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DISTRIBUTION OF TRUNK-INJECTED ¹⁴C
IMIDACLOPRID IN *FRAXINUS* AND *ACER* TREES: A
TEST OF THE SECTORED FLOW HYPOTHESIS.

presented by

Sara Rose Tanis

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of the requirements for the

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ACER TREES: A TEST OF THE SECTORED FLOW HYPOTHESIS.

By

Sara Rose Tanis

A THESIS

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ABSTRACT

DISTRIBUTION OF TRUNK-INJECTED ^{14}C IMIDACLOPRID IN *FRAXINUS* AND *ACER* TREES: A TEST OF THE SECTORED-FLOW HYPOTHESIS.

By

Sara Rose Tanis

Emerald ash borer (*Agrilus planipennis* Fairmore, Coleoptera: Buprestidae) and Asian longhorned beetle (*Anoplophora glabripennis*, Motschulsky, Coleoptera, Cerambycidae) are invasive exotic insect pests that have destroyed millions of trees in the United States and Canada. Imidacloprid applied as a trunk injection is often used as a means of control, but efficacy is variable. The objectives of this study were to determine the (1) spatial movement of imidacloprid in ash (*Fraxinus* spp.) and maple (*Acer* spp.) trees (2) concentration of imidacloprid in tree tissues over two growing seasons and (3) variability of imidacloprid concentration in trees trunk-injected in either spring or fall. Thirty-two ash trees were trunk-injected with ^{14}C imidacloprid in June 2006, eight additional ash and eight maple trees were trunk-injected in September 2006. Leaves, trunk cores, fine roots, and stems were sampled over two growing seasons. Imidacloprid equivalent concentration (IEC) in ash leaves varied with time, and orientation to the injection point. Leaves from trees injected in the June had higher IEC than leaves from trees injected in September 21 DAT. During the year after injection, IEC in leaves from both sets of trees were similar, though some patterns of sectoriality were still present. Imidacloprid equivalent concentration in maple leaves was relatively constant and did not vary between opposite branches or over time. We conclude that ash trees have zigzag xylem architecture patterns and maple trees have integrated xylem architecture patterns.

DEDICATION

I would like to dedicate this thesis to my parents: Dr. Dale and Rose Marie Sutton.

Dad – you gave me an undeniable love and fascination for plants from the moment I could follow you around in the yard. Through the years you taught me everything from plant identification to the ups and downs of xylem and phloem. You always pushed me because you knew I could do better – it's funny how I see that now. It was always nice to know that YOU survived this crazy process and that you understood how I was feeling when I was both struggling and triumphant.

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With all my love and deepest gratitude,

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In Memory of Mapel Russell Sutton and Roland L. Cregg.

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TABLE OF CONTENTS

LIST OF TABLES.....	viii
---------------------	------

LIST OF FIGURES.....	ix
----------------------	----

CHAPTER 1

LITERATURE REVIEW

Introduction.....	1
Emerald Ash Borer.....	2
Asian Longhorned Beetle.....	4
<i>Fraxinus</i> spp.....	6
<i>Acer</i> spp.....	7
Ash and Maple in Urban Forests.....	8
Insect Impacts.....	10
Insect Control.....	11
Containment/Eradication.....	12
Bio-Control.....	12
Chemical Control.....	14
Trunk Injection Application.....	15
Efficacy Variability.....	17
Sectoral Flow.....	20
Radio-labeled Tracers.....	24
Summary.....	27
Bibliography.....	34

CHAPTER 2

DISTRIBUTION OF TRUNK-INJECTED ^{14}C IMIDACLOPRID IN *FRAXINUS* SPP. TREES: A TEST OF THE SECTORED FLOW HYPOTHESIS.

Abstract.....	45
Introduction.....	47
Materials and Methods.....	52
Spring Injection.....	52
Fall Injection.....	54
Sampling.....	54
Statistical Analysis.....	57
Results.....	59
Spring Injection.....	59
Fall Injection.....	61
Spring vs. Fall Injections.....	62
Observations.....	62

Discussion.....	64
Objective One.....	64
Objective Two.....	68
Objective Three.....	69
Bibliography.....	93

CHAPTER 3

DISTRIBUTION OF TRUNK-INJECTED ¹⁴C IMIDACLOPRID IN *ACER* SPP. TREES: A TEST OF THE SECTORED FLOW HYPOTHESIS.

