 61 DAT. Percentages based on unfed reference leaf within each bioassay date. Means with the same letter are not significantly different (alpha = 0.05). Error bars are ± s.e.

Discussion

This research shows that two products formulated for trunk injection, emamectin benzoate and phosphorous acid, can interact depending on injection site and timing. Apple scab incidence was reduced for trees which received injections in different holes, and order of

injection did not have an effect in most circumstances. For injections using the same hole for both products, in which phosphorous acid followed emamectin benzoate injections, incidence of apple scab was also reduced. Injections of emamectin benzoate followed by phosphorous acid in the same hole resulted in greater reduction in scab incidence relative to untreated trees and other treatment combinations. In scab evaluations one year after injections, all injection treatments reduced scab incidence, and there were no between-treatment differences. This suggests that different injection order and pesticide combination is most important for managing scab during the first year.

The variation in treatment efficacy indicates an interaction between emamectin benzoate and phosphorous acid within the trees. When injected together, there is an opportunity for the two chemicals to interact in the injection site reservoir. In addition, products may be able to interact as they travel through the xylem, or when they reach leaves. There was a delay of several hours between injections, and injection order impacted the efficacy of the two same-hole treatments. This may be due to acidification of the tree sap and the timing by which it occurs: when phosphorous acid is injected first, it has time to change the pH in the xylem before emamectin benzoate is introduced. The emamectin benzoate is then subjected to a damaging environment immediately. When emamectin benzoate is injected first, there is some time for it to distribute throughout the canopy.

Interaction between products was observed less in trees with separate injection holes for phosphorous acid and emamectin benzoate, which may be due to limited lateral movement of product in the woody tissue. Pesticides travel quickly up the xylem via translocation, but rely on diffusion to move laterally (Aćimović et al. 2014). This results in xylem pathways above injection ports being somewhat compartmentalized, and preventing injected pesticides from

interacting with each other to the same degree that they might in same-hole injections.

Bioassays with *C. rosaceana* showed a similar trend, with mortality being higher in some cases for trees receiving emamectin benzoate injections first. Larval feeding was lower on leaves from trees which received different-hole injections in 2014, indicating that although mortality was not always high, antifeedant effects were present. Bioassays in 2014 suggested that different-hole injections may have a higher antifeedant effect; however, bioassays in 2015 showed few differences between injection treatments, and an increase in feeding activity for different-hole injections compared with same-hole injections. The mechanism for this is unclear; while it is tempting to speculate on synergism of emamectin benzoate and phosphorous acid in same-hole injections, this effect on feeding was minimal. Additional injection studies should be conducted to further investigate possible interactions.

In future studies, additional treatments of water-only controls, and injections of only emamectin benzoate and phosphorous acid should be included. Sprays of emamectin benzoate and phosphorous acid could also be included for direct comparison of treatments. Pre-mixing of emamectin benzoate and phosphorous acid was not investigated in this study. However, based on the results of same-hole injections, it is unlikely that phosphorous acid and emamectin benzoate would be compatible. In order to reduce the number of injection ports needed for trunk injection, timing for specific injections will be critical.

Conclusion

This study represents a first attempt at combining insect and disease management in apple trees, using trunk injection formulations to treat two primary pests. We demonstrated that when using a single set of injection ports to minimize trunk damage, injecting emamectin

benzoate followed by phosphorous acid produced the best results for control of scab and leafrollers. By showing that these two products can be injected into the same tree during a single season, we have expanded the degree to which trunk injection can be utilized by growers looking for pest management tools that reduce pesticide overspray while comprehensively addressing multiple major apple pests.

CHAPTER 5: CONTROL OF INSECT PESTS USING TRUNK INJECTION IN A NEWLY ESTABLISHED APPLE ORCHARD

Abstract

Nursery apple trees do not produce fruit for several years after first planting. This presents a management issue for growers, as relatively high pesticide inputs are required for delayed fruit output. In this study we evaluated a novel demonstration of nursery tree trunk injection. ‘Gala’ nursery trees with M9 rootstock were injected with a commercially available emamectin benzoate trunk injection formulation. The insecticide was injected below the graft union into either the trunk or the taproot at either 0.01 or 0.001 g a.i. per tree. Sets of trees were injected and planted for two separate years at a research station in southwestern Michigan. Trees were evaluated throughout the planting season and into the following year. Injections into the trunk best delivered emamectin benzoate to the canopy compared with injections into the taproot, and the higher rate reduced insect pests more than the lower rate. In the following year, differences in insect control between trunk and root injections were less pronounced, but the higher rate of emamectin benzoate was more persistent and reduced pests relative to the other treatments or control trees. This study demonstrates a novel use of trunk injection to reduce insect pests in young apple trees, and may be useful to fruit growers in need of lower-input management strategies on non-bearing fruit trees.

Introduction

Newly-established apple trees from nurseries present a unique pest management challenge. Trees in commercial apple production pass through multiple management stages,

starting with a multi-year non-bearing period after nursery trees are first planted in orchards. Apple production in Michigan is valued at $221M (NASS 2014), and protecting crops from insect damage is a high priority. However, apple growers cannot generally justify the same high-input pest management for non-bearing trees as is used for bearing trees. Mature apple orchards are managed using airblast sprayers to protect fruit from direct pests. Overspray from airblast sprayers and the large surface area sprays must cover results in less than 0.1% of the insecticide actually reaching the target pest (Pimentel 1995). Airblast sprayers are also less efficient at covering young apple trees from nurseries compared with mature fruit-bearing trees because of greater open space between tree canopies. Since young apple trees do not bear fruit, management programs for non-bearing trees focus on indirect pests.

Nursery trees are not subject to the same pesticide label requirements as mature trees until they reach fruit bearing stage. Systemic insecticides such as imidacloprid and abamectin has been utilized for pest management on several species of nursery trees. Basal drenches of imidacloprid controlled *Agrilus planipennis* on young white and green ash trees (*Fraxinus americana* and *F. pennsylvanica*) (Smitley, Rebek, et al. 2010). Soil drenches of neonicotinoids controlled *Scirtothrips perseae* in nursery avocado trees (*Persea americana*) (Byrne et al. 2007). Trunk injections of imidacloprid and abamectin have been shown to successfully control *Corythucha cydoniae* on recently established green hawthorn (*Crataegus viridis*) nursery trees (Gill et al. 1999). However, no studies to date have investigated trunk injection in apple nursery trees.

Trunk injection is a method of applying chemicals directly into the vascular system of a tree, first by piercing the bark to access the xylem tissue. The chemicals are then injected and distributed systemically via the xylem sap. Trunk injection is a discriminating delivery system

that targets pests while reducing pesticide inputs and environmental exposure (Wise et al. 2014). It has recently been demonstrated for control of foliar pests and diseases in fruit-bearing apple orchards (VanWoerkom et al. 2014, Aćimović, VanWoerkom, et al. 2016). Trunk injection may be an ideal management technique for nursery trees. This represents a novel area of research with potential benefits to nursery production and the apple industry.

The objective of this study was to evaluate the feasibility and efficacy of nursery tree injection, using a formulation of emamectin benzoate designed for trunk injection (TREE-age®, Arborjet, Woburn MA) into apple (*Malus pumila*). Nursery trees were injected during two field seasons and planted at a Michigan State University research station in southwest Michigan. Treatments were evaluated with multiple leaf residue samples, and whole tree samples of roots, trunks, shoots and leaves. Efficacy of injection treatments was quantified with bioassays of laboratory-reared *Choristoneura rosaceana*, and field evaluations of *Empoasca fabae* and *Phyllonorycter blancardella*. We postulated that due to small canopy size, injecting formulated emamectin benzoate at a low concentration, 1/80 of what would be injected into a mature apple tree, would be as effective as a moderate concentration of 1/8 the rate. We also postulated that injections into the root of nursery trees would perform as well as injections into the trunk.

Materials and Methods

Study Design and Setup

Nursery apple trees (‘Gala’ on M.9 rootstock; Adams County Nursery, Inc., Aspers, PA) approximately 1.5 m tall, with 5 cm diameter rootstock and 2.5 cm diameter scion, were acquired at the beginning of each season and stored in a cold room at 40°C until use. Injections were performed in the laboratory prior to planting. Two holes were drilled, spaced 180° apart on either

side of the tree, 4.7625 mm (0.1875 in) in diameter and 6.35 mm (0.25 in) deep. The two holes were drilled below the graft union, either into the taproot below the first lateral roots, or into the trunk just below and at right angles to the graft union. Injections were performed with a micropipette (Pipetman® Classic, Gilson Inc., Middleton WI). Holes were capped with nylon plugs (Widgetco, Houston TX) and sealed with grafting tape. Trees were then watered and returned to the cold room. On the same day or the day thereafter, trees were planted in cultivated Blount silt loam soil at the Trevor Nichols Research Center, Michigan State University, in Fennville, MI (Soil Survey Staff 2016). They were spaced 1.8 m apart, with rows spaced 4.9 m apart. Treatments were assigned in a randomized complete block design.

