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Research Article

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Trunk Injection: A Discriminating Delivering System for Horticulture Crop IPM

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Abstract

Trunk injection technology represents an alternative delivery system to provide crop protection for horticultural crops of commercial and smallholder farmers in the developed and developing world. Field studies, laboratory bioassays, and residue profile analysis were used to determine the seasonal effectiveness of trunk injected insecticides against key apple insect pests. Insecticides formulated for trunk injection, imidacloprid, rynaxypyr, and emamectin benzoate were injected into semi dwarf Empire apple trees and evaluated for a wide range of insect pests. Imidacloprid controlled piercing and sucking pests, and emamectin benzoate controlled leaf rollers, Oriental fruit moth, and leafhoppers. The residue profiles for insecticides showed that vascular delivery was predominantly to foliage, with fruit residues far below the EPA maximum residue limits These results suggest that trunk injection is a promising delivering system for plant protection materials for control of foliar pests, while minimizing impacts on natural enemies, eliminating spray drift, and reducing the pesticide load in the agro-ecosystem. For smallholder farmers this low-capital investment technology has the potential to significantly reduce the human health risks associated with pesticide use, while protecting high value horticultural crops from pests.

Keywords: Trunk injection; Horticultural crops; Smallholder farmer; Pesticide residues; Integrated pest management

Introduction

The global demand for horticulture crop production is on the rise, largely in response to public awareness of the associated health benefits of fruits and vegetables, as well as for their preventative attributes to various forms of cancer and heart disease [1]. Fruit crops in particular are receiving heightened attention because they are an important source of cholesterol free, fat free, dietary fiber that lowers cholesterol. In support of these facts, the U.S. FDA has reordered fruits and vegetables as to their importance for human dietary intake, as well as their place in the Food Pyramid [2]. This trend is well documented in developed nations like the USA and European Union, but is also gaining attention in developing countries around the world. For Northern hemisphere regions the leading fruit crops include apples, pears, cherries, peaches, grapes and blueberries. For Southern hemisphere regions the leading fruit crops include mangos, citrus, bananas, oranges, avocados and papayas [3].

Profitability in global food markets requires meeting high food quality standards, often through the judicious use of crop protection materials, including pesticides [4]. Corporate buyers, however, increasingly demand blemish free fruit while maintaining extremely tight limits for pesticide residues on incoming produce [5]. This results in a market niche that is very difficult or impossible for many of the world's farmers to attain.

Effective use of pesticides in an Integrated Pest Management (IPM) program requires precise delivery of selected materials to the crop canopy [6,7]. While farmers in developed countries reply upon mechanized sprayers to deliver crop protection materials, smallholder farmers in developing countries often rely upon hand-held sprayers, which are far less efficient. Even with modern mechanized sprayers the efficiency of pesticide delivery is less than ideal. Scientists, like Pimentel [8], estimate that with conventional ground sprayers as little as 0.4% of the pesticide contacts the target pest. Other studies show that air blast 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 [4,9-11] (Figure 1).

Small holder farmers' use of hand-held sprayers is inefficient and often results in excessive worker exposure to the pesticides being applied. Thus the risks of pesticide exposure to the health of farmers and farm workers remain a critical concern in the developing world. Pesticides are estimated to be responsible for 4% of all deaths from all accidental poisonings, and health risks from pesticide exposure are magnified for poor farm workers [12]. Although smallholder men are

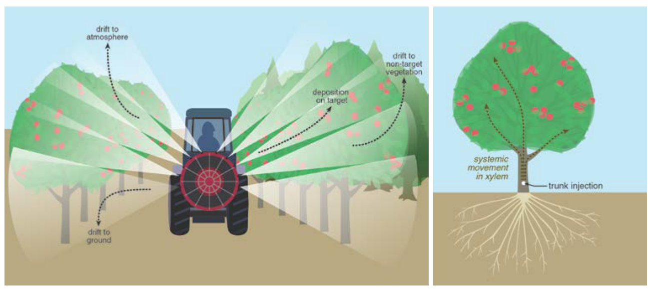

Figure 1: Non-target drift from airblast sprayer foliar sprays versus trunk injection delivery (images by Marlene Cameron).

