TRUNK INJECTION: A NEW AND INNOVATIVE TECHNIQUE FOR PESTICIDE DELIVERY IN TREE FRUITS

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ABSTRACT

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Protecting fruit trees from disease and insect pests is an essential part of an agriculture production system. The classes of insecticides and fungicides used in apple production have evolved rapidly, but the application techniques have changed very little in the last fifty years, creating many ecological, environmental, and health issues. One application method that arborists use commercially, and is of growing interest to agriculturalists is trunk injection. Trunk injection is an environmentally sensitive application technique which was used in this research to apply insecticides and fungicides systemically into Empire apple trees (*Malus domestica* Borkhausen) and semi dwarf MacIntosh (RedMax) at the Michigan State University (MSU) Trevor Nichols Research Center (TNRC) in the growing seasons of 2010 and 2011. The insecticides studied included Imidacloprid (Ima-jet™), rynaxypyr (XCL-r8™), and emamectin benzoate (TREE-age™). The fungicides used were propiconazole (Alamo®), phosphorous acid (Phospho-jet™), and a diluted solution of penthiopyrad (Fontelis™). These compounds are currently formulated for trunk injection and were tested to control the main insect pests and disease of apples. Insect and fungal control along with injury experiments were measured using field studies, laboratory bioassays, and residue profiling of a range of apple tree tissue types. Trunk injection may provide substantial persistence in the vascular system up to 2 seasons, minimizing many off-target effects, but also may cause significant injury to the tree.

Dedicated to Gordon and Deborah VanWoerkom

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

Pest Management in Fruit Production

Protecting crops from disease and insect pests is an essential part of agriculture production systems. Profitability in domestic fruit markets requires meeting high food quality standards, often through the judicious use of crop protection materials, including pesticides (Wise and Whalon 2009). Technological advances in agriculture in the last half century have allowed farmers to grow and protect their crops more efficiently (Wise and Whalon 2009). Integrated pest management (IPM) is an economic and environmentally sensitive technique for pest control, and plays an important role in tree fruit production (Metcalf 1980). Effective use of pesticides in an IPM program requires precise delivery of selected materials to the crop canopy (Landers 2002, McCartney and Obermiller 2008). Conventional airblast sprayers have been very effective for increasing crop, yields, and profits (Devine and Furlong 2007); however the negative impacts can be considerable and limited progress has been made to improve application strategies. Conventional spray methods dot not represent a well rounded IPM program based on the IPM definition, which is an economic and environmentally sensitive technique for pest control. There are many economic and non-economic concerns with the current pesticide application strategies in tree fruits, yet agriculture depends on conventional farming methodologies (Devine and Furlong 2007). Economically, with rising prices of fuel, equipment, pesticides, and other farming essentials, growers are in need of new ways to cut spending and be more efficient in all areas of production. Non-economical issues include of soil compaction, pest resistance, and many non-target effects such as environmental hazards, negative impacts on beneficial insects, health concerns caused by worker/ public exposure. Pesticides are a major expense and inefficiently applied with only 0.1% of the pesticide making contact with the target

pest (Pimentel and Levitan 1986) and 29-56% of the spray deposited on the canopy (Reichard et al 1979). The wasted non-target materials are subject to drift and runoff, which effects many other non-target environments (Damalas and Eleftherohorinos 2011).

Pimentel and Levitan (1986) state that in most cases growers apply more pesticide than recommended to crops to account for the pesticide that does not reach the target crop. The impact of pesticides on non-target organisms is a growing concern in conventional spraying methods. Humans, beneficial insects, and many non-insect organisms are negatively affected in soil, atmosphere, and water systems where there is direct and indirect pesticide exposure. Pesticide residues are found in the atmosphere worldwide (Pimentel and Levitan 1986). Zhu (2006) found that many nurseries and orchards are located in the urban and suburban areas making pesticide contamination a threat to local residents. This off-target contamination may be lethal or sub-lethal to many beneficial organisms such as pollinators, natural enemies, and decomposers (Devine and Furlong 2007). These insects include natural enemies of the pest insects and pollinators.

Many organisms such as fruit trees, insects, and fungi may obtain a certain level of resistance towards foreign invaders. The intense use of conventional pesticide application methods select individuals that carry alleles for resistance. Pesticide resistance is a growing concern in apple production systems (Mota-Sanchez 2008). Resistance is the result of evolutionary processes which include mechanisms of defenses, both metabolic and non-metabolic. The genetic defensive traits which allow the organism to survive the initial chemical application get passed on to their offspring (Georghiou 1986). One way to delay resistance is by constant modification of application methods by rotating pesticides of different compound

classes, which have a different mode of action. To manage resistance application methods are constantly modified with compound class rotation and spray timings.

Environmentally friendly techniques have been developed to lessen the negative impacts of conventional spraying with drift retardants and low-drift nozzles. Spray droplet research shows that polymer-based drift retardants lost effectiveness after they were recalculated through the pumps (Zhu et al. 2006). Results show that drift retardant droplets reduce the leaf's ability to photosynthesize and transpire. In one study, spraying phosphorus and a phosphorus potassium combination on Golden Delicious apples resulted in reduced photosynthesis and transpiration (Veberic et al 2005). Although there are efforts to improve conventional application methods, there is need for paradigm shift.

Trunk Injection-tree physiology, delivery, and translocation

In spite all the risks of spraying, farmers are compelled to apply pesticides to produce fruit that will meet market standards. Trunk injection is a novel alternative to conventional airblast spraying for delivering crop protection materials to tree fruit crops. It is a well established technique in urban tree pest management, but until now has not been considered in tree fruit production systems because of concerns with the cost of time, tree injury, proportional pesticide coverage, and pesticide residues remaining in fruit.

Trunk injection inserts pesticides into the vascular system of the tree by severing the outer bark tissue. Injecting compounds into the vascular system of a tree requires knowledge of a woody plant system and the understanding of how these compounds move throughout the vascular system. The movement of fluids throughout the tree's vascular system is called translocation. Water moves from the soil to the tree, into the atmosphere, and back into the soil.

Water in plants forms a continuous system and depends on high cohesive forces to carry a flow of water throughout the plant (Chaney 2000). When water transpires from leaves through stomata, water tension increases and pulls more water up the tree. Stomata are small openings on the top and underside portions of the leaf surface, which regulate the volume of water which exits the plant. Stomata open and close depending on the water pressure in the guard cell. Transpiration through the leaf surface is regulated by guard cells. Transpiration is the water loss from openings on most plant surfaces. When the overall leaf surface area has peaked, the transpiration rate is high, allowing more water to replace what transpired. With more leaf area there are more stomata, resulting in overall greater transpiration potential. Once water transpires via the stomata the tension continues through the water columns to the roots, sucking water upwards through many small straw-like structures. More leaf area is important, but the amount of vapor pressure in the atmosphere is the major factor, allowing the water in the plant to move from a higher concentration to a lower concentration. With a decrease in vapor pressure, there is greater transpiration rate. This is a very strong and consistent continuum reaching to the tops of the tallest trees, which can be harnesses for pesticide delivery.

There are five main cross-sectional tissue layers which make up the anatomy of a tree trunk, from the exterior to interior are the bark, phloem, cambium, xylem, and heartwood. The vascular system refers to the inner phloem, meristem, and xylem tissues. The bark is dead wood tissue which acts as a protective shield from insects and diseases. The phloem is live tissue which transports sugars, and other chemical substances both up and down the tree with sieve tubes and companion cells through an active process, which requires metabolic energy against a gradient. Phloem transport begins with phloem loading. Adjacent to the phloem is the vascular cambium, which differentiates the phloem and xylem. Cambium has the ability to divide and

extends throughout the entire tree. It produces new phloem toward the outside of the tree and xylem or wood towards the inside of the tree. Adjacent to the vascular cambium is the xylem. This layer of cells moves water and other soluble nutrients from the roots to the crown when mature and dead. The xylem is responsible for replacing water that the tree has expelled through transpiration. Depending on the type of tree, there are four main types of cells moving these fluids in the xylem: vessels, tracheids, fibers, and parenchyma. Vessels and tracheids are similar in some ways with both arranged vertically moving water upward and connected by pits. Pits are small holes in the side walls of the cells connecting the specific cells together (Chaney 1986). When these cells are mature they are dead transportation cells. Tracheids are much smaller than vessels, closed at the ends requiring water to move through the pit pairs to the next tracheid for upward accent. The last two types of cells in the xylem tissue are fibers and parenchyma. Fibers are thick and also connected with pit pairs, but do not conduct water, their function is structural support. Parenchyma cells store carbohydrates needed for growth. They also move water laterally throughout the xylem. They have the ability to divide and are responsible for the callus tissue responding to cuts or wounding. These cells become heartwood tissue when they die. The heartwood is located beneath the xylem and functions as structural support and strength for the tree.

Tree wood types are often defined on the type of xylem, of which there are three types: diffuse porous, ring porous and nonporous. Nonporous gymnosperms include pine, spruce, and firs, which only have tracheid cells. Ring porous angiosperms, such as hickory, chestnut, ash, elm, and locust, contain vessels and tracheids with the vessels mainly in the early wood of each growth ring. Diffuse porous angiosperms such as maple, dogwood, sycamore, and apple contain both tracheids and vessels, which are scattered equally among each other in each annual growth

ring (Chaney 1986). Wood types and structure vary greatly among tree species, which plays a large role in the translocation characteristics of injected compounds.

Factors Influencing Trunk Injection Material Transport

Proper translocation is the most important factor for an effective trunk injection application (Norris 1967). Proportional compound distribution throughout the tree canopy relies on the translocation ability of the vascular system. Many known factors that influence compound uptake need to be considered for the success of injection to occur. Different species have different physiologies and injection techniques should differ according to the way the tree uptakes and moves the compound. This makes injection techniques species specific. A single type of injection technology is not appropriate with the many types of tree physiologies (Perry et al. 1991), and wood types. These wood types determine the ease of injection (Sachs et al 1986). Tree species, tree size, wood types move fluids differently and they also move fluids to different locations in the canopy inconsistently. There are differences in uptake between ring porous and diffuse porous trees and between pome fruit and stone fruit trees (Clifford , Gendle, and Holgate 1987). There are also many differences in vessel size among the species of each tree type and also among species, depending on the environment conditions (Sachs et al 1986). The larger vessels in angiosperms create fast flowing channels, while the small tight vessels of gymnoperms make it difficult to inject larger volumes in less time. There are also differences in wood types which effects translocation of monocotyledons and dicotyledons (Pegg 1990). It has been noted that the radial movement of phosphoric acid is minimal in avocado trees (dicot), but monocotyledons had superior radial distribution from the injection site (Pegg 1990).

In many instances injected materials are located near or in line with the injection point creating uneven distribution in the canopy. Materials move faster longitudinally than tangentially with uneven distribution (Harries 1965). There may be some areas in the tree's vascular system that do not make contact with the compound, causing uneven pest control over the entire canopy. Uneven distribution has been shown to be the limiting factor in the control in insects and mites (Harries 1965).

Tools and techniques may be modified to get the material into the tree canopy proportionally for efficient pest control. The pressure of injection, drill speed (if used) or drill bit type hole location, hole depth, hole angle, number of holes, and injection timing are some crucial notes for attaining proportional distribution throughout the canopy. Injecting at different pressures alters uptake. High pressure forces the compound into the vascular system at higher volumes and rates. This method may also cause several types of injury such as blocked xylem, bark splitting, cambial damage, and bark lesions (Perry et al. 1991). A decrease in pressure will slow the uptake of the compound allowing the tree to use more of its own pressure gradient causing less injury.

Many injection methods require a drilled hole or injection port. The drill speed and drill bit type affects the uptake. Higher drill speeds may cause increased friction to the conducting tissue causing damage and a decrease in uptake (Orr et al., 1988). A sharp brad point drill bit creates a cleaner cut increasing uptake through the conducting tissues as well (Orr et al., 1988). With high speeds there is a cleaner cut through the wood tissue allowing for clearer vascular system access. At low drill speeds there is a greater chance there will be a rough cut with wood shavings left behind blocking access to the vascular system. Injecting into the newest wood where the functional xylem cells are located and avoiding any knots or injury points is best for

translocation. Injecting near a wound or cut, whether above or below may cause the material to exit the system as the injection is taking place. Injecting above or below knots causes the material to take a longer route than needed to the intended site and may cause increased active ingredient binding to sites around the knot area. Depending on the tree species, injecting closer to the roots such as the root flare, increases the translocation rate and lateral movement of the compound and decreases compartmentalization. Injections near the roots or basal trunk obtain the greatest distribution (Norris 1967). Root wood is generally more porous and has more vessel contacts creating increased lateral movement and uniform translocation pattern compared to the trunk wood (Sinclair and Larson 1981).

Hole location as well as the hole depth and number of holes play large role in translocation. This also depends on the injected tree species. Holes were drilled 2 inches deep in this research, penetrating into both early and latewood xylem of the apple trees. Tree physiologies such as the diffuse porous apple tree xylem with vessels and tracheids scattered among the annual rings will benefit from deeper injection points.

Ring porous xylem types with vessels and tracheids located only in the current annual ring benefit from shallow injections. Injections past the current annual ring are not necessary for this type of wood. Proper translocation throughout a diffuse porous apple tree requires an injection through the bark, cambium, phloem, and into the active xylem tissue. With vessels and tracheids being distributed fairly even throughout the each xylem growth ring, injecting into more than one xylem layer is optimal. When the compound reaches the phloem and xylem, it travels up the trunk out to the branches and leaves. It also travels downward through the phloem to the root system.

The hole angle is important for the correct depth to connect with the correct tissues. Creating a port that is perpendicular to the trunk is optimal for injecting into the best wood tissues according to tree species. There is a greater chance of missing the conducting tissues with an increase in hole angle (Davis 1991). Drilling damage may also be caused by a smaller angle. A 5 degree angle downward may cause material to collect such water, active ingredient, fungi, or bacteria. A 5 degree angle upward would drain material but also active ingredient (Orr et al. 1988).

The optimal number of injection points depends on the species because wood grains differ in their accent up the trunk. Some species have a spiral grain causing injected compounds to follow a sectorial winding accent relocating to more locations throughout the canopy and some tree species have a straight grain, causing compounds to follow a sectorial straight accent keeping in line with the injection point (Chaney 1986). This type will generally find areas in the canopy above the injection point. Tree species with the spiral grain wood type do not need as many injection points because of the relocation and spiraling of the compound as it follows the wood grain. Straight ascending wood types require more injection points covering more area of the trunk, increasing the branch distribution for proportional coverage.

The uptake and translocation of pesticides are dependent on solubility (Percival and Boyle 2005). Pesticides are typically prepared in a formulation that includes an active ingredient, oil or water base and various inert ingredients to aid in stability and delivery. Formulations designed for foliar application to the plant canopy are not necessarily compatible with the attributes that will optimize vascular deliver through injection. Choosing the correct compound formulation to travel through these functioning tissues is crucial. Systemic compounds should be liquid formulated for proper distribution. If the compound is not liquid formulated and is a dry

mixture, it must be highly water soluble. The more water-soluble the compound, the more efficient it will distribute. In experiments by Harries (1965), water-soluble compounds translocated more effectively than emulsions. The compounds injected are best if concentrated for low volumes and persistence, systemically mobile or water-soluble for effective translocation. With an improved time saving injection technique and persistence of these concentrated compounds being present in the vascular tissue, it may provide disease or insect pest protection for 1-2 seasons, making trunk injection a cost effective and superior application strategy.

Trunk Injection for Apple Pest Management

Apples are host of 10-15 different disease and insect pests, some of which are direct and indirect. Direct refers to the pests which attack the fruit directly and indirect refers to the pests which primarily attack other portions of the tree effecting the fruit indirectly. Many insect pests and fungi inhabit interior portions of the tree that are below the bark and cannot be effectively reached through external application methodologies (Norris 1967). Systemic trunk injected compounds have advantages over foliar broadcast applications to reach internal pests.

Persistence is another potential expected advantage of trunk injection. Persistence is influenced by metabolism, degradation, and extended uptake, by the plant (Norris 1967). Once the trunk application is made, reapplication may not be needed for an extended period due to the fact that the compound is sheltered in the vascular system from UV degradation. Research with avocado trunk injection has shown that the weather does not inhibit the persistence of the compound. Therefore there is no concern over runoff, wash off, waste of active ingredient, or reapplication due to weather conditions.

Injection timing, which corresponds to tree phenology is important (Giblin et al. 2007) for effective translocation and canopy coverage, and the resulting pest control. Injection timings are generally dependent on phenological stage of the tree and weather conditions, which effect the soil moisture, transpiration rate, translocation, and when pests arrive. Timing effects the location of where the compound will be located throughout the tree. Injecting fungicides in plum and apple trees after harvest suggests movement of the compounds in non-conducting woody tissue by diffusion (Clifford, Gendle, and Holgate 1987). Injecting at flowering time or pre-bud burst causes movement into the conducting tissue (Clifford, Gendle, and Holgate 1987). Injection when fruit bodies are present accelerates translocation, because fruits act as a sink (where carbohydrates are stored or used). This speeds the translocation and photosynthesis, which is associated with greater incidence of stomatal apertures (Hansen 1971). Sunlight allows for increased evaporation and transpiration not only from leaf stomata, but also from buds, fruit, bark, and lenticels.

Injecting when the leaves are fully flushed is crucial. Leaves are the only tissue that can control when water exits the plant tissue with stomata (Coder 1999). Sunlight also causes air and soils to dry, which account for poor translocation. During these conditions trees is conserve water by closing stomata and reducing transpiration. An increase in rainfall post drought increases systemic movement (Orr et al.,1988). Once soil is saturated roots send signals to open stomata, increasing transpiration and translocation rates. This situation takes place when relative humidity (RH) begins to decrease with sun exposure. If the weather continues overcast, with minimal sun exposure, high RH will continue limiting translocation. Rain generally increases the RH, which increases the amount of water in the soil, reducing transpiration (Zamora and Escobar

2000). When the sun is exposed and air dries, a gradient is created causing increased transpiration.

Wind also causes a decrease in RH and dry air. With high winds there is lower vapor pressure because of moisture loss in the air. With dry air there is a need for the plant to transpire and cool down. The water in the tree moves from high concentration to low concentration to the atmosphere, increasing translocation. With low wind there is an increase in vapor pressure allowing moisture buildup. The increase in vapor pressure in the atmosphere changes the concentration gradient, which causes a corresponding decrease in translocation. The atmospheric vapor pressure may be the greatest factor in the transpiration rate. Therefore, observing the tree's phenology stage, weather, and soil moisture are key factors in optimizing the performance of trunk injection. Injection requires practice and experience for a successful application.

Concerns with Agricultural Injections

Current accumulative research provides some concerns about adaptation of trunk injection to apple production systems. The first and possibly most significant damage it may cause to the tree. Without the correct injection techniques, unaffordable injury may be caused by drilling, taping, and movement of the compound formulation throughout the vascular tissue, potentially resulting in compartmentalization, growth ring disruption, rot, decay, girdling, disease infection, insect infestation, embolism, phytotoxicity, chlorosis, and necrosis. The extent of injury depends on the health and size of the tree, depth and diameter of the hole, volume of application, and weather conditions. Detailed concerns of compartmentalization, rot, decay, and girdling are discussed in chapter 4. Injecting foreign compounds under the bark into the vascular system may cause phytotoxic reactions such as leaf burning. If the correct rates are not used

under the recommended conditions leaf burning, leaf yellowing or leaf death may occur. This may cause long term or permanent injury to the tree effecting fruit yield and growth with variable foliar reflush. Correct application methods may prevent this occurrence. Correct methods may also prevent embolism, which is the injection of air pockets into the water conducting system breaking the water columns of the internal vessels. These air pockets disrupt the distribution of the compound creating uneven distribution. Air pockets break the water column in the xylem, causing a decrease in translocation to that area of the tree.

The EPA regulates the amount of foreign substances allowed in food. Rigorous research is the basis for setting tolerances for all pesticides registered for use on food crops in the USA. These tolerances are based on conventional delivery methods, like airblast sprayers, with field residue studies conducted under Good Laboratory Practices (GLP). It cannot be assumed that the residues in apple fruits resulting from trunk injection will be the same as from foliar application. Prelimimary data (Wise et al. unpublished data 2009) suggest that the vast majority of the residues end up in foliage, although detections were also found in the apple fruit. The fungicide imizalil was injected into chestnuts with no residues located in the nuts, indicating that the compound did not move to the fruit tissue (Washington et al. 1998).

Certain pesticides are more toxic to pollinators than others. Imidacloprid is an insecticide which has high acute toxicity to one of the major pollinators, the honeybee *Apis Mellifera* (Suchail, Guez, and Belzunces 1999). Pollinators are exposed to pesticides by contact from drift, but also by sprays that make contact with the target plant as they are foraging. Sprays may be delayed during the prime honeybee foraging time, but there are many other benefecial pollinators affected. Another pollinator exposure factor may be through nectar. Trunk injection may allow certain levels of pesticides into flower tissue, however application timing is a factor. Injection

timing may decrease the amount of residue recovered far below the maximum residue level (MRL) from the fruit and flower tissue. An optimal injection time to reduce the possible residues that may enter the fruit and flower tissue may be post harvest, but needs further research.

With the evidence of minimal residue levels in the fruit, there is concern about wether or not direct fruit pests (insect or pathogen) can be controlled with trunk injection. Effective direct fruit pest control generally requires the insect or fungus to make contact with the compound before infecting or infesting the fruit. Indirect pests ingest/infect foliage, some direct pests also feed on foliage before infesting fruit. There are also some direct pests that infest foliage in one generation, and then fruit in subsequent generations.

There are some procedural injection details that may take extra time and effort, thus the technology may be less appealing. These are equipment maintenance and preparation, trunk diameter or tree measurements, rate calculations, matching the appropriate compound formulation with the right tree species, observing the age and health of the tree, and the injection application itself. Rate calculations are generally related to the diameter or size of the tree, but with maximum insect control and minimum phytotoxicity in mind, rates may be adjusted (Norris 1967). Trees that are or were under diseased, infested, or in poor health may not move compounds at the desired rate or locations throughout the tree due to dead wood, rot, or non-functioning tissue. Normal translocation pathways may be disrupted by insect damage (Holderness 1992). This prevents full compound accumulation in the target plant tissue. Organization is key to getting the correct rates in the corresponding trees. The trees must be scouted and measured individually for rate calculation. The larger the tree the more compound it requires and wounds must be avoided above and below the injection point along with equal spaced injections.

A final concern with the trunk injection technique in fruit production is the feasibility of integrating it into current fruit production systems. Convincing farmers that the success of these new technologies are economically feasible in the orchard and still meeting market standards for fruit production will be challenging. With relative success using traditional application methods, changing gears towards an alternative may be difficult. Pesticide use in the US producing agriculture returns about $4 for every $1 invested for pest control which makes the conventional pest management methods so attractive. (Pimentel et al. 1978).

Past and Present of Injection Research

Some of the earliest injection work done on fruit was recorded by Roach (1939). Injections of powdered spices and colors to kill worms and give flavor to the fruit were made in 1602 by an anonymous author. Mokrzecki (1903) injected inorganic nutrient solutions to control some pest mites, bark beetle, gummosis, and other insects on pears and apples. In 1903 Dementiev made cuts on different locations of the tree and injected “poisonous solutions”, attempting to make it undesirable to insects. He found that the rate of uptake increased with the increase in injection pressure. Bolley (1902), Simon (1906), and Harries (1965) also injected fruit trees. Simon injected cider apples, peaches, pears, potatoes, melons, and cabbage by using tubing and a reservoir held 2 m above ground level. Bolley believed that individual unhealthy trees could be saved by formaldehyde, copper sulphate, and ferrous sulphate injections, which he proved by saving apples suffering from sun-scald. Harrie's injections were successful in controlling the two-spotted spider mite (*Tetranychus urticae*) on pear and cherry with trichlorfon and methyl demeton, the peach silver mite (*Aculus cornutus*) on peach with cycloheximide injections and pear psylla (*Cacopsylla pyricola*) on pear with Bidrin® and Bayer thiophosphate

biotests. Lastly, control of apple aphid (*Aphid pomi*), green peach aphid (*Myzus persica*), and wooly apple aphid (*Eriosoma lanigerum)* was achieved with injections of dimethoate emulsions and water solutions of other compounds. Many injection attempts have been recorded in history by Roach (1939) and Harries (1965). Roach's findings suggest that there have been studies dealing with the timing of liquid injections involving fruits and vascular flow. He mentions the plugging of drilled or tapped holes, which is very similar to current injection studies.

Recent trunk injection research on insect pests and diseases in ornamentals includes studies of the emerald ash borer (*Agrilus Planipennis*) on ash (Smitley 2010), oak wilt disease (*Ceratocystis fagacearum*) on oak (Blaedow and Juzwik 2010), Dutch elm disease on elm (Haugen and Stennes 1999), and wooly adelgid (*Adelges tsugae*) on hemlock (Doccola et al. 2007). Studies involving wounding that injection requires to incorporate compounds into the vascular tissue has been also been done on ash (Doccola et al. 2011), but injection work in agriculture is limited. There are fruit tree studies involving insect pests of avocado thrips (*Scirtothrips perseae Nakahara*), and lace bug (*Pseudacysta perseae*) (Byrne et al. 2009) with some success. Injection work on diseases and injection wound responses include apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*) of apples (Percival 2005), Phytophthora root rot (*Phytophthora cinnamomi*) of avocado (Botha, Skinner, and Hough 2003), and wounding of avocado (Doccola et al. 2011). There has been success in controlling different insect pests and diseases of apples and other fruits, but taking the next step and incorporating this type of application in agriculture requires additional research and improved techniques. Trunk injection studies have continued and discontinued for many years. In the last fifteen years trunk injection has become a standard practice for arborists (Doccola et al. 2011). With the onset of Dutch elm disease in the United States led to a renewed interest in tree injection (Perry et al.

1991). Trunk injection may develop into a beneficial pesticide delivery system in food crops as well as ornamentals.

Current Trunk Injection Techniques

There are several types of trunk injection tools and techniques, which involve penetrating the outer bark tissue to provide an avenue for pesticides to reach the inner vascular tissue, using varying hole depths and diameters. One popular technique is pressure injection which applies enough pressure to directly force the entire application volume into a pre-drilled hole. Pressure injections may use holes of different depths, such as the shallow-pit injection between 3-5 mm deep just below the vascular cambium (Ellmore et al. 1988). A second popular technique is tree I.V. or infusion which allows the tree to use transpirational pull to move the compound from an exterior-held reservoir to the canopy. A third technique that is widely used is a drill and tap method where a deeper cavity is drilled, creating an internal cavity that serves as a reservoir for the liquid solution prior to the movement through the vascular tissue. The full volume is applied relatively quickly, so that the applicator can move on to the next application without delay. These popular techniques use varying hole depths and diameters according to tree species, life stage of the tree, and weather conditions. The drill and tap method may be the most efficient technique with the total application volume injected without having to apply excessive force or wait for the vascular pressure to move the compound.

The different devices used to perform such injections range from simple combinations of bottles, syringes, tubes or even bags, tubes, needles and pumps that are capable of forcing the fluids into the tree (Perry et al. 1991). These techniques are successfully used on the trunk, root flares, and even the branches of different tree species. Injection techniques are often identified by

the depth or width of the injection port, and volume of compound injected. Terms used to describe port size and volume injected include micro and macro-injection. One source differentiates the two types stating, micro-injection causes less injury with an entry port diameter of 3/16 inches or less that penetrates into the xylem tissue 3/4 inches or less (Costonis 1981). They can also differentiated by the volume injected. Macro-injection causes greater injury with an entry port that has a diameter of 3/8" or greater and penetrates into the xylem tissue one or more inches (Costonis 1981). Hole diameter and depths vary according to the type of tree injected.

The next few chapters will discuss the current insecticide and fungicide apple tree injection experiments, wounding caused by the current injection methods, and the future work involved with injection in agriculture. It may also answer a few of the concerns about trunk injection in agriculture and bring us closer to an improved method of pesticide application in orchards.

CHAPTER 2: TRUNK INJECTION-INSECTICIDES IN APPLES

Abstract

Field studies, laboratory bioassays, and residue profiling were used to determine the seasonal effectiveness of trunk injection against key apple insect pests using three insecticides that are currently formulated for trunk injection. Imidacloprid (Ima-jet™), rynaxypyr (XCL-r8™), and emamectin benzoate (TREE-age™) were injected into semi dwarf Empire apple trees (*Malus domestica* Borkhausen) at the Michigan State University (MSU) Trevor Nichols Research Center (TNRC). The insects included in field evaluations were obliquebanded leafroller *(Choristoneura rosaceana)* (Harris),Oriental fruit moth *(Grapholita molesta)* (Busck), codling moth (*Cydia pomonella*) (Linnaeus), plum curculio *(Conotrachelus nenuphar)* (Herbst), Japanese beetle *(Popillia japonica)* (Newman), spotted tentiform leafminer *(Phyllonorycter blancardella)* (Fabr.), rosy apple aphids *(Dysaphis plantaginea)* (Passerini), green apple aphid *(Aphis pomi)* (Passerini), apple maggot *(Rhagoletis pomonella)* (Walsh), potato leafhopper *(Empoasca fabae)* (Harris). For two growing seasons, imidacloprid was highly effective in controlling piercing and sucking pests such as the potato leafhopper and aphids, emamectin benzoate was highly effective in controlling leafrollers and leafhopper, and rynaxypyr was highly effective in controlling Oriental fruit moth and leafrollers. The insects used for bioassays were first instar obliquebanded leafroller larvae, adult plum curculio, and brown marmorated stink bug nymphs (BMSB) *(Halyomorpha halys)* (Stål) nymphs. Imidacloprid was not effective in bioassays, emamectin benzoate was effective on leafrollers, and rynaxypyr had some effect on leafrollers and stink bugs. The residue profiling indicates substantial foliar residue levels for

multi-season foliar pest control, with fruit residues below the EPA MRLs. High wood core residues near the trunk injection point indicates a reservoir effect in wood tissue, and low flower residues suggest safety for pollinators. This study will help expand current knowledge in the science of trunk injection and support an improved method of insecticide application in tree fruits.

Introduction

Protecting apple trees, *Malus domestica* Borkhausen, from insect pests in the USA can require as many as eight insecticide applications per season (Wise et al. 2011). There is a wide array of insecticides used to control insect pests in apple orchards, ranging from organophosphates (OPs) to neonicotinoids, to diamides, and insect growth regulators (IGRs). However, since the US congress passed the Food Quality Protection Act in 1996 (FQPA), insecticides belonging to organophosphate, carbamate, and synthetic pyrethroid classes are under increased scrutiny regarding their continued use within the country. Because of the US Environmental Protection Agency (EPA) phase-out of azinphosmethyl and increasing evidence of resistance, new control strategies are being explored and implemented to control key insect pests. Many of the new pesticides registered in the last decade have received Reduced-Risk status by the U.S. Environmental Protection Agency (USEPA 1997). They have been shown to be effective in controlling key fruit insect pests, but require precise application timing in relation to insect development to maximize efficacy (Wise et al. 2007).

As tree fruit producers enter the Twenty First century it is important to note that even though there has been significant evolution of the tools (ie; reduced-risk pesticide chemistries)

being used in pest management (USEPA 1997), spray equipment has remained relatively unchanged. Scientists, like Pimentel (1986), estimate that with conventional sprayers as little as 0.1% of the pesticide contacts the target pest. Other studies show that airblast sprayers are a relatively inefficient means of delivering pesticides to their target, with only 29 to 56% of the applied spray solution being deposited on the tree canopy, and the remaining product drifting to ground or other off-target end points (Reichard et al. 1979, Zhu et al. 2006, Perry et al. 1998). Some technical advancements have come to conventional ground sprayers, such as adding towers or nozzle sensors (Landers and Farooq 2005, Landers 2002, 2004), but the fundamental elements for delivering materials to the tree canopy have remained the same.

In the United States imidacloprid, emamectin benzoate, and rynaxypyr are reduced-risk insecticides registered for use as foliar sprays in apples for various direct and indirect insect pests (Wise et al 2011). While trunk injection is not currently a technique used in apple production, Imidacloprid and emamectin benzoate, have registered uses in ornamentals using the trunk injection technique for insects such as emerald ash borer (*Agrilus planipennis*) (Fairmaire).

Imidacloprid is a neonicotinoid that is effective by contact or ingestion and acts as a nerve poison by binding to the nicotinic receptor and blocking the acetylcholine receptors resulting in paralysis and death. Imidacloprid is effective on sucking insects such as aphids, leafhoppers, psylla, and leafminers in apples (Wise et al 2009a).

Emamectin benzoate is an avermectin compound that is effective by ingestion and acts as a nerve poison in insects by binding to GABA receptors, opening chloride channels resulting in paralysis and death. Emamectin benzoate is effective on leafrollers, leafminers, Oriental fruit moth, codling moth, and psylla (Wise et al 2009b).

Rynaxypyr is an anthranilic diamide that is effective by ingestion and acts as a muscle disruptor. Rynaxypyr binds to ryanodine receptors causing uncontrolled release of calcium preventing muscle contraction resulting in paralysis and death. Rynaxypyr is effective on leafrollers, codling moth, Oriental fruit moth, and apple maggot (Wise et al 2009b).

Methods and Materials

Insect Material

Field Evaluations

Field evaluations targeted natural populations of apple insect pests at the MSU Trevor Nichols Research Center in Fennville, MI, USA (latitude 42.5951° : longitude -86.1561°). The insects surveyed in the field evaluations included obliquebanded leafroller larval infested shoots, Oriental fruit moth larval infestation of apple terminals (flagging), plum curculio oviposition stings on fruit, Japanese beetle adults, spotted tentiform leafminer larval mines in apple leaves, rosy apple aphids colony numbers, green apple aphid colony numbers, apple maggot larval infestation of fruit, and potato leafhopper nymphs.

Bioassays

The insects used for bioassays were first instar obliquebanded leafroller larva, adult plum curculio, and brown marmorated stink bug nymphs.

Obliquebanded leafroller. For trials evaluating the mortality, behavior, and feeding habits, obliquebanded leafroller larvae were used from a continuous laboratory colony. Male and female adults are allowed to mate and oviposit on wax paper in styrofoam box cages kept at

25°C in a walk-in environmental chamber with a photoperiod of 16:8 (L:D) h. Egg masses are collected from wax paper in the cages and placed on a pinto bean diet in separate containers (4-5 masses per container) until hatching. The first instar larvae are then separated for even dispersal (8-10 per container) to avoid cannibalism until pupation. The pupae are then washed in a diluted bleach solution and placed in a petri dish which is placed in the adult cages for emergence.