In 2014, nursery trees were injected on 4 June. There were five treatments, consisting of (1) an untreated check (UTC), (2) emamectin benzoate into the trunk (TREE-age, Arborjet) at 1/8 the typical field rate, or 0.2325 ml/tree (0.01 g a.i.), (3) the 1/8 rate into the root zone, (4) emamectin benzoate into the trunk at a 1/80 rate, or 0.0233 ml/tree (0.001 g a.i.), and (5) the 1/80 rate into the root zone. Insecticides were diluted with deionized water which helped increase uptake into the tree. Treatments 2 and 3 were injected at a 1:1 dilution, and treatments 4 and 5 were injected at a 1:10 dilution. There were five replications of each treatment. Three additional replications, of treatments 4 and 5 only, were included for the purpose of collecting root, trunk, stem, and leaf residue samples from entire trees. These samples were taken during the second half of the growing season.

On 2 June 2015, nursery trees were injected with one of three treatments: (1) an untreated check (UTC), (2) emamectin benzoate (TREE-age, Arborjet) at 1/8 field rate (0.2325 ml/tree, 0.01 g a.i.) in the trunk, and (3) the same dose in the root zone. Treatments with the 1/80 rate of emamectin benzoate were not included in the 2015 study. Eight replications of each treatment

were included and planted in a randomized complete block design Three of these replications were designated for whole tree residue samples of roots, trunks, stems, and leaves. The first 5 replications were maintained through the following year.

Insecticide Residues

Leaves (20 per sample or approximately 10 g) were collected each year from replications 1-3, at 4 and 8 weeks after injection and planting. The three replications designated for whole tree sampling were dug out and sampled at approximately 8 weeks each year. Roots, trunk, shoot stems, and leaves were cut from the nursery trees using a pair of garden pruners. The trunk and roots were cut into 2.54 cm sections to fit in the jars. Samples were then weighed, stored with dichloromethane in 120 ml graduated round glass bottles (Quorpak®, Berlin Packaging, Chicago IL) and held in a cold room at 40°C until sample cleanup using QuEChERS and analysis with HPLC (Anastassiades et al. 2003). Leaf residues were analyzed in SAS 9.4 using repeated measures mixed models (SAS 2013). Residues of tree parts were analyzed using a two-way glimmix model in SAS with Tukey's multiple comparisons for means separations.

Bioassays

Leaves for bioassays were sampled from nursery trees injected in 2014 at 63 and 358 days after treatment (DAT), and from 2015 trees at 36 and 71 DAT. Eight leaves were collected from replicates 1-5 of each treatment and cut into discs with a cork borer, rinsed with acetone between treatments. In some instances, leaves were too small for the standard leaf disc size (15 mm) so multiple leaves were aggregated to approximate the leaf disc area. Discs were placed into 5.5 cm polystyrene Petri dishes lined with moist filter paper. Five laboratory-reared *C.*

rosaceana larvae (neonate, first or second instar) were placed in each Petri dish. Bioassays were kept in an incubation chamber with 16:8 day:night light cycle. After 7 days, dishes were opened and evaluated for mortality. Data analyses comparing mean numbers of dead larvae were performed in SAS using proc genmod to specify a log-linked Poisson distribution model, with total numbers of larvae as an offset. Means separations were performed with paired t-tests at an alpha of 0.05.

Remaining leaf disc parts were removed from the completed bioassays and cleaned with a moist paper towel. They were then taped to sheets of printer paper, imaged with a flatbed scanner, and saved as JPEG files at 600 dpi. Total area and defoliation of leaf discs was quantified by number of pixels using ImageJ software (Rasband 2017). Analyses of leaf disc area were performed with one-way ANOVA using proc glm in SAS and Fisher's LSD with an alpha of 0.05 was used to construct means separations.

Field Evaluations

Numbers of *Empoasca fabae* nymphs were counted by checking the underside of leaves for 30 s per tree. In 2014, nursery trees were evaluated at 36 DAT, and at 31 DAT for nursery trees injected in 2015. Data analyses comparing mean numbers of nymphs per tree were performed in SAS using proc genmod, specifying a log-linked Poisson distribution model for count data. Means separations were performed with individual t-tests at an alpha of 0.05.

Damage due to *E. fabae* feeding was evaluated by counting numbers of chlorotic and curled leaves as well as total numbers of leaves per shoot, for 20 shoots per tree. Damage was recorded for 2014 injections at 432 DAT. Nursery trees injected in 2015 were also evaluated, at 69 DAT. Data were analyzed to compare numbers of damaged leaves between treatments using

proc genmod in SAS to specify a log-linked Poisson distribution model, with total numbers of leaves per shoot as an offset. Means separations were performed with paired t-tests at an alpha of 0.05.

Infestations of nursery trees by *Phyllonorycter blancardella* were evaluated for 2014 injections 434 DAT, and for 2015 injections at 71 DAT, by counting the number of leaf mines in leaves as well as total numbers of leaves on shoots and spurs. For 2015 injections at 505 DAT, a timed count of 30 s was performed and numbers of leaves with mines were recorded for that time window.

Results

Insecticide Residues

In 2014, leaf residue concentrations between treatments were affected more by injection site than injection rate. Leaves of trunk-injected trees had higher concentrations of emamectin benzoate than root-injected trees for both insecticide rates (F-value = 5.51, df = 1, p-value = 0.0469) (Fig. 16). Residues of emamectin benzoate for root injections at both 1/8 and 1/80 the field rate were low, and insecticide rate was not statistically significant (F-value = 1.95, df = 1, p-value = 0.2002). There were concentration differences between whole tree parts for trees injected at the 1/80 rate, with stems containing the highest overall concentration (F-value = 12.75, df = 3, p-value = 0.0003) (Fig. 17). No significant differences between trunk and root injections were found in emamectin benzoate concentration at the 1/80 rate in 2014.

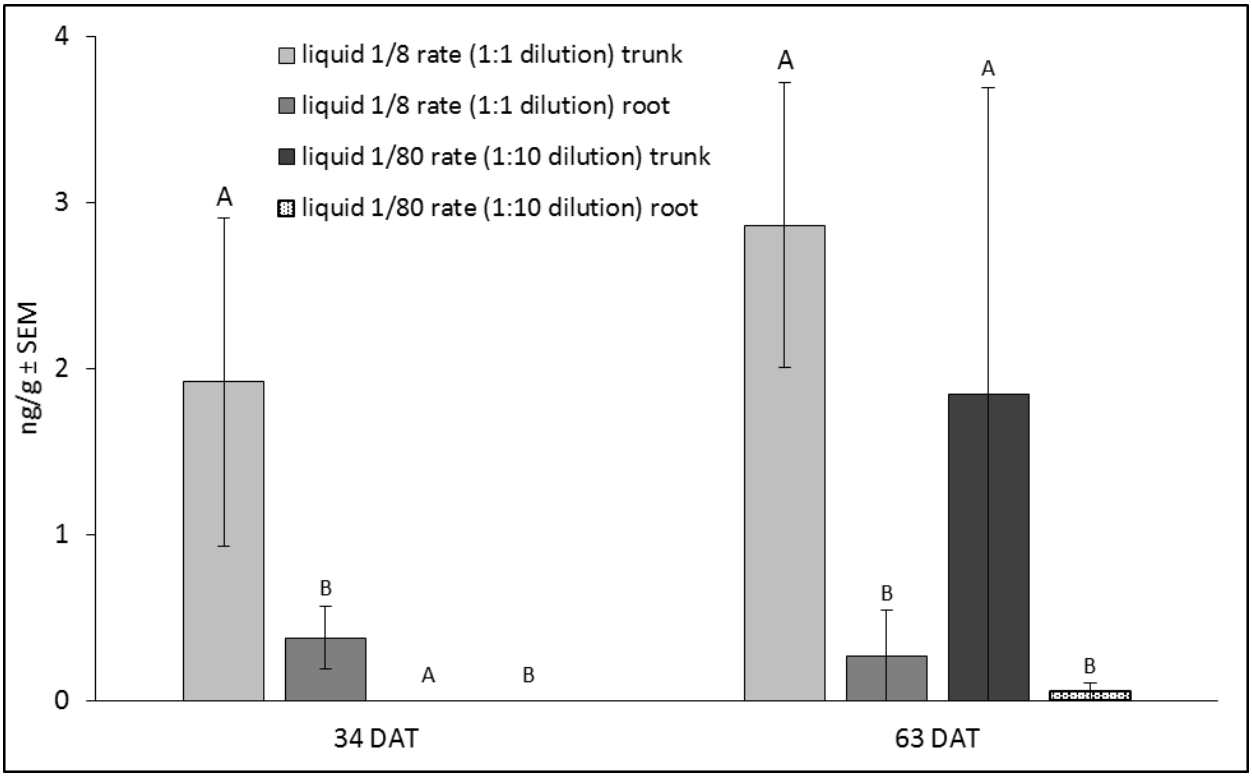

Figure 16. Mean concentrations of emamectin benzoate recovered from nursery tree leaf samples taken in 2014, at 34 and 63 DAT. Error bars are ± s.e. Treatment means with the same letter are not significantly different based on alpha = 0.05.