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at the highest risk of exposure from direct handling of pesticides, since 70% of the field labor tasks are supplied by women, they are at higher long-term health risk from exposure to treated plant materials [13]. Therefore, the smallholder farmer is caught in a very difficult dilemma. In order to produce the high quality horticulture crops demanded in the market place, he/she needs access to crop protection materials. But without effective delivery methods their crop will not meet market standards, thus putting financial self-sufficiency in jeopardy. These small farmers typically do not have the necessary access to capital to purchase the expensive equipment used in modern commercial crop production. Thus, many of them sell to contractors, who hold control over the marketable crop and production practices, and ultimately reduce the smallholder farmers' full potential for profitability. There are additional risks associated with pesticide exposure that can result in short and long-term health problems to the farm family and farm workers.

In developed countries the inefficiencies of mechanized ground sprayers not only contribute to non-target pesticide contamination, but excessive tractor-sprayer use also results in soil compaction and erosion, as well as increased energy and labor costs [14]. The proximity of the commercial fruit production acreage to major population centers both provides economic opportunity related to "local markets", but also brings tensions related to the agriculture-urban interface. Horticulture crop farmers also face increasing US/GAO scrutiny of pesticide use around the sensitive bodies of water like the US Great Lakes: the elimination of toxic pollutants, especially pesticides, is a primary goal of the USA and Canada. As regulatory scrutiny on water quality, carbon abatement, and non-target drift increases, and the high cost of pesticides exacerbates the penalty of wasting active ingredient, the need for creative investigation of alternatives heightens [15].

Trunk injection as an alternative delivery system

Trunk injection represents an alternative delivery system for crop protection materials to horticultural crops. Arborists have developed a variety of techniques for injecting pesticides directly into tree trunks. Most of these methods require delivery of relatively small amounts of a given compound per injection, and are referred to as 'micro-injections'. To be effective the injected compounds must be translocated from the injection site to the canopy location of insect feeding or disease infection (Figure 1). Once in the xylem, chemicals are dependent upon the transpiration stream to move upward and be distributed throughout the tree canopy [16,17].

Trunk injection has been successfully used in protecting ash trees from the Emerald ash borer (EAB) [18-20]. Adult EABs feed on foliage during the summer and larvae feed on phloem (under the bark) until late autumn [21]. Trunk injection of systemic insecticides has become the preferred method for controlling EAB in urban and suburban landscapes because of minimal risks of applicator exposure, drift and impacts on non-target organisms [18]. Trunk injection has also been shown to have positive application to tropical and sub-tropical tree fruit crops, such as of avocado, citrus and palm, addressing serious disease and insect pests of these high value horticulture crops [16,22-24].

Protecting apple trees, *Malus domestica* Borkhausen, from insect pests can require as many as eight insecticide applications per season [25]. The most serious world-wide arthropod pests of apples include codling moth (*Cydia pomonella* (L.), Oriental fruit moth, *Grapholita molesta* (Busck), oblique banded leaf roller, *Choristenura rosaceana* (Harris), rosy apple aphid, *Dysaphis plantaginea* (Passerini), San Jose scale (*Quadraspidiotus perniciosus*), white scale (*Pseudoulacaspis* sp.), European red mite (*Panonychus ulmi* (Koch)), wooly apple aphid (*Eriosoma lanigerum*), and apple fruit moth (*Agyresthia conjugella*) [26]. Many of the new pesticides registered in the last decade have received Reduced-Risk status by the U.S. Environmental Protection Agency [27]. They have been shown to be effective in controlling key fruit insect pests, but require precise application timing in relation to insect development and excellent canopy coverage to maximize efficacy [28,29]. Compared to conventional organophosphate insecticides, Biopesticides and reduced-risk insecticides tend to be short-lived in the environment. Another difference for Biopesticides and reduced-risk insecticides is that delivering the poison to the target pest by ingestion is far more effective than relying on contact activity. The challenge, therefore for modern environmentally safe compounds is to enhance the ingestion exposure of the material to the pest and improve in-plant distribution and longevity of the material in the crop. Trunk injection of reduced-risk insecticides and Biopesticides offers a superior means of delivering these materials to the target pest for optimal exposure.

Broad impacts on smallholder agriculture

Trunk injection represents an alternative delivery system to provide quality crop protection for smallholder apple farmers in the developing world. The potential broad positive impacts on smallholder farmers of horticultural crops world-wide are numerous. *Improved yields and profitability;* the preliminary studies suggest that trunk injection is a low-capital investment means of delivering plant protection materials to fruit trees, providing superior seasonal control of foliar pests, thus allowing smallholder farmers to access high value markets that were previously unavailable because of financial constraints. *Reduced pesticide loading in the environment*; this means of product delivery has the potential of dramatically reducing the amount of pesticide needed to protect crops from pests, thus lowering risks of environmental contamination and minimizing impacts on natural enemies [30]. *Enhancing health and safety of men and women farm workers*; trunk injection eliminates the short and long-term risks associated with pesticide spray drift, and direct exposure to harmful residues on plant surfaces. For smallholder men the risk of direct exposure to spray drift while applying pesticides is eliminated by this delivery system. For smallholder women the long term exposure to pesticide residues on treated crops and the orchard surroundings is eliminated, such that their cultivation and harvest activities can be performed in a comparatively safe environment.