Abstract.....	98
Introduction.....	100
Materials and Methods.....	104
Trunk Injection.....	104
Sampling.....	105
Statistical Analysis.....	108
Results.....	109
Discussion.....	110
Objective One.....	110
Objective Two.....	113
Bibliography.....	127

APPENDICES.....	130
-----------------	-----

LIST OF TABLES

Table		Page
CHAPTER TWO		
Table 2.1	Total number of emerald ash borer (<i>Agrilus planipennis</i>).....larval galleries in ash trees (<i>Fraxinus americana</i> and <i>F. pennsylvanica</i>) and gallery density (number of larvae galleries per meter ² trunk surface) in trees trunk-injected in either spring or fall with ¹⁴ C-imidacloprid	71
APPENDIX		
Table A.1	Analysis of variance of imidacloprid equivalent concentration.....in <i>Fraxinus americana</i> and <i>F. pennsylvanica</i> trees trunk-injected with ¹⁴ C – imidacloprid 28 June 2006.	130
Table A.2	Analysis of variance of imidacloprid equivalent concentration.....in <i>Fraxinus americana</i> and <i>F. pennsylvanica</i> trees trunk-injected with ¹⁴ C – imidacloprid 5 September 2006.	131
Table A.3	Analysis of variance of Imidacloprid equivalent concentration.....in <i>Acer platanoides</i> and <i>A. x. freemanii</i> trees trunk-injected with ¹⁴ C – imidacloprid 5 September 2006.	132

LIST OF FIGURES

Figure		Page
CHAPTER 1		
Figure 1.1	Distribution of Emerald Ash Borer (<i>Agrilus planipennis</i>29 Fairmaire, Coleoptera: Buprestidae) in the North America (www.emeraldashborer.info 2008).	
Figure 1.2	Distribution of ash trees (<i>Fraxinus spp.</i> , Oleaceae) in North.....30 America (USDA NRCS, 2008)	
Figure 1.3	Distribution of native white ash (<i>Fraxinus americana</i> L.,.....31 Oleaceae) in North America (Burns and Honkala, 1990).	
Figure 1.4	Distribution of native green ash (<i>Fraxinus pennsylvanica</i> ,.....32 Marsh. Oleaceae) in North America (Burns and Honkala, 2008).	
Figure 1.5	Distribution of maple trees (<i>Acer spp.</i> Aceraceae) in North.....33 America (USDA NRCS, 2008).	
CHAPTER 2		
Figure 2.1a	Treatment schematic, trees were injected at either 0° or 90° to.....72 the first whorl of branches. Mean distance from injection point to the first whorl = 1.28 m. Mean distance between whorls = 0.18 m.	
Figure 2.1b	Sampling schematic of trees injected at either 0° or 90° to the.....73 first whorl of branches. Branches of the first three whorls were labeled 0°, 180°, L90° or R90° in relation to the injection point. Mean distance from injection point to the first whorl = 1.28 m. Mean distance between whorls = 0.18 m.	
Figure 2.2	Mean ¹⁴ C-Imidacloprid equivalent concentration ± S.E. in whorl.....74 and terminal leader leaves from 0° or 90° (in relation to the first whorl of branches) spring-trunk-injected <i>Fraxinus americana</i> and <i>F. pennsylvanica</i> trees. Branches were labeled 0°, 180°, L90° or R90° (in relation to the injection point. An * denotes leaves collected after abscission.	
Figure 2.3a	Mean ¹⁴ C-Imidacloprid equivalent concentration ± S.E. in whorl.....76 one stems and leaves (0°, 180°, L90° or R90°-in relation to the injection point), trunk cores (0° or 180° in relation to the injection	