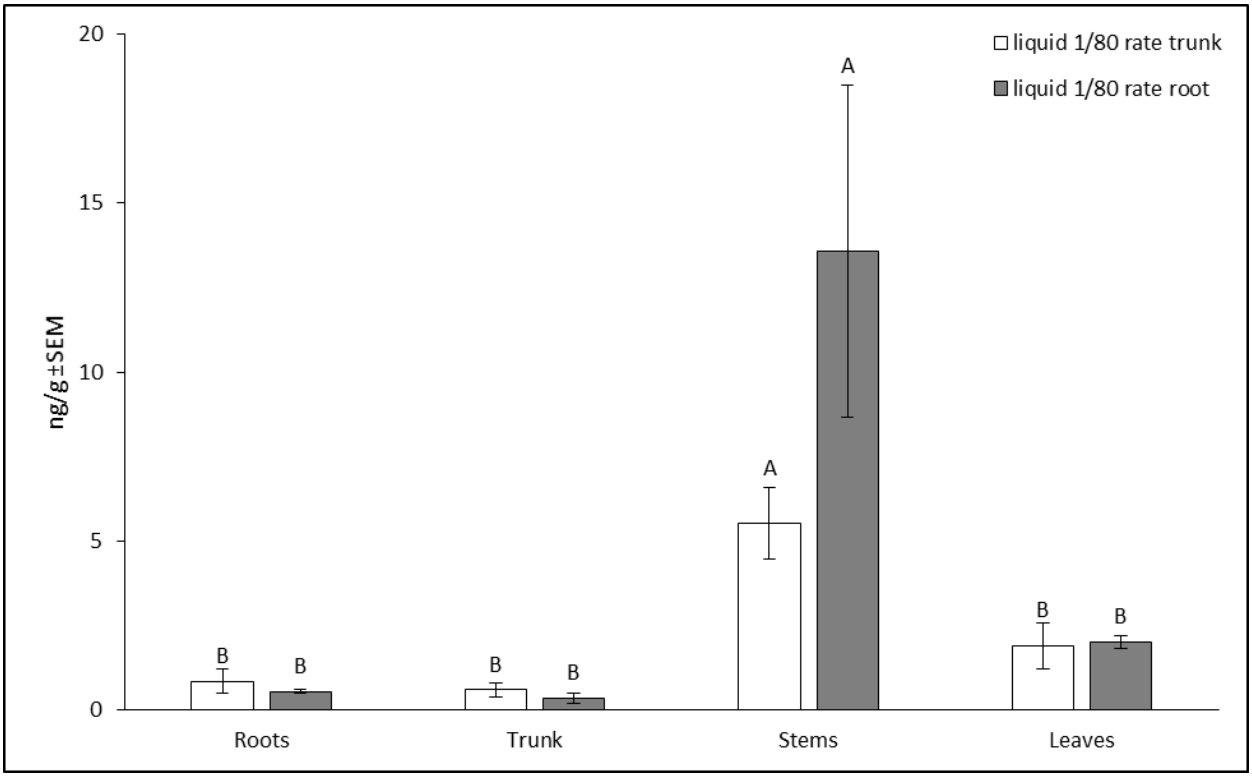

Figure 17. Mean concentrations of emamectin benzoate recovered from nursery trees in 2014 treated at the 1/80 rate. Samples were taken from roots, trunk, stems, and leaves at 58 DAT. Error bars are ± s.e. Means followed by the same letter are not significantly different based on alpha = 0.05.

In 2015, the effect of injection site was less pronounced than in 2014. No differences were found between the 1/8 rate injected into the trunk versus into the root (F-value = 3.24, df = 1, p-value = 0.1462), though there were higher emamectin benzoate concentrations in the leaves at 43 DAT compared to root injections (Fig. 18). Instead, sample date had a significant effect on leaf residues for both trunk and root injections (F-value = 8.97, df = 1, p-value = 0.0401). Emamectin benzoate residues injected at the 1/8 rate had a different distribution pattern within nursery trees compared to the 1/80 rate in 2014. Trees which received root-injected 1/8 rate of emamectin benzoate had the highest concentrations of active ingredient, found in the root tissue,

versus trunk injection (F-value = 7.63, df = 3, p-value = 0.0029) (Fig. 19). Trunk and stem tissues had higher concentrations of emamectin benzoate in trees receiving trunk injections compared with root injections, though this was not a statistically significant effect.

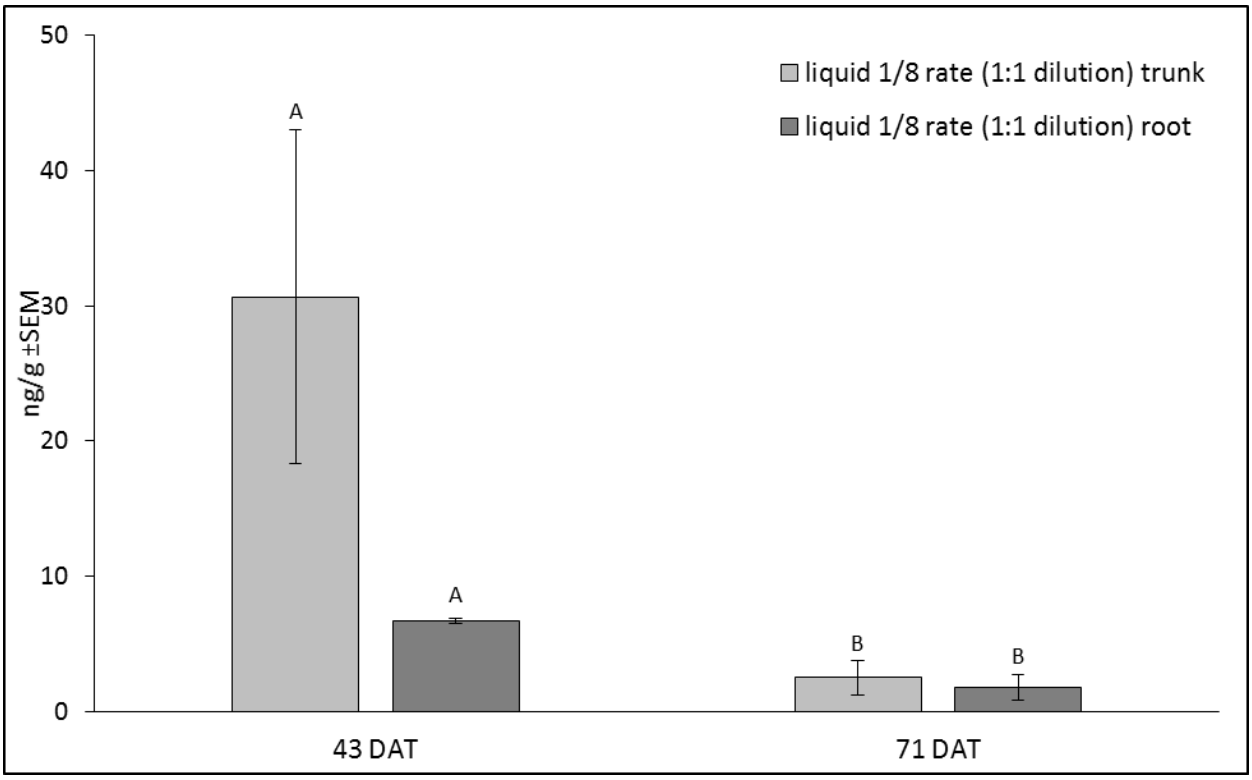

Figure 18. Mean concentrations of emamectin benzoate recovered from nursery tree leaf samples taken in 2015, at 43 and 71 DAT. Error bars are ± s.e. Means followed by the same letter are not significantly different based on alpha = 0.05.