Plum curculio. Southern (nondiapausing) strain plum curculio were used for evaluating mortality, oviposition, behavior, and feeding habits. They were used from a continuous colony. Adults are reared in plastic cages for mating and oviposition on immature apples collected from non-insecticide sprayed orchards. The cages are kept at 25°C in a temperature regulator with a photoperiod of 16:8 (L:D) h. Once females had oviposited, apples were transferred to screen boxes placed on top of trays for larval emergence. The larvae are then collected from the trays and placed in mason jars of dirt with a screen emergence trap on top. Adults were placed in plastic cages on new apples for mating and oviposition.

Northern (diapausing strain). For trials evaluating mortality, oviposition, behavior, and feeding habits. They were used from a continuous laboratory colony at Michigan State University. Adults are reared in plastic cages for mating and oviposition on immature apples collected from TNRC orchards. The cages are kept at 25°C in a temperature regulator with a photoperiod of 16:8 (L:D) h. Once the females had oviposited, apples were then transferred to screen boxes placed on top of trays for larval emergence. Larvae were then collected from trays and placed in mason jars of dirt with a screen emergence trap on top. Adults were placed in plastic cages on new immature apples for mating and oviposition.

Brown marmorated stink bug. For trials evaluating mortality, behavior, and feeding habits. They were used from a continuous colony. Adults are reared in 950 ml plastic containers (Fabri-Kal, Kalamazoo, MI) for mating and egg laying on paper towel. Once the eggs are laid egg masses are separated into 4 oz plastic portion cups (Gordon Food Service, Grand Rapids, MI). The containers are kept at 25°C in a temperature regulator with a photoperiod of 16:8 (L:D). The adults and nymphs fed on green beans that were opened.

Field Plots and Applications

Field Plots, Compounds, and Rates

Treatment applications were made at apple petal fall stage in both 2010 (May 5) and 2011 (May 30), with each of three insecticides injected that are currently formulated for trunk injection: imidacloprid (Ima-jet™, Arborjet Inc. Woburn, MA), and emamectin benzoate (TREE-age™ Arborjet Inc. Woburn, MA), and rynaxypyr (XCL-r8™, Arborjet Inc. Woburn, MA). Injections were made on 5-6 inch DFH (trunk diameter 1 foot above the ground) semi dwarf Empire apple trees (*Malus domestica* Borkhausen) with a five replicate trees per treatment in a randomized block design. Low and high rates of each compound were injected at volumes depending on tree DFH, the high rate corresponding to the maximum seasonal allowance of AI per in apples (Table 1). A low rate of 0.2 g AI (active ingredient) per DFH and high rate of 0.4 g AI per DFH (doubling the low rate) were injected for all treatments (Table 1). The rates were converted from ounces per acre to ml per plug or injection site (Table 1). Trees were chosen based on the overall health of the tree and canopy structure to assure uniform compound delivery

to the canopy. DFH measurements were taken with a forester's D-tape (Lufkin®, Sparks, MD) to provide application calculations for each repetition (Table 1).

Field evaluations, bioassays, and residue analysis were performed in 2010 and 2011 seasons. All the experimentation on the 2010 injection study continued into the 2011 season and is stated as the 2010 continuation.

Injection procedure

Injection data were collected for each tree including the tree DFH, the # injection ports, milliliters per DFH inch, rate (g AI), total volume for the injection solution per tree, total volume of injection solution per plug, and the date of injection. These data are used for organization and rate calculation. The tree DFH was taken prior to any injection by wrapping the D-tape around the diameter of the trunk 12 inches above the ground or at injection height. Next the rate was calculated and the compound was prepared. The injection equipment included the Arborjet Quick-jet™ kit, containing the injector, #4 arbor plugs, and plug tapper (Arborjet Inc. Woburn, MA). A few other tools needed were a hammer, cordless drill, and a 3/8 inch wood drill bit. The injection system was sanitized before each injection with the Arborjet Cleanjet™ solution and water to rinse any residues and to prime in the injection system. Next, the compound was poured into the Arborjet injector holding tank. Extra solution was added to allow priming of the tank. The needle was then attached to the injector tip and the pump was primed so there were no air pockets causing inaccurate applications. The injector was put aside and the holes were drilled into the apple trunk 2 inches deep, 90 degrees horizontal from the trunk, and 12 inches above the ground using the arborjet drill bit. The plugs were staggered and spaced as equally as possible

while strategically placing them under the main scaffold branches of the tree to distribute maximum compound volume throughout the canopy. The holes were not placed above or below any trunk injury to prevent the compound from leaving the tissue through those wounds. The plugs were then tapped in place so the outside rim of the plug was just beneath the bark. The needle was then inserted into the plug so the base of the needle was tight against the lip of the plug. The compound was then injected at the desired rate in each plug. The Arborjet injector has a built-in graduated cylinder so the amount per application is accurate to the nearest quarter of a milliliter. The table below best illustrates how the data was organized prior to applications (Table 1).

Table 1. Tree size, field rates and doses per tree and per injection site for the 2010 and 2011 seasons. All volumes are measured in milliliters. All measurements are means of the 5 repetitions per treatment.

2010								
Trt	Tree DFH (in.)	# ports/tree	Ml/ DFH	Rate (gai)	Tot. vol./ tree	Vol/ plug	Units	Appl. DAT
1 Control	-	-	-	-	-	-	-	-
2 ImiL	3.6	4.0	4.0	0.2	15.1	3.6	ml-DFH	5-5
3 ImiH	4.0	4.0	8.0	0.4	32.0	8.0	ml-DFH	5-5
4 EmaL	3.8	4.0	5.0	0.2	19.0	4.8	ml-DFH	5-5
5 EmaH	4.1	4.0	10.0	0.4	40.8	9.6	ml-DFH	5-5
6 RynL	3.7	4.0	5.0	0.2	18.6	4.7	ml-DFH	5-5
7 RynH	3.5	4.0	10.0	0.4	34.8	8.7	ml-DFH	5-5

2011								
Trt	Tree DFH (in.)	# ports/tree	Ml/ DFH	Rate (gai)	Tot. vol./ tree	Vol/ plug	Units	Appl. DAT
1 Control	-	-	-	-	-	-	-	-
2 ImiL	3.8	4.0	4.0	0.2	15.4	3.8	ml-DFH	5-30
3 ImiH	4.5	4.0	8.0	0.4	36.3	9.1	ml-DFH	5-30
4 EmaL	3.7	4.0	5.0	0.2	18.4	4.6	ml-DFH	5-30
5 EmaH	4.1	4.0	10.0	0.4	40.8	10.2	ml-DFH	5-30
6 RynL	4.1	4.0	5.0	0.2	20.5	5.1	ml-DFH	5-30

7 RynH	3.7	4.0	10.0	0.4	37.2	9.3	ml-DFH	5-30

Field Evaluations

In-season plant protection was measured by conducting a series of field evaluations for the incidence of pests or level of foliar or fruit injury in 2010 and 2011 seasons. All treatment replicates were surveyed at specific days after treatment (DAT) intervals around the entire tree using the same scouting techniques for each of the target insect species in the 2010 and 2011 seasons.

2010 Season

2010 Injection

Plot trees were surveyed for OBLR damage by counting the total number of infested terminals per tree. The OBLR damage (1st generation) was scouted at 7 days after treatment (DAT) (12 May) and 60 DAT (2 July) (2nd generation). At 40 DAT (14 June), the incidence of OFM flagging injury was scouted for a two minute period per tree. At 40 DAT (14 June), STLM leaf mines were scouted for two minutes. At 40 DAT (14 June), the PLH were scouted by observing twenty shoots per replicate and counting the number of nymphs per shoot. The JB were scouted for two minutes at 60 DAT (2 July) and the total number of beetles were tallied per tree.

2011 Season

In the 2010 continuation and 2011 injection plots, field evaluations were conducted with the same insects, but with a few additional insect pests in 2011.

2010 Continuation

OBLR (1st generation) damage was scouted at 1 yr 39 DAT (13 June), OFM flagging at 1 yr 55 DAT (29 June), STLM mines at 1 yr 85 DAT (29 July), PLH at 1 yr 39 DAT (13 June), and JB at 1 yr 85 DAT (29 July). Fifty fruit per tree assessed for plum curculio fruit oviposition damage at 1 yr 55 DAT (29 June) and 1yr 106 DAT (19 Aug). Rosy apple aphids were surveyed for 2 minutes at 1 yr 39 DAT (13 June) and the number of infested shoots were tallied. Codling moth fruit infestation was assessed at harvest time at 1yr 126 DAT (8 Sept) by collecting 25 fruit per rep and dissecting them for larval identification. The number of codling moth larvae and the total number of injured fruit were tallied. Green apple aphid was surveyed for two minutes and scouted at 1 yr 18 DAT (23 May). The number of shoots with live colonies were tallied.

2011 Injection

OBLR (1st generation) damage was scouted at 14 DAT (13 June), OFM flagging at 30 DAT (29 June), STLM mines at 60 DAT (29 July), PLH at 14 DAT (13 June), and JB at 60 DAT (29 July). Fifty fruit per tree assessed for plum curculio oviposition damage at 30 (29 June) and 80 DAT (18 Aug). Rosy apple aphids were surveyed for 2 minutes at 14 DAT (13 June) and the number of infested shoots were tallied. Codling moth fruit infestation was assessed at harvest time at 100 DAT (8 Sept) by collecting 25 fruit per rep and dissecting them for larval

identification. The number of codling moth larvae and the total number of injured fruit were tallied.

Bioassays

There was a foliar OBLR and foliar/ fruit PC bioassay conducted at different time intervals in the 2010 season and foliar OBLR, foliar/ fruit PC, and foliar/ fruit BMSB bioassays in the 2011 season.

2010 Season

2010 Injection

Obliquebanded leafroller-We initiated foliar OBLR bioassays on 7 (12 May), 60 (2 July), and 90 DAT (4 Aug) . Each replicate of the five treatments were tested against first instar OBLR larvae. For each of the 5 reps per treatment there were 8 leaf punches (2.4 cm), which were collected from 2 leaves (high, low) on each cardinal direction side (N,S,E,W) of the tree. Moist filter paper (5.5 cm) was pressed into a 5 cm wide petri dish (Beckton Dickinson & Co., Franklin Lakes, NJ) dish and the leaf punches were placed into the dish. The puncher was dipped in acetone between each treatment for sterilization. Ten larvae were selected from different egg masses, to avoid genetic similarities, and placed on the leaf disks spaced evenly within a dish. The dishes were sealed, labeled by treatment, and stored at a constant temperature and light intensity in a walk-in colony incubator at 25° C with photoperiod of 16:8 (L:D) h. After one week larval mortality was evaluated and leaf area consumed was calculated by laying them out on labeled white copy paper. The treatments were laid out on separate sheets of paper and the 8

leaf disks for all five reps of each treatment were lined up. Clear packaging tape was used to tape them down so they could be scanned for percent defoliation calculations using Photoshop Elements 8.

Plum curculio-We initiated a PC bioassay at 30 DAT (4 June). PC adults were exposed to fruit and leaves for 7 days with one shoot per rep. Each shoot had one fruit with 3-5 leaves. One lush terminal shoot per rep from each treatment were collected and put into labeled Ziploc bags. The shoots were brought back to the lab and clear plastic labeled 950 ml plastic containers (Fabri-Kal, Kalamazoo, MI) were prepared. OASIS floral foam bricks (Smithers-Oasis Co., Kent, OH) were soaked in water and cut ± 1.5 inches thick to fit in the bottom of the containers. Then blocks of gulf wax were double boiled and holes were poked in the lids for air. Next ±1 cm of wax was poured into the containers on top of the foam. As the shoots were taken out of the bags they were cut to the same length in order to fit into the containers with lids on. Before the wax was dry the shoot stems were pushed through the wax and into the foam. Finally, five random male and female PC (southern strain) were put into the containers and sealed.

After 1 week of exposure the bioassays were evaluated by measuring the mortality of the PC. Lastly the number of feeding marks on the leaves and oviposition scars on the fruit were tallied.

2011 Season

OBLR bioassays were set up using the same techniques as the 2010 season with the same materials, DAT intervals, and evaluation methods. The bioassays were set up for both 2010 continuation and 2011 injections with parallel DAT intervals. PC techniques were changed and BMSB bioassays were new to 2011.

2010 continuation and 2011 Injections

Plum curculio-PC bioassays were conducted 21 DAT (20 June) for the 2011 plot and 1 yr 46 DAT (20 June) for 2010 continuation. PC were exposed to three lush terminal shoots for 24 hours then the same beetles were exposed to 3 fruit of the same treatment and repetition for both the 2010-injected and 2011 plots for 72 hours. Once the 24 hours had passed the 3 shoots were removed and replaced with 3 shoots with one fruit per shoot. Five random PC (northern strain) in the 2010 continuation and five random female PC (southern strain) were selected for the 2011 injection. Different beetle stains were used from one season to the next due to the undistinguishable oviposit marks by the southern strain in the 2010 season and 2010 continuation of the 2011 season. Males and females were used for the 2010 continuation due to lack of beetles.

The evaluation process in the 2010 continuation and 2011 injections consisted of measuring the mortality, number of feeding marks, and number of oviposit marks on each fruit. The PC mortality was measured after 24 hours and 72 hours.

Brown marmorated stink bug-Given the importance of the brown marmorated stink bug, a preliminary experiment was conducted 90 DAT (28 Aug) in the 2011 injection plot. The number of nymphs were limited because of access to the colony. Three third instar nymphs per repetition were used in UTC, imidacloprid high, emamectin benzoate high, and rynaxypyr high. This experiment was only conducted once using the same methods and materials as the 2011 PC bioassay. The nymphs were exposed to 3 terminal shoots for 24 hours and moved to 3 fruit for 72 hours. After 24 hours, mortality was evaluated and after 72 hours of fruit exposure mortality was evaluated once more.

Residue Profiles

Sample Preparation

Imidacloprid, emamectin benzoate, and rynaxypyr residues were recovered in leaf, fruit, flower, and wood tissue samples at specific time intervals over the growing season using a specific process. This process began by collecting the samples and storing them in a specific volume of HPLC grade dichloromethane. Next the samples were ground and decanted through 10-25 g of reagent-grade anhydrous sodium sulfate (EMD Chemicals, Inc.) to remove water. The samples were then dried using rotary evaporation and the remaining particles were brought back up with 2 ml of acetonitrile. The 2 ml were transferred a 3 ml syringe (BD LuerLok Tip) with a pipette to be filtered by passing them through a 45-µm Acrodisc 33 mm syringe filter (Pall, East Hills, NY) into the final 2 ml vial (Agilent Technologies) for HPLC analysis. The tables below best describe the sample collection and lab preparation for analysis.

Sample Extraction

Residue levels were quantified using a waters 2695 separator module HPLC equipped with a Waters MicroMass ZQ mass spectrometer detector (Waters, Milford, MA), and a C_{18} reversed phase column (50 by 3.0mm bore, 3.5 µm particle size, (Waters, Milford, MA) (Table 2-4).

Table 2. The mobile phase for each insecticide used for HPLC residue analysis 2010 and 2011.

Chemical	Solvent A	Solvent B	Flow Rate (ml/min)
Emamectin	0.1 % Formic Acid	0.1 % Formic acid in acetonitrile	0.3
Rynaxapyr	0.1 % Formic Acid	0.1 % Formic acid in acetonitrile	0.3
Imidacloprid	0.1 % Formic Acid	0.1 % Formic acid in acetonitrile	0.3

Table 3. The gradient used for each insecticide for HPLC residue analysis 2010 and 2011.

Emamectin/Time	0	4	4.5	4.6	10		
Solvent A	80	10	10	80	80		
Solvent B	20	90	90	20	20		
Rynaxapyr/Time	0	1	4	6	6.1	11	
Solvent A	80	80	20	20	80	80	
Solvent B	20	20	80	80	20	20	
Imidacloprid/Time	0	1	3	3.1	4	4.1	8
Solvent A	80	80	50	20	20	80	80
Solvent B	20	20	50	80	80	20	20

Table 4. The ions (m/z) monitored, detector dwell time, and cone voltages for detection of the insecticides in HPLC residue analysis 2010 and 2011.

Chemical	Channel 1	Channel 2	Dwell (s)	Cone1 (V)	Cone2 (V)
Emamectin	872.2	886	0.5	30	45
Rynaxapyr	284	484.1	0.5	50	25
Imidacloprid	209	175	0.5	55	55

The HPLC level of quantification was 0.08 μg/g (ppm) of active ingredient, and level of detection was 0.038 ppm.

2010 Season

2010 Injection-Sample Collection and preparation

There were leaf, fruit, and wood core residue samples taken over specific time intervals throughout the growing season. Leaf residue samples were taken from three of the seven treatments and from three of the five replicate trees per treatment taken at 2 DAT (7 May), 7 DAT (12 May), 30 DAT (4 June), 60 DAT (2 July), and 90 DAT (4 Aug) intervals (Table 5). At 7 DAT there was a min of 5 g of leaf tissue collected due to the size of the leaf tissue (1/2 inch green apple stage) and time taken to collect them. The rest of the samples were a minimum of 10 g of tissue collected from the N, S, E, W cardinal direction sides of the tree, high/ low and put in labeled jars (Table 5). They were taken out of the jars, weighed and put back in the jars with 100 ml of dichloromethane. Fruit samples were taken at 30 and 90 DAT. At the 30 DAT interval, samples were taken from all seven treatments and from three of the five replicate trees per treatment (Table 5). There were 4 fruit picked from N, S, E, W sides of the tree halfway up the crown and midway deep in the crown. Each fruit was cut into 4 pieces from stem to calyx and two slices were randomly selected from each fruit to equal eight slices for each sample to equal 10 g. At the 90 DAT (4 Aug) interval, samples were taken from all seven treatments and from three of the five replicate trees per treatment. There were 4 fruit picked from N, S, E, W sides of the tree halfway up the crown and midway deep in the crown and put into labeled bags. The fruit was taken out of the bags, each cut into 4 pieces from stem to calyx and there was one slice selected from each fruit to equal four slices for each sample to equal 10 g and put into labeled jars (Table 5). Wood core samples were taken once at 60 DAT (2 July) post application for the first 3 treatments and from three of the five replicate trees per treatment (Table 5). The wood cores were taken from the trunk of the tree, five inches above the injection site. Samples were

taken from three of the seven treatments and from three of the five replicate trees per treatment, and there was one sample taken from each replicate tree. The cores were taken using a foresters wood coring tool. The cores were 2 inches deep, the same depth as the injection. The samples were pushed out of the tool and into labeled Ziploc bags using a metal rod and a tap. The samples were taken out of the bags, weighed and put in labeled jars with 20 ml of dichloromethane. They samples weighed between 1.00 and 1.60 g. After each set of samples were taken, they were stored in labeled 120 ml Qorpak jars, and stored in two dozen count Qorpak boxes, and the boxes were labeled and stored in a cold room. After the field season the samples were taken out of the cold room and transported to the MSU Pesticide Analytical laboratory for HPLC analysis and mean data graphic presentation. The table below best describes the sample collection and lab preparation for analysis.

2010 Continuation Injection-Sample Collection ad preparation

There were leaf, fruit, flower, and wood core residue samples taken. The leaf samples were taken using the same methods as the 2010 season. They were taken at 1 yr 11 DAT (16 May), 1 yr 27 DAT (1 June), 1 yr 32 DAT (6 June), 1 yr 39 DAT (13 June), 1 yr 55 DAT (29 June), 1 yr 85 DAT (29 July), and 1 yr 115 DAT (28 Aug) intervals (Table 5). Fruit samples were taken using the same methods as the 2010 season. They were taken at 1 yr 32 DAT (6 June), 1 yr 39 DAT (13 June), 1 yr 55 DAT (29 June), 1 yr 85 DAT (29 July), and 1 yr 115 DAT (28 Aug) intervals (Table 5). For the 1 yr 32 DAT (6 June), 1 yr 39 DAT (13 June), and 1 yr 55 DAT (29 June) intervals there were 4 fruit selected and each fruit was cut into 4 pieces from stem to calyx and selected 2 slices from each fruit to equal eight slices for each sample to equal 20 g. At the 1 yr 85 DAT (29 July) and 1 yr 115 DAT (28 Aug) intervals each fruit was cut into 8 pieces from stem to calyx (Table 5). There was one slice randomly selected from each fruit to

equal four slices for each sample to equal 40 g (Table 5). Lastly, they were put into the labeled jars with 90 ml of dichloromethane. Wood core samples were taken 1 yr 27 DAT (1 June), 1 yr 55 DAT (29 June), and 1 yr 115 DAT (28 Aug) (Table 5). These were collected and processed using the same tools as the 2010 season, however there were 3 different wood cores taken from each rep. There was one sample taken 6 inches above the injection site, 6 inches below the injection site, and one at one inch below the main scaffold branch above the injection site. Flower samples were collected at 1 yr 11 (16 May) DAT. The table below best describes the sample collection and lab preparation for analysis (Table 5).

Table 5. 2010 continuation injection residue sample collection and preparation for analysis.

DAT and Date collected	Trts Sampled	Sample Type	Sample Weight (g)	# Fruit per rep	# Slices per fruit		Total # fruit slices used	Volume per rep		
					cut	used	per rep	CH_2Cl_2 (ml)	Na_2SO_4 (g)	NaCl (g)
1 yr 11, 16 May	1-7	Flower	5.0	-	-	-	-	100	25	0.5
1 yr 11, 16 May	1-7	Leaf	10.0	-	-	-	-	100	25	1.00
1 yr 27, Jun 1	1-7	Leaf	10.0	-	-	-	-	100	25	1.00
1 yr 27, Jun 1	1-7	Wood Core	1.50	-	-	-	-	20	10	0.5
1 yr 32, Jun 6	1-7	Leaf	10.0	-	-	-	-	100	25	1.00
1 yr 32, Jun 6	1-7	Fruit	20.0	4	4	2	8	50	15	1.00
1 yr 39, Jun 13	1-7	Leaf	10.0	-	-	-	-	100	25	1.00
1 yr 39, Jun 13	1-7	Fruit	20.0	4	4	2	8	50	15	1.00
1 yr 55, Jun 29	1-7	Leaf	10.0	-	-	-	-	100	25	1.00
1 yr 55, Jun 29	1-7	Fruit	20.0	4	4	2	8	50	15	1.00
1 yr 55, Jun 29	1-7	Wood Core	1.50	-	-	-	-	20	10	0.5
1 yr 85, Jul 29	1-7	Leaf	10.0	-	-	-	-	100	25	1.00
1 yr 85, Jul 29	1-7	Fruit	40.0	4	8	1	4	90	25	1.00
1 yr 115, Aug 28	1-7	Leaf	10.0	-	-	-	-	100	25	1.00
1 yr 115, Aug 28	1-7	Fruit	40.0	4	8	1	4	90	25	1.00
1 yr 115, Aug 28	1-7	Wood Core	1.50	-	-	-	-	20	10	0.5

2011 Injection-Sample Collection and preparation

There were leaf, fruit, flower, wood core, fruit core, and fruit seed residue samples taken. The leaf, fruit and wood core samples were taken using the same methods as the 2010 continuation. The fruit core and seed samples were taken 60 DAT (28 July) from UTC, imidacloprid high, emamectin benzoate high, and rynaxypyr high. They were processed using a very unique method with a razor blade, fruit corer, knife, and acetone. Initially there were four apples picked from three of the five replicates on the N, S, E, W sides of the tree halfway up the crown and midway deep in the crown. The fruit were placed in labeled Ziploc bags and stored in the freezer until frozen. Each fruit was then cut in half from stem to calyx. Then randomly selected slices from the fruit were cored twice per half. A 9 mm coring tool was used to core each side of each half from the outside inward. Next each core taken was sliced into four separate slices until the correct weight for each sample was obtained. Skin slices were ±5 g per sample, the 2 mm outer flesh were ± 7 g, the inner 10 mm flesh was ±10 g, and the inner 5 mm flesh to the seed cavity ± 10 g (Table 6). These cuts were made using a razor blade, and each section was placed in its own labeled jar with 20 ml of dichloromethane (Table 6). The blade was sterilized using acetone between each core section. Seed samples were taken from 30 fruit in each treatment, 10 fruit per rep and the three reps were added together to equal one sample per treatment (Table 6). There were ± 160 seeds per treatment and 3-8 seeds per fruit to equal ±10 g per sample. The table below best describes the sample collection and lab preparation for analysis (Table 6).

Table 6. 2011 sample collection and preparation for residue analysis.

DAT and Date collected	Trts Sampled	Sample Type	Sample Weight (g)	# Fruit per rep	# Slices per fruit		Total # fruit slices used	Volume per rep		
					cut	used	per rep	CH_2Cl_2(ml)	Na_2SO_4 (g)	NaCl (g)
2, 1 Jun	1-7	Leaf	10.0	-	-	-	-	100	25.0	1.0
2, 1 Jun	1-7	Wood Core	1.5	-	-	-	-	20	10.0	0.5
7, 6 June	1-7	Leaf	10.0	-	-	-	-	100	25.0	1.0
7, 6 June	1-7	Fruit	20.0	4	4	2	8	50	15.0	1.0
14, 13 Jun	1-7	Leaf	10.0	-	-	-	-	100	25.0	1.0
14, 13 Jun	1-7	Fruit	20.0	4	4	2	8	50	15.0	1.0
30, 29 Jun	1-7	Leaf	10.0	-	-	-	-	100	25.0	1.0
30, 29 Jun	1-7	Fruit	20.0	4	4	2	8	50	15.0	1.0
30, 29 Jun	1-7	Wood Core	1.5	-	-	-	-	20	10.0	0.5
60, 29 Jul	1-7	Leaf	10.0	-	-	-	-	100	25.0	1.0
60, 29 Jul	1-7	Fruit	40.0	4	8	1	4	90	25.0	1.0
60, 29 Jul	1, 3, 5, 7	Fruit Core	Skin 5.0	-	-	-	-	20	10.0	0.5
			Outer 8.0	-	-	-	-	20	10.0	0.5
			Middle 10.0	-	-	-	-	20	10.0	0.5
			Inner 10.0	-	-	-	-	20	10.0	0.5
90, 28 Aug	1-7	Leaf	10.0	-	-	-	-	100	25.0	1.0
90, 28 Aug	1-7	Fruit	40.0	4	8	1	4	90	25.0	1.0
90, 28 Aug	1-7	Wood Core	1.5	-	-	-	-	20	10.0	0.5

Results

Field Evaluations

2010 Season

At 7 DAT (12 May) there was no difference in incidence of leafroller damage compared to the control (F=0.263, df=6, P=0.949) (Table 7). Many larval infestations likely occurred before the injections took place due to the fact that OBLR overwinter as larvae. At 40 DAT (14 June) the incidence of PLH nymphs were significantly lowest in the imidacloprid high rate plots, followed by the emamectin benzoate high rate and rynaxypyr high rate, compared to the untreated control (F=35.945, df=6, P=0.0001) (Table 7). At 40 DAT (14 June) all six treatments reduced the incidence of STLM mines compared to the control (F=8.628, df=6, P=0.0001) (Table 7). At 40 DAT (14 June) the incidence of OFM flagging was significantly lower for imidacloprid high rate, all emamectin benzoate and rynaxypyr treatments, but numbers in the imidacloprid low rate plots were not different than the untreated control (F=11.558, df=6, P=0.0001) (Table 7).

Table 7. 2010 mean number (±SE) of foliar insects or pest injury associated with in-season field evaluations, across the seven treatments.

	7 DAT	40 DAT		
Trt	# leafroller Damaged shoots	# PLH nymphs Per 20 shoots	# STLM mines 2 minute count	# OFM flags 2 minute count
Control	5.0 (0.9)a	1.9 (0.2)a	3.8 (1.3)a	13.6 (3.0)a
ImiL	5.2 (0.6)a	1.4 (0.1)a	0.4 (0.4)b	8.4 (1.5)ab
ImiH	5.2 (1.6)a	0.2 (0.0)c	0.2 (0.2)b	5.0 (0.7)bc
EmaL	4.0 (0.3)a	1.6 (0.1)a	0.0 (0.0)b	2.2 (0.7)c
EmaH	4.8 (0.9)a	0.9 (0.1)b	0.0 (0.0)b	1.4 (0.5)c
RynL	4.2 (1.0)a	1.6 (0.2)a	0.0 (0.0)b	5.4 (1.0)bc
RynH	5.0 (0.7)a	0.7 (0.1)b	0.0 (0.0)b	2.4 (0.5)c

Means followed by same letter do not significantly differ (*P*=.05, Tukey's HSD)
ANOVA performed on square-root transformed data; means shown for comparison

2011 Season

2010 Continuation Injection

At 1 year 18 DAT (23 May) treatments did not affect incidence of GAA compared to the control (*F*=1.445, df=6, *P*=0.2427) (Table 8). At 1 year 39 (13 June, 2011) DAT the incidence of OBLR damaged shoots was significantly lower for rynaxypyr high rate than the untreated control (*F*=4.33, df=6, *P*=0.005) (Table 8). At 1 year 39 (13 June) the incidence of PLH nymphs was significantly lower for both imidacloprid treatments than the control (*F*=7.669, df=6, *P*=0.0002) (Table 8). At 1 year 55 DAT (29 June) the incidence of OFM flagging was significantly lower for both emamectin benzoate and rynaxypyr treatments than the untreated control (*F*=4.601, df=6, *P*=0.0036) (Table 8). At 1 year 85 DAT (29 July) the incidence of STLM mines was significantly lowest in the rynaxypyr high rate plots, then the low rate of imidacloprid, both

emamectin benzoate rates, and low rate of rynaxypyr treatments, followed by the high rate of imidacloprid, and the control (F=6.545, df=6, P=0.0004) (Table 8).

Table 8. 2010 continuation mean number (±SE) of foliar insects or pest injury associated with in-season field evaluations, across the seven treatments.

	1 yr 18 DAT	1 yr 39 DAT		1 yr 55 DAT	1 yr 85 DAT
Trt	# live infested GAA shoots* 2 min count	# OBLR damage	# PLH nymphs 20 shoots	# OFM flags* 2 minute count	# STLM mines
Control	1.8 (1.1)a	49.2 (4.6)a	2.6 (0.3)a	6.0 (1.4)a	7.2 (1.5)a
ImiL	0.0 (0.0)a	46.8 (6.9)a	0.8 (0.2)b	2.4 (0.2)ab	2.2 (1.0)bc
ImiH	0.6 (0.2)a	37.0 (7.6)ab	0.7 (0.1)b	3.2 (0.4)ab	3.0 (1.6)b
EmaL	1.4 (0.2)a	25.8 (6.0)ab	3.0 (0.3)a	1.8 (0.4)b	0.2 (0.2)bc
EmaH	1.9 (1.7)a	35.4 (4.6)ab	2.3 (0.2)a	1.2 (0.4)b	0.9 (0.5)bc
RynaL	0.8 (0.2)a	26.0 (6.0)ab	1.8 (0.2)ab	1.0 (0.3)b	0.8 (0.6)bc
RynaH	0.5 (0.5)a	14.5 (1.7)b	1.5 (0.2)ab	2.6 (1.9)b	0.0 (0.0)c

Means followed by same letter do not significantly differ (P=.05, Tukey's HSD)
ANOVA performed on square-root transformed data; means shown for comparison
*ANOVA may not be valid as the data failed Bartlett's test for homogeneity

2011 Injection

At 14 DAT (13 June) there were no significant differences in the incidence of leafroller damage compared to the control (F=2.77, df=6, P=0.0343) (Table 9). At 14 DAT (13 June, 2011) the incidence of PLH nymphs was significantly lower for all imidacloprid, emamectin benzoate, and rynaxypyr treatments than the untreated control (F=9.244. df=6, P=0.0001) (Table 9). At 30 DAT (29 June) the incidence of OFM flagging was significantly lower for all imidacloprid, emamectin benzoate, and rynaxypyr treatments than the untreated control (F=5.568, df=6, P=0.001) (Table 9). At 60 DAT (29 July) the incidence of STLM mines was

significantly lower for all imidacloprid, emamectin benzoate, and rynaxypyr treatments than the control (F=14.437, df=6, P=0.0001) (Table 9).

At 30 DAT (29 June) there were no significant differences in the mean number of PC fruit stings compared to the control (F=3.899, df=6, P=0.0074) (Table 10). At 100 DAT (7 Sept) there were no significant differences in the means of CM larvae in fruit compared to the control (F=3.899, df=6, P=0.0074) (Table 10). At 100 DAT (7 Sept) the mean number of AM dropped from fruit were significantly different in the emamectin benzoate low treatment compared to the control (F=3.221, df=6, P=0.0183) (Table 10).

Table 9. 2011 mean number (±SE) of foliar insects or pest injury associated with in-season field evaluations, across the seven treatments.

	14 DAT			30 DAT	60 DAT
Trt	# Leafroller damage	# PLH* nymphs Per 20 shoots	# Live infested RAA shoots	# OFM flags 2 min	# STLM mines
Control	44.8 (3.8)a	1.6 (0.2)a	1.2 (0.4)a	5.2 (0.9)a	5.0 (1.0)a
ImiL	43.0 (6.4)a	0.1 (0.0)b	0.0 (0.0)a	1.0 (0.4)b	1.0 (0.5)b
ImiH	45.4 (4.2)a	0.1 (0.0)b	0.0 (0.0)a	1.0 (0.5)b	1.2 (0.6)b
EmaL	30.8 (3.9)a	0.7 (0.1)b	0.2 (0.2)a	0.8 (0.4)b	0.0 (0.0)b
EmaH	30.0 (2.8)a	0.6 (0.1)b	0.2 (0.2)a	0.8 (0.6)b	0.0 (0.0)b
RynaL	33.8 (5.8)a	0.7 (0.1)b	0.8 (0.4)a	1.8 (0.9)b	0.0 (0.0)b
RynaH	28.4 (4.9)a	0.6 (0.1)b	0.6 (0.4)a	1.8 (0.9)b	0.2 (0.2)b

Means followed by same letter do not significantly differ (P=.05, t Test LSD)
ANOVA performed on square-root transformed data; data presented are actual counts
Means followed by same letter do not significantly differ (P=.05, Tukey's HSD)
ANOVA performed on square-root (SQR(X+0.5)) transformed data; means shown for comparison.
*ANOVA may not be valid as the data failed Bartlett's test for homogeneity

Table 10. 2011 mean number (±SE) of direct insects or pest injury associated with in-season field evaluations, across the seven treatments.