The objectives of this 'proof of concept' study in apples were to 1) field test trunk injected insecticides for control of key insect pests, and 2) characterize seasonal residue profiles in plant tissues.

Materials and Methods

Trunk injection experiments in the orchard were conducted at the Michigan State University Trevor Nichols Research Center in Fennville, MI (N42º 35' 40.81", W86º 9' 20.10").

Field test trunk injection of insecticides for control of key insect pests of apples

Injection of insecticides: On 18 June 2009 mature semi-dwarf trees of apple cultivar 'Redmax McIntosh', *Malus domestica* Borkhausen, were injected with each of two insecticides formulated for trunk injection: imidacloprid 5% (Ima-jet™, Arborjet Inc., Woburn, MA), and emamectin benzoate 4% (TREE-age™ Arborjet Inc., Woburn, MA), using a dose of 0.2 g of active ingredient (a. i.) per 25.4 mm of trunk DFH (diameter at 30.48 cm or 1 ft of trunk height). Trees were approximately 15 cm in trunk DFH. Imposed temporal distribution treatment included trunk injection delivery through four delivery ports, equally distanced along the trunk circumference. Individual dose per tree was divided equally among the four delivery ports. Each of the experiments was arranged as a completely randomized design (CRD), with three replicate trees per treatment.

In a separate set of trees, treatment applications were made at apple petal fall stage on 5 May 2010 with imidacloprid 5% (Ima-jet™), and emamectin benzoate 4% (TREE-age™). Injections were conducted on 12.7-15.24 cm DFH semi dwarf Empire apple trees (*Malus domestica* Borkhausen); with five replicate trees per treatment in a completely randomized design. Predetermined low and high rates of each compound were injected at volumes depending on tree DFH. A low rate of 0.2 g Active Ingredient (AI) per 25.4 mm of trunk DFH and high rate of 0.4 g AI per 25.4 mm of trunk DFH were injected for all insecticides. All the experiments initiated in 2010 continued to be evaluated into the 2011 season, are referred to as 2010 continuation.

Trunk injections were performed by drilling delivery ports into the xylem trunk tissue of 25.4 mm (1 in) in depth and of 9.53 mm (3/8 in) in diameter, and treatment solutions injected via needle of Quik-jet inserted through the one-way valve silicone septum accessible from the outer side of Arborplugs (Arborjet Inc., Woburn, MA), into the freshly drilled reservoir. All of the delivery ports were compass oriented according to the cardinal direction sides (N, S, E, and W), positioned approximately 30.48 cm (1 ft.) above the ground level, and proportionally separated between the opposing port pairs.

Field evaluations of season-long insect pest control: 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 2009, 2010 and 2011 seasons. All treatment replicates were rated at specific days after treatment (DAT) intervals around the entire tree using the same evaluation techniques for each of the target insect species. Trees were rated for Oriental fruit moth (OFM) apple terminal "flagging" injury and spotted tentiform leafminer *(Phyllonorycter blancardella)* (Fabr.) (STLM) leaf mines after two minutes of observation, and presence of potato leafhopper *(Empoasca fabae)* (Harris) (PLH) was measured by randomly selecting twenty shoots per replicate and counting the number of live nymphs per shoot. Statistical comparisons were made on square root transformed (SQR(X+0.5)) data, with treatment comparisons using ANOVA in ARM 7.2 [31]. Mean separations were done using Tukey's HSD ($\alpha = 0.05$).

Temporal performance bioassays: Semi-field bioassays were conducted at three different time intervals in 2010 and 2011, to provide a controlled temporal of performance over the season. For each of the five tree replicates per treatment there were 8 leaf punches (2.4 cm) collected from two leaves (upper crown, lower crown) on each cardinal direction side (N, S, E and 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) and the punched leaf sections were placed inside. The puncher was sterilized in acetone between each treatment. Oblique banded leaf roller (OBLR) larvae from a continuous laboratory colony, maintained at 25°C in a walk-in environmental chamber with a photoperiod of 16:8 (L: D) h, were used for the bioassays. Five OBLR 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 and placed in a walk-in environmental chamber at 25°C with photoperiod of 16:8 (L: D) h. After one week leaf area consumed was calculated. The eight leaf disks for all five tree replicates of each treatment were computer scanned against white copy paper and percent damage calculation was made available. Using Photoshop Elements 8, the number of white pixels (consumed region) and green pixels (intact foliage) were measured. The pixel data were transferred to Microsoft Excel and percent foliage consumed was calculated. Statistical comparisons were made on arcsine square-root percent transformed data transformed data, with treatment comparisons using ANOVA in ARM 7.2. Mean separations were done using Tukey's HSD ($\alpha = 0.05$).