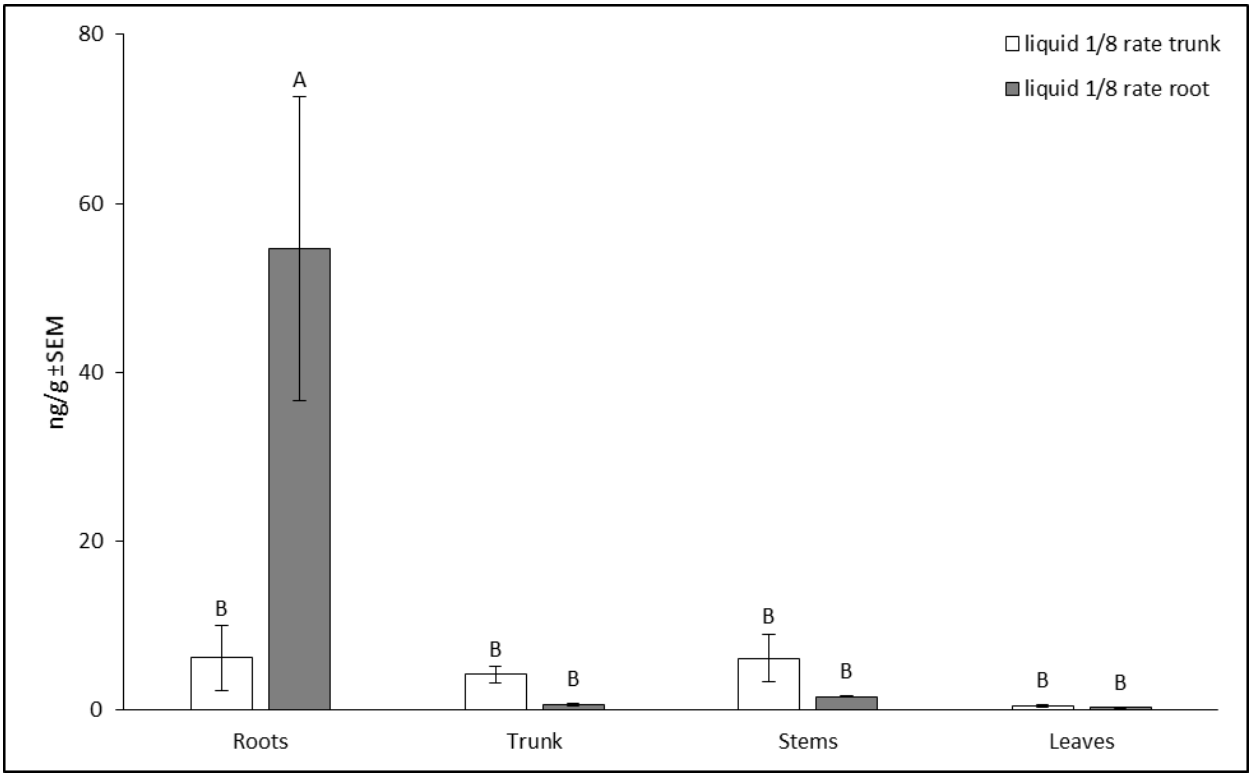

Figure 19. Mean concentrations of emamectin benzoate recovered from nursery trees in 2015 treated at the 1/8 rate. Samples were taken from roots, trunk, stems, and leaves at 72 DAT. Error bars are ± s.e. Means followed by the same letter are not significantly different based on alpha = 0.05.

Bioassays

In 2014, larval mortality in the 63 DAT *C. rosaceana* bioassay did not differ between insecticide treatments and the UTC, and mortality was high across all treatments. There were no differences in larval feeding between injection treatments and the UTC at 63 DAT, although the model was significant overall (F-value = 2.38, df = 8, p-value = 0.0183) (Fig. 20). Larval feeding was slightly lower on leaves which received root injections at both rates, compared to trees with 1/80 rate trunk-injections (F-value = 2.22, df = 4, p-value = 0.0684). However, no treatments were different from the UTC.

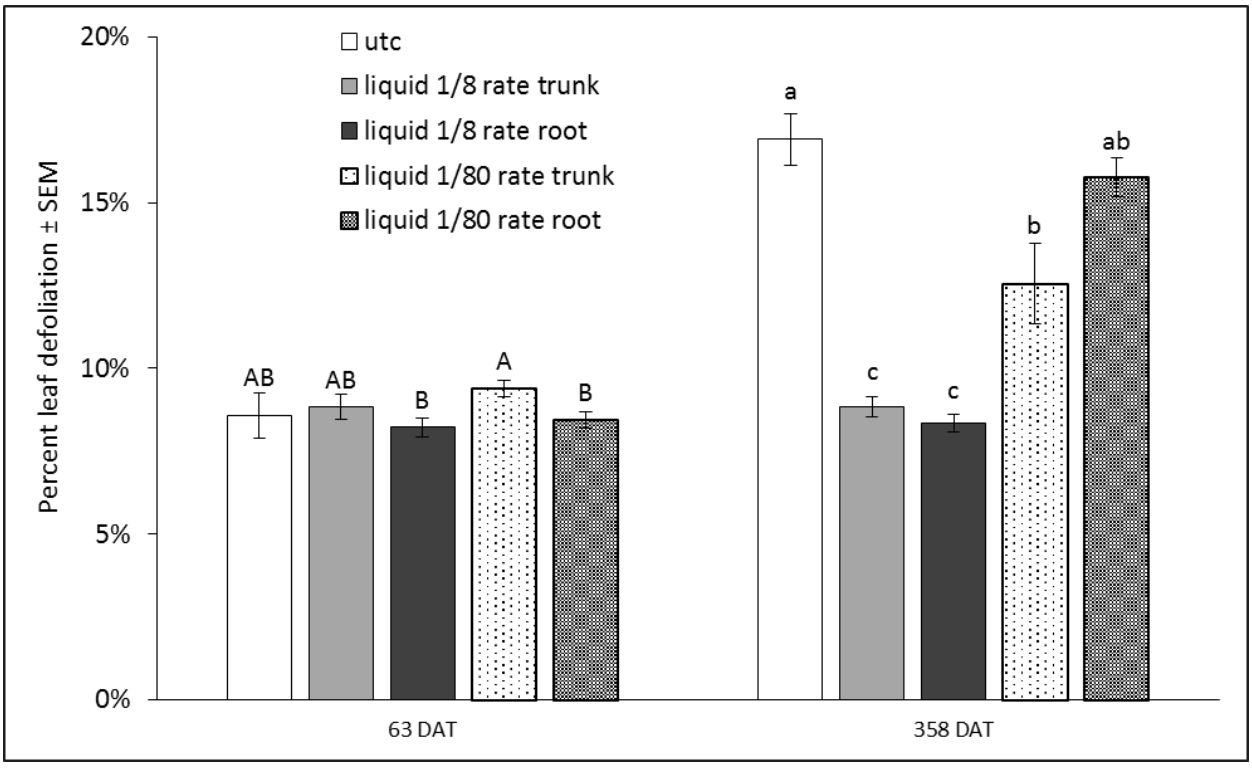

Figure 20. Percent defoliation of leaf discs in *C. rosaceana* bioassays taken in 2014 at 63 and 358 DAT. Percent defoliation was calculated to show relative degree of feeding by using unfed leaf discs within each sample date as reference. Error bars are ± s.e. Treatment means within a given date with the same letter are not significantly different based on alpha = 0.05.

For the 2014 injection bioassay conducted at 358 DAT, mortality was relatively high in the two 1/8 rate emamectin benzoate injections (Fig. 21). Zero mortality across all replicates in the 1/80 rate treatments and the UTC precluded statistical analysis as there was no within-treatment variance from which to draw comparisons. Larval feeding was also lowest for the two 1/8 rate treatments compared to other treatments (F-value = 8.53, df = 4, p-value < 0.0001) (Fig. 21). Larval feeding on leaves from 1/80 rate trunk injected trees was also lower than the UTC.

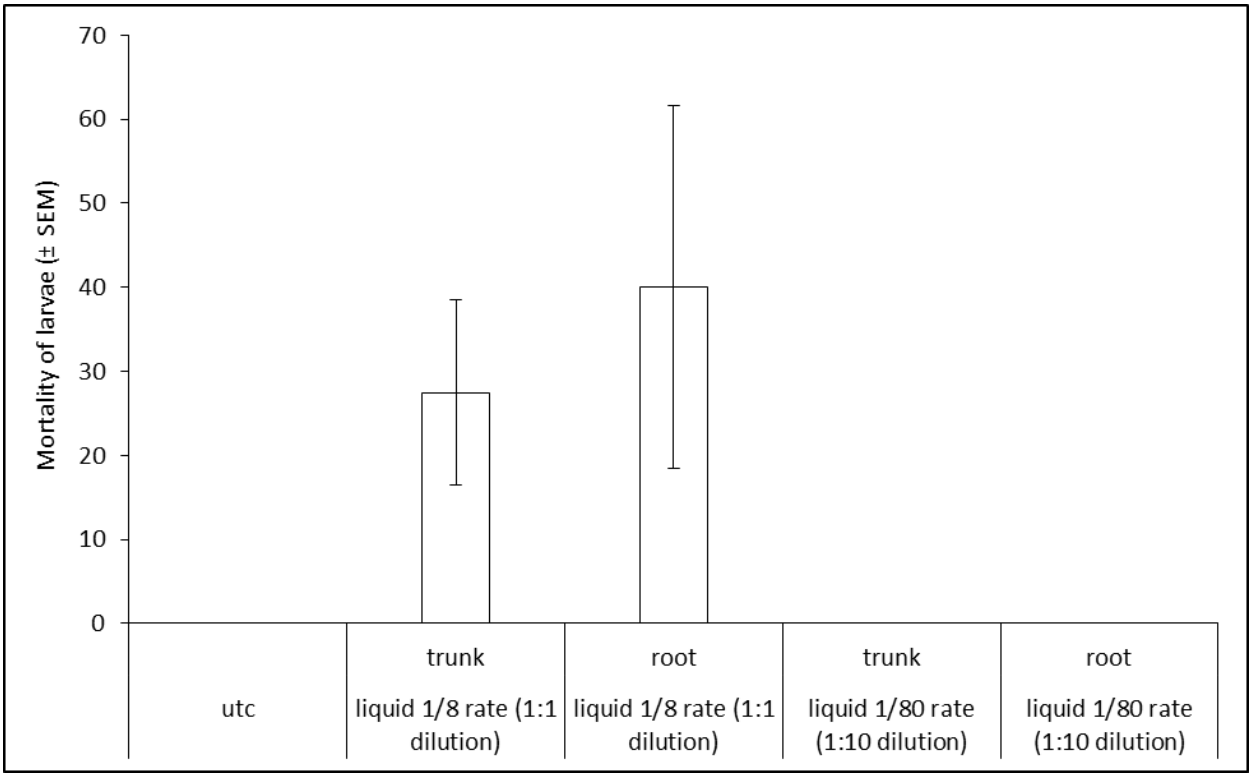

Figure 21. Mortality of *C. rosaceana* larvae in bioassays conducted in 2014 at 358 DAT. Error bars are ± s.e. Statistical analyses and means separations were not calculated due to zero variance within treatments.