	point) and roots of 0° and 90° (in relation to the first whorl of branches) spring-trunk-injected <i>Fraxinus americana</i> and <i>F. pennsylvanica</i> trees, samples taken 60 days after treatment. Inset graphs have leaf data bars removed.	
Figure 2.3b	Mean ¹⁴ C-Imidacloprid equivalent concentration ± S.E. in stem.....78 samples taken from the first three branch whorls of 0° and 90° (in relation to the first whorl of branches) spring-trunk-injected <i>Fraxinus americana</i> and <i>F. pennsylvanica</i> trees 60 days after treatment. Stem samples were labeled 0°, 180°, L90° or R90° in relation to the injection point.	
Figure 2.4	Mean ¹⁴ C-imidacloprid equivalent concentration ± S.E. in.....80 leaves and stems from first whorl branches (0°, 180°, L90° or R90° in relation to the injection point), coarse and fine roots. Samples taken from 0° or 90° (in relation to the first whorl of branches) spring-trunk-injected <i>Fraxinus americana</i> and <i>F. pennsylvanica</i> trees 60 days after treatment. Inset graphs have leaf data bars removed.	
Figure 2.5	Mean ¹⁴ C-Imidacloprid equivalent concentration ± S.E. in 2006.....82 and 2007 stem sections from 0° and 90° (in relation to the first whorl of branches) spring-trunk-injected <i>Fraxinus americana</i> and <i>F. pennsylvanica</i> trees. Stems were harvested in October 2007 from the first six branch whorls (where available) and labeled 0°, 180°, L90° or R90° in relation to the injection point.	
Figure 2.6	Mean ¹⁴ C-imidacloprid equivalent concentration ± S.E. in84 stained and unstained trunk tissue taken at varying heights above the center of the injection scar from 0° or 90° (in relation to the first whorl of branches) spring-trunk-injected <i>Fraxinus americana</i> and <i>F. pennsylvanica</i> trees harvested in October, 2007. Inset graphs have the stained cross section data bars removed.	
Figure 2.7	Mean ¹⁴ C-imidacloprid equivalent concentration ± S.E. in whorl86 and terminal leader leaves from 0° or 90° (in relation to the first whorl of branches) fall-trunk-injected <i>Fraxinus americana</i> and <i>F. pennsylvanica</i> trees. Branches were labeled 0°, 180°, L90° or R90° (in relation to the injection point. An * denotes leaves collected after abscission.	
Figure 2.8	Mean ¹⁴ C-imidacloprid equivalent concentration ± S.E. in leaves.....88 and stems from first whorl branches (0°, 180°, L90°, or R90° in relation to the injection point) and coarse and fine roots. Samples were taken during destructive harvest from 0° or 90° (in relation to the first whorl of branches) fall-trunk-injected <i>Fraxinus</i>	

americana or *F. pennsylvanica*.

- Figure 2.9 Mean ^{14}C -imidacloprid equivalent concentration $\pm$ S.E. in90
stained and unstained trunk tissue taken at varying heights
above the center for the injection scar from 0° and 90°
(in relation to the first whorl of branches) fall-trunk-injected
Fraxinus americana and *F. pennsylvanica* trees harvest in
October, 2007. Inset graph has the stained data bar removed.
- Figure 2.10 Mean ^{14}C -imidacloprid equivalent concentration $\pm$ S.E. in leaves91
from the first three branch whorls of 0° and 90° (in relation to
the first whorl of branches) of spring- and fall-trunk-injected
Fraxinus americana and *F. pennsylvanica* trees. Values were
averaged across whorls. Branches labeled 0°, 180°, L90° or R90°
in relation to the injection point. Samples were taken 21 days
after treatment (19 July 2006 and 26 September 2006).
- Figure 2.11 Mean ^{14}C -imidacloprid equivalent concentration $\pm$ S.E. in92
leaves from the first three branch whorls of 0° and 90° (in
relation to the first whorl of branches) of spring- and fall-trunk-
injected *Fraxinus americana* and *F. pennsylvanica* trees. Values
were averaged across whorls. Branches labeled 0°, 180°, L90° or
R90° in relation to the injection point. Samples were harvested on
11 June 2007.