Characterize residue profiles in plant tissues.

Season-long residue profile in fruit and leaves: Residue samples were collected in 2009 from each treatment plot (replicate trees 1-3) at 1, 7, 14, 30, 45 and 75 days after application (DAT). Leaf (10 g) and fruit (20 g) samples for each treatment (reps 1-3) were collected and held in dichloromethane for HPLC analysis at the MSU Pesticide Analytical laboratory. Residue samples were analyzed with a Waters 2695 Separator Module HPLC equipped with Waters Micro Mass ZQ mass spectrometer detector (Waters, Milford, MA), and Waters X-Bridge C18 reversed phase column (50×3.0 mm bore, 3.5 µm particle size, Waters Corp., Milford, MA) (Bayer, 1998). The mobile phase, solvent A, was water with 0.1% formic acid, and solvent B was acetonitrile with 0.1% formic acid, and was initially held at 80% solvent A and 20% solvent B and followed by a gradient. Column temperature was 40°C. Monitored ions for imidacloprid were 209 and 179 m/z (Da), and ions for emamectin benzoate were 872.2 and 886 m/z (Da). The HPLC level of quantification for imidacloprid was 0.08 parts per million (ppm) of AI, and level of detection was 0.016 ppm. The HPLC level of quantification for emamectin benzoate was 0.00065 ppm of AI, and level of detection was 0.00013 ppm.

Wood tissue samples: In 2011 a series of wood tissue residue samples were taken from three of five replicates of the 2010-continuation treatment trees, and combined as composite samples for residue analysis. Wood cores were extracted from the trunk of the tree, 15.24 cm inches above and below the injection site, and also from scaffold limbs using a foresters wood coring tool (Mattson®, UK). The cores were 50.8 mm (2 in) 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. Each core sample weighed between 1-2 g. Samples were stored in 120 ml Qorpak jars, labeled and stored in a cold room until being transported to the MSU Pesticide Analytical laboratory for HPLC analysis. Chemical analysis followed procedures described above.

Results

2009 field evaluations

At 21 DAT (9 July, 2009) the incidence of PLH nymphs were significantly lower in the imidacloprid and emamectin benzoate treated trees compared to the untreated control (F=11.117, df=2, P=0.005) (Table 1). At 60 DAT imidacloprid and emamectin benzoate treatments reduced the incidence of STLM mines compared to the control

	21 DAT 60 DAT	
	# PLH nymphs	# STLM mines
	20 shoots	2 minute count
Control	1.2 (0.6)a	26.6 (7.6)a
Imidacloprid	0.2 (0.7)b	8.4 (6.6)b
Emamectin benzoate	0.4 (0.8)b	6.6 (3.6)b

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
Table 1. 2009 mean number (±SE) of foliar insects or pest injury associated with in-season field evaluations, across the five treatments.

(*F*=12.67, df=2, *P*=0.003).

2010 field evaluations

At 40 DAT (14 June, 2010) the incidence of PLH nymphs were significantly lowest in the imidacloprid high rate treated trees, followed by imidacloprid low rate, and then the emamectin benzoate high rate, compared to the untreated control (*F*=60.572, df=4, *P*=0.0001) (Table 2). At 40 DAT all treatments reduced the incidence of STLM mines compared to the control (*F*=8.495, df=4, *P*=0.0007). At 40 DAT the incidence of OFM flagging was significantly lower for imidacloprid high rate and both all emamectin benzoate treatments, but numbers in the imidacloprid low rate plots were not different than the untreated control (*F*=12.039, df=4, *P*=0.0001).

2010 continuation field evaluations

At 1 year 39 (13 June, 2011) the incidence of PLH nymphs was significantly lower for both imidacloprid treatments than the control (*F*=9.532, df=4, *P*=0.0005) (Table 3). At 1 year 55 DAT the incidence of OFM flagging was significantly lower for both emamectin benzoate treatments than the untreated control (*F*=13.258, df=4, *P*=0.0001). At 1 year 85 DAT the incidence of STLM mines was significantly lower within all emamectin benzoate-treated plots, than the control (*F*=5.724, df=4, *P*=0.0053).