The 2015 *C. rosaceana* bioassay conducted at 43 DAT experienced high larval mortality in the UTC, and there were no differences between treatments. However, larval feeding was greater on the UTC leaves compared to trunk and root-injected leaves (F-value = 24.41, df = 2, p-value < 0.0001) (Fig. 22). At 71 DAT, larval mortality on leaves from 1/8 rate trunk-injected nursery trees was higher than on leaves from the UTC or root-injected trees (UTC: $\chi^2 = 4.36$, p-value = 0.0314; trunk-injected: $\chi^2 = 3.70$, p-value = 0.0544; root-injected: $\chi^2 = 6.30$, p-value = 0.0121) (Fig. 23). Similarly, larval feeding was greatest on leaves from the UTC compared to trunk and root-injected emamectin benzoate treatments (F-value = 43.95, df = 2, p-value < 0.0001) (Fig. 22).

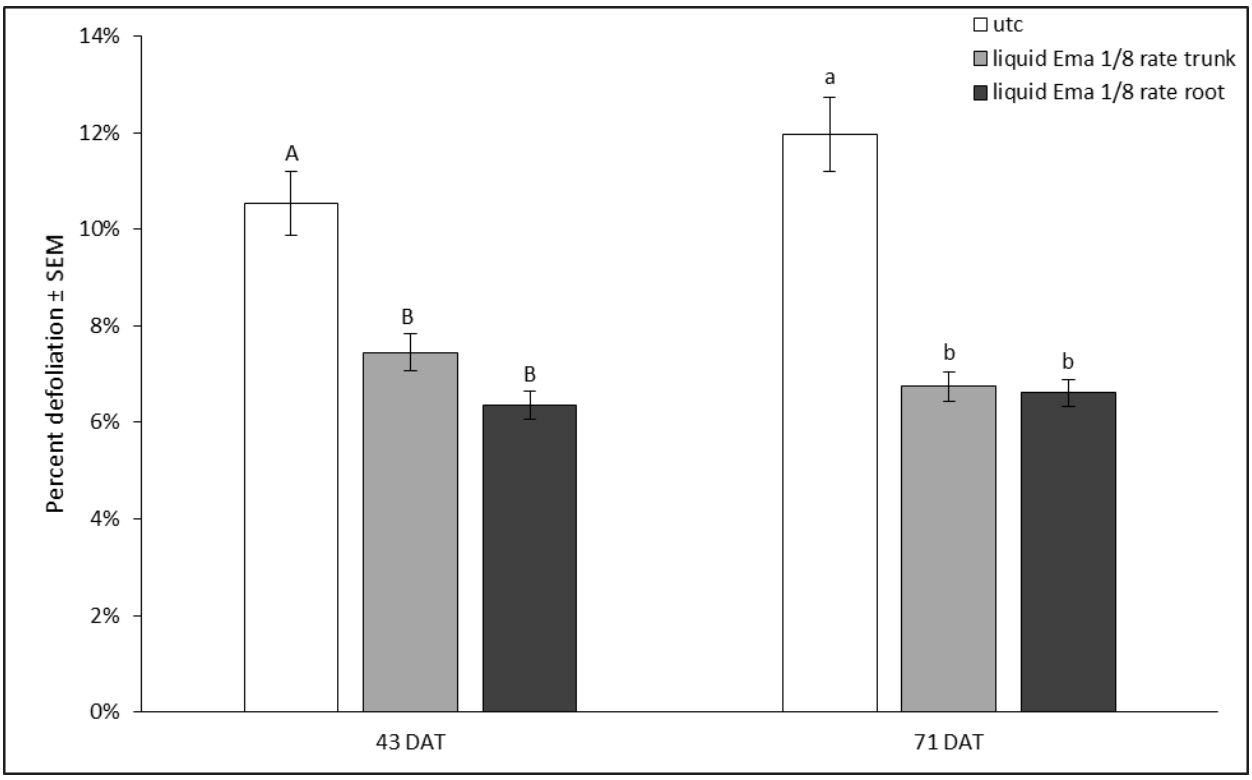

Figure 22. Percent defoliation of leaf discs (area of leaf removed) in *C. rosaceana* bioassays taken in 2015 at 43 and 71 DAT. Percent defoliation was calculated to show relative degree of feeding by using unfed leaf discs within each sample date as reference. Error bars are ± s.e. Treatment means within a given date with the same letter are not significantly different based on alpha = 0.05.

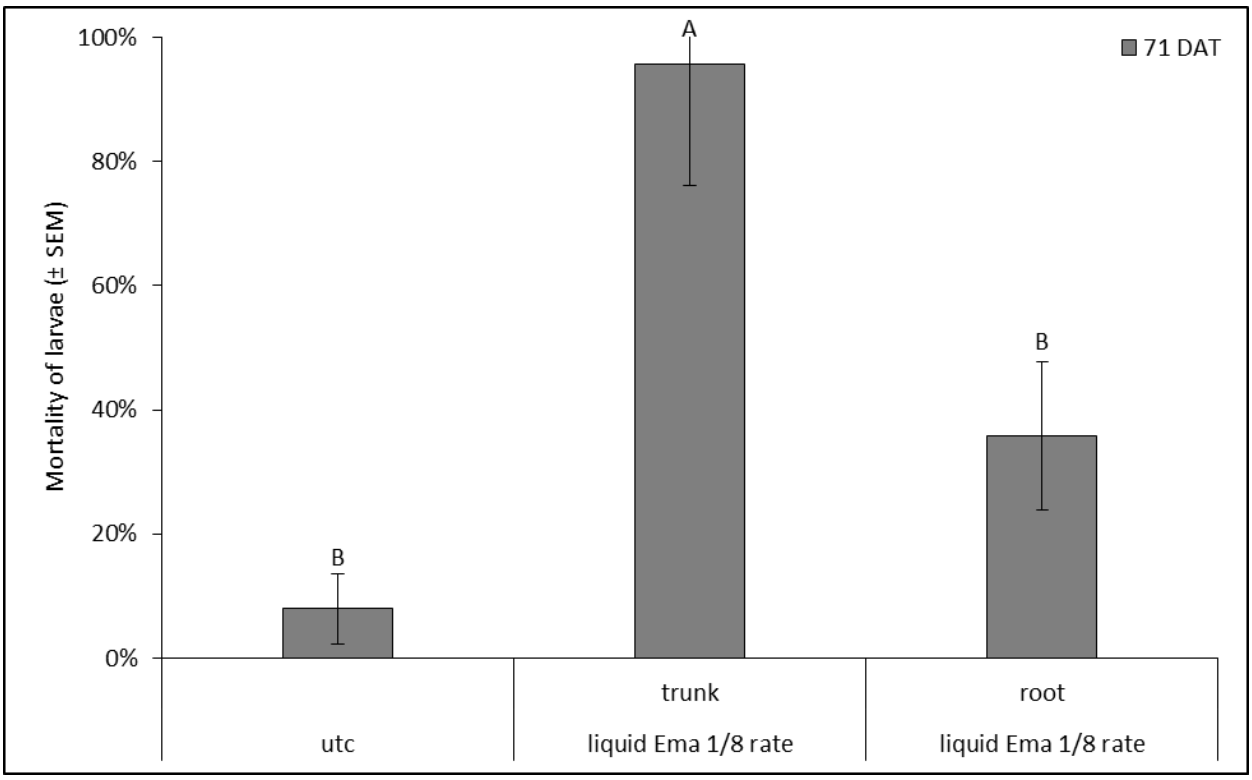

Figure 23. Mortality of *C. rosaceana* larvae in bioassays conducted in 2015 at 71 DAT. Error bars are ± s.e. Data are back-transformed from log-linked Poisson distribution. Means followed by the same letter within each sample date are not significantly different based on alpha = 0.05.