CHAPTER 3

- Figure 3.1a Treatment schematic, trees were injected at either 0° or 90° to116
the first whorl of branches. Mean distance from injection point
to the first whorl = 1.43 m. Mean distance between whorls =
0.18 m.
- Figure 3.1b Sampling schematic of trees injected at either 0° or 90° to the117
first whorl of branches. Branches of the first three whorls were
labeled 0°, 180°, L90° or R90° in relation to the injection point.
Mean distance from injection point to the first whorl = 1.43 m.
Mean distance between whorls = 0.18 m.
- Figure 3.2 Mean ^{14}C -Imidacloprid equivalent concentration $\pm$ S.E. in whorl118
and terminal leader leaves from 0° or 90° (in relation to the first
whorl of branches) trunk-injected *Acer x freemanii* and *Acer*
platanoides trees. Branches were labeled 0°, 180°, L90° or R90°
(in relation to the injection point).

- Figure 3.3 Mean ^{14}C -Imidacloprid equivalent concentration $\pm$ S.E. in whorl120
one leaves and stems (0° , 180° , $L90^\circ$ or $R90^\circ$ -in relation to the
injection point), trunk cores (0° or 180° in relation to the injection
point) and roots of 0° and 90° (in relation to the first whorl of
branches) trunk-injected *Acer x freemanii* and *Acer platanoides*
trees, samples collected during destructive harvest. Inset graph
has leaf data bars removed.
- Figure 3.4a Mean ^{14}C -Imidacloprid equivalent concentration $\pm$ S.E. in 2006121
and 2007 stem sections from 0° (in relation to the first whorl of
branches) trunk-injected *Acer x freemanii* and *Acer platanoides*
trees. Stems were harvested in October 2007 from the first three
branch whorls and labeled 0° , 180° , $L90^\circ$ or $R90^\circ$ in relation to the
injection point.
- Figure 3.4b Mean ^{14}C -Imidacloprid equivalent concentration $\pm$ S.E. in 2006123
and 2007 stem sections from 90° (in relation to the first whorl of
branches) trunk-injected *Acer x freemanii* and *Acer platanoides*
trees. Stems were harvested in October 2007 from the first
three branch whorls and labeled 0° , 180° , $L90^\circ$ or $R90^\circ$ in relation
to the injection point.
- Figure 3.5 Mean ^{14}C -imidacloprid equivalent concentration $\pm$ S.E. in leaves125
from the first three branch whorls of 0° and 90° (in relation to the
first whorl of branches) of trunk-injected *Acer x. freemanii* and
Acer platanoides trees. Branches were labeled 0° , 180° , $L90^\circ$ or
 $R90^\circ$ in relation to the injection point. Samples were taken 21 and
280 days after treatment.

CHAPTER 1

Literature Review

Introduction

Emerald ash borer (EAB, *Agrilus planipennis* Fairmore) (Coleoptera: Buprestidae) and Asian longhorned beetle (ALB, *Anoplophora glabripennis* Motschulsky) (Coleoptera: Cerambycidae) are exotic insects from Asia. They arrived in the United States on separate occasions in solid wood packaging material (Cappaert et al., 2005b, Smith et al., 2001). Emerald ash borer was first identified near Detroit, Michigan in 2002 (Siegert et al., 2007). Asian longhorned beetle was first discovered in New York City, New York in 1996 (Haack, 1997) and Chicago, Illinois in 1998 (Poland et al., 1998). They have similar life cycles and tree damage results from larval feeding in the phloem (Hajek and Bauer, 2007). As the larvae feed, they create galleries beneath the bark of the tree that interrupt water and nutrient flow. In high density populations, these galleries effectively girdle the infested tree, causing death. Since its arrival, EAB has killed over 25 million ash trees in Southeast Michigan, seven additional states, and Ontario, Canada (www.emeraldashborer.info, 2008). Asian longhorned beetle eradication efforts have resulted in the removal of almost 30,000 trees of various species in Chicago, New York City (Haack, 2003), and New Jersey (Richmond, 2008). Together, these two exotic beetles have caused millions of dollars of damage for homeowners and local, state, and federal governments. Federal quarantines are in effect which restrict the movement of

nursery stock, logs, chips and firewood from infested areas. If these insects are not immediately and successfully controlled, there will be irreparable damage to forest and urban forest ecosystems, not to mention billions of dollars in economic losses.