Trt	30 DAT	100 DAT	
	# PC stings Per 50 fruit	fruit harvest # CM larvae Per 25 fruit	# AM larvae emerged from fruit* Per 30 fruit
Control	0.4 (0.0)a	0.8 (0.4)abc	21.4 (7.2)a
ImiL	0.3 (0.0)a	2.2 (0.7)a	8.6 (2.8)ab
ImiH	0.6 (0.1)a	1.8 (0.6)ab	7.4 (2.6)ab
EmaL	0.4 (0.0)a	0.2 (0.2)bc	4.2 (1.2)b
EmaH	0.4 (0.0)a	0.8 (0.6)abc	6.4 (3.3)ab
RynL	0.3 (0.0)a	0.4 (0.2)abc	16.0 (0.8)ab
RynaH	0.3 (0.0)a	0.0 (0.0)c	14.8 (4.2)ab

Means followed by same letter do not significantly differ (P=.05, Tukey's HSD)
ANOVA performed on square-root transformed data; means shown for comparison
*ANOVA may not be valid as the data failed Bartlett's test for homogeneity

Bioassays

2010 Season

Obliquebanded leafroller-The mean survival of OBLR larvae exposed to leaves 7 DAT (12 May, 2010) was significantly lower for the emamectin benzoate treatments than the control and other treatments (F=27.364, df=6, P=0.0001) (Table 11). The mean survival of OBLR larvae exposed to leaves 60 DAT (2 July) was significantly lower for the emamectin benzoate treatments and rynaxypyr high rate than the control and other treatments (F=16.365, df=6, P=0.0001) (Table 11). Only the low and high rates of emamectin benzoate reduced mean survival of OBLR larvae exposed to leaves 90 DAT (3 Aug), compared to the control (F=5.256, df=6, P=0.0014) (Table 11)**.** Though OBLR larvae exposed to imidacloprid treated leaf discs did

not result in reduced survival, qualitative observations noted that larval development and spinneret activity were minimal.

The mean percent of leaf disc areas consumed by larvae when exposed to leaves 7 DAT (12 May, 2010) were significantly lower for the emamectin benzoate treatments and rynaxypyr high rate than the control (F=13.883, df=6, P=0.0001) (Table 12). The mean proportion of leaf disc areas consumed by larvae when exposed to leaves 60 DAT (2 July) were significantly lower for the emamectin benzoate treatments, imidacloprid low rate, and rynaxypyr treatments than the control (F=14.474, df=6, P=0.0001) (Table 12). The mean proportion of leaf disc areas consumed by larvae when exposed to leaves 90 DAT (3 Aug) were significantly lower for imidacloprid, emamectin benzoate, and rynaxypyr treatments than the control (F=10.777, df=6, P=0.0001) (Table 12).

Table 11. 2010 mean (±SE) survival of OBLR larvae exposed to treated leaves 7, 60, and 90 DAT across the seven treatments**.**

	# Live OBLR Larvae		
Trt	7 DAT*	60 DAT	90 DAT*
Control	7.2 (1.1)a	4.0 (0.6)ab	4.6 (0.2)a
ImiL	4.8 (0.4)a	3.2 (0.5)ab	2.4 (1.0)ab
ImiH	6.0 (0.8)a	5.0 (0.3)a	2.6 (0.8)ab
EmaL	0.0 (0.0)b	0.0 (0.0)d	0.0 (0.0)b
EmaH	0.6 (0.6)b	0.6 (0.6)cd	0.0 (0.0)b
RynaL	6.0 (0.5)a	1.6 (0.7)bc	1.4 (0.7)ab
RynaH	5.8 (1.5)a	0.4 (0.2)cd	1.2 (1.0)ab

Means followed by same letter do not significantly differ (P=.05, Tukey's HSD)
ANOVA performed on arcsine square-root transformed data; means shown for comparison.

*ANOVA may not be valid as the data failed Bartlett's test for homogeneity

Table 12. 2010 mean (±SE) percent of leaf area consumed by OBLR larvae when exposed to seven treatments 7, 60, and 90 DAT.

	Percent Consumed		
Trt	7 DAT	60 DAT*	90 DAT
Control	63.3 (6.9)a	7.8 (2.8)a	1.1 (0.2)a
ImiL	52.5 (8.7)ab	0.9 (0.1)b	0.5 (0.1)b
ImiH	52.6 (9.0)ab	4.9 (1.4)a	0.4 (0.1)b
EmaL	4.0 (1.8)c	0.1 (0.1)b	0.1 (0.0)b
EmaH	8.4 (5.8)c	0.1 (0.1)b	0.1 (0.0)b
RynaL	40.6 (6.7)ab	0.2 (0.1)b	0.1 (0.0)b
RynaH	27.9 (8.8)bc	0.2 (0.1)b	0.3 (0.1)b

Means followed by same letter do not significantly differ (P=.05, Tukey's HSD) ANOVA performed on arcsine square-root transformed data; means shown for comparison.

*ANOVA may not be valid as the data failed Bartlett's test for homogeneity

Plum curculio- There were no significant differences in the mean survival of PC adults exposed to leaves for 1 week, 30 DAT (4 June-11 June) compared to the control (F=0.971, df=6, P=0.4659) (Table 13). There also were no significant differences in the mean number of adult PC foliar feeding marks when exposed to leaves for 1 week 30 DAT (4 June-11 June) compared to the control (F=0.661, df=6, P=0.6818) (Table 13). The mean number of adult PC fruit feeding marks was significantly higher for emamectin benzoate low and rynaxypyr low treatments compared to the control (F=3.353, df=6, P=0.0152) (Table 13).

Table 13. 2010 mean (±SE) survival of live PC and feeding marks of both leaf and fruit exposed for 1 week 30 DAT across the seven treatments.

	30 DAT		
Trt	# Live	# Leaf Feeding Marks[s]	# Fruit Feeding Marks[s]
Control	3.4 (0.4)a	3.8 (1.4)a	49.8 (9.9)b
ImiL	4.2 (0.4)a	2.8 (1.7)a	44.0 (17.5)b
ImiH	3.4 (1.0)a	2.2 (1.2)a	37.2 (9.9)b
EmaL	3.2 (0.4)a	0.8 (0.2)a	87.2 (10.5)a
EmaH	4.6 (0.2)a	2.0 (0.9)a	66.0 (5.9)ab
RynaL	3.4 (0.6)a	2.0 (0.8)a	88.6 (9.3)a
RynaH	4.2 (0.4)a	1.6 (0.5)a	62.8 (5.1)ab

Means followed by same letter do not significantly differ (P=.05, LSD)
[a]ANOVA performed on arcsine square-root transformed data; data presented are actual counts
[s]ANOVA performed on square-root transformed data; data presented are actual counts
*ANOVA may not be valid as the data failed Bartlett's test for homogeneity

2011 Season

2010 Continuation Injection

Obliquebanded leafroller-The mean survival of OBLR larvae exposed to leaves 1 year 32 DAT (6 June, 2011) were significantly lower for the emamectin benzoate treatments than the control (F=12.201, df=6, P=0.0001) (Table 14). The mean survival of OBLR larvae exposed to leaves 1 year 85 DAT (29 July) were significantly lower for the emamectin benzoate treatments than the control (F=5.677, df=6, P=0.0011) (Table 14). The mean survival of OBLR larvae exposed to leaves1 year 115 DAT (28 Aug) were significantly lower for the emamectin benzoate treatments and rynaxypyr high rate than the control (F=10.377, df=6, P=0.0001) (Table 14)**.** Though OBLR larvae exposed to imidacloprid treated leaf discs did not result in reduced

survival, qualitative observations noted that larval development and spinneret activity were minimal.

The mean percent of leaf disc areas consumed by larvae when exposed to leaves at 1 year 32 DAT (6 June, 2011) were significantly lower for the emamectin benzoate treatments and rynaxypyr high rate than the control (F=14.282, df=6, P=0.0001) (Table 15). The mean proportion of leaf disc areas consumed by larvae when exposed to leaves 1 year 85 DAT (29 July) DAT were significantly lower for the emamectin benzoate treatments, imidacloprid low rate, and rynaxypyr treatments than the control (F=1.906, df=6, P=0.1249) (Table 15). The mean proportion of leaf disc areas consumed by larvae when exposed to leaves 1 year 115 DAT (28 Aug) were significantly lower for imidacloprid, emamectin benzoate, and rynaxypyr treatments than the control (F=7.416, df=6, P=0.0002) (Table 15).

Table 14. 2010 continuation mean (±SE) survival of OBLR larvae exposed to treated leaves 7, 60, and 90 DAT across the seven treatments**.**

	# Live OBLR Larvae		
Trt	7 DAT*	60 DAT	90 DAT
Control	4.4 (0.2)a	3.0 (0.9)a	5.0 (0.0)a
ImiL	4.0 (0.5)a	1.0 (0.6)abc	4.0 (0.8)a
ImiH	3.2 (0.4)a	1.4 (0.9)abc	4.0 (0.8)a
EmaL	1.0 (0.5)bc	0.0 (0.0)c	0.6 (0.4)bc
EmaH	0.7 (0.5)c	0.0 (0.0)bc	0.9 (0.5)bc
RynL	3.0 (0.5)a	2.2 (0.7)a	2.8 (1.0)ab
RynH	1.9 (0.4)ab	1.7 (0.3)ab	0.0 (0.0)c

Means followed by same letter do not significantly differ (P=.05, Tukey's HSD)
ANOVA performed on arcsine square-root transformed data; means shown for comparison.

*ANOVA may not be valid as the data failed Bartlett's test for homogeneity

°Rynaxypyr high means fixed to 0 due to a negative means, does not change ANOVA

Table 15. 2010 continuation mean (±SE) percent of leaf area consumed by OBLR larvae when exposed to seven treatments 7, 60, and 90 DAT.

	Percent Consumed		
Trt	7 DAT*	60 DAT*	90 DAT
Control	8.8 (0.7)a	8.3 (1.3)a	14.6 (4.0)a
ImiL	9.9 (1.3)a	5.2 (2.7)a	7.0 (2.3)ab
ImiH	7.0 (1.7)ab	13.2 (7.9)a	7.3 (2.1)ab
EmaL	2.9 (0.4)bc	1.2 (0.5)a	0.6 (0.2)b
EmaH	1.3 (0.4)c	3.7 (0.8)a	1.0 (0.5)b
RynL	2.3 (1.2)c	5.0 (1.4)a	3.4 (1.5)b
RynH	1.5 (0.2)c	3.3 (1.2)a	0.5 (0.3)b

Means followed by same letter do not significantly differ (*P*=.05, Tukey's HSD) ANOVA performed on arcsine square-root transformed data; means shown for comparison.

*ANOVA may not be valid as the data failed Bartlett's test for homogeneity

Plum curculio- There were no significant differences in the mean survival of PC adults exposed to leaves for 24 hours 21 DAT (26 May) compared to the control (*F*=1.048, df=6, *P*=0.4221) (Table 16). There were no significant differences in the mean survival of PC adults exposed to leaves for 1 week, 21 DAT (26 May-32 May) compared to the control (*F*=2.359, df=6, *P*=0.0653) (Table 16). The mean number of oviposition stings when exposed to fruit for 1 week DAT (26 May-32 May) were significantly higher in the rynaxypyr high treatment compared to the control (*F*=5.556, df=6, *P*=0.0012) (Table 16). The mean number of adult PC fruit feeding marks when exposed to fruit for 1 week DAT (26 May-32 May) were significantly higher for emamectin benzoate and rynaxypyr high treatments compared to the control (*F*=3.064, df=6, *P*=0.0247) (Table 16).

Table 16. 2010 continuation mean (±SE) survival of live PC adults after 24 hours, live PC adults after 1 week, oviposition stings after 1 week, and fruit feeding marks after 1 week when exposed to leaf and fruit tissue at 21 DAT 26 May across the seven treatments.

	21 DAT			
Trt	# Live after 24 hrs[a]	# Live after 1 week[a]	# oviposition stings after 1 week[s]*°	# fruit feeding marks after 1 week[s]
Control	4.2 (0.6)a	1.0 (0.3)a	0.4 (0.4)b	6.6 (2.8)b
ImiL	2.6 (0.5)a	1.0 (0.3)a	0.8 (0.6)b	7.8 (3.2)b
ImiH	2.4 (0.7)a	1.2 (0.4)a	0.4 (0.4)b	8.8 (4.2)b
EmaL	3.8 (0.7)a	2.8 (1.0)a	0.0 (0.0)b	21.8 (7.3)a
EmaH	3.3 (0.9)a	2.6(0.8)a	0.2 (0.2)b	23.1 (5.8)a
RynL	3.0 (0.4)a	3.0 (0.4)a	0.0 (0.0)b	13.0 (2.5)ab
RynH	2.8 (0.5)a	2.7 (0.7)a	27.1 (15.5)a [e]	20.4 (5.4)a

Means followed by same letter do not significantly differ (P=.05, LSD)
[a]ANOVA performed on arcsine square-root transformed data; data presented are actual counts
[s]ANOVA performed on square-root transformed data; data presented are actual counts
*ANOVA may not be valid as the data failed Bartlett's test for homogeneity
°Emamectin benzoate high means fixed to 0 due to a negative means, does not change ANOVA
[e]outlier due to the skewed number of female PC, females outnumbered males 4:1

2011 Injection

Obliquebanded leafroller-The mean survival of OBLR larvae exposed to leaves 7 DAT (6 June, 2011) showed no significant differences compared to the control (F=5.325, df=6, P=0.0013) (Table 17). The mean survival of OBLR larvae exposed to leaves 60 DAT (29 July) were significantly lower for the emamectin benzoate treatments and rynaxypyr high rate than the control (F=8.81, df=6, P=0.0001) (Table 17). The mean survival of OBLR larvae exposed to leaves 90 DAT (28 Aug) were significantly lower for the emamectin benzoate and rynaxypyr

treatments than the control (F=13.883, df=6, P=0.0001) (Table 17)**.** Though OBLR larvae exposed to imidacloprid treated leaf discs did not result in reduced survival, qualitative observations noted that larval development and spinneret activity were minimal.

The mean percent of leaf disc areas consumed by larvae when exposed to leaves 7 DAT (6 June) were significantly lower for the emamectin benzoate treatments and rynaxypyr high rate than the control (F=10.124, df=6, P=0.0001) (Table 18). The mean proportion of leaf disc areas consumed by larvae when exposed to leaves 60 DAT (29 July) were significantly lower for the imidacloprid high rate than the control (F=9.94, df=6, P=0.0001) (Table 18). The mean proportion of leaf disc areas consumed by larvae when exposed to leaves 90 DAT (28 Aug) were significantly lower for emamectin benzoate, and rynaxypyr treatments than the control (F=13.883, df=6, P=0.0001) (Table 18).

Table 17. 2011 mean (±SE) survival of OBLR larvae exposed to treated leaves 7, 60, and 90 DAT across the seven treatments**.**

	# Live OBLR Larvae		
Trt	7 DAT	60 DAT*	90 DAT*
Control	1.8 (0.9)abc	2.4 (0.8)ab	4.4 (0.4)a
ImiL	2.4 (0.6)ab	1.8 (1.1)abc	3.0 (0.3)ab
ImiH	3.2 (0.9)a	3.8 (0.4)a	3.0 (0.8)ab
EmaL	0.0 (0.0)c	0.0 (0.0)c	0.0 (0.0)c
EmaH	0.0 (0.0)c	0.0 (0.0)c	0.0 (0.0)c
RynL	0.6 (0.2)abc	0.2 (0.2)bc	1.2 (0.7)bc
RynH	0.2 (0.2)bc	0.0 (0.0)c	0.4 (0.2)c

Means followed by same letter do not significantly differ (P=.05, Tukey's HSD)

ANOVA performed on arcsin square-root transformed data; means shown for comparison.

Table 18. 2011 mean (±SE) percent of leaf area consumed by OBLR larvae when exposed to seven treatments 7, 60, and 90 DAT.

	Percent Consumed		
Trt	7 DAT*	60 DAT*	90 DAT*
Control	10.3 (0.6)a	3.5 (0.7)bc	10.7 (3.2)a
ImiL	10.3 (2.3)a	6.4 (2.9)ab	6.8 (2.4)a
ImiH	8.9 (3.6)ab	10.6 (1.7)a	5.6 (1.3)a
EmaL	2.0 (1.2)bc	0.5 (0.1)c	0.2 (0.1)b
EmaH	0.6 (0.1)c	0.5 (0.2)c	0.7 (0.2)b
RynL	0.5 (0.2)c	0.5 (0.1)c	0.2 (0.1)b
RynH	1.4 (0.6)c	1.9 (1.1)bc	0.1 (0.0)b

Means followed by same letter do not significantly differ (*P*=.05, Tukey's HSD)
ANOVA performed on arcson square-root transformed data; means shown for comparison.
*ANOVA may not be valid as the data failed Bartlett's test for homogeneity

Plum curculio-There were no significant differences in the mean survival of PC adults exposed to leaves for 24 hours 30 DAT (4 June) compared to the control (*F*=7.423, df=6, *P*=0.0001) (Table 19). There were no significant differences in the mean survival of PC adults exposed to leaves for 1 week, 30 DAT (4 June-11 June) compared to the control (*F*=1.131, df=6, *P*=0.3743) (Table 19). The mean number of oviposition stings when exposed to fruit for 1 week were significantly higher in the rynaxypyr high treatment compared to the control (*F*=1.915, df=6, *P*=0.1195) (Table 19). The mean number of adult PC fruit feeding marks were significantly higher for emamectin benzoate and rynaxypyr high treatments compared to the control (*F*=4.483, df=6, *P*=0.0035) (Table 19).

Table 19. 2011 mean (±SE) survival of PC and feeding marks of both leaf and fruit exposed for 1 week 21 DAT across the seven treatments.

	21 DAT			
Trt	# Live after 24 hrs[a]	# Live after 1 week[a]	# oviposition stings after 1 week	# fruit feeding marks after 1 week
Control	4.6 (0.2)a	2.4 (0.5)a	44.0 (1.6)a	48.8 (4.1)a
ImiL	5.0 (0.0)a	1.0 (0.5)a	15.8 (5.1)a	38.0 (9.2)a
ImiH	4.4 (0.2)a	1.2 (0.6)a	14.0 (6.0)a	15.8 (3.7)b
EmaL	3.8 (0.6)ab	0.4 (0.2)a	10.8 (6.8)a	11.8 (3.2)b
EmaH	3.6 (0.4)ab	1.4 (0.6)a	19.6 (9.5)a	16.6 (6.9)b
RynL	1.2 (0.6)c	1.6 (1.0)a	27.6 (15.0)a	14.2 (4.9)b
RynH	2.6 (0.5)b	1.0 (0.3)a	34.2 (11.7)a	10.0 (3.5)b

Means followed by same letter do not significantly differ (P=.05, LSD)
[a]ANOVA performed on arcsine square-root transformed data; data presented are actual counts
[s]ANOVA performed on square-root transformed data; data presented are actual counts.

Residue Profiles

2010 Season

Imidacloprid

Imidacloprid was first detected at 3.19 ppm in low rate leaf samples at 30 DAT (4 June), increased to a maximum level of 16.24 ppm at 60 DAT (2 July), and decreased to a minimum 1.22 ppm at 90 DAT (3 Aug) (Figure 1). High rate foliar residues were also first recovered at 30 DAT (4 June) at 8.94 ppm, increased to a maximum level of 19.13 ppm at 60 DAT (2 July), and decreased to a minimum of 2.08 ppm 90 DAT (3 Aug) (Figure 1).

There were no residues recovered from the low rate imidacloprid fruit tissue at 30 DAT (4 June), but 1.22 ppm was detected at 90 DAT (3 Aug) (Table 20). There were 0.11 ppm of residue recovered from high rate imidacloprid fruit tissue at 30 DAT (4 June) and 2.08 ppm recovered at 90 DAT (3 Aug) at the end of the growing season (Table 20).

Low rate imidacloprid wood core residue samples collected 6 inches above the injection site showed 11.22 ppm and 42.22 ppm from high rate samples at 60 DAT (2 July) (Table 20).

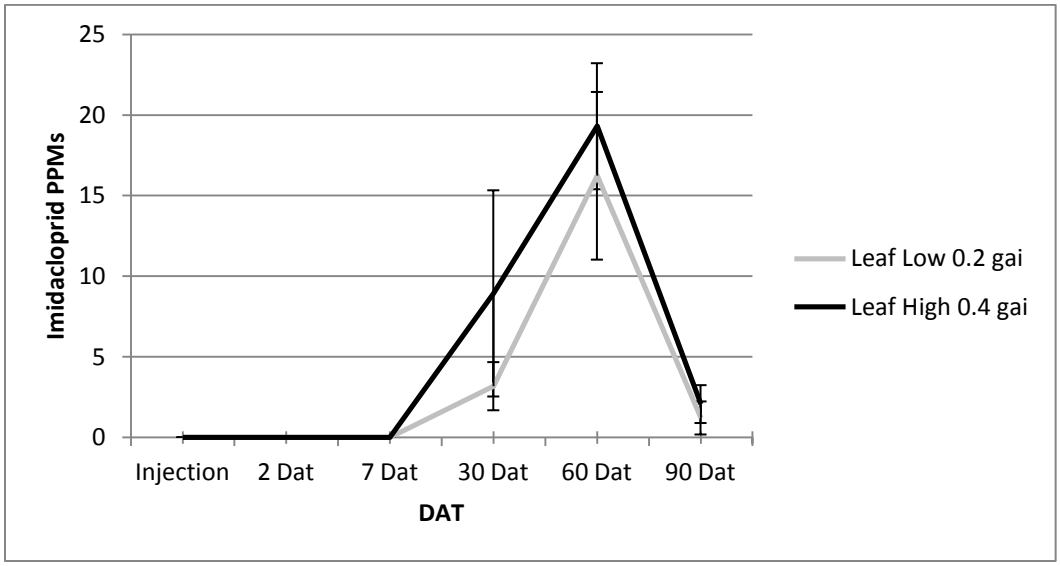

Figure 1. Mean residue recovery (ppm) from leaf tissues for 2010 imidacloprid low and high rate injected trees. Injections were on 5 May, 2 DAT was 7 May, 7 DAT was 12 May, 30 DAT was 4 Jun, 60 DAT was 2 Jul, and 90 DAT was 4 Aug 2010.

Table 20. Mean residue recovery (ppm) from fruit and wood core tissues for 2010 imidacloprid low and high rate injected trees.

Type	Rate	DAT [a]	Mean residue Recovered (ppm)
Fruit	Low	30	0
Fruit	High	30	0.11
Wood Core [b]	Low	60	11.22
Wood Core [b]	High	60	42.22
Fruit	Low	90	1.22
Fruit	High	90	2.08

[a] 30 DAT was 4 June, 60 DAT was 2 Jul, and 90 DAT was 3 Aug 2010.
[b] Wood core samples taken 6 in above the injection point.

Emamectin Benzoate

Emamectin benzoate was first detected at 0.439 ppm in low rate fruit samples at 30 DAT (4 June) and increased to a maximum level of 0.504 ppm at 90 DAT (4 Aug) (Figure 2). High rate fruit residues were also first recovered at a maximum of 0.321 ppm at 30 DAT (4 June) and decreased to 0.183 ppm at 90 DAT (3 Aug) (Figure 2).

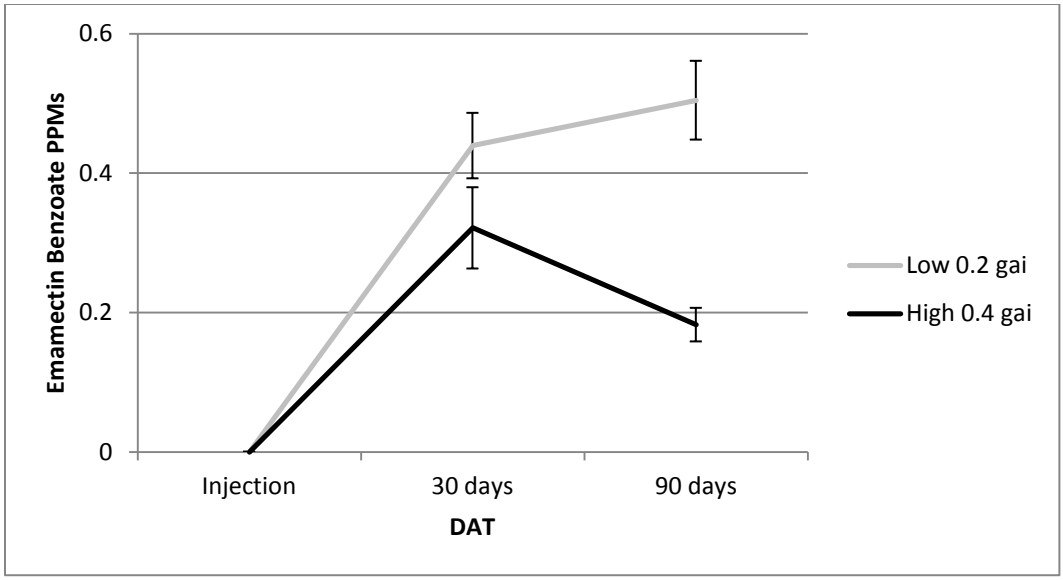

Figure 2. Mean residue recovery (ppm) from fruit tissues for 2010 emamectin benzoate low and high rate injected trees. Injections were on 5 May, 30 DAT was 4 Jun, and 90 DAT was 3 Aug 2010.

Rynaxypyr

Rynaxypyr was first detected at a maximum of 0.038 ppm in low rate fruit samples at 30 DAT (4 June) and decreased to 0.018 ppm at 90 DAT (3 Aug) (Figure 3). High rate fruit residues were also first detected at a maximum of 0.022 ppm at 30 DAT (4 June) and decreased to 0.015 ppm at 90 DAT (3 Aug) (Figure 3).

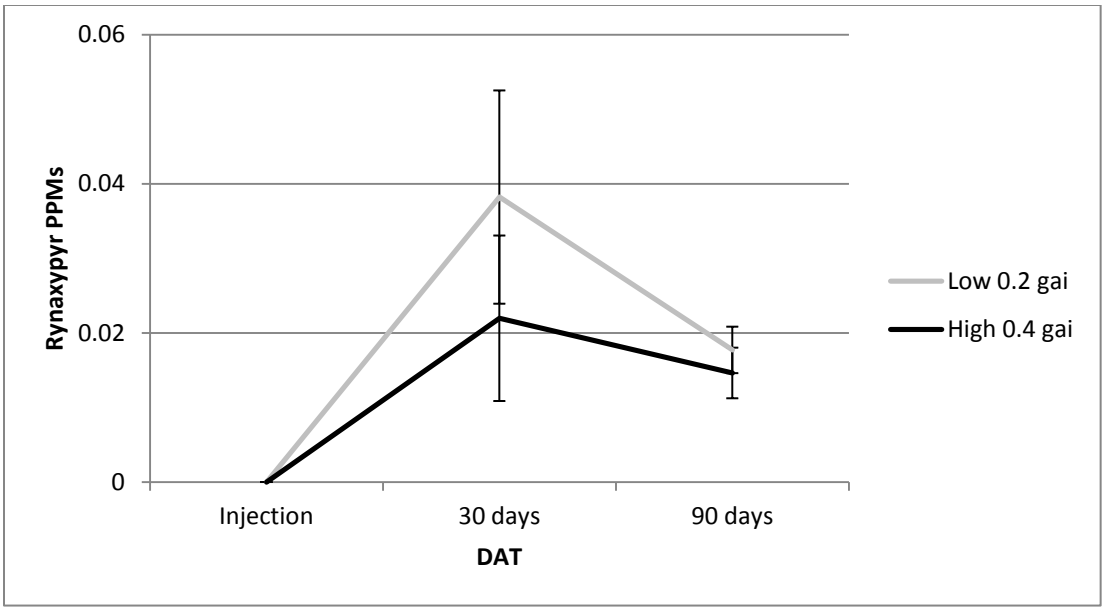

Figure 3. Mean residue recovery (ppm) from fruit tissues for 2010 rynaxypyr low and high rate injected trees. Injections were on 5 May, 30 DAT was 4 Jun, and 90 DAT was 3 Aug 2010.

2011 Season

2010 Continuation Injection

Imidacloprid

Imidacloprid was first detected at 0.16 ppm in low rate foliar samples at 1 year 11 DAT (16 May) and increased to a maximum level of 0.56 ppm at 1 year 27 DAT (1 June) (Figure 4). Residue levels then decreased to 0.03 ppm at 1 year 115 DAT (28 Aug) (Figure 4). High rate foliar residues were also first detected at 1 year 11 DAT (16 May) at 0.38 ppm and increased to a maximum level of 0.88 ppm at 1 year 55 DAT (29 June).(Figure 4). Residue levels decreased to 0.08 ppm at 1 year 115 DAT (28 Aug) (Figure 4).

There was no low rate residue recovered from fruit tissue (Figure 4). High rate fruit residues were first detected at 0.01 ppm at 1 year 32 DAT (6 June) (Figure 4). Residues then

increased to a maximum of 0.03 ppm at 1 year 39 DAT (13 June) (Figure 4). There was no residue recovered the rest of the growing season (Figure 4).

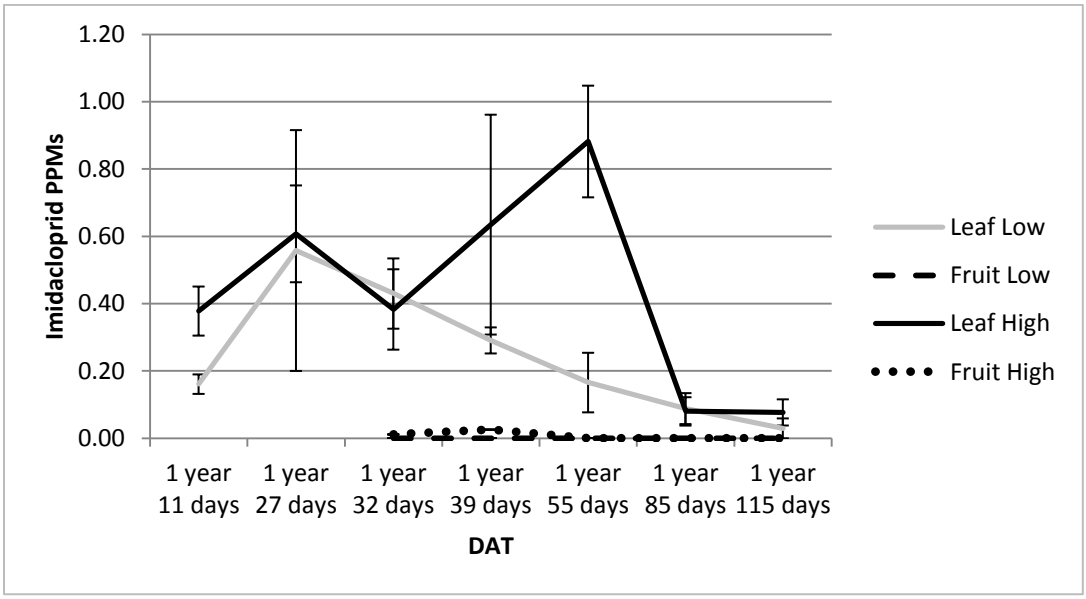

Figure 4. Mean residue recovery (ppm) from 2011 foliar and fruit residue for imidacloprid low and high rates. The injections were on May 5, 2010, 1 year 11 DAT was 16 May, 1 year 27 DAT was 1 June, 1 year 32 DAT was 6 June, 1 year 39 DAT was 13 June, 1 year 55 DAT was 29 June, 1 year 85 DAT was 29 July, and 1 year 115 DAT was 28 Aug.

There was no flower residue recovered from low or high rates the full growing season.

Imidacloprid was first detected at 16.82 ppm in low rate wood core samples recovered 6 inches below the injection site at 1 year 27 DAT (1 June), 38.33 ppm at 1 year 55 DAT (29 June), and 111.60 ppm at 1 year 115 DAT (28 Aug) (Figure 5). Residues detected 6 inches above the injection site were 15.09 ppm at 1 year 27 DAT (1 June), 48.98 ppm at 1 year 55 DAT (29 June), and 64.89 ppm at 1 year 115 DAT (28 Aug) (Figure 5). Residues detected at the base of the main scaffold branch were 3.22 ppm at 1 year 27 DAT (1 June), 0.66 ppm at 1 year 55 DAT (29 June), and 2.30 ppm at 1 year 115 DAT (28 Aug) (Figure 5). Imidacloprid was also first detected at 30.28 ppm in high rate wood core samples recovered 6 inches below the injection site at 1 year 27 DAT (1 June), 197.65 ppm at 1 year 55 DAT (29 June) and 94.83 ppm at 1 year 115

DAT (28 Aug) (Figure 6). Residue detected 6 inches above the injection site was 52.84 ppm at 1 year 27 DAT (1 June), 132.72 ppm at 1 year 55 DAT (29 June), and 97.58 ppm at 1 year 115 DAT (28 Aug) (Figure 6). Residue recovered from the base of the main scaffold branch were 6.96 ppm at 1 year 27 DAT (1 June), 3.53 ppm at 1 year 55 DAT (29 June), and 7.49 ppm at 1 year 115 DAT (28 Aug) (Figure 6).