Field Evaluations

Counts of *E. fabae* nymphs at 36 DAT in 2014 injections were lowest on UTC trees, while 1/8 rate root-injected trees had relatively higher numbers of nymphs (p-value < 0.0001) (Fig. 24). The other three injection treatments were not different from either the root-injected 1/8 rate or the UTC. At 394 DAT, there were not enough leafhopper nymphs present on the nursery trees to conduct an analysis. Leaf damage due to leafhopper feeding was evaluated for the 2014 injections at 432 DAT (Fig. 25). The two 1/8 rate treatments and the 1/80 rate trunk-injected treatment had less leafhopper damage than the UTC (p-value < 0.0001). Counts of *E. fabae* nymphs at 31 DAT in the 2015 injections were not different between the trunk and root

injections or the UTC. At 69 DAT, there were not enough leaves on the nursery trees to conduct a leafhopper damage analysis.

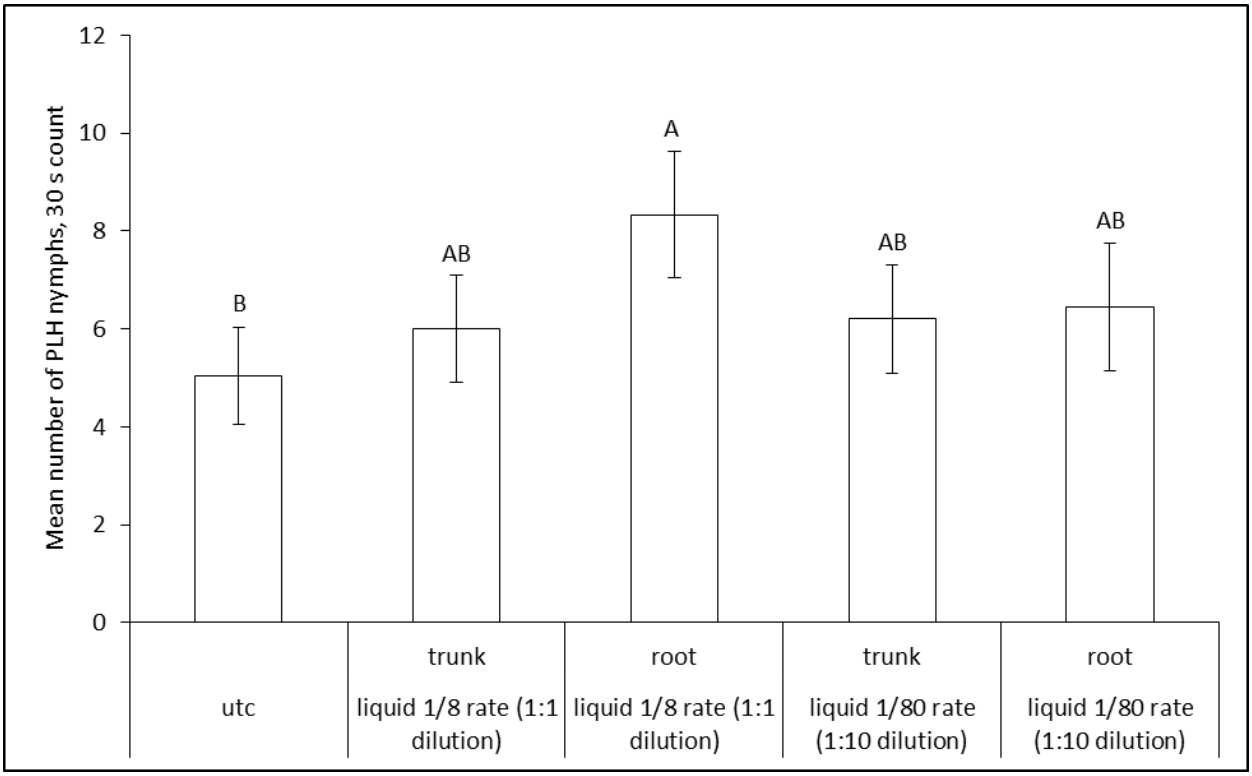

Figure 24. Mean numbers of *E. fabae* nymphs counted in 2014 at 36 DAT. Error bars are ± s.e. Means followed by the same letter within each sample date are not significantly different based on alpha = 0.05. Data are back-transformed from Poisson log-linked distribution used for analysis.

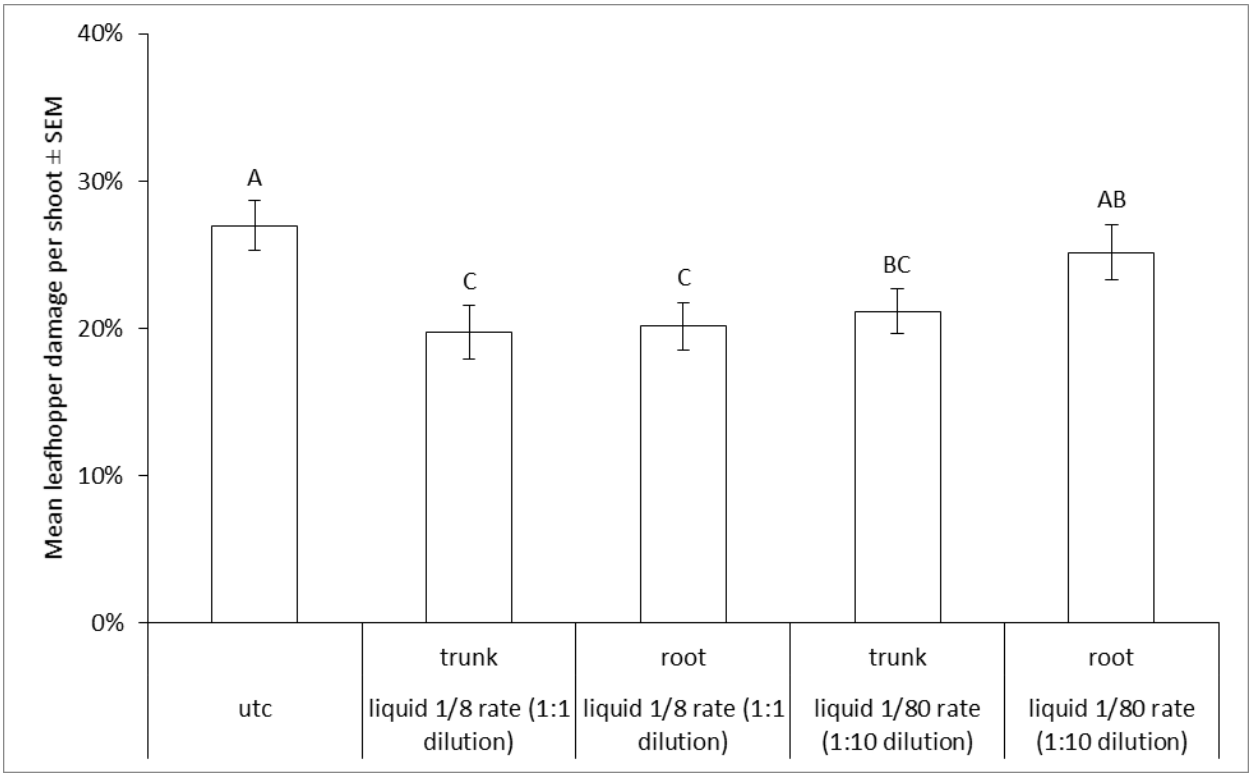

Figure 25. Mean percent of leaves damaged *E. fabae* nymphs on nursery trees planted in 2014, counted at 432 DAT. Error bars are ± s.e. Means followed by the same letter within each sample date are not significantly different based on alpha = 0.05. Data are back-transformed from Poisson log-linked distribution used for analysis.

At 434 DAT, counts of *P. blancardella* leaf mines on trees injected in 2014 were lowest for trees which received trunk-injected 1/8 rate emamectin benzoate, followed by root-injected 1/8 rate emamectin benzoate (p-value < 0.0001) (Fig. 26). Numbers of leaf mines on the two 1/80 rate injection treatments were not different from the UTC. In 2015, leaf mines at 71 DAT were too few to conduct a complete analysis. In the year following the 2015 injections, at 505 DAT, there were no differences in numbers of *P. blancardella* leaf mines between treatments or the UTC.