Unfortunately, there are currently no insecticides that are 100% effective against either ALB or EAB. Imidacloprid applied as a trunk injection provides high levels of control but efficacy against both of these insects is variable (Harrell, 2006, McCullough et al, 2004, Wang et al., 2004). Trunk-injected insecticide variability could be a result of various physical and environmental factors.

This study used ^{14}C -labeled imidacloprid applied as a trunk injection to track the movement of insecticide within *Fraxinus* spp. and *Acer* spp. trees over time. The objectives were to determine the (1.) spatial movement of imidacloprid in trees; (2.) concentration of imidacloprid in tree tissues over two growing seasons; (3.) variability of imidacloprid concentration in trees trunk-injected in either spring or fall.

1. Emerald Ash Borer:

Emerald ash borer is a metallic phloem-boring beetle from Asia. It arrived in the United States near Detroit, Michigan in the early 1990's (Siegert et al., 2007) and was first identified in 2002 after reports from homeowners of dead and dying ash trees. Emerald ash borer is native to China, Japan, Taiwan, Korea, Mongolia and Eastern Russia (Cappaert et al., 2005b). It appears to be host specific and thus far has attacked only trees in the genus *Fraxinus* in North America (Anulewicz et al., 2008). Emerald ash borer is not considered a pest in its native range and very little was known about the beetle before it gained notoriety in the United States (Liu et al., 2003). Current distribution of EAB in North America covers over 10,000,000 hectares (40,000 sq miles)

and includes nine states and parts of Ontario, Canada (Figure 1.1)

(www.emeraldashborer.info, 2008).

In Michigan, EAB usually has a one year life cycle, although in some outlier populations, larvae have been known to develop over two or even three years (McCullough et al., 2005b). Adult EAB emerge in late May through June and feed on the leaves of ash trees, rarely causing noticeable damage. Adults mate one week after emergence and females lay between 60 and 90 eggs approximately one week later (McCullough and Katovich, 2004). The female EAB oviposits individual eggs on the rough bark of one or more ash trees. During the first years of infestation, most eggs are laid in the upper canopy of trees but as population densities build and canopy dieback occurs, the trunk of the tree is colonized. Adults generally live six to eight weeks. Eggs hatch beginning in July and larvae develop through four instars. They feed on the nutrient-rich tree phloem from late summer through fall, and create serpentine galleries throughout the cambium and phloem of the tree. During feeding they also score the outermost layer of sapwood. Larvae over-winter as non-feeding pre-pupae in the xylem or in bark. Pupation occurs in mid-April through May, and adult emergence takes place approximately three weeks later. Emerald ash borers produce one generation of offspring per year except in the cases where it takes two years for larvae to develop (Cappaert et al., 2005b).

Emerald ash borer dispersal takes place at two levels: natural and human assisted. Natural dispersal capabilities are strongest in mated females which were capable of flying up to 1.7 km/yr, in flight mill studies, two times farther than unmated females (Taylor et al., 2007). In low density outlier populations, most EAB rarely travel more than 1 km/yr.

Human assisted dispersal occurs when people move infested firewood, nursery stock, saw logs, or other wood products into previously un-infested areas. Humans have been the main cause of EAB spread across the US (Cappaert et al., 2005b)

Since its arrival, EAB has decimated forest and urban landscapes. In low population densities, trees can withstand minor infestations and exhibit few external symptoms (McCullough and Siegert, 2007). However, in high density populations, heavily infested trees may exhibit one or more of the following symptoms: epicormic shoots, wood pecker holes along the trunk, “D” shaped exit holes, and longitudinal bark cracking and blistering. Canopy die-back can be as high as 30% after only two years and death can occur, even in mature healthy trees, within three to four years of initial infestation (McCullough and Katovich, 2004). Emerald ash borers preferentially attack stressed trees (Poland et al., 2004) but also readily colonize healthy trees, 2.5 to 200 cm in diameter (McCullough and Seigert, 2007).