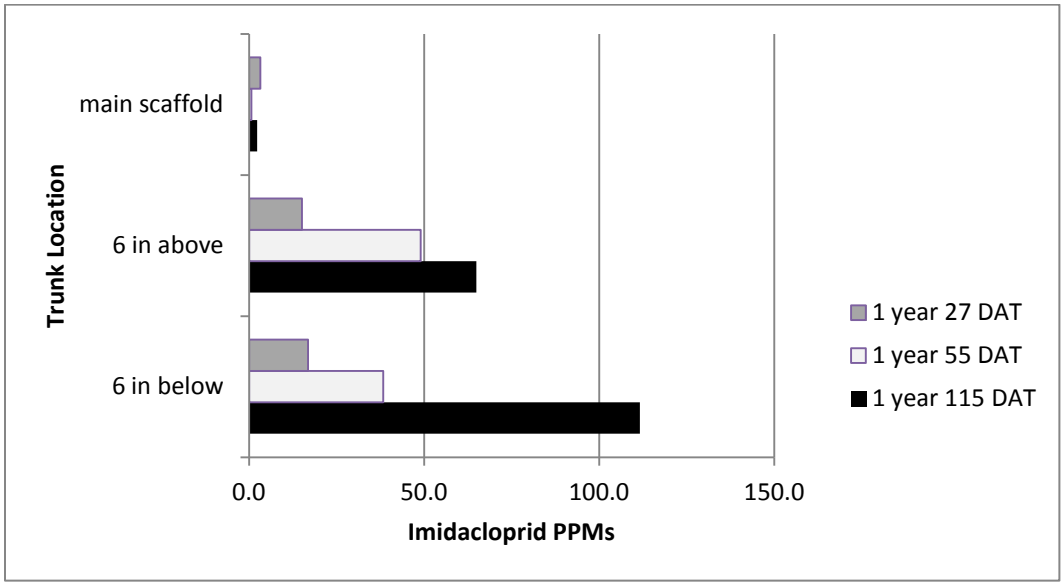

Figure 5. Mean residue recovery (ppm) from low rate imidacloprid residue samples of wood core tissue, 6 inches below injection, 6 inches above injection, and at the base of the main scaffold branch. The injections were on May 5, 2010, 1 year 27 DAT was 1 June, 1 year 55 was 29 June, and 1 year 115 DAT was 28 Aug.

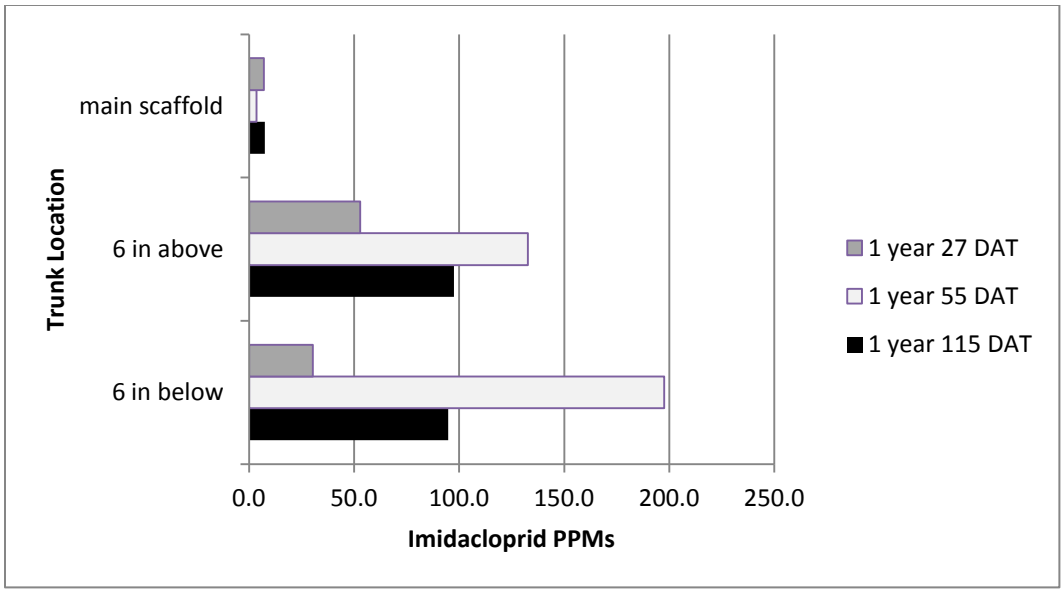

Figure 6. Mean residue recovery (ppm) from high rate imidacloprid residue samples of wood core tissue, 6 inches below injection, 6 inches above injection, and at the base of the main scaffold branch. The injections were on May 5, 2010, 1 year 27 DAT was 1 June, 1 year 55 was 29 June, and 1 year 115 DAT was 28 Aug.

Emamectin Benzoate

Emamectin benzoate was first detected at maximum of 0.0045 ppm in low rate foliar samples at 1 year 11 DAT (16 May) (Figure 7). Residues decrease to a minimum of 0.0008 ppm at 1 year 32 DAT (6 June) (Figure 7). Residue levels increased to 0.003 ppm at 1 year 115 DAT (28 Aug) (Figure 7). Emamectin benzoate was also first detected at 0.004 ppm in low rate foliar samples at 1 year 11 DAT (16 May) and decreased to a minimum level of 0.0009 ppm at 1 year 32 DAT (6 June) (Figure 7). Residues increased to 0.002 ppm at 1 year 115 DAT (28 Aug) (Figure 7).

Emamectin benzoate was first detected at 0.0001 ppm in low rate fruit samples at 1 year 32 DAT (6 June) (Figure 7). Residues decreased to a minimum of 0 ppm at 1 year 39 DAT (13 June) then increased to a maximum of 0.0023 ppm at 1 year 115 DAT (28 Aug) (Figure 7).

Emamectin benzoate was also first detected at a maximum of 0.0039 ppm in high rate fruit samples at 1 year 32 DAT (6 June), then deceased to a minimum of 0.0007 ppm at 1 year 55 DAT (29 June) (Figure 7). Residues then increased to 0.0011 ppm at 1 year 115 DAT (28 Aug) (Figure 7).

Emamectin benzoate was first detected at 0.002 ppm in low rate flower samples and high rate samples at 0.002 ppm at 1 year 11 DAT (16 May) (Table 21).

Table 21. Mean residue recovery (ppm) from flower tissue for 2011 emamectin benzoate low and high rate injected trees.

Type	Rate	DAT	Mean Residue recovered (ppm)
Flower	Low	1 year 11 days	0.002
Flower	High	1 year 11 days	0.002

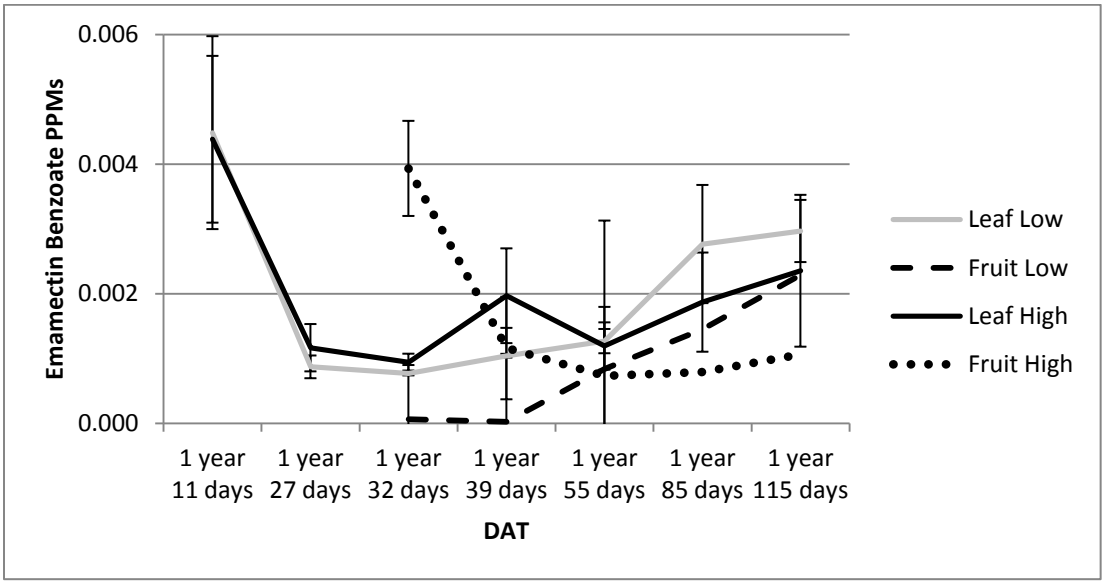

Figure 7. Mean residue recovery (ppm) from 2011 foliar and fruit residue samples of emamectin benzoate low and high rates. The injections were on May 5, 2010, 1 year 11 DAT was 16 May, 1 year 27 DAT was 1 June, 1 year 32 DAT was 6 June, 1 year 39 DAT was 13 June, 1 year 55 DAT was 29 June, 1 year 85 DAT was 29 July, and 1 year 115 DAT was 28 Aug.

Emamectin benzoate was first detected at 7.90 ppm in low rate wood core residue recovered 6 inches below the injection site at 1 year 27 DAT (1 June), 19.04 ppm at 1 year 55 DAT (29 June), and 16.33 ppm at 1 year 115 DAT (28 Aug) (Figure 8). Residues detected 6 inches above the injection site were 0.55 ppm at 1 year 27 (1 June) DAT, 9.14 ppm at 1 year 55 DAT (29 June), and 8.07 ppm at 1 year 115 DAT (28 Aug) (Figure 8). Residues detected at the base of the main scaffold branch were 0.55 ppm at 1 year 27 DAT (1 June), 0.28 ppm at 1 year 55 DAT (29 June), and 0.46 ppm at 1 year 115 DAT (28 Aug) (Figure 8). Emamectin benzoate was also first detected at 24.13 ppm high rate wood core residue recovered 6 inches below the injection site at 1 year 27 DAT (1 June), 8.91 ppm at 1 year 5 DAT (29 June), and 38.69 ppm at 1 year 115 DAT (28 Aug) (Figure 9). Residues detected 6 inches above the injection site were 5.95 ppm at 1 year 27 DAT (1 June), 26.33 ppm at 1 year 55 DAT (29 June), and 26.61 ppm at 1 year 115 DAT (28 Aug) (Figure 9). Residues detected from the base of the main scaffold branch were 0.44 ppm at 1 year 27 DAT (1 June), 2.27 ppm at 1 year 55 DAT (29 June), and 2.23 ppm at 1 year 115 DAT (28 Aug) (Figure 9).

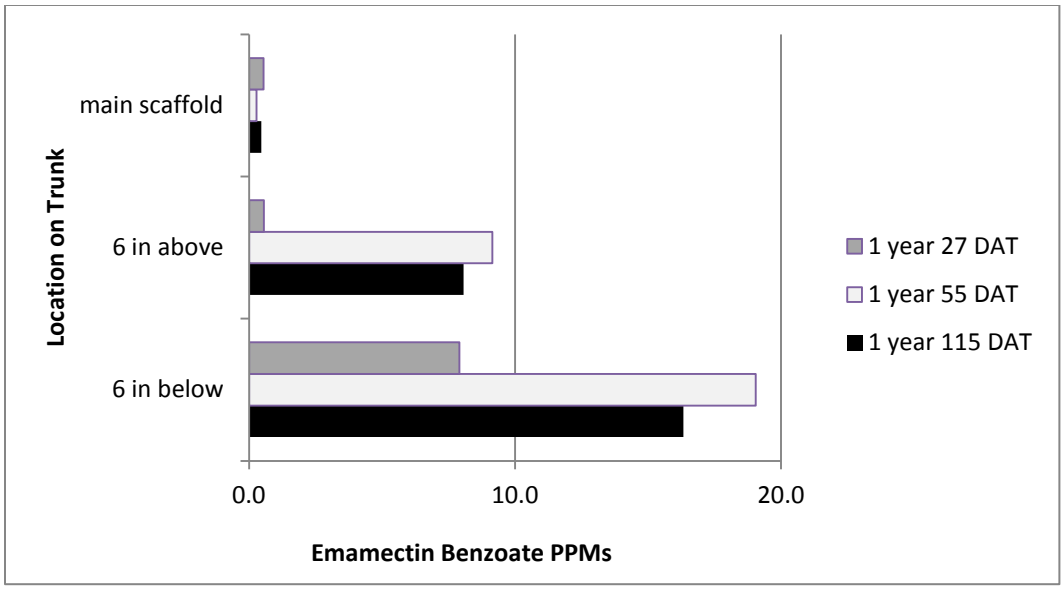

Figure 8. Mean residue recovery (ppm) from 2011 wood core tissue samples, 6 inches below injection, 6 inches above injection, and at the base of the main scaffold branch for emamectin benzoate low rate. The injections were on May 5, 2010, 1 year 27 DAT was 1 June, 1 year 55 was 29 June, and 1 year 115 DAT was 28 Aug.

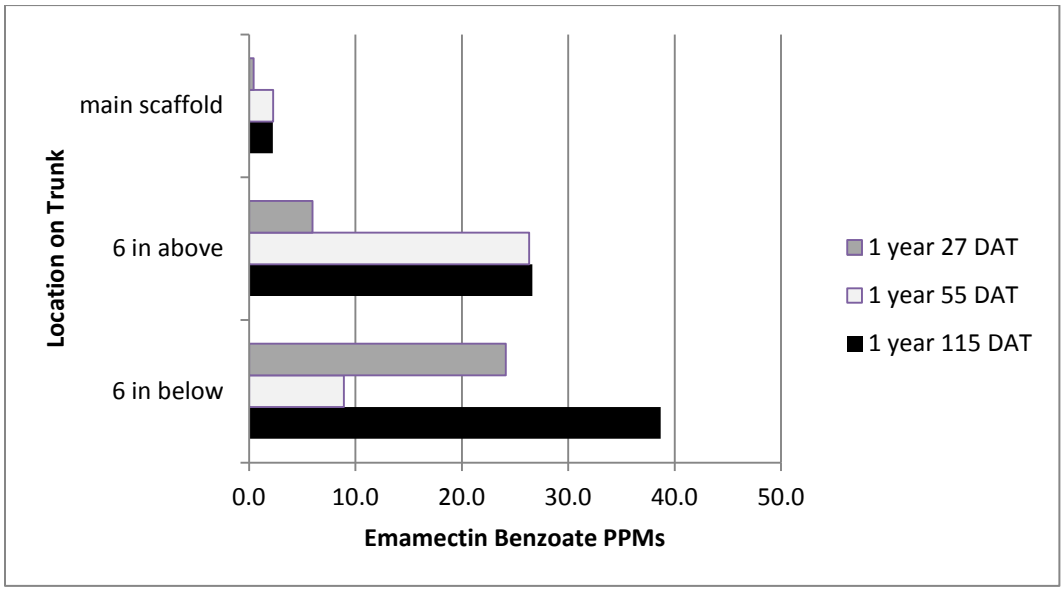

Figure 9. Mean residue recovery (ppm) from 2011 wood core tissue samples, 6 inches below injection, 6 inches above injection, and at the base of the main scaffold branch for emamectin benzoate high rate. The injections were on May 5, 2010, 1 year 27 DAT was 1 June, 1 year 55 was 29 June, and 1 year 115 DAT was 28 Aug.

Rynaxypyr

Rynaxypyr was first detected at 0.05 ppm in low rate foliar samples at1 year 11 DAT (16 May) and decreased to a minimum of 0.02 ppm at 1 year 27 DAT (2 June) (Figure 10). Residues then increased to a maximum level of 0.09 ppm at 1 year 115 DAT (28 Aug) (Figure 10). Rynaxypyr was also first detected at 0.10 ppm in high rate foliar samples at 1 year 11 DAT (16 May) and decreased to a minimum of 0.06 ppm at 1 year 27 DAT (1 June) (Figure 10). Residues then increased to 0.10 ppm at 1 year 32 DAT (6 June), decreased to 0.07 ppm at 1 year 39 DAT (13 June), increased to maximum of 0.15 ppm at 1 year 85 DAT (29 July), and decreased to 0.08 ppm at 1 year 115 DAT (28 Aug) (Figure 10).

Rynaxypyr was detected in low rate flower samples at 0.11 ppm and 0.10 ppm in high rate flower samples at 1 year 11 DAT (16 May) (Table 22).

Table 22. Mean residue recovery (ppm) from flower tissue for 2011 rynaxypyr low and high rate injected trees.

Type	Rate	DAT	Mean Residue recovered (ppm)
Flower	Low	1 year 11 days	0.11
Flower	High	1 year 11 days	0.10

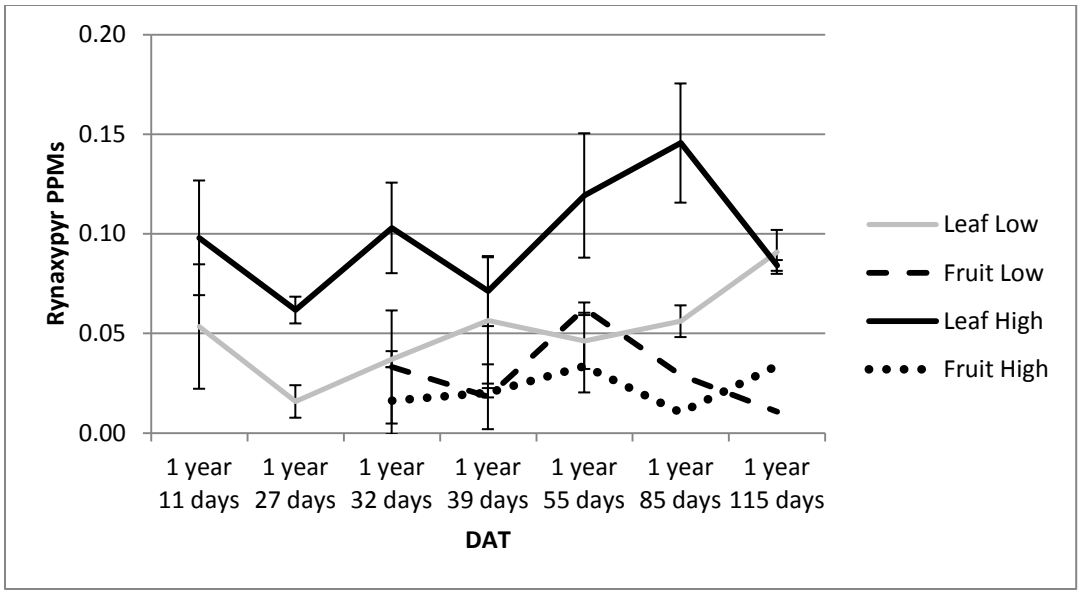

Figure 10. Mean residue recovery (ppm) from 2011 foliar and fruit residue samples of rynaxypyr low and high rates. The injections were on May 5, 2010, 1 year 11 DAT was 16 May, 1 year 27 DAT was 1 June, 1 year 32 DAT was 6 June, 1 year 39 DAT was 13 June, 1 year 55 DAT was 29 June, 1 year 85 DAT was 29 July, and 1 year 115 DAT was 28 Aug.

Rynaxypyr was first detected at 146.40 ppm in low rate wood core residue recovered 6 inches below the injection site at 1 year 27 DAT (1 June), 93.50 ppm at 1 year 55 DAT (29 June), and 237.07 ppm at 1 year 115 DAT (28 Aug) (Figure 11). Residues detected 6 inches above the injection site were 66.80 ppm at 1 year 27 DAT (1 June), 103.08 ppm at 1 year 55 DAT (29 June), and 32.57 ppm at 1 year 115 DAT (28 Aug) (Figure 11). Residues detected at the base of the main scaffold branch were 1.47 ppm at 1 year 27 DAT (1 June), 1.08 ppm at 1 year 56 DAT (29 June), and 1.27 ppm at 1 year 115 DAT (28 Aug) (Figure 11). Rynaxypyr was also first detected at 204.08 ppm in high rate wood core residue recovered 6 inches below the injection site at 1 year 27 DAT (1 June), 481.94 ppm at 1 year 55 DAT (29 June), and 579.49 ppm at 1 year 115 DAT (28 Aug) (Figure 12). Residues detected 6 inches above the injection were 172.99 ppm at 1 year 27 DAT (1 June), 69.68 ppm at 1 year 55 DAT (29 June), and 52.17 ppm at 1 year 115 DAT (28 Aug) (Figure 12). Residues detected from the base of the main

scaffold branch were 7.13 ppm at 1 year 27 DAT (1 June), 7.46 ppm at 1 year 55 DAT (29 June), and 12.78 ppm at 1 year 115 DAT (28 Aug) (Figure 12).

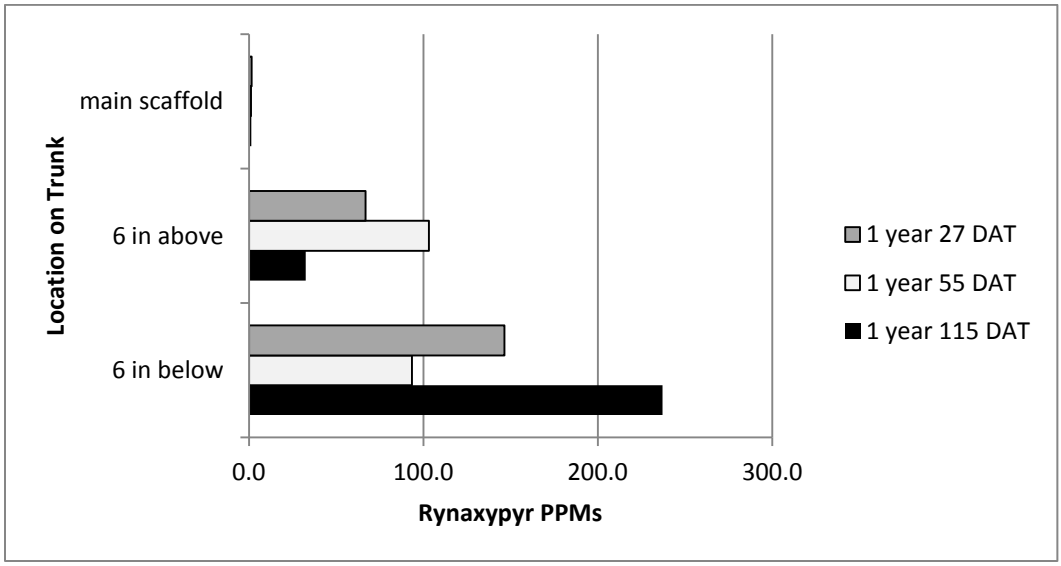

Figure 11. Mean residue recovery (ppm) from 2011 wood core tissue samples, 6 inches below injection, 6 inches above injection, and at the base of the main scaffold branch for rynaxypyr low rates. The injections were on May 5, 2010, 1 year 27 DAT was 1 June, 1 year 55 was 29 June, and 1 year 115 DAT was 28 Aug.

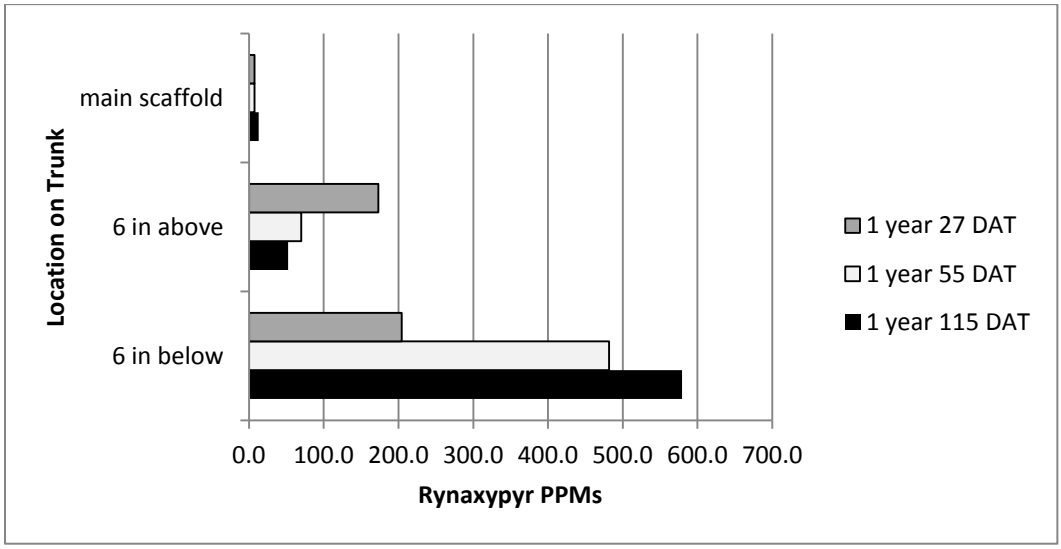

Figure 12. Mean residue recovery (ppm) from 2011 wood core tissue samples, 6 inches below injection, 6 inches above injection, and at the base of the main scaffold branch for rynaxypyr high rates. The injections were on May 5, 2010, 1 year 27 DAT was 1 June, 1 year 55 was 29 June, and 1 year 115 DAT was 28 Aug.

2011 Injection

Imidacloprid

Imidacloprid was first detected at 0.63 ppm in low rate foliar samples at 2 DAT (1 June) and increased to a maximum of 19.58 ppm at 14 DAT (13 June) (Figure 13). Residues decreased to a minimum of 0.33 ppm at 90 DAT (28 Aug) (Figure 13). Imidacloprid high rate was also first detected at 5.17 ppm 2 DAT (1 June), and increased to a maximum of 32.85 ppm at 14 DAT (13 June) (Figure 13). Residues decreased to 0.64 ppm at 90 DAT (28 Aug) (Figure 13).

Imidacloprid was first detected at 0.30 ppm in low rate fruit samples at 7 DAT (6 June), decreased to 0.12 ppm at 14 DAT (13 June), and increased to 0.28 ppm at 30 DAT (29 June) (Figure 13). There were no residues detected at 60 DAT (29 July) and 90 DAT (28 Aug) (Figure 13). Imidacloprid was also first detected at 1.37 ppm in high rate samples at 7 DAT (6 June) (Figure 13). Residues increased to a a maximum of 0.85 ppm at 14 DAT (13 June) (Figure 13). Residues decreased to a minimum of 0.02 ppm at 90 DAT (28 Aug) (Figure 13).

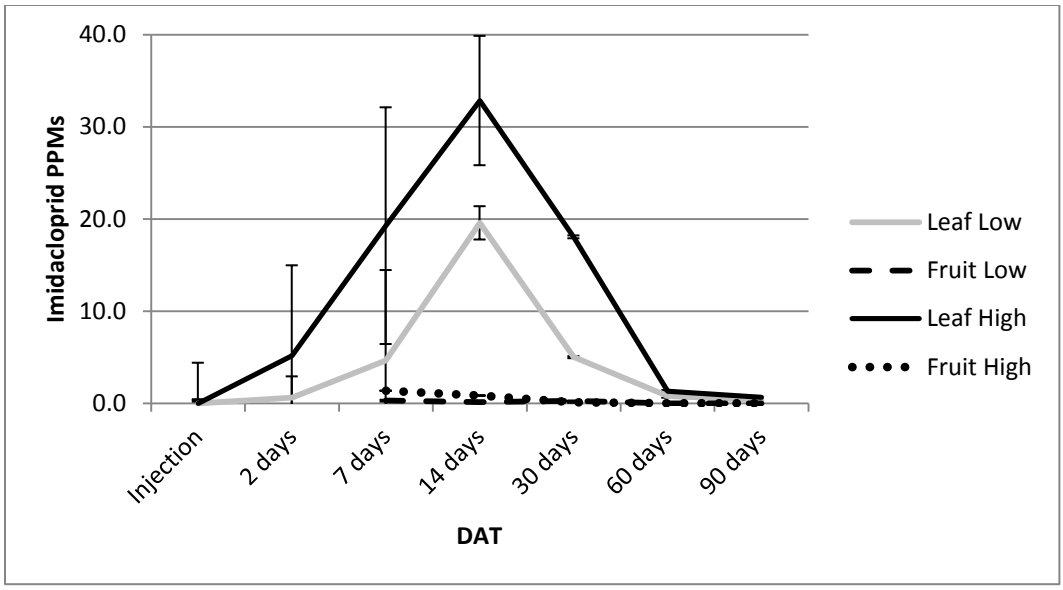

Figure 13. Mean residue recovery (ppm) from 2011 foliar and fruit residue samples of imidacloprid low and high rates. The injections were on May 30, 2011, 2 DAT was 1 June, 7 DAT was 6 June, 14 DAT was 13 June, 30 DAT was 29 June, 60 DAT was 29 July, and 90 DAT was 28 Aug.

Imidacloprid was first detected at 237.63 ppm in low rate wood core residue recovered 6 inches below the injection site at 2 DAT (1 June), 75.43 ppm at 30 DAT (29 June), and 400.61 ppm at 90 DAT (28 Aug) (Figure 14). Residues detected 6 inches above the injection site were 188.96 ppm at 2 DAT (1 June), 139.43 ppm at 30 DAT (29 June), and 265.90 ppm at 90 DAT (28 Aug) (Figure 14). Residues detected at the base of the main scaffold branch were 5.69 ppm at 2 DAT (1 June), 0.11 ppm at 30 DAT (29 June), and 0.74 ppm at 90 DAT (28 Aug) (Figure 14). Imidacloprid detected at for high rate wood core samples recovered 6 inches below the injection site were 303.22 ppm at 2 DAT (1 June), 105.80 ppm at 30 DAT (29 June), and 395.0 ppm at 90 DAT (28 June) (Figure 15). Residues detected 6 inches above the injection were 85.84 ppm at 2 DAT (1 June), 221.23 ppm at 30 DAT (29 June), and 101.80 ppm at 90 DAT (28 Aug) (Figure 15). Residues detected from the base of the main scaffold branch were 4.24 ppm at 2 DAT (1 June), 6.31 ppm at 30 DAT (29 June), and 9.56 ppm at 90 DAT (28 June) (Figure 15).

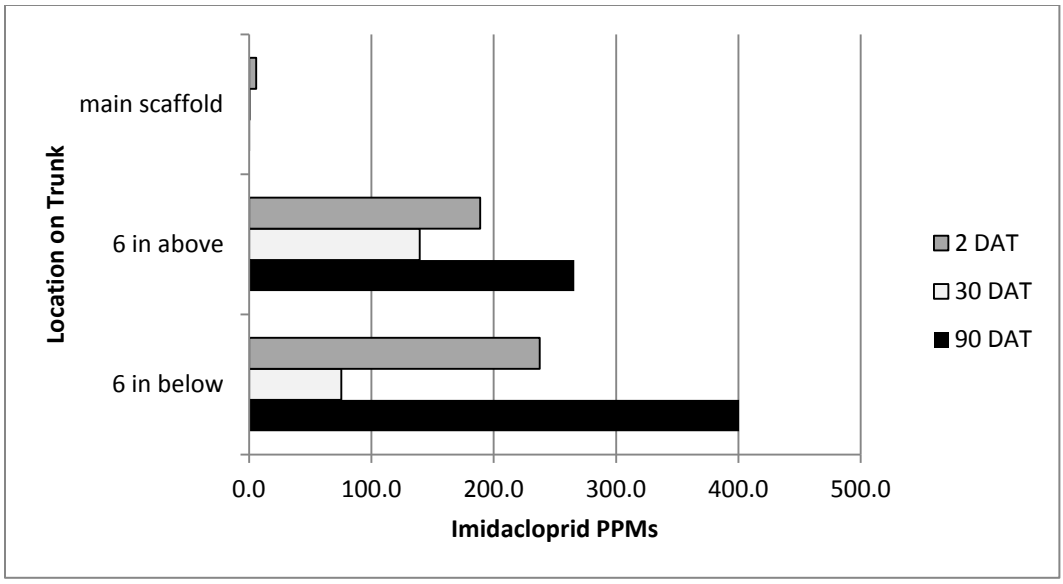

Figure 14. Mean residue recovery (ppm) from 2011 wood core tissue, 6 inches below injection, 6 inches above injection, and at the base of the main scaffold branch for imidacloprid low rate.. The injections were on May 30, 2011, 2 DAT was 1 June, 30 DAT was 29 June, and 90 DAT was 28 Aug.

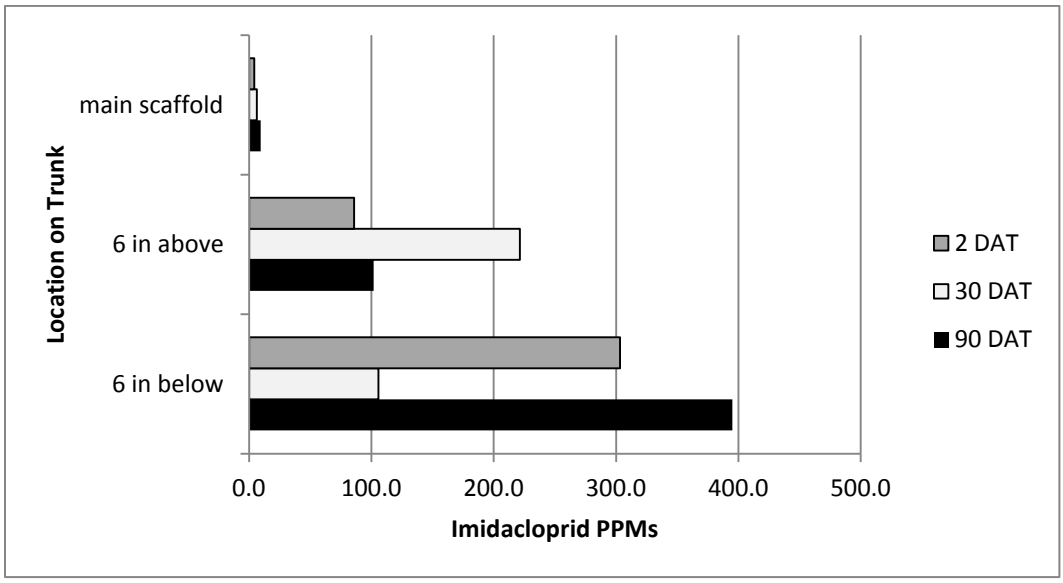

Figure 15. Mean residue recovery (ppm) from 2011 wood core tissue, 6 inches below injection, 6 inches above injection, and at the base of the main scaffold branch for imidacloprid high rate. The injections were on May 30, 2011, 2 DAT was 1 June, 30 DAT was 29 June, and 90 DAT was 28 Aug.

Emamectin benzoate

Emamectin benzoate was first detected at 0.01 ppm in low rate foliar samples at 2 DAT (1 June) and increased to 0.02 ppm at 7 DAT (6 June) (Figure 16). Residues decreased to 0.01 ppm at 14 DAT (13 June), increased to a maximum of 0.03 ppm at 30 DAT (29 June), and decreased to 0.02 ppm at 90 DAT (28 Aug) (Figure 16). Emamectin benzoate was also first detected at a maximum of 0.06 ppm in high rate foliar samples at 2 DAT (1 June), decreased to 0.004 ppm 30 DAT (29 June), and increased to 0.04 ppm at 90 DAT (28 Aug) (Figure 16).

Emamectin benzoate was first detected at 0.002 ppm in low rate fruit samples at 7 DAT (6 June), increased to a maximum 0.003 ppm at 30 DAT (29 June), then decreased to minimum 0.0005 ppm at 60 DAT (29 July) (Figure 16). Residues dencreased to a minimum to 0.0009 ppm at 90 DAT (28 Aug) (Figure 16). Emamectin benzoate was also first detected at 0.0009 ppm in high rate fruit samples at 7 DAT (6 June) (Figure 16). Residues increased to a maximum of 0.001 ppm at 30 DAT (29 June), decreased to a minimum of 0.0004 ppm at 60 DAT (29 July), then decreased to 0.0006 ppm at 90 DAT (28 Aug) (Figure 16).