2010 OBLR Bioassays

The mean percent of leaf disc areas consumed by OBLR larvae when exposed to leaves 7 DAT (12 May, 2010) were significantly lower for the emamectin benzoate treatments than the control (*F*=25.163, df=4, *P*=0.0001) (Table 4). The mean proportion of leaf disc areas consumed by larvae when exposed to leaves 60 DAT were significantly lower for the emamectin benzoate treatments and imidacloprid low rate, than the control (*F*=12.918, df=4, *P*=0.0001). The mean proportion of leaf disc areas consumed by larvae when exposed to leaves 90 DAT were significantly lowest in the emamectin benzoate-treated trees, followed by the imidacloprid treatments, compared to the control (*F*=16.673, df=4, *P*=0.0001).

Trt	40 DAT		
	# PLH nymphs	# STLM mines	# OFM flags
	20 shoots	2 minute count	2 minute count
Control	1.9 (0.2)a	3.8 (1.3)a	13.6 (3.0)a
Imidacloprid L	1.4 (0.1)b	0.4 (0.4)b	8.4 (1.5)ab
Imidacloprid H	0.2 (0.0)d	0.2 (0.2)b	5.0 (0.7)bc
Emamectin L	1.6 (0.1)ab	0.0 (0.0)b	2.2 (0.7)c
Emamectin H	0.9 (0.1)c	0.0 (0.0)b	1.4 (0.5)c

Table 2. 2010 mean number (±SE) of foliar insects or pest injury associated with in-season field evaluations, across the five treatments.
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

	1 yr 39 DAT	1 yr 55 DAT	1 yr 85 DAT
Trt	# PLH nymphs	# OFM flags	# STLM mines
	20 shoots	2 minute count	2 minute count
Control	2.6 (0.3)a	6.0 (1.4)a	7.2 (1.5)a
Imidacloprid L	0.8 (0.2)b	2.4 (0.2)bc	2.2 (1.0)ab
Imidacloprid H	0.7 (0.1)b	3.2 (0.4)b	3.0 (1.6)ab
Emamectin L	3.0 (0.3)a	1.8 (0.4)bc	0.2 (0.2)b
Emamectin H	2.3 (0.2)a	1.2 (0.4)c	0.9 (0.5)b

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

Table 3. 2010 continuation mean number (±SE) of foliar insects or pest injury associated with in-season field evaluations, across the five treatments.

	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
Imidacloprid L	52.5 (8.7)a	0.9 (0.1)bc	0.5 (0.1)b
Imidacloprid H	52.6 (9.0)a	4.9 (1.4)ab	0.4 (0.1)b
Emamectin L	4.0 (1.8)b	0.1 (0.1)c	0.1 (0.0)c
Emamectin H	8.4 (5.8)b	0.1 (0.1)c	0.1 (0.0)bc

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.

Table 4. 2010 mean (±SE) percent of leaf area consumed by OBLR larvae when exposed to five treatments 7, 60, and 90 DAT.

	Percent Consumed		
Trt	1 yr 32 DAT *	1 yr 85 DAT *	1 yr 115 DAT
Control	8.8 (0.7)a	8.3 (1.3)a	14.6 (4.0)a
Imidacloprid L	9.9 (1.3)a	5.2 (2.7)a	7.0 (2.3)ab
Imidacloprid H	7.0 (1.7)ab	13.2 (7.9)a	7.3 (2.1)ab
Emamectin L	2.9 (0.4)bc	1.2 (0.5)a	0.6 (0.2)b
Emamectin H	1.3 (0.4)c	3.7 (0.8)a	1.0 (0.5)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

Table 5. 2010 continuation mean (±SE) percent of leaf area consumed by OBLR larvae when exposed to seven treatments 1 yr 32, 1 yr 85, and 1 yr 115 DAT.

2010 continuation OBLR Bioassays

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 than the control (*F*=13.051, df=4, *P*=0.0001) (Table 5). The mean proportion of leaf disc areas consumed by larvae when exposed to leaves 1 year 85 DAT were not significantly different than the control (*F*=2.103, df=4, *P*=0.1311). The mean proportion of leaf disc areas consumed by larvae when exposed to leaves 1 year 115 DAT (28 Aug) were significantly lower for emamectin benzoate, than the control (*F*=7.646, df=4, *P*=0.0014).