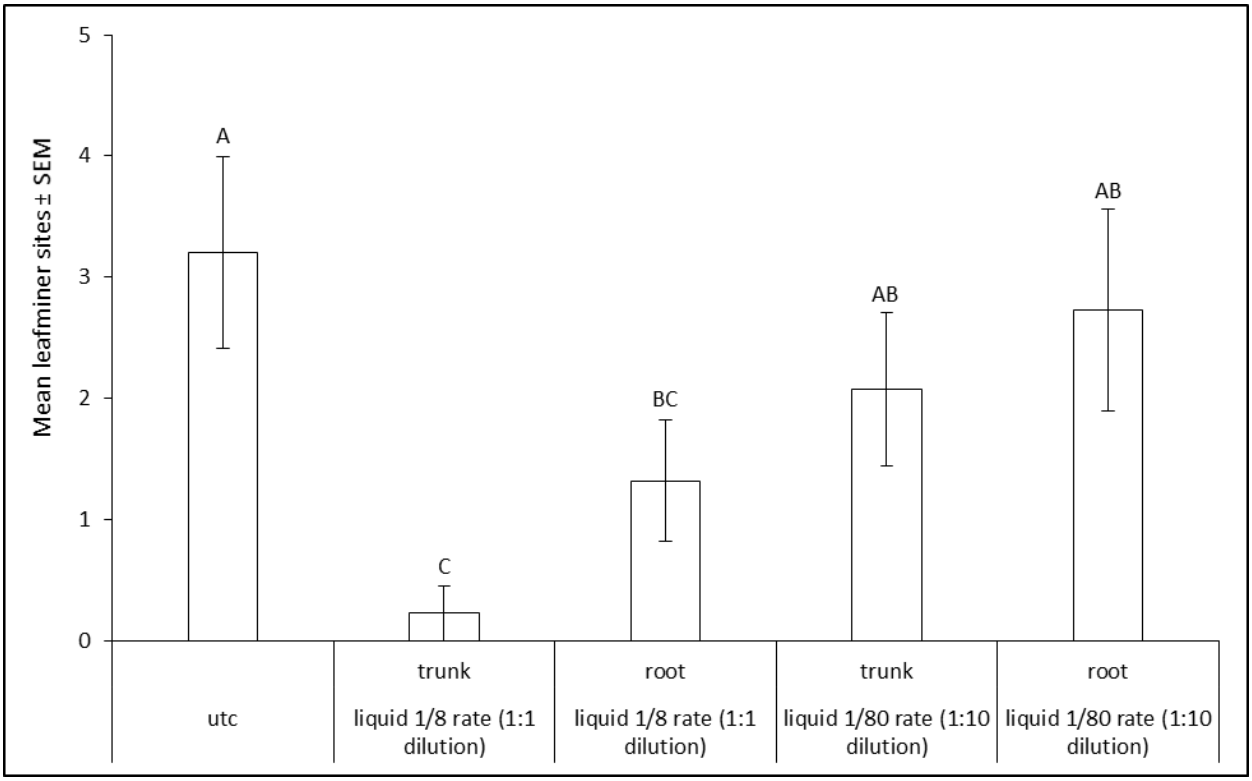

Figure 26. Mean numbers of leaves with P. blancardella mines on nursery trees planted in 2014, counted at 434 DAT. Error bars are ± s.e. Means followed by the same letter within each sample date are not significantly different based on alpha = 0.05. Data are back-transformed from Poisson log-linked distribution used for analysis.

Discussion

In this study, we demonstrated that injecting emamectin benzoate into the trunks of nursery trees can reduce insect pests. Also, emamectin benzoate accumulated most in the stems during the latter half of the growing season. Rates of emamectin benzoate injected into the trunk resulted in higher leaf residues compared to root injections, and reduced numbers of pest insects more than root injections. Moderate doses of emamectin benzoate reduced pests, although this effect was greater and more persistent at the 1/8 rate compared to the 1/80 rate.

Trunk injections yielded better results for distributing emamectin benzoate throughout

nursery trees, compared to root injections. Injecting in the root zone negatively impacts movement of emamectin benzoate. In 2015, emamectin benzoate concentrations in leaves were higher for trunk injections compared to root injections in the first month of the study, but in the latter half of the season there was very little movement to the leaves and injection site had no effect on concentration. Leaf residue concentration was only significant between sample dates in 2015, and not for injection site. Leaf concentrations were not different between the 1/8 and 1/80 rates in 2014, although the 1/8 rate impacted pests more than the 1/80 rate. Therefore a 1/8 rate of emamectin benzoate injected into the trunk portion of the rootstock appears to be the most efficacious out of the treatments included in this study.

Residues were primarily accumulated in the stems when sampled in 2014. This may be due to trees pulling the insecticide out of the leaves along with endogenous compounds toward the end of the season, which indicate that emamectin benzoate moved via the phloem. However, across both years there were variations in distribution patterns. In 2015, trees injected in the root zone had their residues mostly concentrated in the roots. Residues from trunk-injected trees were higher in the stem and trunk compared with root-injected trees in 2015. In 2014, conversely, residues were somewhat higher in stems for root-injected trees, which may be due to a lower viscosity than the higher rate, allowing for better discernment between treatments. Whole-tree sampling was only performed for the 1/80 rate in 2014, and the 1/8 rate in 2015, which somewhat limits the ability to make inferences year to year. Future studies should include metabolites of emamectin benzoate to provide a complete picture of insecticide movement within the vascular system of nursery trees.

Considering that we found high insecticide residues in woody tissue, trunk injection of nursery trees may be valuable for control of the black stem borer, *Xylosandrus germanus*. Young

fruit trees are at the greatest risk of injury, although because the beetles feed on ambrosia fungi rather than plant tissue, systemic insecticides are not recommended for control of *X. germanus* (Haas et al. 2016). However, the systemic fungicide propiconazole has been demonstrated to control disease-causing fungi associated with ambrosia beetles in redbay trees (*Persea borbonia*) (Mayfield et al. 2008). Injections of systemic fungicides into young apple trees may potentially help prevent establishment of *X. germanus* and control other common fungal pathogens such as apple scab.

In the one year follow-up 2014 bioassay, mortality of *C. rosaceana* was high and larval feeding was low for trunk and root injected 1/8 rates of emamectin benzoate compared with UTC trees. This indicates long persistence of emamectin benzoate residues in the nursery trees, and would require fewer injections during the nonbearing stage of nursery trees. Although the 2014 trunk-injected 1/80 rate treatment had zero mortality, it had less larval feeding compared to the UTC. Similarly in 2015, the percent leaf defoliation for root-injected 1/8 rate leaves was equal to the trunk-injected leaves, despite larval mortality being lower between treatments. Numbers of *E. fabae* nymphs were higher on root-injected 1/8 rate trees than on the UTC in 2014, but feeding damage was found to be lower for both 1/8 rates at 432 DAT. These results suggest that at sublethal doses emamectin benzoate can still act as a feeding deterrence to foliar pests.

In this study we demonstrated that trunk injections can be successfully performed on nursery trees. This represents a novel approach to pest management in nursery tree plantings. Injections of emamectin benzoate at reduced doses can reduce numbers of insect pests for multiple seasons. In future studies, the long term survivability of trunk injected nursery trees should be investigated. Injections of systemic fungicides should also be tested to expand the utility of trunk injections in young trees. Comparisons with conventional nursery tree

management should be conducted to refine injection methods, and adapt current and future technologies for use in nursery systems.

CHAPTER 6: CONCLUSION

Trunk injection is currently being researched to address pests in many crop bearing trees (Murphy and Briscoe 1999, Byrne et al. 2012, 2014, Salem et al. 2014, VanWoerkom et al. 2014, Wise et al. 2014). This technology is simple and effective on a small scale and may benefit small holder farmers in developing countries who rely on hand-held sprayers (Wise et al. 2014). Throughout the previous four studies we expanded the number of pesticide candidates for trunk injection in apple trees; compared commercially available injection tools; tested new applications for trunk injection with insecticide-fungicide combinations and nursery tree injections; and investigated pollinator exposure risk. This conclusion will address each chapter as a whole and I will provide some of my own insights into trunk injection and its future directions.

Expanding the List of Pesticides for Trunk Injection in Apple

The neonicotinoids imidacloprid and dinotefuran reduced numbers of piercing sucking insects such as *Aphis pomi* and *Empoasca fabae*. While imidacloprid was present in leaves in fairly high concentrations, dinotefuran was not. This was unexpected, because dinotefuran is more soluble than imidacloprid, and I expected it to be more xylem mobile. Small concentrations of dinotefuran were still effective at managing pests. Injections of emamectin benzoate and abamectin were effective at controlling *Choristoneura rosaceana* and *Phyllonorycter blancardella*. Abamectin also showed efficacy against *E. fabae,* for which it is not labelled. Generally, avermectins are effective against lepidopteran pests, and need to be ingested. Evidently the route of exposure improved the efficacy of abamectin on *E. fabae*. Trunk injection of emamectin benzoate and imidacloprid has been investigated before (VanWoerkom et al. 2014,

Wise et al. 2014); however in these chapters I used lower doses, suggesting that much less product could be used for control.

Abamectin is labelled as a miticide in apple trees, so this finding may be useful for developing management programs that combine multiple groups of pests. Further research should be done to evaluate abamectin as a trunk-injected miticide and whether it may cause mite flaring. Chlorantraniliprole was also found to be effective at controlling lepidopteran pests. This is a systemic insecticide and, while not OMRI listed, is considered to be a reduced risk insecticide for honey bees (Krupke et al. 2014). Spinosad was not effective at controlling pests, due to poor translocation within apple trees. As such the formulation used is not recommended for trunk injection.