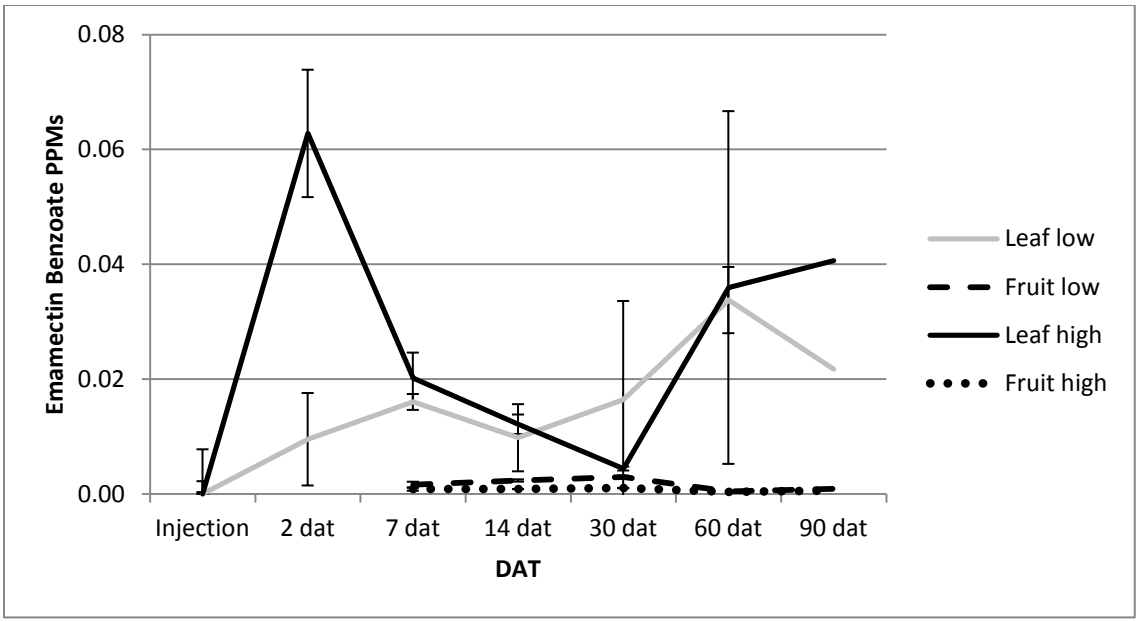

Figure 16. Mean residue recovery (ppm) from 2011 foliar and fruit residue of emamectin benzoate low and high rates. The injections were on May 30, 2011, 2 DAT was 1 June, 7 DAT was 6 June, 14 DAT was 13 June, 30 DAT was 29 June, 60 DAT was 29 July, and 90 DAT was 28 Aug.

Emamectin benzoate was first detected at 40.42 ppm in low rate wood core residue recovered 6 inches below the injection site at 2 DAT (1 June), 158.64 ppm at 30 DAT (29 June), and 93.13 ppm at 90 DAT (28 Aug) (Figure 17). Residues detected 6 inches above the injection site were 25.18 ppm at 2 DAT (1 June), 79.52 ppm at 30 DAT (29 June), and 28.32 ppm at 90 DAT (28 Aug) (Figure 17). Residues detected at the base of the main scaffold branch were 0.96 ppm at 2 DAT (1 June), 2.79 ppm at 30 DAT (29 June), and 2.26 ppm at 90 DAT (28 Aug) (Figure 17). Emamectin benzoate was also first detected at 159.51 ppm in high rate wood core residue recovered 6 inches below the injection site at 2 DAT (1 June), 28.84 ppm at 30 DAT (29 June), and 113.93 ppm at 90 DAT (28 Aug) (Figure 18). Residues detected 6 inches above the injection site were 60.06 ppm at 2 DAT (1 June), 29.04 ppm at 30 DAT (29 June), and 38.80 ppm at 90 DAT (28 Aug) (Figure 18). Residues detected from the base of the main scaffold

branch were 9.92 ppm at 2 DAT (1 June), 4.39 ppm at 30 DAT (29 June), and 4.22 ppm at 90 DAT (28 Aug) (Figure 18).

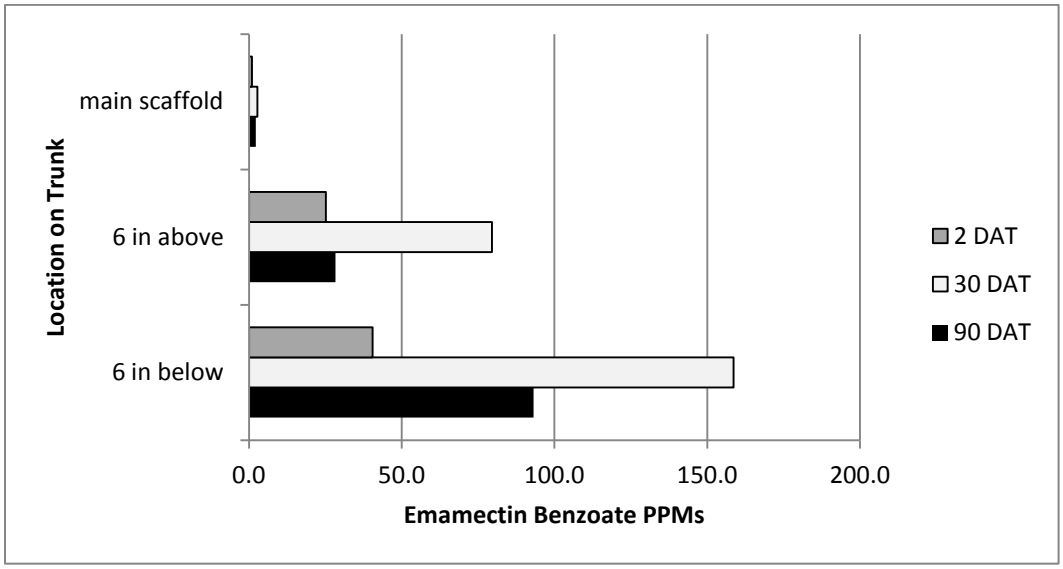

Figure 17. Mean residue recovery (ppm) from 2011 wood core tissue samples, 6 inches below injection, 6 inches above injection, and at the base of the main scaffold branch for emamectin benzoate low rate. The injections were on May 30, 2011, 2 DAT was 1 June, 30 DAT was 29 June, and 90 DAT was 28 Aug.

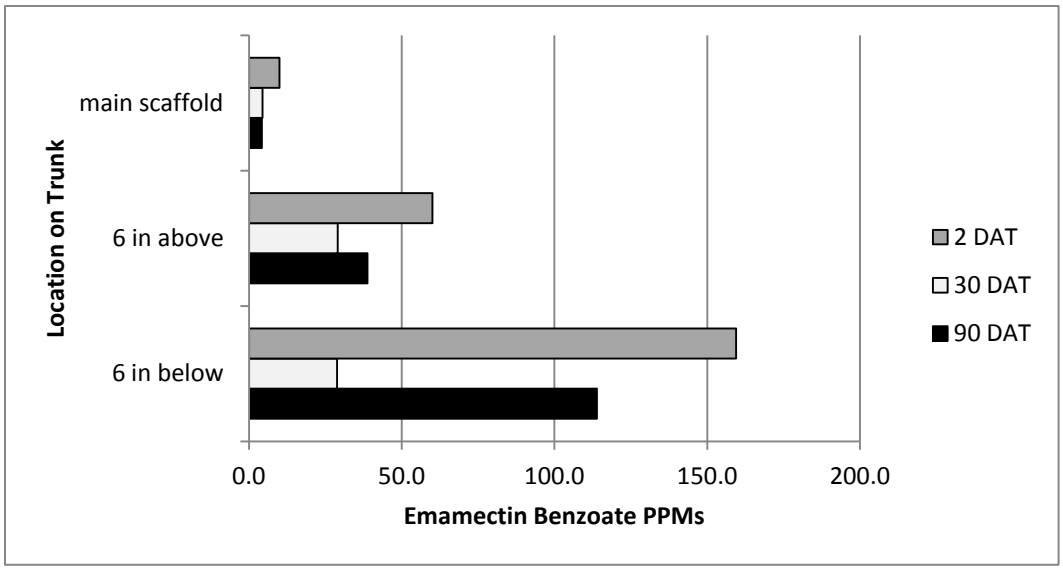

Figure 18. Mean residue recovery (ppm) from 2011 wood core tissue samples, 6 inches below injection, 6 inches above injection, and at the base of the main scaffold branch for emamectin benzoate high rate. The injections were on May 30, 2011, 2 DAT was 1 June, 30 DAT was 29 June, and 90 DAT was 28 Aug.

Rynaxypyr

Rynaxypyr was first detected at 0.35 ppm in low rate foliar samples at 2 DAT (1 June), at and increased to maximum of 0.89 ppm at 14 DAT (13 June) (Figure 19). Residues then decreased to a minimum of 0.07 ppm at 90 DAT (28 Aug) (Figure 19). Rynaxypyr was also first detected at 0.16 ppm in high rate foliar samples at 2 DAT (1 June) and increased to a maximum of 2.15 ppm at 14 DAT (13 June) (Figure 19). Residues then decreased to a minimum of 0.41 ppm at 90 DAT (28 Aug) (Figure 19).

Rynaxypyr was first detected at a maximum of 0.08 ppm in low rate fruit samples 7 DAT (6 June), decreased to minimum of 0.015 ppm at 60 DAT (29 July), then increased to 0.02 ppm at 90 DAT (28 Aug) (Figure 19). Rynaxypyr was also first detected at 0.05 ppm in high rate foliar samples at 7 DAT (6 June) at decreased to 0.04 ppm at 14 DAT (13 June), increased to a maximum of 0.08 ppm at 30 DAT (29 June), decreased to minimum of 0.031 ppm at 60 DAT (29 July), then increased to 0.033 ppm at 90 DAT (28 Aug) (Figure 19).

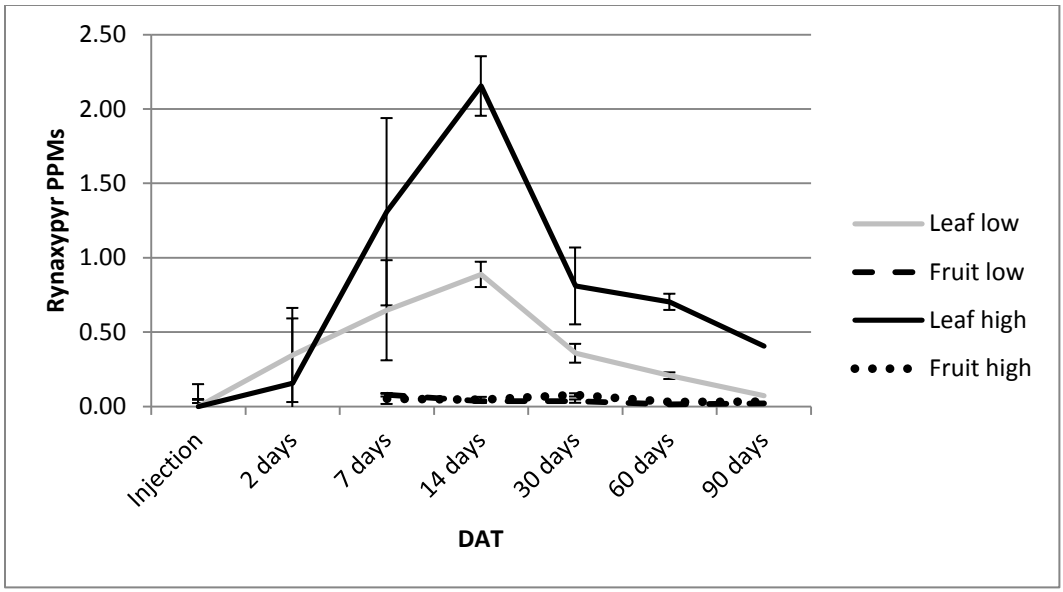

Figure 19. Mean residue recovery (ppm) from 2011 foliar and fruit residue samples of rynaxypyr low and high rates. The injections were on May 30, 2011, 2 DAT was 1 June, 7 DAT was 6 June, 14 DAT was 13 June, 30 DAT was 29 June, 60 DAT was 29 July, and 90 DAT was 28 Aug.

Rynaxypyr was first detected at 46.92 ppm in low rate wood core samples 6 inches below the injection site at 2 DAT (1 June), 268.30 ppm at 30 DAT (29 June), and 202.0 ppm at, 90 DAT (28 Aug) (Figure 20). Residues detected 6 inches above the injection site were 111.39 ppm at 2 DAT (1 June), 168.51 ppm at 30 DAT (29 June), and 243.27 ppm at 90 DAT (28 Aug) (Figure 20). Residues detected at the base of the main scaffold branch were 1.62 ppm at 2 DAT (1 June), 1.40 ppm at 30 DAT (29 June), and 6.44 ppm at 90 DAT (28 Aug) (Figure 20). Rynaxypyr was also first detected at 322.71 ppm in high rate wood core samples 6 inches below the injection site at 2 DAT (1 June), 263.46 ppm at 30 DAT (29 June), and 546.08 ppm at 90 DAT (28 Aug) (Figure 21). Residues detected 6 inches above the injection site were 197.29 ppm at 2 DAT (1 June), 254.79 ppm at 30 DAT (29 June), and 317.18 ppm at 90 DAT (28 Aug) (Figure 21). Residues detected from the base of the main scaffold branch were 2.35 ppm at 2 DAT (1 June), 10.67 ppm at 30 DAT (29 June), and 24.74 ppm at 90 DAT (28 Aug) (Figure 21).

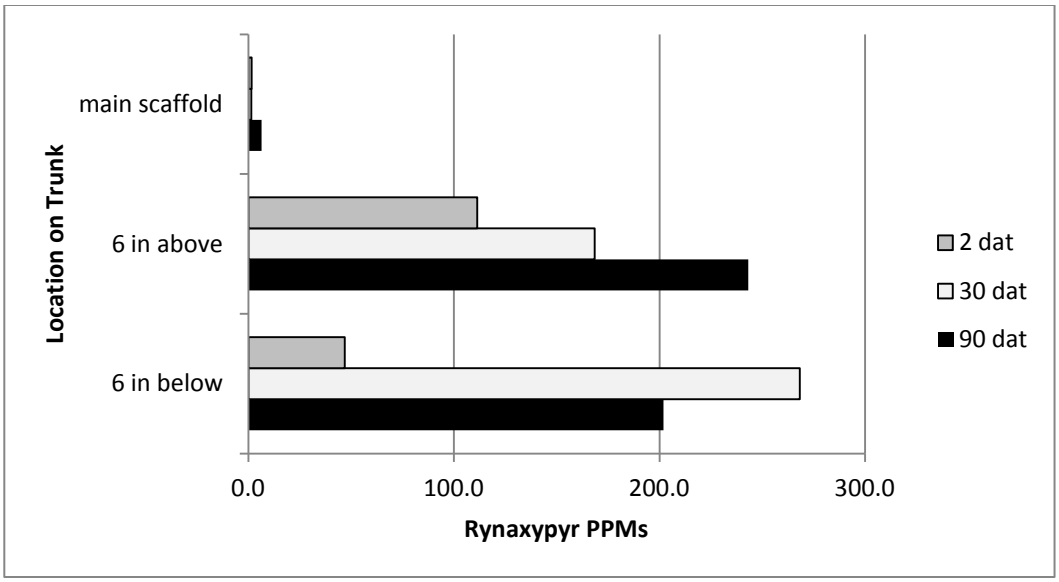

Figure 20. Mean residue recovery (ppm) from 2011 wood core tissue samples, 6 inches below injection, 6 inches above injection, and at the base of the main scaffold branch for rynaxypyr low rate. The injections were on May 30, 2011, 2 DAT was 1 June, 30 DAT was 29 June, and 90 DAT was 28 Aug.

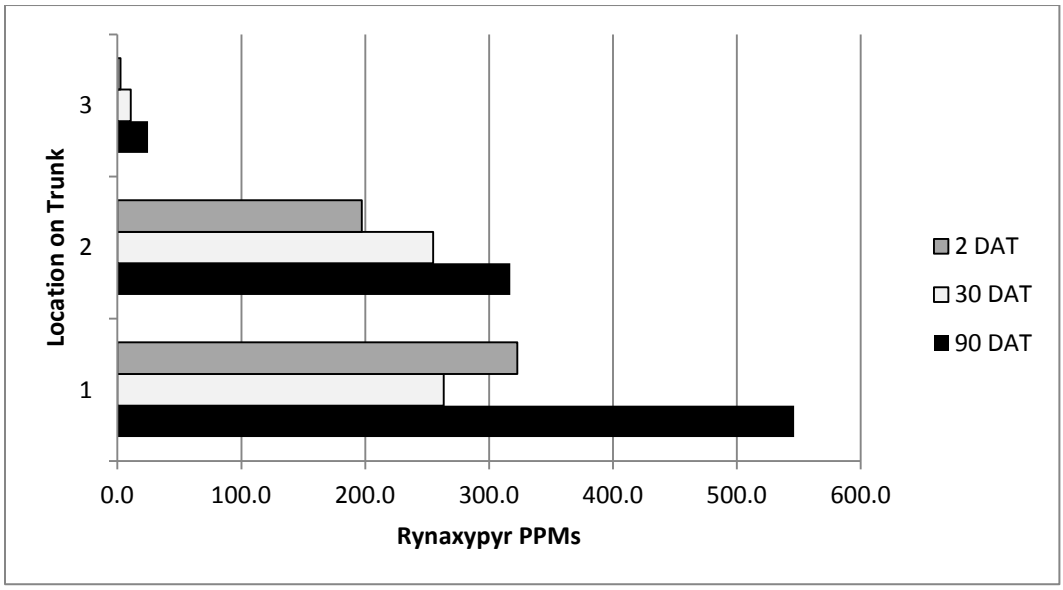

Figure 21. Mean residue recovery (ppm) from 2011 wood core tissue samples, 6 inches below injection, 6 inches above injection, and at the base of the main scaffold branch for rynaxypyr high rate. The injections were on May 30, 2011, 2 DAT was 1 June, 30 DAT was 29 June, and 90 DAT was 28 Aug.

Imidacloprid, Emamectin benzoate, and Rynaxypyr

Imidacloprid was detected at different levels throughout the fruit tissue in fruit core samples in the high rate treatment at 60 DAT (29 July) (Figure 22). The proportion of residues throughout the fruit were calculated at 14% in the skin, 9% in the outer 2 mm flesh, 21% in the inner 10 mm flesh, 15% in the inside 5 mm to the core, and 41% in the seed tissue (Figure 22).

Emamectin benzoate was detected at different levels throughout the fruit tissue in fruit core samples in the high rate treatment 60 DAT (29 July) (Figure 22). The proportion of residues throughout the fruit were calculated at 16% in the skin, 56% in the outer 2 mm flesh, 11% in the inner 10 mm flesh, 8% in the inside 5 mm to the core, and 9% in the seed tissue (Figure 22).

Rynaxypyr was detected at different levels throughout the fruit tissue in fruit core samples in the high rate treatment 60 DAT (29 July) (Figure 22). The proportion of residues throughout the fruit were calculated at 20% in the skin, 24% in the outer 2 mm flesh, 34% in the inner 10 mm flesh, 15% in the inside 5 mm to the core, and 7% in the seed tissue (Figure 22).

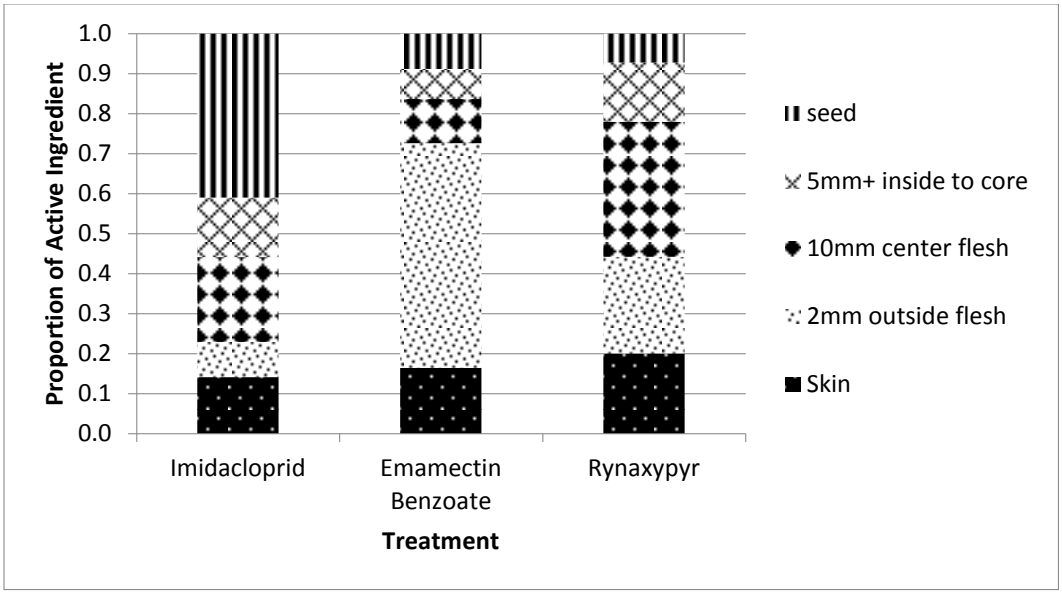

Figure 22. Mean residue recovery (ppm) from 60 DAT 2011 fruit core tissue samples consisting of skin, 2mm outer flash, 10mm center flesh, 5mm inside to the core, and seeds. for imidacloprid, emamectin benzoate, and rynaxypyr high rate. The injections were on May 30, 2011, 60 DAT was 29 July.

Foliar Phytotoxicity

Visual qualitative photographical data estimations were recorded for the extent of foliar phytotoxicity from the imidacloprid, emamectin benzoate, and rynaxypyr. We were able to conclude that weather conditions played a role with translocation and phytotoxicity, but differences between seasons were minimal since weather was very similar with cold overcast conditions in April. All photographs were taken of maximum phytotoxic effects during the growing season. Phytotoxic effects were dependent on weather conditions and rates. Insecticides injected on May 5, 2010 under overcast conditions resulted in little to no phytotoxicity. With limited phytotoxic effects, the difference between low and high rates were minimal. Insecticide injections made on May 30, 2011 under sunny conditions resulted in increased phytotoxicity for imidacloprid high rate, emamectin benzoate high rate, and rynaxypyr high rate (Figure 23-25).

Figure 23. Extent of phytotoxicity of rynaxypyr high rate from the 2010 injection 15 DAT (20 May). For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

Figure 24. Extent of phytotoxicity of imidacloprid high rate from the 2011 injection 3 DAT (3 June) (left) and extent of phytotoxicity of emamectin benzoate high rate from the 2011 injection 3 DAT (3 June) (right) (**Figure 25**).

Discussion

This research contributes important new information about the potential of trunk injection for delivering insecticides to control apple insect pests. The results show up to two seasons of control of indirect pests with a single injection application, and selective activity on direct pests, depending on the biology and feeding behavior of each. Imidacloprid, emamectin benzoate, and rynaxypyr showed positive results in insecticidal activity on many insect pests and sufficient residue levels on several tree tissue types.

Imidacloprid provided two seasons of control of indirect pests, like PLH, RAA and STLM, that are currently listed on the US Federal label for apple. Imidacloprid provided seasonal control of several direct pests, including OFM, which is not currently listed on the apple label. This suggests that trunk injection may be a superior delivery system, compared to the conventional for imidacloprid, and provides enhanced exposure to the pest, resulting in more effective pest control. Imidacloprid did not, however, control the key direct insect pests OBLR, CM and PC. The foliar imidacloprid residues from the 2010 injections were sufficient for control of foliar pests in the 2010 and 2011 seasons with residue levels below 1 ppm throughout the second growing season. This suggests that the high and low rates tested in this study may be reduced substantially if the objective is to achieve a single season of control of indirect pests. The residue profiles showed that imidacloprid generally had fast translocative abilities to the foliage with detectable residue levels one to seven days after application, reaching peak levels within 14 to 60 DAT, then dropping to a minimum level 90 DAT. The leaf residue profiles in the following season follow the same general trend, but at much lower levels. The trunk core residue samples provide evidence for a reservoir effect, with woody tissue being the source of

imidacloprid for translocation to the canopy in the second season. The residue recovered in apple fruit were much lower than those in leaves, which likely explains the lack of control of direct fruit pests like CM and PC. One surprise, however, were the lower infestations apple maggot in fruit. Since AM infests fruit only, the explanation for control is that either there was sufficient residues in fruit to kill larvae, or there is sufficient partitioning of residues to leaf surfaces to be ingested by AM adults that feed on bird droppings and other ammonia sources in the canopy before laying eggs in fruit. The fruit residue levels, although detectable, persisted at levels far below the 5 ppm maximum levels (MRLs) set by the US EPA for apples.

Emamectin benzoate provided two seasons of control of direct and indirect pests, like OBLR, STLM, CM, and OFM that are currently listed on the US federal label for apple. Emamectin benzoate also provided control of insect pests, including AM and PLH in some instances, which are not currently listed on the apple label. This suggests that trunk injection may be a superior delivery system for emamectin benzoate, which provides enhanced exposure to the pest, resulting in more effective pest control. Emamectin benzoate provided seasonal control of certain direct and indirect pests. The foliar residues from the 2010 injections were sufficient for the control of indirect pests over the 2010 and 2011 seasons with residues below 0.01 ppm throughout the 2011 growing season. This suggests that the high and low rates tested in this study may be reduced substantially if the objective is to achieve a single season of control of indirect pests. In the 2011 injection the residue profiles showed that emamectin benzoate had fast translocative abilities to the foliage with maximum levels being reached 0 to 2 days after application. Residues begin to drop dramatically 2 to 30 days after application reaching the minimum level at 30 days. Leaf residue profiles in the following season showed a similar trend with residues maximizing early in the season, but at much lower levels. The trunk core residue

samples provide evidence for a reservoir effect, with woody tissue being the source of emamectin benzoate for translocation to the canopy in the second season. The residue recovered in apple fruit were much lower than those in leaves, which likely explains the lack of control of direct fruit pests like CM. One surprise, however, was significantly lower infestations apple maggot in fruit. Since AM infests fruit only, explanation for control is that either there was sufficient residues in fruit to kill larvae, or there is sufficient partitioning of residues to leaf surfaces to be ingested by AM adults that feed on bird droppings and other ammonia sources in the canopy before laying eggs in fruit. The fruit residue levels, although detectable, persisted at levels far below the 0.02 ppm maximum levels (MRLs) set by the US EPA for apples.

Rynaxypyr provided two seasons of control of direct and indirect pests like OFM, OBLR, and CM that are currently listed on the US federal label for apple. Rynaxypyr also provided control of insect pests, including STLM and PLH in some instances, which are not listed on the apple label. This suggests that trunk injection may be a superior delivery system for rynaxypyr, which provides enhanced exposure to the pest, resulting in more effective pest control. Rynaxypyr provided seasonal control of certain direct and indirect pests. The foliar residue profiles from the 2010 injections were sufficient for the control of indirect pests over the 2010 and 2011 seasons with residues below 0.2 ppm throughout the 2011 growing season. This suggests that the high and low rates tested in this study may be reduced substantially if the objective is to achieve a single season of control of indirect pests. In the 2011 injection the residue profiles showed that rynaxypyr had good translocative abilities to the foliage with detectable levels being reached same day to 2 days after application. Maximum levels are reached 14 DAT and begin to drop dramatically until 30 days after application reaching the minimum level at 60-90 DAT. Leaf residue profiles in the following season showed irregular

residue patterns with increases and decreases over the growing season at low levels. The trunk core residue samples provide evidence for a reservoir effect, with woody tissue being the source of rynaxypyr for translocation to the canopy in the second season. The inconsistency of residue patterns for the 2010 continuation season shows evidence of the possibility of rynaxypyr binding to wood tissue not releasing consistent material for translocation to the canopy. The residue recovered in apple fruit were much lower than those in leaves, which likely explains the lack of control of direct fruit pests like PC and AM. The fruit residue levels, though detectable, persisted at levels far below the 0.3 ppm maximum levels (MRLs) set by the US EPA for apples.

These data collected in the 2010 and 2011 seasons give information on each of their translocative abilities, pest control, and persistence. Each compound gave the best control for what they are labeled for, but also was effective on other pests suggesting that trunk injection may broaden the use of specific insecticides.

Foliar residue levels of all three compounds exceeded the volume necessary for pest control. Imidacloprid maximum low dose residue levels in the 2010 season are approximately 16.2 ppm with significant control of specific insect pests in the field and laboratory. The following 2010 continuation from the 2011 season, foliar residue levels were lower from the same low rate with a maximum of 0.6 ppm, but still had significant control of specific insect pests. This wide range of residue levels with consistent pest control indicates that pest control is achievable over 2 seasons with a lower dose and rate of imidacloprid. It also indicates that there was a waste of active ingredient for both low and high doses/ rates. The 4 ml/ DFH inch low dose and 0.2 gai rate exceeds the amount necessary for pest control of specific insect pests. Emamectin benzoate maximum low dose residue level from the 2011 season was 0.03 ppm and

2010 continuation low dose and rate maximum foliar residue level was lower at 0.005 ppm, but still had significant control of specific insect pests in the field and laboratory. This indicates that pest control is achievable over 2 seasons with a lower dose and rate of Emamectin benzoate. It also indicates there was a waste of active ingredient for low and high doses/ rates. Rynaxypyr maximum foliar low dose residue level from the 2011 season was 0.9 ppm. The rynaxypyr low dose and rate 2010 continuation maximum foliar residue level was 0.09 ppm. Both the low/ high dose and rate achieved some insect pest control in the field of laboratory in 2010 continuation and 2011 seasons indicating insufficient dose and rate, poor translocation, or compound formulation drawbacks.

Flower residue levels for the 2010 continuation have the potential to negatively affect beneficial pollinators. There was no residue recovered from imidacloprid, a maximum of 0.0015 µg recovered from emamectin benzoate, and a maximum of 0.1117 µg recovered from rynaxypyr. Imidaclprid has an oral LD 50 of about .041 µg per bee (Nauen, Ebbinghaus-Kintscher, Schmuck 2001). Emamectin benzoate acute toxicity of 0.0035 µg per bee, and rynaxypyr has an oral LD 50 of about 114 µg (Bassi, Rison, and Wiles 2009). This provides evidense that the injected rates are not a hazard to pollinators such as the honeybee. The total residues detected in the composite flower samples are likely higher than what would be found in pollen or nectar alone.There are economic and non-economic negative impacts of trunk injection and the conventional application methods, of which trunk injection may have the least impact. Non-economically, the negative effects on worker exposure and on natural enemies from trunk injection are expected to be lower than that of the conventional application methods. The negative off-target effects of conventional application methods ,such as drift, runoff, and leaching, increase the worker exposure to pesticides and negative direct effects on natural

enemies. With the direct source application method of trunk injection there is no off-target drift, runoff, or leaching, as the convention methods will have.

Economically, trunk injection compared to conventional methods may be the most cost effective in the long run. At this point the initial cost of the injection equipment, extra labor, and price of concentrated formulated compounds, injection may cause loss of interest. The end of the field season may reveal that trunk injection is the most cost effective. With only a single injection application trunk injection may provide up to 2 seasons of persistent pest protection. The concentrated compounds remain in the internal plant tissues for up to 2 seasons withstanding weather conditions with minimal fruit residues.

This research provides an important proof of concept for trunk injection in apples, but there are more questions to be answered. Future research is necessary for additional information on compound persistence, volumes and rates, compound mode of actions, compound transport to fruit and flower tissue, and injection timings. Information is needed for new compound formulations and how they translocate, persist, and act on the insect pest. Specific injection compound formulations need to be continually modified for improved for translocative abilities so they can act efficiently on the pest at the correct doses persistently. With an efficient formulation, accurate application volumes and rates can be calculated along with overall cost of application. Injection timings need to be modified for efficient pest control. The compound needs to be in the target plant tissue when the pests arrive. Additional information on the translocation to the non-target tissue such as the fruit and flower are also needed. It is known that after 2 seasons there are minimal residues in fruit and flower tissue, but future seasons and new formulations may yield different results.

With continued research there is great potential adopting this new application technology to the agricultural system with much success. It may lead to improved tools, compound formulations, and injection techniques. Injection technology may be a cost, time, and labor efficient pest control method in agriculture, safe for the applicator and the environment.

CHAPTER 3: TRUNK INJECTION-FUNGICIDES IN APPLES

Abstract

Field studies and residue profiling were used to determine the seasonal effectiveness of trunk injection against apple scab (*Venturia inaequalis),* using two fungicides that are currently formulated for trunk injection: propiconazole (Alamo®), phosphorous acid (Phospho-jet™), and a diluted solution, penthiopyrad (Fontelis™). They were injected into semi dwarf MacIntosh apple trees (RedMax) at the Michigan State University (MSU) Trevor Nichols Research Center (TNRC). Through one growing season, propiconazole and penthiopyrad showed limited control of apple scab, while phosphojet showed scab control once the phytotoxic effects had subsided. In the 2011 season there were new unique injection regimes tested to overcome the phytoxicity risks of phosphorous acid and poor translocative abilities of propiconazole and penthiopyrad. With these new regimes, propiconazole and penthiopyrad showed little difference in the control of apple scab. Phosphorous acid showed significant scab control compared to the untreated control. There was also reduced phytotoxicity compared to the 2010 season as a result of reduced rates. Propiconazole and penthiopyrad residue profiles showed limited foliar residue levels and persistence for two seasons with low fruit residues below the EPA MRLs. There were high residue levels in wood cores near the injection point, indicating persistence and poor translocative abilities of propiconazole and penthiopyrad in vascular wood tissue. Phosphorous acid residue profiles showed good translocative abilities, scab control, and high risk of phytotoxicity. This study will help gain further knowledge in the science of trunk injection and bring us closer to an improved method of fungicide application in tree fruits.

Introduction

Protecting apples trees (*Malus domestica*) (Borkhausen) from diseases in the USA takes a wide array of fungicides, ranging from triazoles to carboxamides, and difenoconazoles. These fungicides have been effective in controlling key diseases, but require precise application timing according to infection time to maximize control.