Season-long residue profiles of fruit and leaves

The 2009 season long residue analysis for emamectin benzoate and imidacloprid showed for both that the vast majority (40-fold difference for emamectin benzoate, 400-fold difference for imidacloprid) of residue were found in the apple foliage versus apple fruit. For imidacloprid the peak residue levels were found at 29 DAT on 17 Jul (20 ppm in leaf, .05 ppm in fruit), declining by the 2 Sep harvest (1.6 ppm in leaf, 0.04 ppm in fruit) (Figure 2). For emamectin benzoate the peak residue levels were found at 7 DAT on 25 Jun (0.389 ppm in leaf, 0.006 ppm in fruit), declining by the 2 Sep harvest (0.0245 ppm in leaf, 0.0004 ppm in fruit) (Figure 3). In neither case did residue levels in fruit reach the US EPA tolerance for apples (imidacloprid 0.5 ppm MRL; emamectin benzoate 0.02 ppm MRL).

Wood tissue samples

In the 2010 continuation plots imidacloprid was detected at 16.82 ppm in low rate treatment 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 4A).

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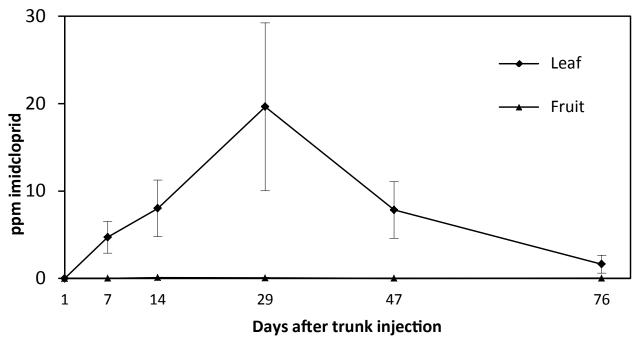

Figure 2: Temporal distribution profile of trunk-injected imidacloprid in apple canopy during 2009 based on residue concentration in leaves and fruits. *MRL: maximum residue limit for imidacloprid in apple fruits is 0.5 ppm, set by the US Environmental Protection Agency (EPA).

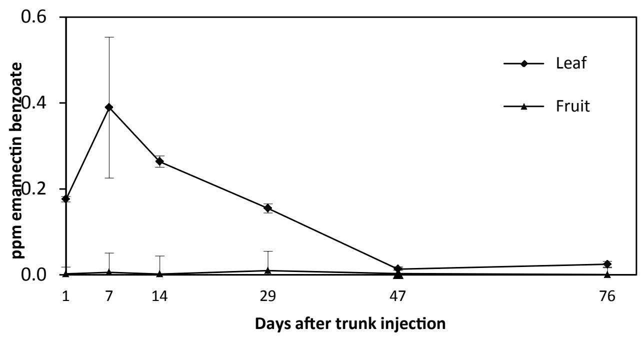

Figure 3: Temporal distribution profile of trunk-injected emamectin benzoate in apple canopy during 2009 based on residue concentration in leaves and fruits. *MRL: maximum residue limit for imidacloprid in apple fruits is 0.02 ppm, set by the US Environmental Protection Agency (EPA).

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). Residues detected at the base of the main scaffold branch were 3.22 ppm at 1 year 27 DAT, 0.66 ppm at 1 year 55 DAT (29 June), and 2.30 ppm at 1 year 115 DAT. Imidacloprid was also detected at 30.28 ppm in high rate treatment wood core samples recovered 6 inches below the injection site at 1 year 27 DAT, 197.65 ppm at 1 year 55 DAT and 94.83 ppm at 1 year 115 DAT (Figure 4B). Residue detected 6 inches above the injection site was 52.84 ppm at 1 year 27 DAT, 132.72 ppm at 1 year 55 DAT, and 97.58 ppm at 1 year 115 DAT (28 Aug). Residue recovered from the base of the main scaffold branch were 6.96 ppm at 1 year 27 DAT, 3.53 ppm at 1 year 55 DAT, and 7.49 ppm at 1 year 115 DAT.

In the 2010 continuation plots Emamectin benzoate was detected at 7.90 ppm in low rate treatment wood core samples 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 5A). Residues detected 6 inches above the injection site were 0.55 ppm at 1 year 27 DAT, 9.14 ppm at 1 year 55 DAT, and 8.07 ppm at 1 year 115 DAT. Residues detected at the base of the main scaffold branch were 0.55 ppm at 1 year 27 DAT, 0.28 ppm at 1 year 55 DAT, and 0.46 ppm at 1 year 115 DAT. Emamectin benzoate was also detected at 24.13 ppm high rate wood core treatment samples recovered 6 inches below the injection site at 1 year 27 DAT, 8.91 ppm at 1 year 5 DAT, and 38.69 ppm at 1 year 115 DAT (Figure 5B). Residues detected 6 inches above the injection site were 5.95 ppm at 1 year 27 DAT, 26.33 ppm at 1 year 55 DAT, and 26.61 ppm at 1 year 115 DAT. Residues detected from the base of the main scaffold branch were 0.44 ppm at 1 year 27 DAT, 2.27 ppm at 1 year 55 DAT, and 2.23 ppm at 1 year 115 DAT.