2. Asian Longhorned Beetle:

The Asian longhorned beetle is a wood-boring invasive pest from Asia. It was first identified in New York City (Haack et al., 1997) and populations were subsequently discovered in Chicago (Poland et al., 1998) Jersey City, New Jersey, Toronto, Ontario, Canada (Wang et al., 2005) and in 26 warehouses in 14 states across the US (Nowak et al., 2001). The ALB is native to SE Asia and China where it is rated as one of the top ten economic insect pests because of the damage it causes to *Populus* plantations (Li and Wu, 1993).

In contrast to EAB, ALB has an extremely wide host range including *Acer*, *Populus*, *Prunus*, *Malus*, *Salix*, *Ulmus* and 21 other tree species (MacLeod et al., 2002).

The economic implications of widespread dispersal are potentially devastating. Apple, cherry, maple syrup, and nursery production industries might all become victims of this invasive insect, not to mention millions of residential and urban forest trees (Ludwig et al., 2002).

In urban landscapes, ALB prefers host trees in the genus *Acer* and will attack both healthy and stressed trees (Nowak et al., 2001). Asian Longhorned Beetle can produce one or two generations per year, depending on the time of adult emergence (Yang, 2005). Adults usually emerge June through August but timing is variable. They feed on bark, young stems, and occasionally leaves (Dubois et al., 2004a). In most cases, beetles stay on the tree from which they emerge but they are capable of flying up to 1,000 m to find a suitable host tree (Wang et al., 2005). Human assisted dispersal is also a problem (Nowak et al., 2002). Adults mate a few days after emergence and eggs are laid one week later. The female chews a small slit into the tree bark and oviposits a single egg underneath. Females can lay eggs up to 32 eggs during a period lasting about five weeks (MacLeod et al., 2002). Oviposition location on trees varies, but normally females will lay eggs in tree crowns during initial infestation and as trees become more heavily populated, eggs will be deposited further down the trunk (Dubois et al., 2004a). Eggs hatch in 15 days (Keena, 2000). The first, second, and third instar larvae feed on nutrient rich phloem just beneath the surface of the bark and create galleries that disrupt nutrient and water translocation within the tree. In areas with high density ALB populations, this can cause tree mortality within two to four years. As larvae grow and develop, they tunnel deeper into the xylem heartwood to feed and then over winter. In doing so, they create weak spots in the branches. Weakened branches may succumb to wind or snow load and cause injury to

pedestrians or damage vehicles, houses, and other property (MacLeod et al., 2002).

Larvae pupate just under the bark in early spring (Poland et al., 2006a)

3. *Fraxinus* spp.

Fraxinus spp. trees are widely distributed across Michigan and the United States in both urban and forest settings (Figure 1.2) The USDA Forest Service estimates that there are over 804 million ash trees in Michigan's forests (Poland, 2007). There are at least 16 species of ash in North America (Poland and McCullough, 2006), and all are likely susceptible to EAB infestation (Herms et. al, 2003). For this study, we used the two most widely distributed and commonly occurring ash in the US; *Fraxinus americana* L. and *Fraxinus pennsylvanica* Marsh, white and green ash.

White ash is the most commercially important native ash tree in North America (Cappaert et al., 2005b). Its wood is used for tool handles, baseball bats, furniture, and flooring (MacFarlane and Meyer, 2005). White ash is usually found on moderately moist uplands in the northeast and north central parts of the United States (Farrer, 1995) (Figure 1.3). Though not a dominant tree in the forest it is an important species in several forest ecosystems (MacFarlane and Meyer, 2005).

Green ash is the most widely distributed ash species (Figure 1.4) and also of economic importance. Its wood is generally of lower quality than white ash but it is also used for tool handles, furniture and flooring. Its seeds are a source of food for many types of wildlife. It is also a popular ornamental tree. Green ash grows best on fertile, moist, well drained soil, but it is also successful in a variety of less than ideal environments (Farrer, 1995) and is probably the most adaptable ash species. Green ash is

widely used in the landscape because of its adaptability, quick growth rate, and beautiful form.