As tree fruit producers enter the Twenty First century it is important to note that even though there has been significant evolution of the tools (ie; reduced-risk pesticide chemistries) for pest management (USEPA 1997), spray equipment has remained relatively unchanged. Studies show that airblast sprayers are a relatively inefficient means of delivering pesticides to their target, with only 29 to 56% of the applied spray solution being deposited on the tree canopy, and the remaining product drifting to ground or other off-target end points (Reichard et al. 1979, Zhu et al. 2006, Perry et al. 1998). Some technical advancements have made additions to the conventional ground sprayer, such as towers or nozzle sensors (Landers and Farooq 2005, Landers 2002, 2004), but the fundamental elements for delivering materials to the tree canopy have remained the same.

In the Unites States Propiconazole, phosphorous acid, and penthiopyram are all registered for use as foliar sprays in apples, but do not have registered uses for trunk injection in apples. Propiconazole (Alamo®) and phosphorous acid (Phospho-jet™) however have registered uses for trunk injection in ornamentals on oak wilt (*Ceratocystis fagacearum*) (Bretz), dutch elm disease (*Ophiostoma ulmi*) (Buism.), blights, and root rots. The three compounds have various degrees of effectiveness as foliar sprays in apples and injections for multiple diseases in ornamentals.

Propiconazole is triazole fungicide effective on Ascomycetes and Basidiomycetes, with preventative and curative properties, which is effective on apple scab, rusts, and powdery mildew (*Podosphaera leucotricha*) (Ell. & Evherh.). Propiconazole slows or stops the growth of the fungus by slowing the ergosterol production, which is essential for the production of the cell wall.

Penthiopyrad is a broad spectrum carboxamide fungicide, preventative and curative properties, which is effective on powdery mildew (*Podosphaera leucotricha*) (Ell. & Evherh.), grey mold (*Botrytis spp*), and apple scab. Penthiopyrad acts on the mitochondrial respiratory chain and interrupts the electron transport so the fungus cannot produce vital energy in the form of ATP.

Phosphorous acid has direct and indirect effects on oomycete pathogens, apple scab, fire blight (*Erwinia amylovora*) (Burrill), and downy mildews. The indirect effect is identified as systemic acquired resistance (SAR). Phosphorous acid causes the plant to use its own natural protectants by initiating pathogenesis-related (PR) proteins, and salicylic acid (Schilder 2005).

The test subject of this study was apple scab, caused by the fungus Venturia inaequalis, which affects the leaves, petioles, blossoms, and fruit (Jones 1996). The symptoms are most noticeable on the fruit and leaves of apple. The infection has a velvety-brown blotchy appearance to begin, then forms a harder darker brown blotch. The symptoms appear very similar on both leaves and fruit. Apple scab may result in leaf defoliation, fruit deformation, cracking, and uneven growth. Severe infection can cause permanent damage to the entire tree. This results in reduced bloom and poor fruit yield, which continues into the following year. The purpose of this

study was to determine the effectiveness of these three fungicides to control apple scan via trunk injection in apples.

Methods and Materials

The Fungal Disease

Venturia inaequalis

Field Plots and Applications

2010 Season

2010 Injection

At MSU TNRC, treatment applications were made at green tip/ silver tip stage of apple, on April 2, 2010 before the first apple scab infection. Each of two fungicides tested were formulated for trunk injection: Phosphorous acid (Phospho-jet™, Arborjet Inc. Woburn, MA), and low and high rate of propiconazole (Alamo®, Arborjet Inc. Woburn, MA). Injections were made on 5-7 inch DFH (diameter of the trunk 1 foot above the ground) semi dwarf MacIntosh (RedMax) apple trees (*Malus domestica* Borkhausen) with a five replicate trees per treatment (15 trees, 1 control) in a randomized block design. Low and high rates of Alamo® and an estimated rate of phosphorous acid were injected at volumes depending on DFH. A high rate of phosphorous acid was injected at 9.6 g AI, and low rate of propiconazole at 7.1 g/ AI (Table 1). The high rate of propiconazole was double the low rate. Due to compound availability there was

no low rate of phosphorous acid injected. Trees were chosen based on overall health of the tree and canopy structure to assure uniform compound delivery to the canopy. DFH measurements were taken with a forester's D-tape (Lufkin®, Sparks, MD) to provide application calculations for each repetition. Colored ribbons were used to separate the four treatments with a total of 25 trees.

Field evaluations and residue analysis were performed in 2010 and 2011 seasons. All the experimentation on the 2010 injection study continued into the 2011 season and is stated as the 2010 continuation.

Injection procedure

Pre-injection data were collected for each tree including the tree DFH, the # injection ports, milliliters per DFH inch, rate (g AI) , total volume for the injection solution per tree, total volume of injection solution per plug, and the DATe of injection. These data are used for organization and rate calculation. The tree DFH was taken prior to any injection by wrapping the D-tape around the diameter of the trunk 12 inches above the ground or at injection height. Next the rate is calculated and the compound is prepared. The injection equipment included an Arborjet Quick-jet™ injector, #4 arbor plugs (Arborjet Inc. Woburn, MA), plug tapper, hammer, cordless drill, and a 3/8 inch wood drill bit. The injection system was sanitized before each injection with Arborjet Cleanjet™ solution and water to rinse any residues and get the pump primed. The drill bit was also sterilized between each rep to prevent spread of infection. Next, the compound was poured into the Arborjet injector holding tank. Extra solution was added to allow priming of the tank. The needle was then attached to the injector tip and the pump

was primed so there were no air pockets that might cause inaccurate applications. The injector was put aside and the holes were drilled into the apple trunk 2 inches deep, 90 degrees horizontal from the trunk, and 12 inches above the ground. The holes were strategically placed under main scaffold branches to orient with the vascular physiology of the tree. The plugs were equally spaced and staggered around the trunk for optimal delivery to the foliage. The holes were not placed above or below any trunk injury as to prevent compound from leaving the tissue through those wounds. The plugs were then tapped in place so the outside rim of the plug was just beneath the bark. The needle was then inserted into the plug so the base of the needle was tight against the lip of the plug. The compound was then injected at the desired rate in each plug. The Arborjet injector has built-in graduated cylinder so the amount per application is accurate to the nearest quarter of a milliliter. The table below best illustrates how the data was organized prior to applications (Table 23).

Table 23. Tree size, field rates and doses per tree and per injection site for the 2010 season. All volumes are measured in milliliters. All measurements are means of the 5 repetitions per treatment.

Trt	Tree DFH	# ports/tree	Ml/ DBH	Tot. vol./ tree	Vol/ plug	Units	Appl. Date
1 Control	-	-	-	-	-	-	-
2 phosH	5.9	4	27.6	162.6	40.7	ml-dbh	4-14
3 propL	6.2	4	8.3	51.2	12.9	ml-dbh	4-14
4 propH	5.3	4	16.6	87.4	21.9	ml-dbh	4-14

Measurements are means of the 5 repetitions per treatment. Volumes are measured in milliliters.

Field Evaluations

Field evaluations targeted natural populations of apple scab (*Venturia inaequalis*) at the MSU Trevor Nichols Research Center in Fennville, MI, USA (latitude 42.5951° : longitude -86.1561°).

2011 Season

2011 Injection

At silver tip stage on April 14, 2011 before the first scab infection, fungicides were injected into 50 trees. The 2011 fungicide injection compared multiple-application regimes and additional compound. There were 10 treatments that were compared to an untreated non injected control. Injections were made to 5-7 inch DFH trees with a five replicate trees per treatment in a randomized block design in the same 2010 MacIntosh plot. Treatments 2-5 were a low and high rate of Phosphorous acid (Phospho-jet™, Arborjet Inc. Woburn, MA), treatments 6-9 were a low and high rate of propiconazole (Alamo®, Arborjet Inc. Woburn, MA), and treatments 10-11 a high rate of penthiopyrad (Fontelis™, DuPont Inc. Wilmington, DE) (Table 24). These injection regimes, maintained a common total volume, but comparing single and multiple injections over time. The first regime injected the total volume into the same set of four holes as in the 2010 injection (Trt 2 & 3) (Table 24). The second regime injected at two different times, with each application in a different sets of four holes. The first set of injection holes were located one foot above the ground and the second set were 5 inches above the first. Twenty percent of the total volume was injected for the first injection at silver tip and the remaining eighty percent of the volume was applied when the foliage was pink stage (Trt 4, 5, 8, 9, & 11) (Table 24). The third

regime was splitting up the total volume into two separate applications with another twenty and eighty percent split, but injecting into the same holes as the first application. The first application was at silver tip and the second was applied when the foliage was fully flushed (Trt 6, 7, & 10) (Table 24). All three injection techniques were used for phosphorous acid and propiconazole with low and high rates. Penthiopyrad used regime two and three with one high rate and a 50/50 total injected volume split instead of a 20/80 split. Milliliters per DFH inch was not calculated for the penthiopyrad treatments. The table below illustrates how the treatments were organized (Table 24). Penthiopyrad was diluted to 24% penthiopyrad, 76% water due to the high viscosity and low "injectability" potential.

Table 24. Tree size, field rates and doses per tree and per injection site for the 2011 season.

Trt	Tree DFH (in.)	# ports/ tree	# Trt/ tree	Appl. same or diff ports	Ml/ DFH	Tot. vol./ inj./ tree		Vol/ plug		Tot inj vol	Appl. date	
						1st inj. 20%	2nd inj. 80%	1st inj. 20%	2nd inj. 80%		1st inj	2nd inj
1 Control	-	-	-	-	-	-	-	-	-	-	-	-
2 phosL	6.7	4	1	same	2.59	17.3	none	4.3	none	17.3	14-Apr	none
3 phosH	6.3	4	1	same	5.17	32.8	none	8.2	none	32.8	14-Apr	none
4 phosL	6.7	8	2	diff	2.59	3.5	13.9	0.89	3.5	17.4	14-Apr	12-May
5 phosH	6.6	8	2	diff	5.17	6.8	27.3	1.7	6.8	34.1	14-Apr	12-May
6 propL	7	4	2	same	8.3	11.6	46.2	2.9	11.6	57.8	14-Apr	12-May
7 propH	6.3	4	2	same	16.6	20.8	83.1	5.2	20.8	104	14-Apr	12-May
8 propL	6.4	8	2	diff	8.3	10.6	42.5	2.6	10.6	53.1	14-Apr	12-May
9 propH	6.1	8	2	diff	16.6	20.4	81.5	5.1	20.4	103	14-Apr	12-May
10 pen	6.4	4	2	same	-	20	20	5	5	40	14-Apr	12-May
11 pen	6.5	8	2	diff	-	20	20	5	5	40	14-Apr	12-May

Treatments 10-11 are a 50/50% injection with 20 ml/acre rate.

All volumes are measured in milliliters.
All measurements are means of the 5 repetitions per treatment.

Injection day April 14, 2011 the total volumes for all the treatments were injected. The second injection of treatments 6-9 and 11on May 12, the final volume could not be injected. Because of the life stage of the tree and weather conditions, the maximum volume tree uptake would allow was below 8 ml. There was a maximum of ±8ml per injection site for this second injection. Treatments 2-5 and 10 took up the total intended rates, for those volumes were under 8 ml.

Field evaluations and residue analysis was performed in 2010 and in 2011. All the experimentation on the 2010 injection study continued into the 2011 season and is stated as the 2010 continuation.

Field Evaluations

Season-long plant protection was measured by conducting a series of field evaluations for the incidence of apple scab (*Venturia inaequalis*) in 2010 and 2011. Evaluations took place when the first symptoms we expected to be visible according to Eniroweather scab predictor. Information regarding the prediction of and actual apple scab infections was found on the Michigan State University Enviro-weather *http://www.enviroweather.msu.edu/homeMap.php*. All treatment replicates were surveyed at specific intervals post application or days after treatment (DAT) and around the entire tree. On each of 20 shoots per rep, the total number of leaves were counted, and of those how many infected leaves there were.

2010 Season

2010 Injection

There were a total of four foliar evaluations with the first evaluation at 42 DAT (14 May). The second was 46 DAT (18 May), the third was 48 DAT (20 May), and the fourth was 56 DAT (28 May). The first 42 day evaluation included treatments 1, 3, 4 and did not include phosphorous acid treatment 2 because of significant phytotoxicity with limited available foliage. The 46 day evaluation included treatments 1, 3, and 4 again because of the continued phytotoxicity of the phosphorous acid treatment 2. The 48 day evaluation included only treatments 1 and 2. This evaluation was only comparing the phosphorous acid treatment with significant phytotoxicity to the untreated check. This was primarily because the foliage was beginning to reflush on treatment 2 at the upper portion of the canopy. The available shoots were included in the evaluation. The 56 day evaluation included all treatments since treatment 2 had available foliage around the entire canopy.

2011 Season

2010 Continuation

There were a total of four foliar evaluations with the final 1 year 102 DAT (13 July) evaluation including fruit. The first evaluation at 1 year 48 DAT (20 May). The second was 1 year 62 DAT (3 June), the third was 1 year 69 DAT (10 June), and the fourth was 1 year 102 DAT (13 July). The 2010 continuation plot included all four treatments 1-4 for all the foliar evaluations and the 2011 plot included all treatments 1-11 for all the foliar evaluations. The 1 year 102 DAT (13 July) evaluation included a fruit evaluation of 25 fruit per rep. These fruit were randomly selected from N, S, E, W cardinal direction sides of the tree both low and high

portions of the canopy. The total number of scab lesions were counted on each fruit. The fruit evaluation for the 2010 continuation plot treatment 2 only included a rep 4. The rest of the repetitions did not produce sufficient fruit yield because of the 2010 phosphorous acid phytotoxicity.

2011 Injections

There were a total of four foliar evaluations with the final 90 DAT (July 13) evaluation including fruit. The first evaluation at 36 DAT (20 May). The second was 50 DAT (3 June), the third was 57 DAT (10 June), and the fourth was 90 DAT (13 July). The 2010 continuation plot included all four treatments 1-4 for all the foliar evaluations and the 2011 plot included all treatments 1-11 for all the foliar evaluations. The 90 DAT (13 July) evaluation included a fruit evaluation of 25 fruit per rep. These fruit were randomly selected from N, S, E, W cardinal direction sides of the tree both low and high portions of the canopy. The total number of scab lesions were counted on each fruit. The fruit evaluation for the 2010 continuation plot treatment 2 only included a rep 4. The rest of the repetitions did not produce sufficient fruit yield because of the 2010 phosphorous acid phytotoxicity.

Residue Profiling

Sample Preparation

Propiconazole, phosphorous acid, and penthiopyrad residues were recovered in leaf, fruit, flower, and wood tissue samples at specific time intervals over the growing season using a specific process. This process began by collecting the samples and storing them in a specific volume of HPLC grade dichloromethane (Burdick & Jackson, Muskegon, MI). Next the samples were ground and decanted through 10-25 g of reagent-grade anhydrous sodium sulfate (EMD

Chemicals, Inc.) to remove water. The samples were then dried using rotary evaporation and the remaining particles were brought back up with 2 ml of acetonitrile. The 2 ml were transferred a 3 ml syringe (Becton & Dickinson, Franklin Lakes, NJ) with a pipette to be filtered by passing them through a 45-µm Acrodisc 33 mm syringe filter (Pall, East Hills, NY) into the final 2 ml vial (Agilent Technologies, Santa Clara, CA) for HPLC analysis. The tables below best describe the sample collection and lab preparation for analysis.

Phosphorous acid samples used a different extraction method. Depending on the sample size a specific volume of 1% H_2SO_4 sulfuric acid water solution was added to the sample and crushed. The crushed sample was decanted onto a round bottom flask. Depending on the sample size a specific volume of isopropanol (Burdick & Jackson, Muskegon, MI) was added to the decanted sample, crushed, and decanted a second time with isopropanol and 1% sulfuric acid combined in the round bottom flask.. The sample was rotovaped to dryness and brought up with 2 ml of the 1% H_2SO_4 solution. The solution was transferred to a 3 ml syringe (Becton & Dickinson, Franklin Lakes, NJ) with a pipette to be filtered by passing them through a 45-µm Acrodisc 33 mm syringe filter (Pall, East Hills, NY) into the final 2 ml vial (Agilent Technologies, Santa Clara, CA) for HPLC analysis. The table below best describes the phosphorous acid sample preparation for the different sample types (Table 25).

Table 25. 2011 phosphorous acid residue sample preparation for analysis.

Sample Type	Sample Size (g)	Volume 1% H_2SO_4 (ml)	Volume Isopropanol (ml)
Leaf	10	30	18
Fruit	20	30	30
Fruit	40	30	30
Wood Core	1.5	10	6

Sample Extraction

Samples were analyzed for fungicide residue with a waters 2695 separator module HPLC equipped with a Waters MicroMass ZQ mass spectrometer detector (Waters, Milford, MA), and a C_{18} reversed phase column (50 by 3.0mm bore, 3.5 µm particle size, (Waters, Milford, MA) (Table 26-28).

Table 26. The mobile phase for each fungicide used for HPLC residue analysis 2010 and 2011.

Chemical	Solvent A	Solvent B	Flow Rate (ml/min)
Penthiopyrad	0.1 % Formic Acid	0.1 % Formic acid in acetonitrile	1.0
Phos. acid	0.1 % Formic Acid	0.1 % Formic acid in acetonitrile	0.30
Fluopyram	NA	NA	GC/MSD
Propiconazole	NA	NA	GC/MSD

Table 27. The gradient used for each fungicide for HPLC residue analysis 2010 and 2011.

Penthiopyrad/Time	0	4.5	6.5	7.0	10.00
Solvent A	30	30	30	70	70
Solvent B	70	70	70	30	30
Phostrol/Time	0	1.00	9.00	10	10.5
Solvent A	90	90	10	10	90
Solvent B	10	10	90	90	10
GC/MSD Parameters	Oven			Inlet	
Temperatures (C)	Start	Ramp	End		
Fluopyram	70	20	320		
Propiconazole	79	20	320		

Table 28. The ions (m/z) monitored, detector dwell time, and cone voltages for detection of the fungicides in HPLC residue analysis 2010 and 2011.

Chemical	Channel 1	Channel 2	Dwell (s)	Cone1 (V)	Cone2 (V)
Penthiopyrad	254 nm	NA	NA	NA	NA
Fluopyram	223.1	393.2	NA	NA	NA
Propiconazole	173.1	259.1	NA	NA	NA
Phosphorous acid			0.50	50	25

The HPLC/MSD level of quantification was 0.08 µg/g (ppm) of active ingredient, and level of detection was 0.038 ppm. The HPLC/UV level of quantification was 0.12 µg/g (ppm) of active ingredient, and level of detection was 0.6 ppm.

GC/MSD level of quantification was 0.01 ug/g of ai, and level of detection was 0.05 ug/g.

2010 Season

2010 Injection-Sample collection preparation

Leaf residue samples were taken from all four treatments and from three of the five replicate trees per treatment 7 DAT (9 April), 21 DAT (23 April), 35 DAT (7 May), 63 DAT (4 June) at, 90 DAT (1 July), and 120 DAT (31 July) intervals. Treatment 2 phosphorous acid at 120 DAT was not collected. The leaf samples were a minimum of 10 g of tissue collected from the N, S, E, W sides of the tree, high/ low and put in labeled jars. They were taken out of the jars, weighed and put back in the jars with 100 ml of dichloromethane. Fruit samples were taken from treatment 4 and from three of the five replicate trees per treatment 120 DAT (31 July). There were 4 fruit picked from N, S, E, W cardinal direction sides of the tree halfway up the crown and midway deep in the crown and put into labeled bags. The fruit was taken out of the bags, cut into 8 pieces from stem to calyx and selected random slices from each fruit to equal 10 g and put into labeled jars with 50 ml of dichloromethane. Wood core samples were taken from all four treatments and from three of the five replicate trees per treatment 83 DAT (24 June). The wood cores were taken from the trunk of the tree, 6 inches above the injection site. Samples were taken from three of the seven treatments and from three of the five replicate trees per treatment, and there was one sample taken from each replicate tree. The cores were taken using a foresters wood coring tool (Forestry suppliers Inc, Jackson, MS). The cores were 2 inches deep, the same depth as the injection. The samples were pushed out of the tool and into labeled Ziploc bags using a metal rod and a tap. The 1.50 g samples were taken out of the bags, weighed and put in labeled jars with 20 ml of dichloromethane. After each set of samples were taken they were stored in labeled 120 ml sample jars (Qorpak, Bridgeville, PA), the jars were stored in two dozen count Qorpak boxes, and the boxes were labeled and stored in a refrigeration cold room. After

the field season the samples were taken out of the cold room and transported to the MSU Pesticide Analytical laboratory (East Lansing, MI) for HPLC analysis and mean data graphic presentation. Flower samples were taken from all four treatments and from three of the five replicate trees per treatment 21 DAT (23 April). These flower samples were a minimum of 5 g of tissue collected from the N, S, E, W cardinal direction sides of the tree, high/ low and put in labeled jars. They were taken out of the jars, weighed and put back in the jars with 50 ml of dichloromethane. All phosphorous acid samples for all tissue types for both 2010 and 2011 season were collected using labeled plastic Ziploc bags and frozen (Table 29).

Table 29. 2010 fungicide residue sample collection and preparation for HPLC analysis.

Date sample	Trts	Sample	Sample	# Fruit	# Slices per fruit		Total # fruit slices used	Volume per rep		
collected	Sampled	Type	Weight (g)	per rep	cut	used	per rep	CH_2Cl_2 (ml)	Na_2SO_4 (g)	NaCl (g)
7 DAT April 9	1-4	Leaf	5.0	-	-	-	-	40	25.0	-
21 DAT April 23	1-4	Leaf	10.0	-	-	-	-	100	25.0	-
21 DAT April 23	1-4	Flower	5.0	-	-	-	-	50	25.0	-
35 DAT May 7	1-4	Leaf	10.0	-	-	-	-	100	25.0	-
63 DAT June 4	1-4	Leaf	10.0	-	-	-	-	100	25.0	-
83 DAT June 24	1-4	Wood Core	1.50	-	-	-	-	20	25.0	-
90 DAT July 1	1-3	Leaf	10.0	-	-	-	-	100	25.0	-
120 DAT July 31	1-4	Leaf	10.0	-	-	-	-	100	25.0	-
120 DAT July 31	1, 2, 4	Fruit	10.0	4	4	1	3	50	25.0	-

2011 Season

2010 Continuation-Sample collection and preparation

Compound residue was recovered from leaf, fruit, and wood core tissue over specific time intervals throughout the growing season. Leaf residue samples were taken from all eleven treatments and from three of the five replicate trees per treatment 1 year 41 DAT (13 May), 1 year 62 DAT (3 June), 1 year 92 DAT (3 July), 118 DAT (29 July), and 1 year 146 DAT (26 August) intervals. The leaf samples were a minimum of 10 g of tissue collected and processes just as the 2010 season. Fruit samples were taken from all eleven treatments and from three of the five replicate trees per treatment at 1 year 92 DAT (3 July), 118 DAT (29 July), and 1 year 146 DAT (26 August) intervals. Samples were a minimum of 20 g for the 1 year 41 DAT (13 May), 1 year 62 DAT (3 June), and 1 year 92 DAT (3 July). Samples were a minimum of 40 g for the 118 DAT (29 July), and 1 year 146 DAT (26 August). These were collected and processed using the same methods as the 2010 season. Wood core samples were taken from all eleven treatments and from three of the five replicate trees per treatment at 1 year 41 DAT (13 May), 1 year 92 DAT (3 July), and 1 year 146 DAT (26 August). These samples were collected and processed using the same methods as the 2010 season (Table 30).

Table 30. 2010 continuation fungicide residue sample collection and preparation for HPLC analysis.

Date sample collected	Trts Sampled	Sample Type	Sample Weight (g)	# Fruit per rep	# Slices per fruit cut	# Slices per fruit used	Total # fruit slices used per rep	Volume per rep CH_2Cl_2 (ml)	Volume per rep Na_2SO_4 (g)	Volume per rep NaCl (g)
1 year 41 DAT May 13	1-4	Leaf	10.0	-	-	-	-	100.0	25.0	1.0
1 year 41 DAT May 13	1-4	Wood Core	1.5	-	-	-	-	20.0	10.0	0.5
1 year 62 DAT June 3	1-4	Leaf	10.0	-	-	-	-	100.0	25.0	1.0
1 year 92 DAT July 3	1-4	Leaf	10.0	-	-	-	-	100.0	25.0	1.0
1 year 92 DAT July 3	1-4	Fruit	20.0	4	4	1	4	50.0	25.0	1.0
1 year 118 DAT July 29	1-4	Leaf	10.0	-	-	-	-	100.0	25.0	1.0
1 year 118 DAT July 29	1-4	Fruit	40.0	4	4	1	4	90.00	25.0	1.0
1 year 146 DAT Aug 26	1-4	Leaf	10.0	-	-	-	-	100.0	25.0	1.0
1 year 146 DAT Aug 26	1-4	Fruit	40.0	4	4	1	3	90.00	25.0	1.0
1 year 146 DAT Aug 26	1-4	Wood Core	1.5	-	-	-	-	20.0	10.0	0.5

2011 Injection-sample collection and preparation

Compound residue was recovered from leaf, fruit, and wood core tissue over specific time intervals throughout the growing season. Leaf residue samples were taken from all eleven treatments and from three of the five replicate trees per treatment 15 DAT (29 April), 29 DAT (13 May), 50 DAT (3 June), 80 DAT (3 July), 106 DAT (29 July), and 134 DAT (26 August) intervals. The leaf samples were a minimum of 10 g of tissue collected, except for the 15 DAT sample, which were leaf buds and 5 g were collected. The rest were collected and processed just as the 2010 season. Fruit samples were taken from all eleven treatments and from three of the five replicate trees per treatment at 80 DAT, 106 DAT, and 134 DAT intervals. Samples were a minimum of 20 g for the 29 DAT, 50 DAT, and 80 DAT. Samples were a minimum of 40 g for the 106, and 134 DAT. These were collected and processed using the same methods as the 2010 season. Wood core samples were taken from all eleven treatments and from three of the five replicate trees per treatment at 29 DAT, 80 DAT, and 134 DAT. These samples were collected and processed using the same methods as the 2010 season (Table 31).

Table 31. 2011 fungicide residue sample collection and preparation for HPLC analysis.

Date sample collected	Trts Sampled	Sample Type	Sample Weight (g)	# Fruit per rep	# Slices per fruit cut	# Slices per fruit used	Total # fruit slices used per rep	Volume per rep CH_2Cl_2 (ml)	Volume per rep Na_2SO_4 (g)	Volume per rep NaCl (g)
15 DAT April 29	1-11	Leaf	5.0	-	-	-	-	50.0	25.0	1.0
29 DAT May 13	1-11	Leaf	10.0	-	-	-	-	100.0	25.0	1.0
29 DAT May 13	1-11	Wood Core	1.5	-	-	-	-	20.0	10.0	0.5
50 DAT June 3	1-11	Leaf	10.0	-	-	-	-	100.0	25.0	1.0
80 DAT July 3	1-11	Leaf	10.0	-	-	-	-	100.0	25.0	1.0
80 DAT July 3	1-11	Fruit	20.0	4	4	1	4	100.0	25.0	1.0
80 DAT July 3	1-11	Wood Core	1.5	-	-	-	-	20.0	10.0	0.5
106 DAT July 29	1-11	Leaf	10.0	-	-	-	-	100.0	25.0	1.0
106 DAT July 29	1-11	Fruit	40.0	4	4	1	4	90.0	25.0	1.0
134 DAT Aug 26	1-11	Leaf	10.0	-	-	-	-	100.0	25.0	1.0
134 DAT Aug 26	1-11	Fruit	40.0	4	4	1	3	90.0	25.0	1.0
134 DAT Aug 26	1-11	Wood Core	1.5	-	-	-	-	20.0	10.0	0.5

Results

Field Evaluations

2010 Season

At 42 DAT (14 May, 2010) the percent infection rate was significantly lower for propiconazole treatments compared to the control (F=9.372, df=2, P=0.008) (Table 32). No data were available for the phosphorous acid treatment because of significant foliar phytotoxicity. At 46 DAT (18 May) the percent infection rate was significantly lower in the propiconazole low treatment compared to the control (F=4.824, df=2, P=0.0422) (Table 32). No data was available for the phosphorous acid treatment because of significant foliar phytotoxicity. At 48 DAT (20 May) the percent infection rate was significantly lower for the phosphorous acid treatment compared to the control (F=389.361, df=1, P=0.0001) (Table 32). A 56 DAT (28 May) the percent infection rate was significantly lower for the phosphorous acid treatment, lower portion of the tree compared to the control (F=85.157, df=3, P=0.0001) (Table 32). At 56 DAT (28 May) the percent infection rate was significantly lower for the phosphorous acid treatment, upper portion of the tree compared to the control (F=113.76, df=3, P=0.0001) (Table 32).

Table 32. 2010 mean ± SE percentage of foliar tissue infected with apple scab out of 20 shoots per rep.

Trt	42 DAT	46 DAT	48 DAT	56 DAT*[L]	56 DAT*[U]
			Percent Infected		
Control	3.2 (0.8)a	24.6 (2.3)a	29.5 (2.7)a	67.9 (1.7)a	70.9 (2.1)a
Phos.	PH	PH	5.9 (2.3)b	9.6 (1.7)b	3.0 (0.8)b
PropL	0.7 (0.3)b	13.0 (1.8)b	N/D	63.8 (2.0)a	69.4 (2.3)a
PropH	0.4 (0.2)b	20.1 (2.4)ab	N/D	55.6 (1.5)a	64.5 (2.1)a

Means followed by same letter do not significantly differ (P=.05, Tukey's HSD)
ANOVA performed on arcsine square-root transformed data; means shown for comparison
*ANOVA may not be valid as the data failed Bartlett's test for homogeneity
[L]Lower portion of the tree scouted
[U]Upper portion of the tree scouted
PH significant phytotoxicity and foliar data could not be collected
N/A there is no data available and data was not collected or compared

2011 Season

2010 Continuation Injection

Foliar. At 1 year 48 DAT (20 May, 2011) there were no significant differences in foliar percent infection rates for all treatments compared to the control (F=0.249, df=3, P=0.8606), likely because of the overall low incidence of lesions at the early season DATe (Table 33). At 1 year 62 DAT (3 June) the percent infection rate was significantly lower for phosphorous acid and propiconazole low treatments (F=61.412, df=3, P=0.0001) (Table 33). At 1 year 69 DAT (10 June) the percent infection rate was significantly lower for the phosphorous acid treatment compared to the control (F=293.099, df=3, P=0.0001) (Table 33). At 1 year 102 DAT (13 July) the percent infection rate was significantly lower for the phosphorous acid treatment compared to the control (F=101.196, df=3, P=0.0001) (Table 33).

Fruit. At 36 DAT (19 May) the percent fruit infection rates were significantly lower for phosphorous acid and propiconazole low treatments compared to the control (F=78.331, df=3, *P*=0.0001) (Table 34).

Table 33. 2010 continuation mean (± SE) percentage of foliar tissue infected with apple scab out of 20 shoots per rep.

Treatment	1 yr 48 DAT	1 yr 62 DAT	1 yr 69 DAT	1 yr 102 DAT*
		Percent infected		
Control	0.5 (0.3)a	24.9 (1.6)a	50.8 (2.0)ab	72.8 (1.6)a
Phos.	0.2 (0.2)a	0.0 (0.0)c	1.7 (0.6)c	9.5 (1.3)b
PropL	0.3 (0.2)a	15.3 (1.5)b	57.3 (2.1)a	68.3 (2.0)a
PropH	0.3 (0.2)a	20.4 (1.5)ab	46.1 (2.0)b	73.6 (1.9)a

Mean percent of apple scab infection per 20 shoots throughout the growing season
Means followed by same letter do not significantly differ (*P*=.05, Tukey's HSD)
ANOVA performed on arcsine square-root transformed data; means shown for comparison
*ANOVA may not be valid as the data failed Bartlett's test for homogeneity

Table 34. 2010 continuation mean (± SE) percentage of fruit tissue infected with apple scab out of 25 fruit per rep 90 DAT.

Treatment	1 yr 102 DAT
	# of Infections
Control	24.6 (1.1)a
Phos.	7.9 (1.5)c
PropL	19.2(0.9)b
PropH	21.6 (1.0)ab

Means followed by same letter do not significantly differ (*P*=.05, Tukey's HSD)
ANOVA performed on square-root transformed data; data presented are actual counts

2011 Injection

Foliar. At 36 DAT (20 May) there were no significant differences in foliar percent infection rates for all treatments compared to the control (F=0.94, df=10, P=0.508), likely because of the overall low incidence of lesions at the early season DATe (Table 35). At 50 DAT (3 June) the percent infection rate was significantly lower for phosphorous acid treatments compared to the control (F=13.49, df=10, P=0.0001) (Table 35). At 57 DAT (10 June) there were no significant differences in foliar percent infection rates for all treatments compared to the control (F=4.336, df=10, P=0.0004) (Table 35). At 90 DAT (13 July) the percent infection rate was significantly lower for the phosphorous acid high/ 1 injection, phosphorous acid low/ 2 injections, phosphorous acid high/ 2 injection treatments compared to the control (F=6.439, df=10, P=0.0001) (Table 35).

Fruit. At 90 DAT (12 July) the percent fruit infection rates were significantly lower for the phosphorous acid treatments compared to the control (F=19.133, df=10, P=0.0001) (Table 35).

Table 35. 2011 mean (± SE) percentage of foliar tissue infected with apple scab out of 20 shoots per rep 36 (20 May), 50 (3 June), 57 (10 June), and 90 (12 July) DAT.