Discussion

Our study establishes a successful 'proof of concept' for the use of trunk injection to control insect pests in apples. The field evaluations showed effective control of indirect pests from Lepidoptera (*Phyllonorycter blancardella*) and Homoptera (*Empoasca fabae*) orders. Direct pests, such as Oriental fruit moth, oblique banded leaf roller, that feed on foliage in addition to fruit were also controlled. Direct pests, like codling moth, which feed/infest solely the apple fruit with little or no exposure to foliage, are not controlled [32].

This study points to several key advantages of trunk injection for delivery of insecticides, over conventional foliar spraying methods. First, is the duration of time that insecticides are active on a target pest is greatly lengthened. Whereas a foliar spray of most registered insecticides can be expected to provide only about 14 days of control [28], a single injection in our study showed activity on a range of apple pests 40 – 90 days after application. This is likely a result of where trunk injection delivers compounds in the tree canopy [33]. Foliar sprays deposit insecticides as surface residues on the plant, thus exposing them to maximum UV degradation and environmental loss (precipitation, volatilization, etc.) [4,34]. Trunk injection transports compounds through the vascular system of the tree, depositing material through the symplastic and apoplastic regions of the plant [35]. This minimizes environmental exposure and degradation, and lengthens the duration

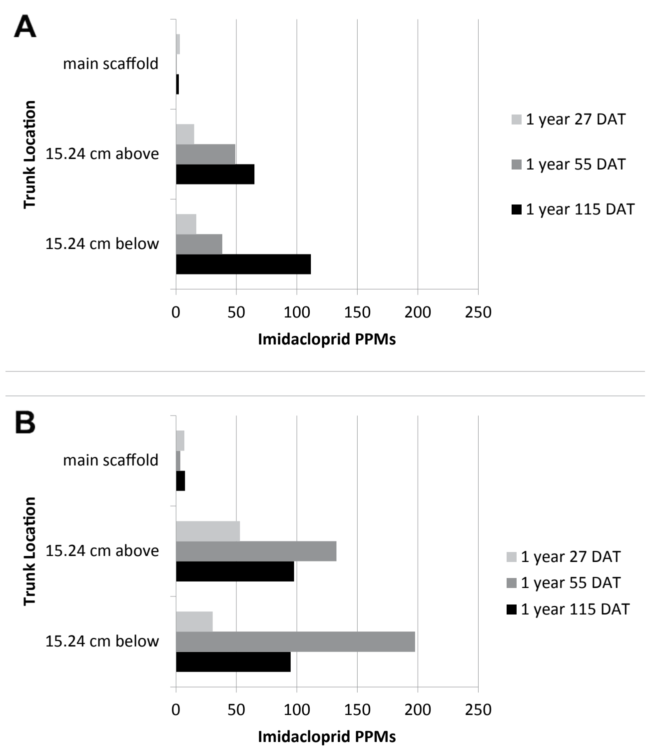

Figure 4: Mean residue recovery (ppm) from low (A) and high (B) treatment rates of imidacloprid residue samples of wood core tissue, 6 inches below injection, 6 inches above injection, and at the base of the main scaffold branch. The injections were on May 5, 2010, 1 year 27 DAT was 1 June, 1 year 55 was 29 June, and 1 year 115 DAT was 28 Aug.