4. *Acer* spp.:

There are approximately 100 species of maple (*Acer* spp.) trees and shrubs worldwide, 13 of which are native to North America (Figure 1.5). Numerous maple cultivars exhibit a vast array of sought after aesthetic and physiological traits. Maple trees are common in the landscape and are often planted for their beautiful fall color (Farrar, 1995). Maple is also an important forest products tree. Its wood is used for a variety of commodities including flooring, furniture, musical instrument panels, and baseball bats. A unique product of maple trees is syrup and confections that are produced from the sap that is collected in early spring (van Gelderen et al., 1994).

Norway maple (*Acer platanoides* L.), is native to Eastern Europe but is a popular tree in North American landscapes (Farrar, 1995). It is one of the top five shade trees produced annually. Its hardy nature, ease of transplanting, and diverse cultivars have made it an over-used favorite of homeowners and city planners (Dirr, 1998). Norway maple grows best in moist, well-drained soil but it too is highly adaptable and can overcome a wide variety of stresses including: soil compaction, heat, and air pollution. Norway maple is a non-native tree with prolific habits and has been described as invasive in some areas. (Nowak and Rowntree, 1990).

Freeman maple (*Acer x freemanii* E. Murray) is found throughout the United States; it is a hybrid cross between *A. rubrum* L. and *A. saccharinum* L., red maple and silver maple. It was hybridized by Oliver Freeman but was later discovered to be occurring naturally in forests where both parents are present (van Gelderen et al., 1994).

Its cultivars are said to combine the beauty of red maple with the hardiness of silver maple. It is more drought tolerant and can grow up to four times faster than the species from which it is derived (Dirr, 1998). These traits, in addition to its vibrant fall color, make it a popular ornamental tree.

5. Ash and Maple in Urban Forests:

Approximately 3.8 billion trees (Nowak et al., 2002) make up the urban forests that cover approximately 3% of the United States (Dreistadt et al., 1990). These trees are an often overlooked resource valued at \$2.4 trillion (Nowak et al., 2002). In addition to monetary value, urban forests also provide elements that positively affect human health, biodiversity, and wildlife habitat (Schwaab et al., 1995). They regulate urban heat effects, sequester carbon, and remove pollutants from the atmosphere (Galvin, 1999). Urban forests are highly vulnerable to invasive forest insect pests and pathogens because diversity generally decreases as urbanization increases and trees in urban environments are often under high degrees of environmental stress (Dreistadt et al, 1990).

The over use of a single tree species and the consequences of doing so are not new concerns to urban foresters. In 1930, Dutch elm disease (*Ceratocystis ulmi* Buisman) was introduced to the US. At the time, over 60% of urban trees were American elms (*Ulmus americana* L.) (Karnosky, 1979). The disease swept through the United States and led to the removal of over 100 million trees (Carley, 2008). This disease changed the face of landscapes throughout the country (Karnosky, 1979), but for a variety of reasons, only a few tree species were utilized to replace elms. Of the 3.8 billion trees currently found in urban forests, 75% represent only nine genera. Trees in

the *Acer* and *Fraxinus* genera comprise five of the ten most common urban trees (Dreistadt et al., 1990).

Fraxinus trees were an easy choice for elm replacement, because of their ability to tolerate a wide variety of stresses in urban environments. For nursery growers, ash was an easy tree to produce. It was also easy to sell to homeowners because it requires little maintenance, has relatively few pests, and a very aesthetic growth habit. New cultivars were introduced that were easy to maintain and aesthetically attractive. Examples include: ‘Marshall’s Seedless’ green ash, a cloned seedless cultivar that reduced fall cleanup, ‘Autumn Applause’, and ‘Autumn Purple” white ash cultivars with striking purple fall foliage. With the introduction of new cultivars, ash quickly gained popularity and became one of the most dependable and widely over-planted standards in the landscaping industry. Between 2000 and 2003, ash trees comprised 70% of the canopy trees bought from Ohio nurseries (Sydnor et al., 2007). Conservative estimates predict that 12-14% of urban and suburban landscape trees in the United States are in the genus *Fraxinus*. In Michigan cities and Chicago, ash trees comprise an average of 15–20% of the urban street trees (BenDor, 2006). Today, as a result of increased ash in urban landscapes, an epidemic equal to or greater than Dutch elm disease could very well take place throughout the United States and Canada as a result of the EAB infestation.