Comparing Injection Tools

In Chapter 3, I showed that blade type injectors are able to deliver insecticide from the injection sooner and in higher concentrations than a drill type. It was anticipated that blade type injectors would be faster for uptake based on earlier work in the Wise lab by Aćimović (2014). By the end of the season for both imidacloprid and emamectin benzoate there were still some variations between the two tools. Blade type injectors delivered their product in the highest concentration early in the season, while concentrations peaked later in the season for drill type injection. The residue profile of drill type injectors was flattened and lasted longer over the season. In the subsequent year there was little difference in insecticide performance between the two injection methods. Other research has shown that blade type injectors heal more quickly than drill type (Aćimović, Cregg, et al. 2016). The appropriate tool should be determined by management need. Seasonal timing of the insect pest can be matched to a tool's residue profile

along with the appropriate insecticide. Rate of healing for the tree should also be considered. Trees will heal using either method but risk of infection increases the longer the bark is open.

Testing New Applications for Trunk Injection

In Chapter 4, I injected the fungicide phosphorous acid into the tree alongside emamectin benzoate. Injecting the two pesticides into separate ports versus the same port made little difference with regards to pest control. There were several combination options, and I found that injecting emamectin benzoate first, followed by phosphorous acid into the same hole, worked best. This suggests that products can interact very differently depending on how they are introduced into a tree. Combining products in the injection port is similar to tank mixing and needs to be done on a case-by-case basis. Ideally formulated combinations of fungicides and pesticides could be developed which could ensure compatibility of two active ingredients. With a fungicide and insecticide combination, it will be possible to reduce the need for spraying and increase the utility of trunk injection.

Nursery trees were injected in Chapter 5. We found that injections were not too invasive to young trees, which was an initial concern. Also, injections in the trunk below the graft union were more effective than the roots. Multiple seasons of control can be achieved with a single injection, which greatly reduces inputs required by growers. Nursery trees are non-bearing for several years. This poses a burden to growers who must manage the young plantings with no returns. Sprayers are less efficient for small canopies and must be specially designed for effective canopy coverage (Fox et al. 2008). Trunk injection is a method with which growers can protect young apple tree plantings efficiently. Future research should extend management beyond 2 years and into the fruit-bearing period to look at long term effects of repeated

injections and how management strategies may need to shift.

Exposure Risk to Pollinators

My research has shown that concentrations found in nectar are below the 25 ppb threshold for imidacloprid (EPA 2016). This suggests that with the doses used there is a low risk to pollinators. Methods for nectar and pollen extraction were developed throughout my research program, finally selecting one described in Knäbe et al. (2014). This method, which involves centrifuging nectaries and sieving dried pollen in a tumbler, is much more efficient than earlier work with MicroCaps and vacuum pumps. Nectar and pollen could be collected more quickly with the later method and thus larger experiments could be designed. By using multiple research stations in Michigan, future researchers could take advantage of the delayed bloom times at different latitudes. This would allow for several weeks of nectar and pollen collection from trees injected with different insecticides.

Further research should be conducted to expand on nectar and pollen research presented in Chapter 2 as well as in VanWoerkom et al. (2014). Though concentrations of injected imidacloprid were under the threshold, an *a priori* conclusion that there is no risk to pollinators should be avoided. No experiments were conducted with live pollinators to assess exposure risks. To my knowledge there have been no experiments of this sort to look at exposure via trunk injection. Field experiments using honey bees in a trunk injected orchard could be designed in several ways. Dwarf apple trees are small enough that a tent structure could be built to contain them, along with honey bee hives. This would be a no-choice experiment investigating lethal and sublethal exposure of nectar and pollen only from injected trees. It might be more practical to conduct a laboratory bioassay with field-sampled nectar and pollen. An open field design can be

designed to look at exposure risk in a real-world scenario. Hives are commonly placed at the margins or in the middle of orchards for pollination, so the field experiment would do the same. All trees in the orchard would be injected with either a radiolabeled insecticide or some nontoxic radiolabeled chemical. This would allow the researcher to evaluate active ingredient concentration in nectar and pollen, quantify the amount of insecticide being brought into the colonies (and distinguish between insecticide from the experiment or from other foraging sources), quantify the overall amount of nectar and pollen collected from the experimental orchard, and calculate the proportion of foraging from trees injected with insecticide versus the nontoxic control.

Considering sublethal effects there remains a serious concern over systemic insecticide exposure. Reducing overall environmental exposure through trunk injection rather than spraying may not prove to be a valuable tradeoff when using imidacloprid, due to a direct exposure via nectar and pollen. There is widespread anticipation that the entire class of neonicotinoids will be banned in the US in the near future. Emamectin benzoate is also highly toxic to honey bees, which would also present a barrier to labelling it for trunk injection in flowering plants such as apple trees. Neonicotinoids and avermectins are valuable insecticides for demonstration of trunk injection, but may not available for long, so I anticipate we will need to begin looking at other compounds in order to continue trunk injection research and eventual labelling.

Some systemic insecticides exhibit low toxicity to bees, such as the neonicotinoids acetamiprid and thiacloprid, the anthranilic diamide chlorantraniliprole (Dinter et al. 2009), and the pyridinecarboxamide flonicamid. Organic insecticides with systemic properties may also be beneficial. In a bio-rational trunk injection study, we injected two formulations which contained azadirachtin and pyrethrins or pyrethrins alone (Azera, PyGanic 5.0 EC II, *respectively*; Valent

U.S.A. LLC, Walnut Creek, CA) (Coslor and Wise 2013, *unpublished study*). We found that pyrethrins were more systemic within apple trees and both compounds showed activity on *C. rosaceana* and *P. blancardella*. A survey of pesticides that are known to be relatively nontoxic to bees may yield new candidates for trunk injection. Starting with a list of relatively nontoxic insecticides and fungicides such as listed in Krupke et al. (2014), several candidates could be chosen and formulated for trunk injection.

Resistance Management with Trunk Injection

Because injected insecticides can be persistent in trees for multiple years (Smitley, Doccola, et al. 2010), pests are exposed to the same mode of action compounds for a much longer time. In this way, trunk injected trees are not like sprayed trees that 'reset' every week or two from environmental exposure. Trunk injection is functionally more similar to genetically modified crops producing insecticides like *Bacillus thuringiensis* (Bt). Resistance management is a current field of study in biotechnology. Recommendations for Bt corn include designing them to carry multiple strains of the toxin, planting refuges, and planting random mixtures of Bt and non-Bt corn (Carrière et al. 2016). The resistance management practices of GM crops easily be translated to an injection program in an apple orchard. Trees can be injected randomly, or with planned refuge areas, and they can also be injected with multiple active ingredients. There are many possible ways to design resistance management into a trunk injection program.

Trunk injected trees have several advantages over GM crops. First, trees are long-term plantings, so although injections last multiple seasons they are relatively ephemeral. The pesticides carried by individual trees can be changed. This would only possible for a GM crop by replanting it; or, by investing capital into developing a new GM crop with a different pesticide.

Low cost is another advantage of trunk injection – most of the necessary pieces are already available. In addition, GM crops that carry insecticides are limited to those which can be biosynthesized. Trunk injection is not limited to organic sources, so there is a larger library of specific insecticides from which to choose.

Future of Trunk Injection in Apple

In developed countries it is customary to use airblast sprayers powered by tractors which can treat entire orchards quickly, if not efficiently. Adoption of trunk injection by growers may be possible, but will first require demonstrations of its scalability. It is not possible to extrapolate the required resources based on the time required to inject a single experimental plot. To use these tools as they exist now would require a relatively large crew of employees to treat an orchard in a timely manner, as opposed to a sprayer with which an individual can treat a field in a short amount of time. Mechanized equipment could bridge between small and large scale. Further research is required to address the utility of trunk injection on production-scale orchards. Trunk injection is very efficient for treating individual trees and managing pests where there is need for a low cost solution, a concern of environmental exposure, or where sprays may otherwise not be feasible.

CCC

May 2017

APPENDIX

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: 2017-05

Author and Title of Dissertation:

Charles Clark Coslor

"Refining Trunk Injection Strategies for Control of Foliar Insect Pests and Disease in Michigan Apple Orchards"

Museum(s) where deposited:

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

Table 2. List of voucher specimens.

Family	Genus/Species	Life Stage	Quantity	Preservation
Aphididae	*Aphis pomi*	Adult	10	Ethanol
Cicadellidae	*Empoasca fabae*	Immature	10	Ethanol
Gracillariidae	*Phyllonorycter blancardella*	Immature	10	Ethanol
Tortricidae	*Choristoneura rosaceana*	Adult	10♀ 10♂	Pinned
Tortricidae	*Choristoneura rosaceana*	Immature	10	Ethanol
Tortricidae	*Cydia pomonella*	Immature	10	Ethanol

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