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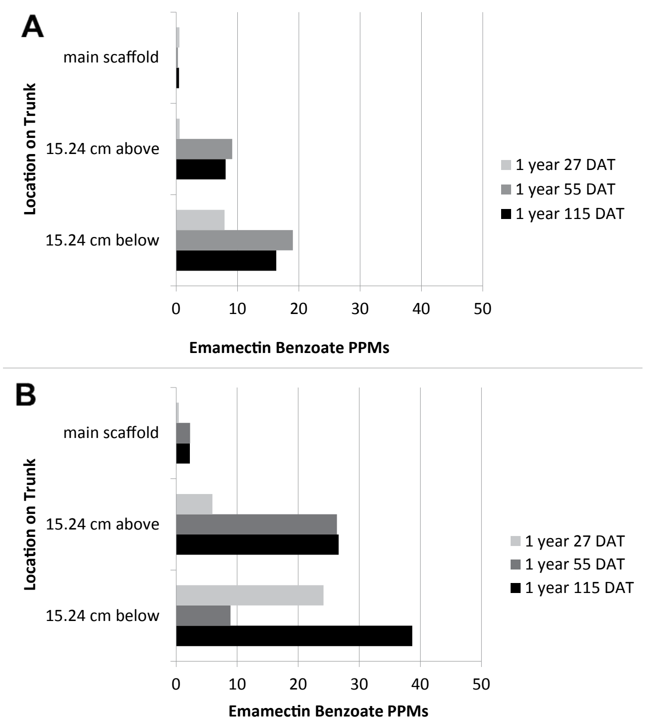

Figure 5: Mean residue recovery (ppm) from low (A) and high (B) treatment rates of emamectin benzoate residue samples of wood core tissue, 6 inches below injection, 6 inches above injection, and at the base of the main scaffold branch. The injections were on May 5, 2010, 1 year 27 DAT was 1 June, 1 year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

of time that insecticides are in an active state for pest control.

Another advantage of trunk injection, based on our results, is the apparent expansion of the pest spectrum for some compounds. Most insecticides are labeled for a range of target pests based on meeting certain thresholds of performance based on foliar spray trials. Our study provided evidence for expanding the "activity spectrum" for imidacloprid and emamectin benzoate, based on trunk injection's enhanced delivery of these compounds. For example, imidacloprid is well known to be active primarily on Homopteran, Hemipteran and Coleopteran insect pests of apple, and is not labeled for most Lepidopteran insects, like *C. rosaceana*, because of poor performance in foliar spray trials. Our 2010 OBLR bioassays showed significant reduction of leaf consumption by *C. rosaceana* larvae on imidacloprid-injected trees, compared to the untreated check. Similarly, emamectin benzoate is well known to be active primarily on Lepidopteran insect pests of apple, and is not labeled for Homopteran pests like *E. fabae*, because of poor performance in foliar spray trials. In 2009 and 2010 field evaluations, trunk injected emamectin benzoate showed significant activity on this leafhopper pest. The expansion of pest spectrum seen with trunk injection is likely a function of the enhanced ingestive exposure of the compounds to the pest, as compared to contact-active surface residues of foliar sprays.

Beyond the season-long pest protection that we documented for trunk injected compounds, evidence for multi-year activity is additionally encouraging. Similar multi-year activities have been documented in other studies, for which a "reservoir effect" in trunk woody tissue is credited as the source of active ingredient for transport into the tree canopy [36]. Our wood core residue data support this theory, with generous amounts of imidacloprid and emamectin benzoate being present in the trunk one year after injection, available for transport in xylem sap initiated by foliar transpiration in the growing season [35]. These residues found in the tree trunk may also be a means of controlling wood-boring pests, such as the dogwood borer, *Synanthedon scitula* Harris, in apples [26].

For high-value horticulture crops, trunk injection should be viewed as one tool in the IPM toolbox, that when integrated can enhance the overall program. For example, selective tools like pheromone mating disruption are likely to compliment trunk injection because they target key direct fruit pests like codling moth and Oriental fruit moth, which are difficult to control with injection delivery. Injection technology also allows farmers and IPM practitioners to readily treat "hot-spots" in orchards as pest thresholds are reached, as opposed to spraying the entire farm. Although we anticipate with injection a reduced negative impact on beneficial organisms, compared to traditional foliar delivery of pesticides, more research is needed to confirm this assertion. Impact on pollinators must also be carefully considered. Although our preliminary work in apples [32] suggests that trunk injection does not increase the risk of insecticide residues in flower nectar or pollen, further research is needed to confirm.

We demonstrated a range of insecticide compounds can be delivered via trunk injection for protection of apple trees from a wide range of insect pests. The compound rate studies showed that reduced rates of pesticides can be used with trunk injection to provide season-long, and in some cases multiple seasons of pest control. Economic analysis is needed to show that trunk injection can be economically competitive for commercial apple production. The residue analysis clearly demonstrates the trunk injection delivery is safe for farmers, farm workers and consumers, while reducing pesticide drift, worker exposure and risks to the environment. We believe that trunk injection can bring significant benefits in terms of farm worker health and will improve the ability of smallholder farmers world-wide to transition to environmentally-friendly production systems, improve plant protection, while becoming more ecologically and economically sustainable.

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