Trt	36 DAT	50 DAT	57 DAT	90 DAT
Control	0.9 (0.4)a	27.1 (1.5)a	40.3 (2.1)abc	78.0 (1.7)a
PhosL, 1 inj	1.1 (1.0)a	10.7 (1.3)bc	30.7 (2.0)bc	61.0 (2.1)abc
PhosH, 1 inj	2.2 (1.2)a	7.9 (1.1)c	29.4 (1.9)c	56.1 (1.8)bc
PhosL, 2 inj	1.0 (0.4)a	8.2 (1.2)c	28.4 (2.2)c	58.2 (1.9)bc
PhosH, 2 inj	0.2 (0.2)a	9.4 (1.3)c	32.9 (1.8)abc	45.9 (2.0)c
PropL, 1 inj	0.4 (0.2)a	20.5 (1.3)a	35.5 (2.2)abc	73.4 (1.6)ab
PropH, 1 inj	1.3 (0.4)a	19.4 (1.4)a	42.5 (2.1)abc	72.8 (1.8)ab
PropL, 2 inj	2.2 (0.7)a	22.9 (1.6)a	46.9 (2.0)a	72.8 (2.1)ab
PropH, 2 inj	1.3 (0.5)a	21.7 (1.4)a	41.4 (2.3)abc	69.8 (2.0)ab
Pen., 1 inj	1.0 (0.4)a	19.9 (1.6)a	40.7 (2.1)abc	73.5 (1.8)ab
Pen., 2 inj	1.4 (0.9)a	19.2 (1.5)ab	44.7 (1.8)ab	72.8 (2.0)ab

Means followed by same letter do not significantly differ (P=.05, Tukey's HSD)
ANOVA performed on arcsine square-root transformed data; means shown for comparison

Residue Profiling

2010 Season

Propiconazole

Foliar. Propiconazole was first detected in low rate foliar samples at 0.09 ppm at 7 DAT (9 April) and increased to 0.77 ppm at 21 DAT (23 April) (Figure 26). Residues decreased to 0.09 ppm at 35 DAT (7 May) and increased to a maximum level of 2.51 ppm at 120 DAT (31 July) (Figure 26).

Propiconazole was first detected in high rate foliar samples at 0.20 ppm at 7 DAT (9 April) and increased to 0.97 ppm at 21 DAT (23 April) (Figure 26). Residues then decreased to

0.34 ppm at 35 DAT (7 May) and increased to a maximum level of 1.37 ppm at 120 DAT (31 July) (Figure 26).

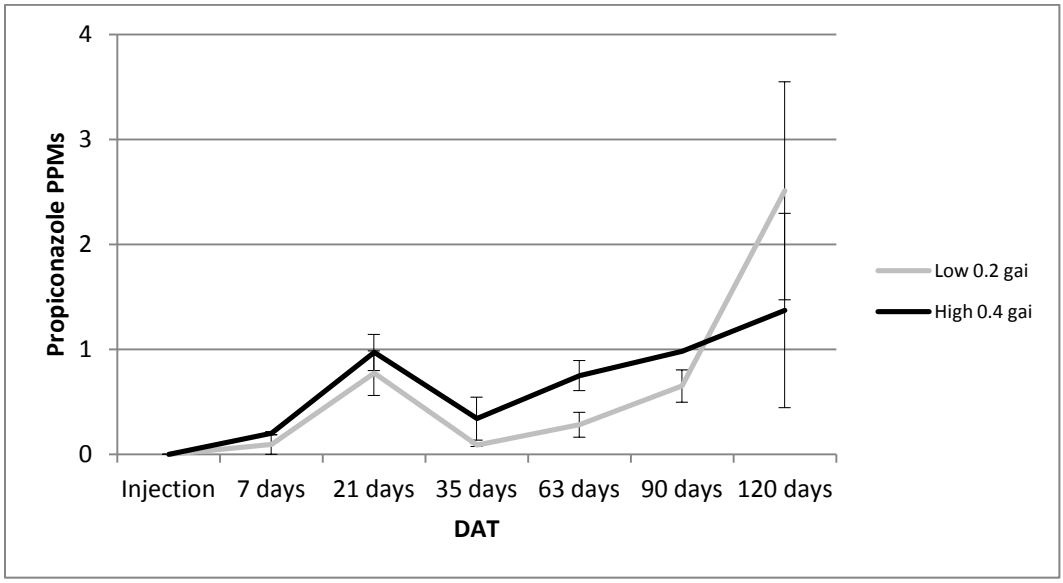

Figure 26. Mean residue recovery (± SE) from leaves for propiconazole low and high rates over the growing season. The injections were on April 2, 2010, 7 DAT was 9 April, 21 DAT was 23 April, 35 DAT was 7 May, 63 DAT was 4 June, 90 DAT was 1 July, and 120 DAT was 31 July. The 90 DAT (28 July) is the mean of the 60 DAT (27 May) and 120 DAT (28 July) due to sampling error.

Fruit. Propiconazole was detected in high rate fruit samples at 5.38 ppm at 120 DAT (31 July) (Table 36).

Flower. Propiconazole was detected in low and high rate flower samples at 21 DAT (23 April). Low rate recovery was 0.06 ppm and high rate was 0.09 ppm (Table 36).

Wood Core. Propiconazole was detected in low and high rate wood core samples at 83 DAT (24 June). Low rate recovery was 10399.41 ppm and high rate was 9057.11 ppm (Table 36).

Table 36. 2010 mean numbers of flower, fruit, and wood core residue for propiconazole low and high rates.

Type	Rate	DAT, Date	Mean Residue Recovered (ppm)
Flower	Low	21, 23-Apr	0.06
Flower	High	21, 23-Apr	0.09
Fruit	High	120, 31-Jul	5.38
Wood Core	Low	83, 24-Jun	10399.41
Wood Core	High	83, 24-Jun	9057.11

Phosphorous acid

Foliar. Phosphorous acid was first detected at a maximum of 127.62 ppm foliar samples at 7 DAT (9 April) and decreased to 3.55 ppm at 14 DAT (16 April) (Figure 27). Residues increased to 4.47 ppm, decreased to a minimum of 1.43 ppm at 42 DAT (14 May), and increased to 5.65 ppm at 49 DAT (21 May) (Figure 27). Lastly, residues decreased to 1.66 ppm at 90 DAT (1 July) (Figure 27).

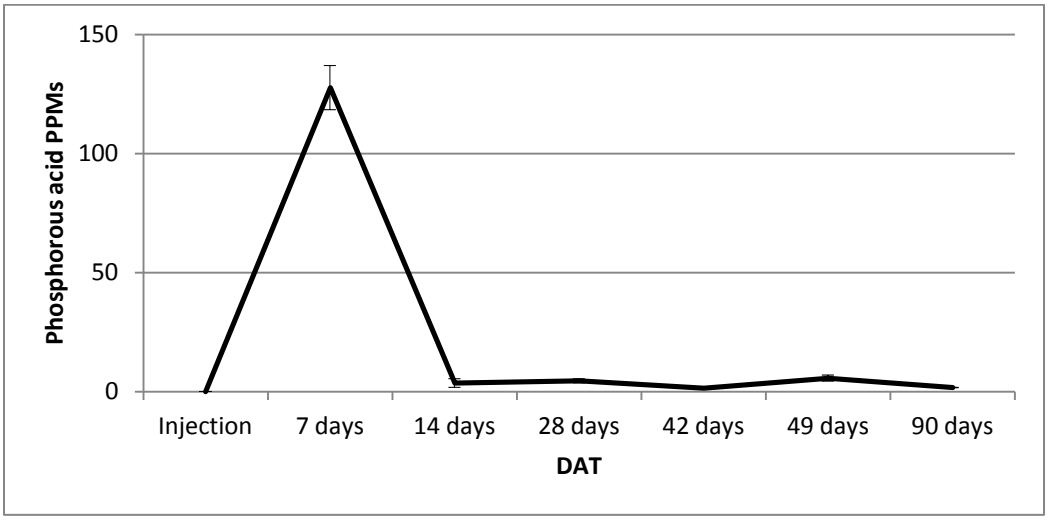

Figure 27. Mean residue recovery (ppm) from 2011 foliar residue samples for phosphorous acid rate. The injections were on April 2, 2010 and samples were taken at 7 DAT (9 April), 14 DAT (16 April), 28 DAT (30 April), 42 DAT (14 May), 49 DAT (20 May) and 90 DAT (1 July).

Flower. Phosphorous acid was detected at 74.36 ppm at 21 DAT (23 April).

Wood core. Phosphorous acid was detected at 13.29 ppm at 83 DAT (24 June).

2011 Season

2010 Continuation Injection

Propiconazole

Foliar. Propiconazole was first detected at a minimum of 0.0002 ppm in low rate foliar samples at 1 year 41 DAT (13 May) and increased to a maximum of 0.0005 ppm at 1 year 92 DAT (3 July) (Figure 28). Residues then decreased to a minimum of 0.02 ppm at 1 year 118 DAT (29 July) and increased to 0.0004 ppm at 1 year 146 DAT (26 Aug) (Figure 28). High rate foliar residues were first detected at 1 year 41 DAT (13 May) at maximum of 0.0003 ppm, decreased to a minimum of 0.0001 ppm at 1 year 92 DAT (3 July), then increased to a maximum of 0.0003 ppm at (Figure 28). By the end of the growing season 1 year 146 DAT (26 Aug) residue decreased to 0.0002 ppm (Figure 28).

Propiconazole was first detected at maximum of 0.000042 ppm in low rate fruit samples at 1 year 92 DAT (3 July), decreased to a minimum of 0.000028 ppm at 1 year 118 DAT (29 July), then increased to 0.000038 ppm at 1 year 146 DAT (26 Aug) (Figure 28). High rate fruit residues were first detected at a maximum of 0.000023 ppm at 1 year 92 DAT (3 July), decreased to 0.00001 ppm at 1 year 118 DAT (29 July), then increased to 0.000015 ppm at 1 year 146 DAT (26 Aug) (Figure 28).

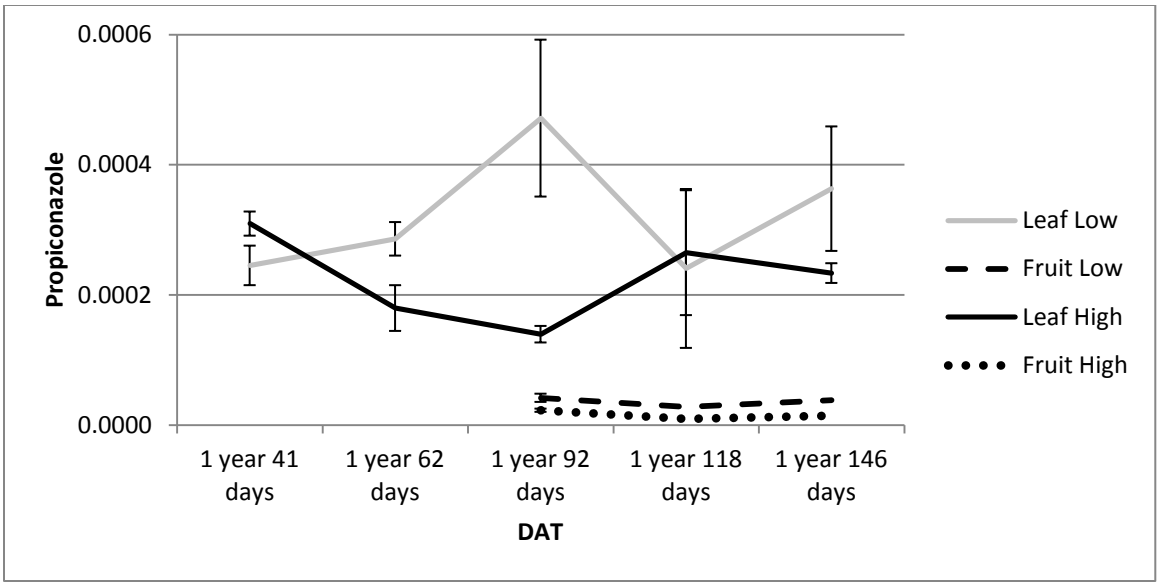

Figure 28. Mean residue recovery (ppm) (± SE) from 2011 foliar and fruit residue samples for propiconazole low and high rates. The injections were on April 2, 2010 and samples were taken at 1 year 41 DAT (13 May), 1 year 62 DAT (3 June), 1 year 92 DAT(3 July), 1 year 118 DAT (29 July), and 1 year 146 DAT (26 Aug).

Wood Core. Propiconazole was first detected at 3.45 ppm in low rate wood core samples recovered 6 inches below the injection site at 1 year 41 DAT (13 May), 1.49 ppm at 1 year 92 DAT (3 July), and 5.60 ppm at 1 year 146 DAT (26 Aug) (Figure 29). Residues detected 6 inches above the injection site were 3.86 ppm at 1 year 41 DAT (13 May), 2.81 ppm at 1 year 92 DAT (3 July), and 2.76 ppm at 1 year 146 DAT (26 Aug) (Figure 29). Residues detected at the base of the main scaffold branch were 0.0054 ppm at 1 year 41 DAT (13 May), 0.0043 ppm at 1 year 92 DAT (3 July), and 0.0019 ppm at 1 year 146 DAT (26 Aug) (Figure 29).

Propiconazole was first detected at 2.05 ppm in high rate wood core samples recovered 6 inches below the injection site at 1 year 41 DAT (13 May), 1.85 ppm at 1 year 92 DAT (3 July), and 2.23 ppm at (1 year 146 DAT (26 Aug) (Figure 30). Residue detected 6 inches above the injection site 1 year 41 DAT (13 May) was 2.43 ppm, 2.40 ppm at 1 year 92 DAT (3 July), and

2.60 ppm at 1 year 146 DAT (26 Aug) (Figure 30). Residue detected at the base of the main scaffold branch were 0.31 ppm at 1 year 41 DAT (13 May), 0.02 ppm at 1 year 92 DAT (3 July), and 0.03 ppm at 1 year 146 DAT (26 Aug) (Figure 30).

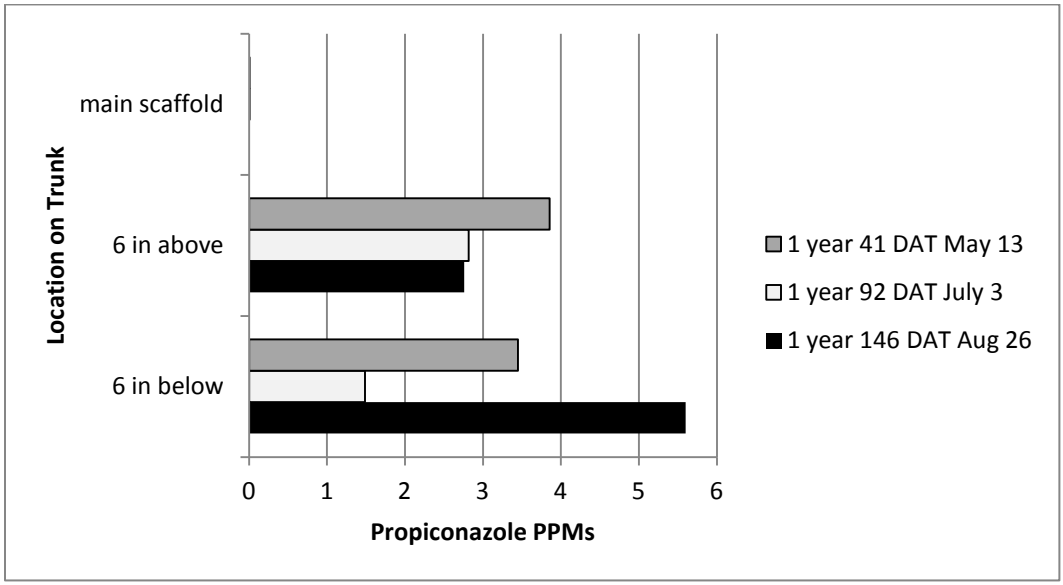

Figure 29. Mean residue recovery (ppm) from propiconazole low rate residue samples of wood core tissue, 6 inches below injection, 6 inches above injection, and at the base of the main scaffold branch. The injections were on April 2, 2010 and samples were taken at 1 year 41 DAT (13 May), 1 year 92 DAT (3 July), and 1 year 146 DAT (26 Aug).

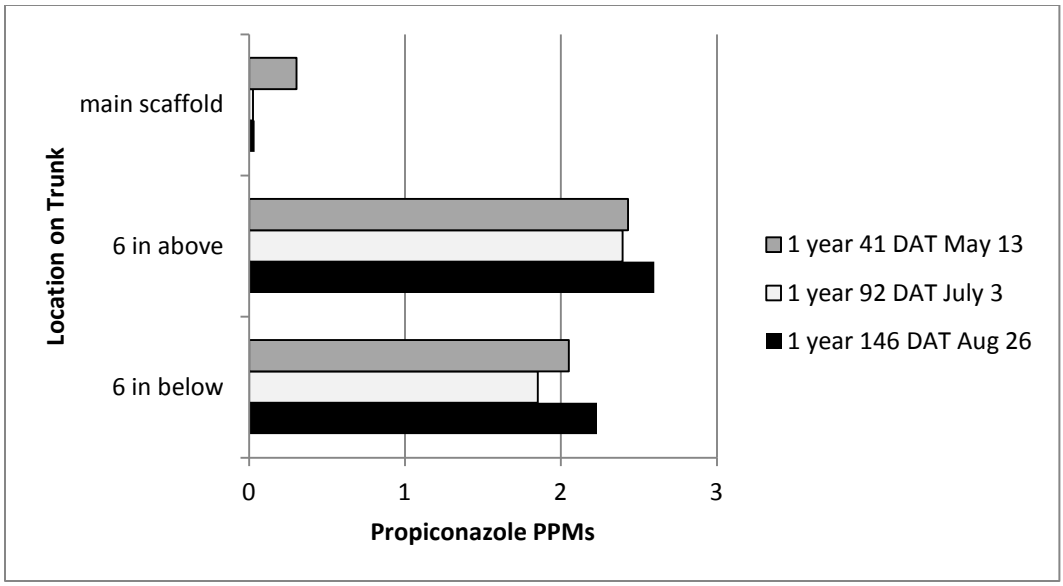

Figure 30. Mean residue recovery (ppm) from propiconazole high rate residue samples of wood core tissue, 6 inches below injection, 6 inches above injection, and at the base of the main scaffold branch. The injections were on April 2, 2010 and samples were taken at 1 year 41 DAT (13 May), 1 year 92 DAT (3 July), and 1 year 146 DAT (26 Aug).

Phosphorous acid-Residue data not available.

2011 Injection

Propiconazole Low Rate

Foliar. Propiconazole was first detected at 7.60E-06 ppm in low rate 1 injection (full vol. injected with 1 appl.) foliar samples at 80 DAT (3 July) and increased to a maximum of 1.84E-06 ppm at 106 DAT (29 July) (Figure 31). Residue levels then decreased to 2.43E-05 ppm at 134 DAT (26 Aug) (Figure 31). Low rate 2 injection (20% and 80% appl. split) foliar residues were first detected at 15 DAT (29 April) at 1.29E-05 ppm and increased to 0.00013 ppm at 106 DAT (29 July) (Figure 31). Residue levels decreased to 1.06E-05 ppm at 50 DAT (3 June) and increased to a maximum of 0.00047 at 134 DAT (26 Aug) (Figure 31).

Fruit. Propiconazole was first detected at 7.81E-07 ppm in low rate 1 injection fruit samples at 80 DAT (3 July) and increased to 7.65E-07 ppm at 106 DAT (29 July) then increased to a maximum of 2.09E-06 at 134 DAT (26 Aug) (Figure 31). Low rate 2 injection fruit residues were first detected at 106 DAT (29 July) at 3.43E-07 ppm and increased to a maximum of 1.21E-06 at 134 DAT (26 Aug) (Figure 31).

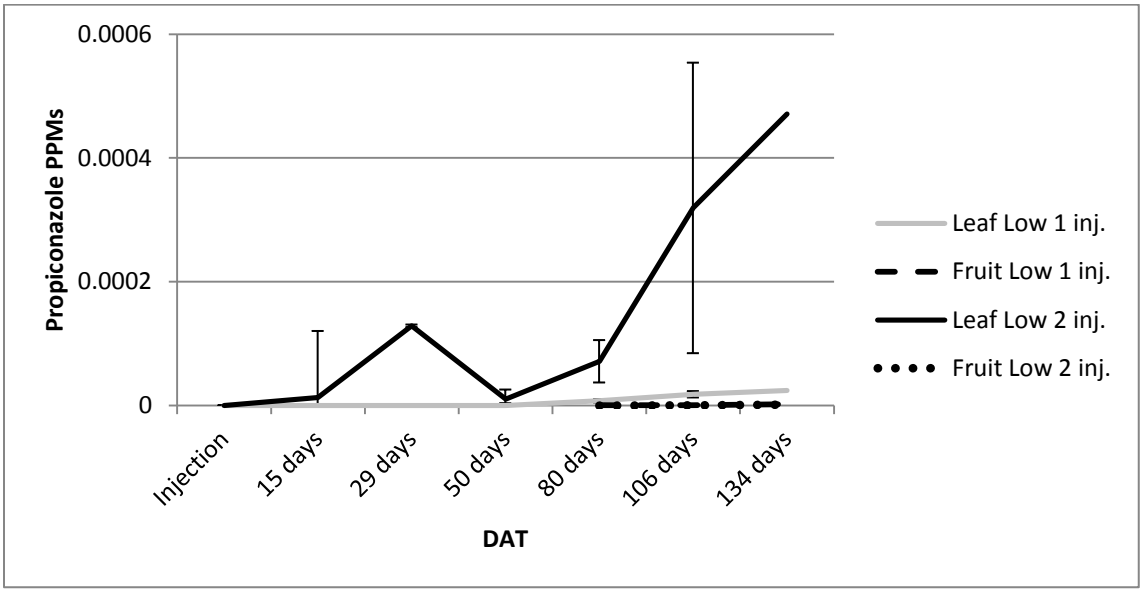

Figure 31. Mean residue recovery (ppm) from 2011 foliar and fruit residue for propiconazole 1 injection and propiconazole 2 injections low rates. The injections were on April 14, 2011 and samples were taken on 15 DAT (29 April), 29 DAT (13 May), 50 DAT (3 June), 80 DAT (3 July), 106 DAT (29 July), and 134 DAT (26 Aug).

Wood Core. Propiconazole was first detected at 0.93 ppm in 1 injection low rate wood core samples recovered 6 inches below the injection site at 29 DAT (13 May), 1.73 ppm at 80 DAT (3 July), and 3.19 ppm at 134 DAT (26 Aug) (Figure 32). Residues detected 6 inches above the injection site were 1.12 ppm at 29 DAT (13 May), 1.40 ppm at 80 DAT (3 July), and 2.73 ppm at 134 DAT (26 Aug) (Figure 32). Residues detected at the base of the main scaffold branch

were 0.15 ppm at 29 DAT (13 May), 0.17 at 80 DAT (3 July), and 0.44 ppm at 134 DAT (26 Aug) (Figure 32).

Propiconazole was first detected at 0.80 ppm in 2 injections low rate wood core samples recovered 6 inches below the injection site at 29 DAT (13 May), 2.03 ppm at 80 DAT (3 July), and 2.05 ppm at 134 DAT (26 Aug) (Figure 33). Residues detected 6 inches above the injection site were 0.02 ppm at 29 DAT (13 May), 0.96 ppm at 80 DAT (3 July), and 1.87 ppm at 134 DAT (26 Aug) (Figure 33). Residues detected at the base of the main scaffold branch were 0.05 ppm at 29 DAT (13 May), 0.09 ppm at 80 DAT (3 July), and 0.08 at 134 DAT (26 Aug) (Figure 33).

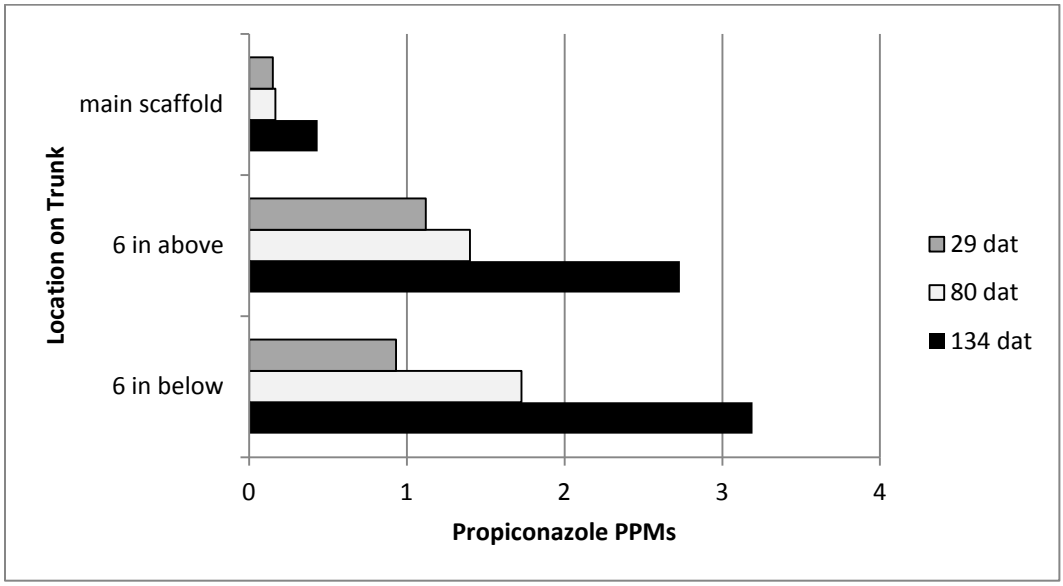

Figure 32. Mean residue recovery (ppm) from propiconazole 1 injection low rate residue samples of wood core tissue, 6 inches below injection, 6 inches above injection, and at the base of the main scaffold branch. The injections were on April 14, 2011 and samples were taken at 29 DAT (13 May), 80 DAT (3 July), and 134 DAT (26 Aug).

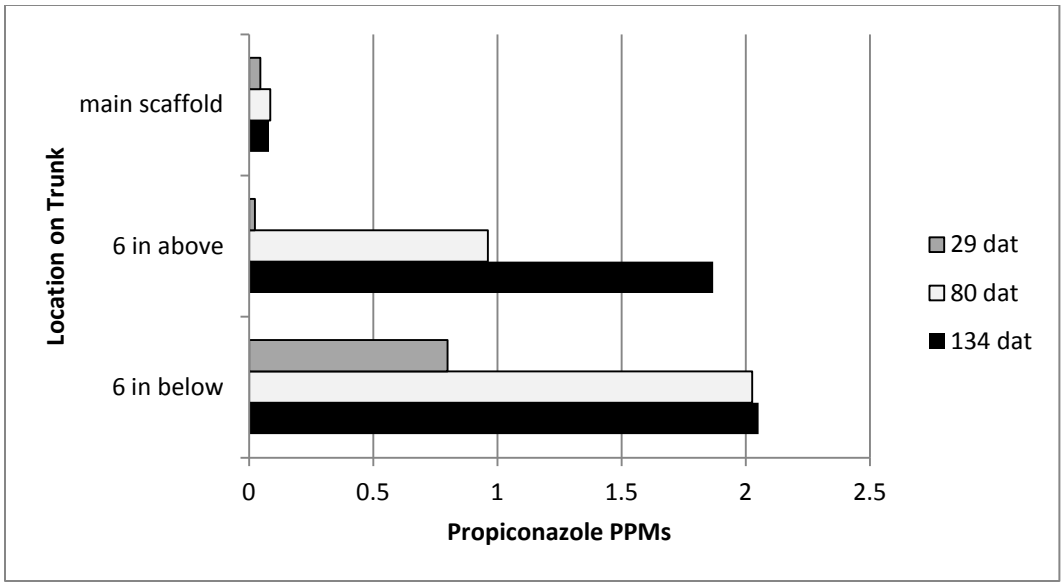

Figure 33. Mean residue recovery (ppm) from propiconazole 2 injections low rate residue samples of wood core tissue, 6 inches below injection, 6 inches above injection, and at the base of the main scaffold branch. The injections were on April 14, 2011 and samples were taken at 29 DAT (13 May), 80 DAT (3 July), and 134 DAT (26 Aug).

Propiconazole High Rate

Foliar. Propiconazole was first detected at 2.38E-06 ppm in high rate 1 injection (full vol. injected with 1 appl.) foliar samples at 15 DAT (29 April) and decreased to a minimum of 9.89E-07 ppm at 29 DAT (13 May) (Figure 34). Residue levels then increased to 1.72E-05 ppm at 80 DAT (3 July), decreased to 1.14E-5 ppm at 106 DAT (29 July), then increased to a maximum of 1.81E-05 ppm at 134 DAT (26 Aug) (Figure 34). High rate 2 injection (20% and 80% appl. split) foliar residues were first detected at 1.04E-06 ppm at 29 DAT (13 May) and increased to 9.81E-07 ppm at 50 DAT (3 June).(Figure 34). Residue levels increased to a maximum of 1.30E-05 ppm at 80 DAT (3 July) and decreased to 0.0 ppm at 134 DAT (26 Aug) (Figure 34).

Fruit. Propiconazole was first detected at 1.76E-07 ppm in low rate 1 injection fruit samples at 80 DAT (3 July) and increased to a maximum of 7.32E-07 ppm at 106 DAT (29 July)

then decreased to a minimum of 1.12E-07 ppm at 134 DAT (26 Aug) (Figure 34). Low rate 2 injection fruit residues were first detected at 2.73E-07 ppm at 106 DAT (29 July) and increased to a maximum of 2.58E-06 at 134 DAT (26 Aug) (Figure 34).

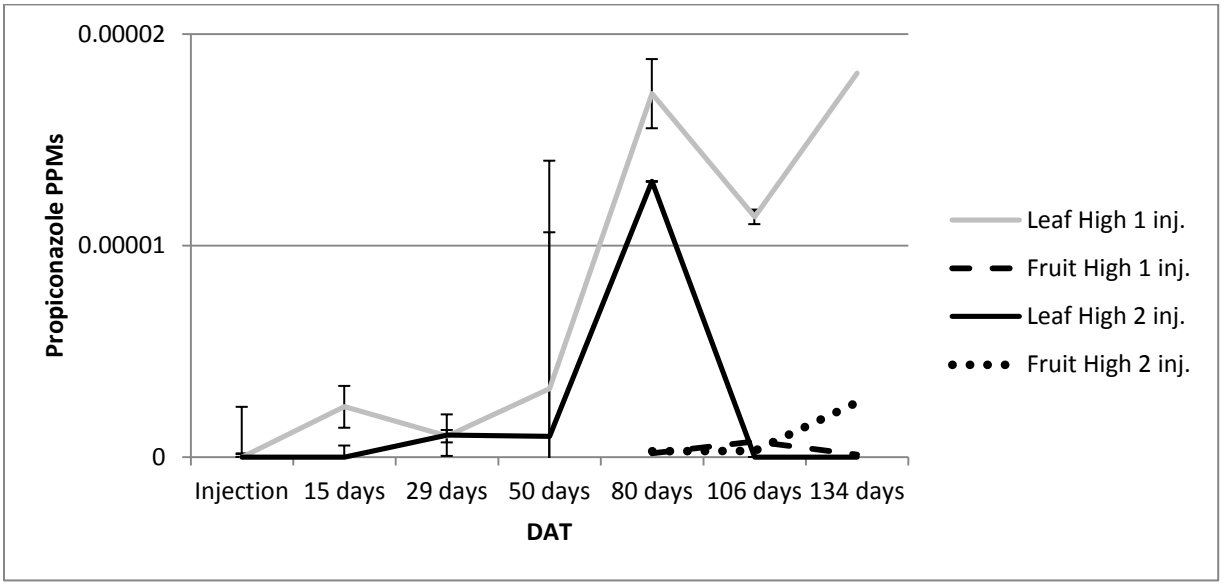

Figure 34. Mean residue recovery (ppm) from 2011 foliar and fruit residue for propiconazole 1 injection and propiconazole 2 injections high rates. The injections were on April 14, 2011 and samples were taken on 15 DAT (29 April), 29 DAT (13 May), 50 DAT (3 June), 80 DAT (3 July), 106 DAT (29 July), and 134 DAT (26 Aug).

Wood Core. Propiconazole was first detected at 2.03 ppm in 1 injection high rate wood core samples recovered 6 inches below the injection site at 29 DAT (13 May), 1.31 ppm at 80 DAT (3 July), and 1.97 ppm at 134 DAT (26 Aug) (Figure 35). Residues detected 6 inches above the injection site were 0.78 ppm at 29 DAT (13 May), 2.29 ppm at 80 DAT (3 July), and 1.89 ppm at 134 DAT (26 Aug) (Figure 35). Residues detected at the base of the main scaffold branch were 0.004 ppm at 29 DAT (13 May), 0.003 ppm at 80 DAT (3 July), and 0.009 at 134 DAT (26 Aug) (Figure 35).

Wood Core. Propiconazole was first detected at 1.55 ppm at in 2 injections high rate wood core samples recovered 6 inches below the injection site at 29 DAT (13 May), 1.77 ppm at 80 DAT (3 July), and 1.44 ppm at 134 DAT (26 Aug) (Figure 36). Residues detected 6 inches above the injection site were 0.95 ppm at 29 DAT (13 May), 1.90 ppm at 80 DAT (3 July), and 1.40 ppm at 134 DAT (Aug 26) (Figure 36). Residues detected at the base of the main scaffold branch were 0.03 ppm at 29 DAT (13 May), 0.001 ppm at 80 DAT (3 July), and 0.009 at 134 DAT (26 Aug) (Figure 36).

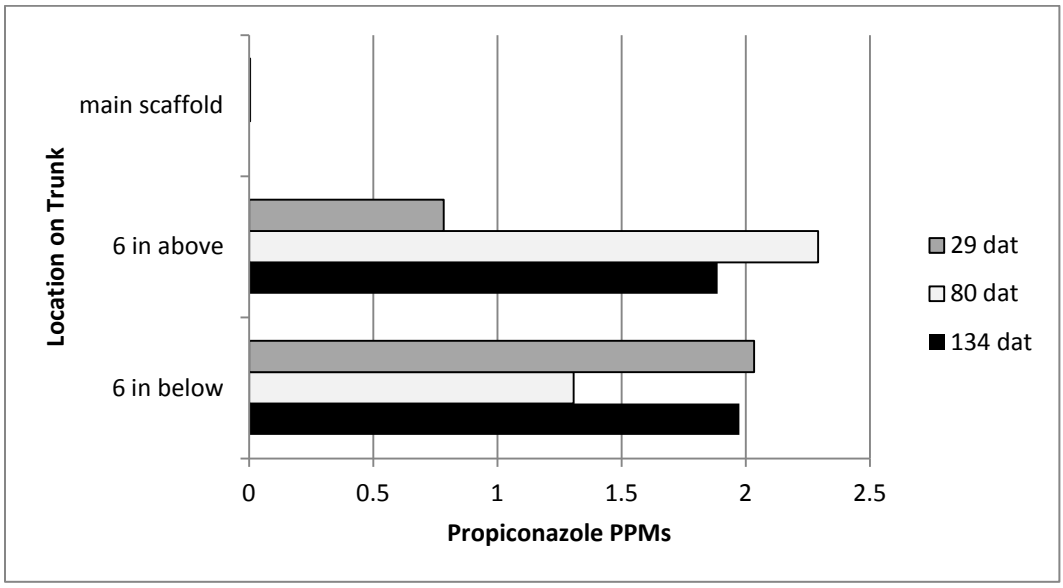

Figure 35. Mean residue recovery (ppm) from propiconazole 1 injection high rate residue samples of wood core tissue, 6 inches below injection, 6 inches above injection, and at the base of the main scaffold branch. The injections were on April 14, 2011 and samples were taken at 29 DAT (13 May), 80 DAT (3 July), and 134 DAT (26 Aug).

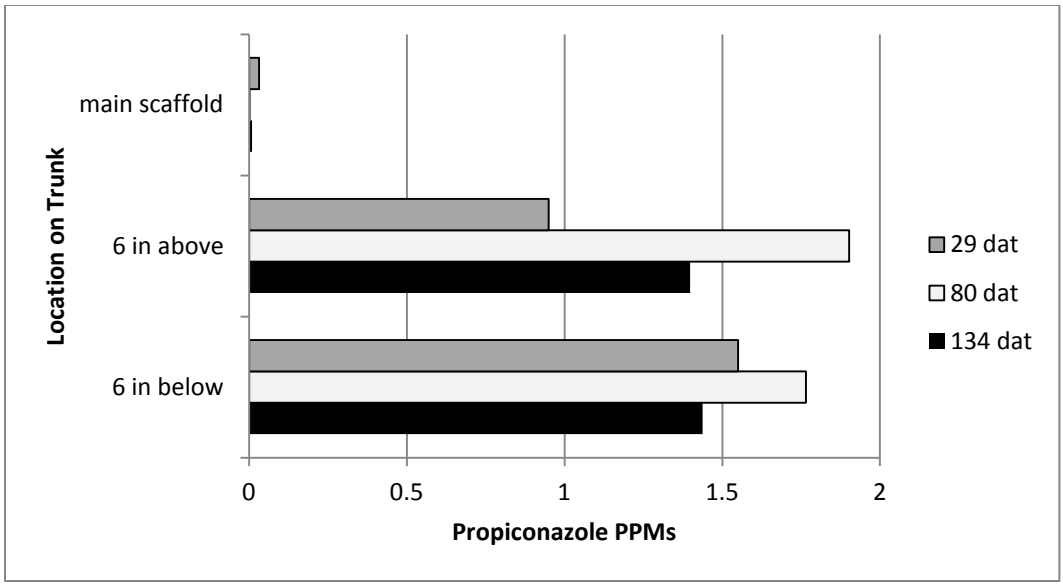

Figure 36. Mean residue recovery (ppm) from propiconazole 2 injections high rate residue samples of wood core tissue, 6 inches below injection, 6 inches above injection, and at the base of the main scaffold branch. The injections were on April 14, 2011 and samples were taken at 29 DAT (13 May), 80 DAT (3 July), and 134 DAT (26 Aug).

Phosphorous acid-Residue data not available.

Penthiopyrad

Foliar. Penthiopyrad was first detected at 2.23 ppm in the 1 set of injections (injected 50%/50% into one set of holes) foliar samples at 29 DAT (13 May) and decreased to 0.12 ppm at 50 DAT (3 June) (Figure 37). Residues increased to 0.20 ppm at 80 DAT (3 July) and 106 DAT (29 July) then decreased to 0.0 ppm at 134 DAT (26 Aug) (Figure 37).

Penthiopyrad was first detected at 0.07 ppm in the 1 sets of injections (injected 50%/50% into 2 different sets of holes) foliar samples at 15 DAT (29 April) and increased to 0.59 ppm at 29 DAT (13 May) (Figure 37). Residues decreased to a minimum of 0.0 ppm at 80 DAT (3 July), increased to a maximum of 1.29 ppm at 106 DAT (29 July), and decreased back to 0.0 ppm at 134 DAT (26 Aug) (Figure 37).

Fruit. There were no fruit residues detected for the 1 set of injections and 2 sets of injections.

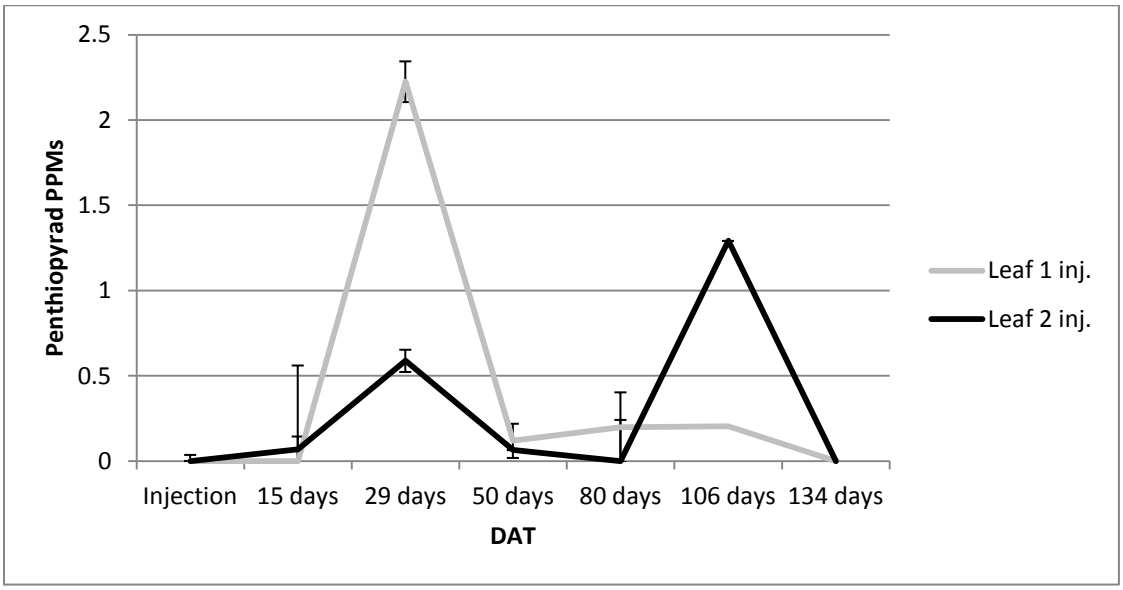

Figure 37. Mean residue recovery (ppm) from 2011 foliar residue for penthiopyrad 1 injection and penthiopyrad 2 injections. The injections were on April 14, 2011 and samples were taken on 15 DAT (29 April), 29 DAT (13 May), 50 DAT (3 June), 80 DAT (3 July), 106 DAT (29 July), and 134 DAT(26 Aug).

Wood Core. Penthiopyrad was first detected at 1.64 ppm in 1 injection (injected 50%/50% into 2 different sets of holes) wood core samples recovered 6 inches below the injection site at 29 DAT (13 May), 9.39 ppm at 80 DAT (3 July), and 20.57 ppm at 134 DAT (26 Aug) (Figure 38). Residues detected 6 inches above the injection site were 3.96 ppm at 29 DAT (13 May), 18.32 ppm at 80 DAT (3 July), and 40.54 ppm at 134 DAT (26 Aug) (Figure 38). Residues detected at the base of the main scaffold branch were 1.08 ppm at 29 DAT (13 May), 1.33 ppm at 80 DAT (3 July), and 1.65 ppm at 134 DAT (26 Aug) (Figure 38).

Propiconazole was first detected at 0.99 ppm at in 2 injections (injected 50%/50% into 2 different sets of holes) wood core samples recovered 6 inches below the injection site at 29 DAT (13 May), 1.42 ppm at 80 DAT (3 July), and 1.30 ppm at 134 DAT (26 Aug) (Figure 39).

Residues detected 6 inches above the injection site were 0.0 ppm at 29 DAT (13 May), 2.00 ppm at 80 DAT (3 July), and 2.75 ppm at 134 DAT (Aug 26) (Figure 39). Residues detected at the base of the main scaffold branch were 0.0 ppm at 29 DAT (13 May), 0.68 ppm at 80 DAT (3 July), and 0.67 ppm at 134 DAT (26 Aug) (Figure 39).

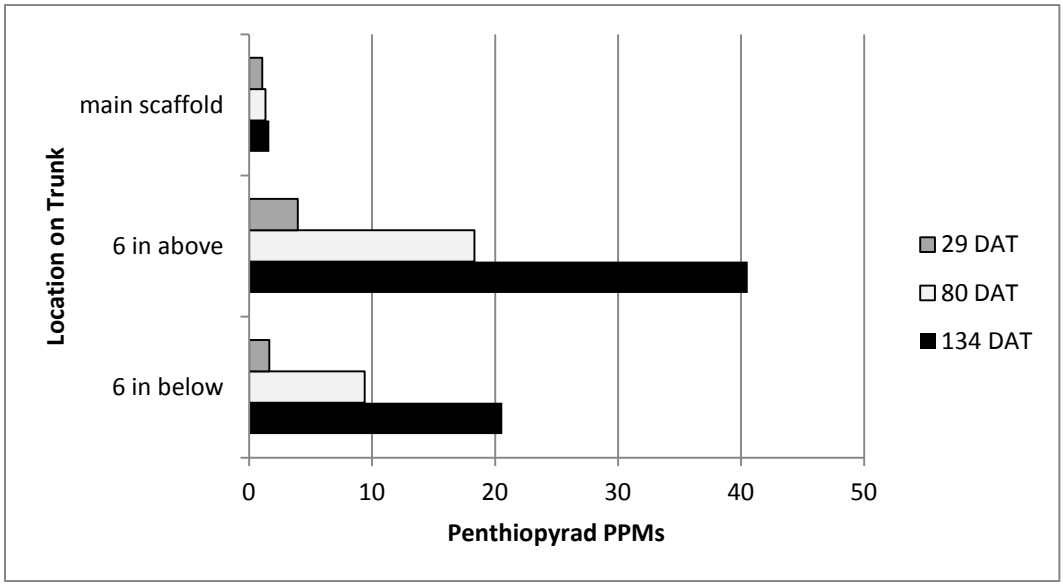

Figure 38. Mean residue recovery (ppm) from penthiopyrad 1 set of injections (injected 50%/50% into 2 different sets of holes) residue samples of wood core tissue, 6 inches below injection, 6 inches above injection, and at the base of the main scaffold branch. The injections were on April 14, 2011 and samples were taken at 29 DAT (13 May), 80 DAT (3 July), and 134 DAT (26 Aug).

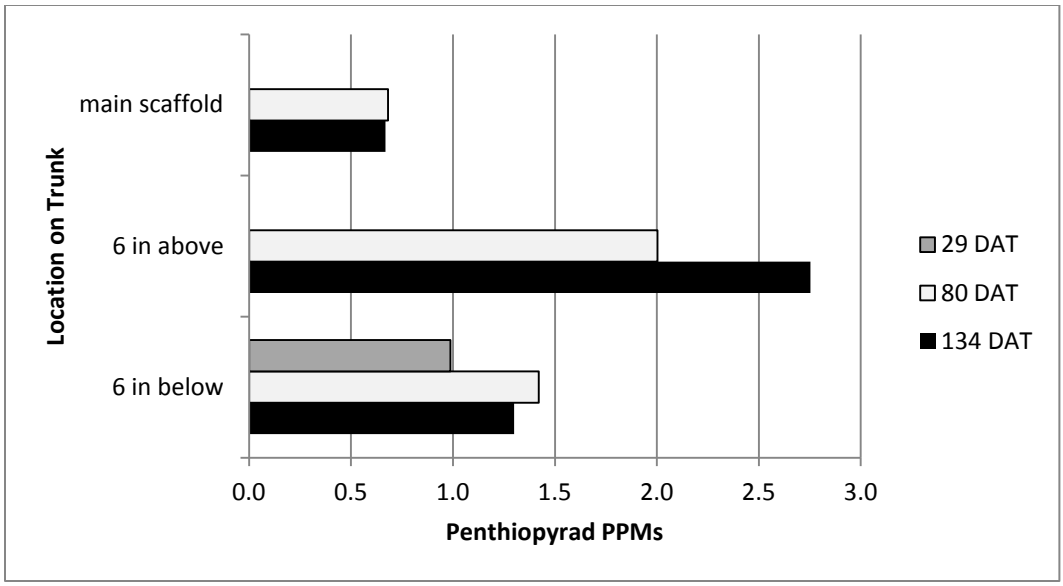

Figure 39. Mean residue recovery (ppm) from penthiopyrad 2 sets of injections (injected 50%/50% into 2 different sets of holes) residue samples of wood core tissue, 6 inches below injection, 6 inches above injection, and at the base of the main scaffold branch. The injections were on April 14, 2011 and samples were taken at 29 DAT (13 May), 80 DAT (3 July), and 134 DAT (26 Aug).

Foliar Phytotoxicity

Visual qualitative photographical data estimations were recorded for the extent of foliar phytotoxicity from the fungicides propiconazole and phosphorous acid caused. We were able to conclude with the fungicides, the single injection regime with high rates caused increased phytotoxicity for propiconazole and phosphorous acid on the Rome apple tree (Figure 40-45). Weather conditions played a role with translocation and phytotoxicity, but differences between seasons were minimal on account the weather was very similar with cold overcast conditions in April. All photographs were taken of maximum phytotoxic effects during the growing season (Figure 40-45).

Figure 40. Extent of phytotoxicity of phosphorous acid (right) compared to propiconazole (left) from the 2010 injection 38 DAT (May 10).

Figure 41. Phosphorous acid terminal shoot foliar phytotoxicity from the 2010 injection 38 DAT (May 10).

Figure 42. Extent of phytotoxicity of phosphorous acid (left) compared to propiconazole (right) from the 2010 continuation injection 1 year 62 DAT (3 June).

Figure 43. Extent of phytotoxicity of phosphorous acid (right) compared to propiconazole (left) from trees with a larger DFH more capable of withstanding the full application of phosphorous acid from the 2010 continuation injection 1 year 89 DAT (12 July).

Figure 44. Extent of phytotoxicity of phosphorous acid single injection full volume from the 2011 injection 35 DAT (19 May).

Figure 45. Extent of phytotoxicity of propiconazole 2 injection 20/80% split high rate from the 2011 injection 35 DAT (19 May).

Information on the foliar phytotoxicity of specific rates using alternate injection regimes of compound formulations such as phosphorous acid and propiconazole were discovered. Phosphorous acid was shown to have maximum phytotoxicity and propiconazole was shown to have minimum phytotoxicity with total volumes injected with a single application. The 2011 injection results indicate that reduced rates and/ or a multiple injection regime (20%/80% split) may reduce the foliar phytotoxicity effects of phosphorous acid and propiconazole. Reduced rates also indicated reduced persistence and scab control. This indicates that multiple small dose

injection applications are necessary for scab control and minimum phytoxicity. New fungicidal injection formations with increased persistence may alter this conclusion with reduced applications.

Discussion

This research contributes important new information about the potential of trunk injection for delivering fungicides to apples to control fungal pathogens such as the apple scab fungus (Venturia inaequalis). The results from 2010 and 2011 show initial variable control between the three fungicides: propiconazole, phosphorous acid, and penthiopyrad. The phosphorous acid treatment regimes provided variable, but significant levels of control, better than what would be expected from a foliar delivery system. Propiconazole showed some significant activity on apple scab, but with a limited duration of control. Penthiopyrad showed very low activity on apple scab, far less than what would be expected with conventional foliar sprays.

Phosphorous acid injected in the 2010 season initially severe foliar phytotoxicity, but provided high levels of plant protection for two field seasons. In fact the levels of apple scab control in the second season were better than what was seen in the 2011 freshly injected trees. This suggests that when optimally distributed in the tree, lower rates of phosphoric acid are adequate for apple scab control. This may also be a function of SAR effects contributing to the overall plant protection. The 2010 foliar residue profiles show high maximum residue levels of 127.6 ppm at 7 DAT (9 April), representing very fast translocative to the foliage, but is also responsible for the observed phytotoxicity. Residues then fall 7 days later and remain under 5 ppm the entire season, which suggests again that minimal levels in foliage are needed for control.

The 2011 injection regimes of injecting the total volume into the same set of holes or injecting 20% volume into one set and 80% into the second set of holes showed much less phytotoxicity with less volume being injected at one time, but with minimal scab control. It is not clear what performance constraints are responsible for the overall weaker scab control in the 2011 injection plots. The residue data for the 2011 phosphorous acid study is not availble.

The limited activity of trunk injected propiconazole on apple scab suggests there were performance constraints related to resistance, persistence, translocation, or timing. The residue profiles show that propiconazole has slow translocative abilities with detectable residue levels 15 DAT and peak levels between 100 and 130 DAT of which there is minimal active ingredient located in the foliage. The leaf residue profiles in the following season show a similar trend in the low rate, but at much lower levels. The trunk core residue samples provide evidence for a reservoir effect, with woody tissue being the source of propiconazole, but there is minimal translocation to the canopy in the second season. This suggests there is limited reservoir effect "feeding" the canopy. The residues recovered in apple fruit were much lower than those in leaves. The low fruit and foliar residues likely explains the lack of control of leaf and fruit scab. Fruit residue levels were also far below the EPA MRL for apple.

Penthiopyrad showed minimal scab control over the 2011 growing season. There were virtually no differences in the percent infection compared to the control with both injection regimes. The leaf residue profiles of the single and double injection show that the first detectable residue is 15 DAT, which indicates slow translocation. The active ingredient is translocated to the foliage at a maximum level of 2.23 ppm at 29 DAT then decreases rapidly to 0.12 ppm 20 days later at 50 DAT, leaving minimal foliar residue. The residue profiles of the 2 injection, 20/

80% split shows more consistent foliar residue levels with 2 residue peaks, the first maximizing at 29 DAT and the second at 106 DAT. The wood core residue profiles show low levels of active ingredient above and below the injection point 29 DAT, but high levels at 80 and 134 DAT suggesting delayed translocation. There were also residue levels found in the scaffold branch that are higher than the other compounds and no residues found in the fruit indicating the slow movement through the vascular system unable to translocate to the foliage. The cause of minimal foliar and fruit scab control with the different injection regimes is likely the result of limited active ingredient in the foliage caused by poor translocation. The high viscosity of penthiopyrad and propiconazole are likely responsible for the poor translocation and injecting difficulties. These types of compounds may perform better if diluted in water, but may also result in increased tree injury and labor time with a greater volume injected.

Apple scab protection in apples requires application before the first main infection period, which generally occurs at the first spring rain in late March or April when green tissue is present. The combination of limited green tissue, and cold and overcast days results in minimal xylem translocation. To overcome this obstacle the number of applications and application volumes should be minimized. Preparing active ingredients in formulations designed for optimal translocation, low in viscosity and high concentration of active ingredient, will also help.

Trunk injection is generally considered non-economical for commercial fruit production, because of the time efficiency of tractor-driven ground sprayers for pesticide delivery. At this point in time the upfront cost of the injection equipment, labor, and price of concentrated formulated compounds make it uncompetitive. If injection technology can be redesigned to address the constraints of commercial fruit production, then the benefits may be more attractive.

Limited applications, no concern for wash-off or worker exposure, long duration of control with minimal fruit residues continues to be attractive benefits of this delivery system. The 2010 and 2011 injection research answers many questions, but more research is needed to address other problems. Future research is necessary for additional information on compound persistence, volumes and rates, compound mode of actions, compound transport to fruit and flower tissue, and injection timings. Information is needed for new compound formulations and how they translocate, persist, and act on the disease pest. Specific injection compound formulations need to be continually modified for improved for translocative abilities so they can act efficiently on the pest at the correct doses persistently. With an efficient formulation, accurate application volumes and rates can be calculated along with overall cost of application. Injection timings need to be modified for efficient pest control. The compound needs to be in the target plant tissue before the infection. Additional information on the translocation to the non-target tissue such as the fruit and flower are also needed. It is known that after 2 seasons there are minimal residues in fruit and flower tissue, but future seasons and new formulations may yield different results.

With continued research there is great potential adopting this new application technology to the agricultural system with much success. It may lead to improved tools, compound formulations, and injection techniques. Injection technology may be a cost, time, and labor efficient pest control method in agriculture, safe for the applicator and the environment.

CHAPTER 4: TRUNK INJECTION-VASCULAR MOVEMENT AND WOUND HEALING

Abstract

With the current commercial injection tools, trunk injection allows the opportunity for negative impacts on tree health. These impacts include hole drilling, phytotoxicity, embolism, and damage to vascular tissues in the trunk. With the current compound formulations, there is substantial persistence, but in time there is the need for additional injection applications to be made, which means more holes or injuries to the tree. One way to minimize negative impacts is to decrease the number of injuries by injecting into the same ports for additional applications. In this dye injection study, results showed that there is possibility of injecting compounds into the same ports for up to 14 days after the original application before translocation is comprimized. The volume or exact distance traveled by the dye is unknown, but dye was located between 1 and 5 inches above and below the original injection site after each injection interval. This study helps expand the current knowledge on the science of trunk injection and support an improved method of pesticide application in tree fruits.

Introduction

With conventional drill-based trunk injection systems there is considerable wounding to the tree. Three major long and short term risks for injury include destruction and loss of woody tissue, the resulting presence of air in the cavity, and risk of insect and disease infestation. Different trees species react in different ways to the injection wounds and to the compounds

injected (Santamour, F. S., Jr 1986, sited by Perry 1991). Fruit trees are particularly vulnerable to trunk injection injury due to their smaller size and trunk diameter.

Drilling a hole through the outer bark and exposing the inner vascular tissue creates risk of disease and insect infestation. The bark tissue is the outer "skin" that protects the tree from foreign invaders. Breaking or severing the bark in any way may allow foreign invaders, such as chemicals, insects, and diseases, to hinder it. As Perry states, "New populations of organisms can invade the tree at each injection site. The populations can multiply rapidly, damage the tree, and also induce the secretion of phenols and other substances (1991)". Second, the drilling is removing an essential part of the tree stem and replacing it with air. This area of the tree is damaged or lost and water/ nutrient flow can no longer pass through the xylem stream freely. Once the wound is made, the area undergoes a process called compartmentalization, which is the process of wound healing and a response to the injury. When the inner (below the bark) tissue is wounded the tree naturally heals itself by changing the cells and forming a wall around the wound to prevent the spread of any disease or further injury. The tree then grows around this healed area. This results in a disruption of the natural directional stream of xylem tissue and flow of material. After the primary injection, a secondary injection port is eventually required as a result of wound healing and compartmentalization of the drilled injection port. Trunk injections are typically associated with columns of compartmentalized xylem (Perry 1991).

This wounding setback may be resolved with adapting injection tools that cause minimal injury to the tree."We must develop alternate techniques for applying or inducing the uptake of substances into trees without wounding or harming them" (Perry 1991). At this point in time trunk injection may induce long term harmful effects to the overall health of the fruit tree. Injecting does not necessarily mean the plant has to be harmed in the process. Injecting plant

tissue is known to be non injurious. According to Roach, "When a liquid is introduced into a plant through a cut or hole in one of its organs this is also called injection, even when little or no force is used. Again, a plant may be injected in this sense without recourse to wounding of any kind" (Roach 1939). Continued research on application techniques is necessary to adapt this technology to agriculture with minimal injury. The purpose of this study was to determine the extent of uptake and translocation of the drilled injection wound after the primary injection, which occurs immediately after the drilling process. This information suggests a healing time of the injection wound and number of injection applications that a single injection site allows.

Methods and Materials

Field Plots and Applications

Four Rome apple trees (*Malus domestica* Borkhausen) at the MSU Trevor Nichols Research Center in Fennville, MI, USA (latitude 42.5951° : longitude -86.1561°) were injected with trunk dye (brand, city, state) at different time intervals to determine the length of time after drilling that trunk xylem will actively translocate solutions. The injections began July 5, 2011 when the trees were fully flushed with normal fruit load. The intervals were 0 DAT (treatment 1) (July 5 or same day), 1 DAT (treatment 2) (6 July), 7 DAT (treatment 3) (12 July), and 14 DAT (treatment 4) (19 July). To begin all four trees were injected with a 1 ml equal dose water injection so the trees had a primary injection before the secondary dye injection, which was a 3 ml equal dose injected into the same hole. The trunk dye was purple consisting of 1% basic

fuchsin. After the water injections on all four trees, the first dye injection took place for the 0 DAT treatment approximately 30 min post water injection.

The injection equipment included an Arborjet Quick-jet™ injector, #4 arbor plugs (Arborjet Inc. Woburn, MA), plug tapper, hammer, cordless drill, and a 3/8 inch wood drill bit. The injection system was sanitized before each injection with the Arborjet Cleanjet™ solution and water to rinse any residues or pathogens and get the pump primed. Next, the dye was poured into the Arborjet injector holding tank. Extra solution was added to allow priming of the tank. The needle was then attached to the injector tip and the pump was primed so there were no air pockets causing inaccurate applications. The injector was put aside and the holes were drilled into the apple trunk 2 inches deep, 90 degrees horizontal from the trunk, and 12 inches above the ground using the Arborjet drill bit. The holes were strategically placed under main scaffold branches to orient with the vascular physiology of the tree. The holes were not placed above or below any trunk injury as to avoid the dye from leaving the tissue through those wounds. The plugs were then tapped in place so the outside rim of the plug was just beneath the bark. The needle was then inserted into the plug so the base of the needle was tight against the lip of the plug. The dye was then injected at the desired rate into the plug. The Arborjet injector has built-in graduated cylinder so the amount per application is accurate to the nearest quarter of a milliliter.

Field Evaluations

In order to locate where the dye had translocated, the trees were destructively dissected. Each of the four treatments was evaluated one week post dye injection. All four treatments were analyzed using the same methods as the results were unpredictable for each. The process began

by pulling the entire tree with root system out of the ground on the corresponding evaluation day with a back hoe and brought to a storage barn for further study. All of the smaller branches (less than 1 in diameter) were trimmed off using pruning loppers in order to have easy access to the main trunk and scaffold branches. The trunk and scaffold branches were cut horizontally into one inch segments (cookies) using a Sawzall reciprocating saw. To cut the one inch slices equally the entire length of the trunk was measured and marked above and below the injection point with a sharpie marker. A solid line was also drawn vertically up the trunk along the injection point to keep all the slices spatially in line with the vascular system. The first cut was at the injection point, which was cut straight across the hole and through the plug. The drilled hole which creates a cylinder cavity was cut in half creating two half cylinders in the cookies. From there the slices were cut below the injection point all the way to the roots, which was between 11-13 inches or 11 cookies. These were stacked in order as they were cut to keep them organized. The trunk was sectioned off to the roots and a small slice was taken from one of the larger roots. Then the above-injection-point cuts were made, which were sliced until the trunk split off into scaffold branches. There were between 60-80 slices above the injection point on all four trees. One slice was taken from one of the main branches. All the cookie slices were stacked in order and lined up with the sharpie line. They were brought back to the lab for photographic documentation and comparative analysis. Each cookie was placed on paper with a red line in order from closest to injection point to furthest. The red line was lined up with the black sharpie line on the cookie before being photographed to keep track where the injection point was and locate any dye or movement of dye. After the first photograph each cookie was flipped over and photographed a second time to record both sides. After both sides of the each whole cookie was photographed they were quartered and all of the inside edges were photographed. The cookies were all held

with the injection point facing a specific direction to keep track where the dye is expected to be located. The treatments were compared visually by locating where and how far the dye traveled. The volume was also compared visually to see which treatment allowed the most dye to be translocated.

Results

Field Evaluations

Visual qualitative photographical data estimations were recorded for the location and distance of dye that traveled through the vascular system. We were able to conclude that the physiology of the Rome apple tree allows dye translocation for a certain distance and volume into the same port for 2 weeks after the original injection. All photographs were taken of dye locations found at the maximum distance from the injection site.

The results were limited with no quantifiable data for the total distance translocated throughout the tree, the location or path the dye traveled in the upper portions of the tree, or how long will the injection port allowed translocation before the wound heals and slows or turns off all translocative abilities.

Treatment 1 (0 DAT) resulted in translocation up to 1 inch above the injection point. Beyond 1 inch the dye was not visible (Figure 1). Translocation downward occurred 4 inches below the injection point. Beyond 4 inches the dye was not visible. Figure 2 shows the dye location 3 inches below the injection site.

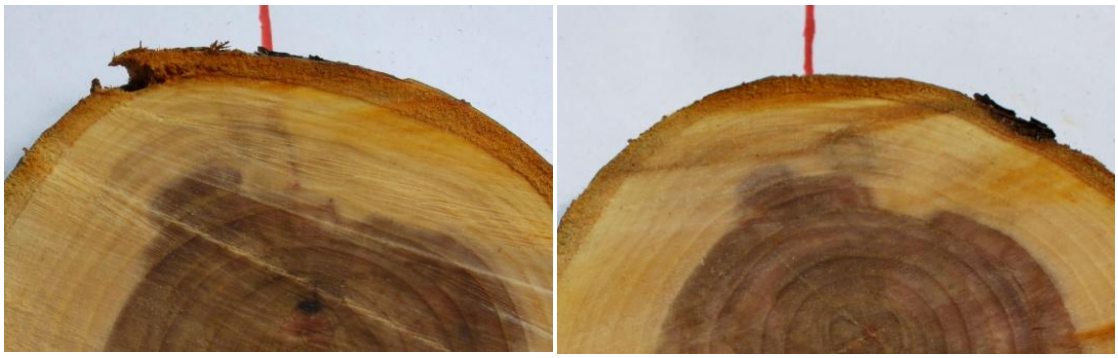

Figure 46. Dye location 1 inch above the injection site (left) and dye location 3 inches below the injection site (**Figure 47**) (right). The red line indicates the injection location.

Treatment 2 (1 DAT) resulted in translocation up to 1 inch above the injection point through the xylem and 3 inches above the injection point through the pith of the heartwood (Figure 3). Beyond 1 inch the dye was only located in the heartwood pith. Translocation downward occurred up to 4 inches below the injection point, which is not yet into the root system or root flare. Below 4 inches the dye was not visible. Figure 4 indicates the extensive movement downward at 1 inch below the injection site.

Figure 48. Dye location from a quartered cookie, 1 inch above the injection site (left) and dye location from a halved cookie, 1 inch below the injection site (**Figure 49**) (right).

Treatment 3 (7 DAT) resulted in translocation up to 3 inches above the injection point. The dye was located in the xylem and pith of the heartwood tissue. Beyond 3 inches the dye was not located in xylem or pith. Translocation downward occurred up to 1 inch below the injection point. There was dye found in the xylem and pith of the heartwood. Below 1 inch of the injection point there was no dye found in the xylem or pith (Figure 5).

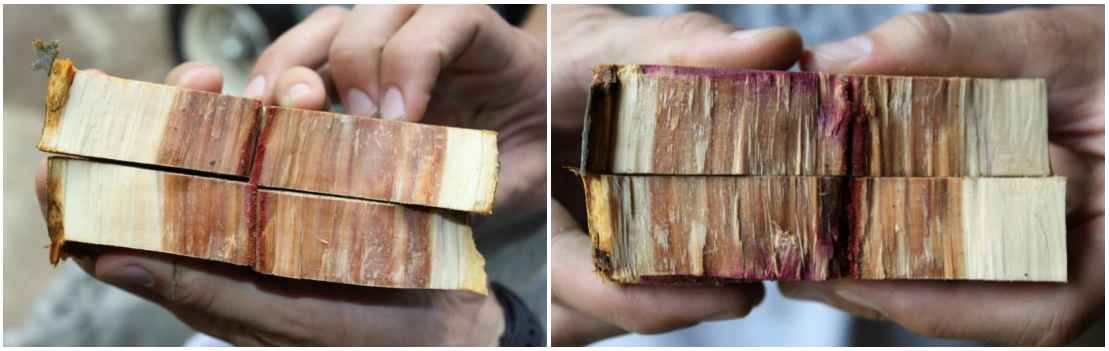

Figure 50. Dye location from cookie cut in half, 1 inch above the injection site and dye location from cookie cut in half 3 inches below the injection site (**Figure 51**).

Treatment 4 (14 DAT) resulted in translocation up to 5 inches above the injection point. The dye was located in the xylem and pith of the heartwood tissue. Beyond 5 inch the dye was not visible (Figure 7). Downward translocation of dye was not visible in any tissues (Figure 8).

Figure 52. Dye location 5 inches above the injection site (left) and no dye located 1 inch below the injection site (**Figure 53**) (right).

Discussion

The dye injection study contributes information about the wounding associated with the trunk injection technology. With the results obtained conclusions were made that the injection wound caused by drilling begins to slow wound translocation after 1 week. Seven and fourteen days of healing time caused portions of the dye injection to get pulled to the center of the heartwood or pith, which may be caused by the water concentration gradient. The center heartwood acts as a "sponge" absorbing the fluids that cannot be translocated through the healed or compartmentalized xylem tissue.

These data collected in the 2011 answers many questions and leaves many to be answered on the healing time of the drilled injection wound and the translocative abilities of the compartmentalizing wood tissue. Future research is necessary for additional information on less injurious injection techniques, compartmentalization process, compartmentalization time, compound persistence, volumes and rates, compound translocation through wounded tissue, injection timings, and the extent of wounding the injection method causes. Information is needed for new compound formulations and how they translocate, persist, and act on the wood and foliar tissue in terms of phytotoxicity and decay. Specific injection compound formulations need to be continually modified for improved for translocative abilities and reduced negative effects and act efficiently on the pest at the correct doses persistently. Additional information on the translocation and negative effects to the non-target tissue such as the fruit and flower are also needed.

With continued research there is great potential adopting this new application technology to the agricultural system with much success. It may lead to improved tools, compound

formulations, and injection techniques. Injection technology may be a cost, time, and labor efficient pest control method in agriculture, safe for the applicator and the environment

APPENDICES

APPENDIX 1

Record of Deposition of Voucher Specimens

The specimens listed below have been deposited in the named museum(s) as samples of those species or other taxa which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached to all specimens deposited.

Name: Anthony VanWoerkom Voucher No. 2012-05

Title of Thesis: Trunk Injection: A New and Innovative Technique for Pesticide Delivery in Tree Fruits

Museum(s) where deposited:

A.J. Cook Arthropod research Collection, Michigan State University (MSU)

Other Museums: None

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Curculionidae	*Conotrachelus nenuphar*	adult	10 ♂, 10 ♀	Pinned
Tortricidae	*Choristoneura roseceana*	adult	10 ♂, 10 ♀	Pinned
Tortricidae	*Cydia pomonella*	adult	10 ♂, 10 ♀	Pinned

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