The heavy reliance on maple trees in urban landscapes is also a concern. In many communities maple trees make up 50% or more of the tree population. Commonly used species include: *A. platanoides* L., *A. saccharum* Marsh., *A. saccharinum* L., and *A. rubrum* L. (Bassuk, 1990). Four of the top ten trees used in landscapes are in the genus *Acer* (Dreistadt et al., 1990), a genus highly preferred by ALB (Nowak et al., 2001).

Unfortunately, maple is not the only susceptible tree; ALB has a host range of over 24 species. In some cities, these species may make up as much as 62% of the urban tree landscape (Antipin and Dilley, 2004). If all ALB susceptible trees were infested as much as 68% canopy loss could occur in some cities (Jersey City, New Jersey) (Nowak et al., 2002).

6. Insect Impacts:

Emerald ash borer likely attacks all North American ash species (*Fraxinus spp.*) which have not evolved resistance against it. If left unchecked, EAB could be responsible for virtually eliminating all native ash in both urban and forest environments.

Ash is an important natural resource in Michigan and North America. Of the 16 ash species native to North America, six have economic significance (Poland and McCullough, 2006) and are used for products as diverse as Native American baskets and major league baseball bats. The U.S.D.A. Forest Service estimates that 8 billion ash trees in the U.S. are threatened by EAB, representing 7.5% of U.S. hardwood saw timber. If lost, there would be at least \$25.1 billion of economic loss with a compensatory value of \$282.3 billion dollars. In addition to forest products, loss of landscape trees is of equal concern. The estimated value of ash in the landscape is \$565 million with potential loss estimated at \$20-60 billion, a figure that includes tree removal but not replacement (Poland and McCullough, 2006).

In Chicago and New York City cost of removing ALB infested trees was over \$25 million (Nowak et al., 2002). Economically, ALB could represent \$669 billion in compensatory damage throughout the 48 contiguous states. The national tree impact in urban areas could reach 34.9% canopy cover loss and 30.3% tree loss if all potential hosts

were attacked (Nowak et al., 2001). In addition, urban forests sequester 700 million metric tons of carbon a figure equal to \$460 million per year (Nowak et al., 2002). Asian longhorned beetle could also wreak havoc on forest, fruit tree, maple syrup, and seed production industries, in addition to forest ecosystems (Ludwig et al., 2002).

7. Insect Control:

In its native range, EAB is considered only a minor pest. When EAB was first discovered very little literature existed to guide researchers in areas of control or even life history (Poland, 2007). In contrast, ALB was a well researched pest in China, so aspects of its life history had already been documented before its U.S. invasion (MacLeod et al., 2002).

Wood- and phloem-boring insects are often difficult to detect in low level populations because their larvae are cryptic and inaccessible (Frank and Mizell, III., 2006) and the insects can exist in low density populations for several years before outbreak levels are reached (Shigesada and Kawasaki, 1997). Frequently, outbreaks are not discovered until it is too late to eradicate the pest because humans have inadvertently transported infested materials away from the site of initial invasion. In addition, exotic wood- and phloem-boring pests are particularly dangerous because their new host trees have no co-evolved resistance against them. For example, healthy, native birch trees (*Betula* spp.) have evolved mechanisms to compartmentalize native bronze birch borer (BBB, *Agilus anxius*, Gory) larvae, while European birch used in the landscape trade do not have the mechanism to compartmentalize to defend against BBB and therefore must receive insecticide treatments to survive (Muilenburg et al., 2007). The same situation is true for ash trees. In the EAB's native range, ash trees have evolved resistance against

the beetle, but N. American trees have little or no co-evolved defense mechanisms (Herms et al., 2003). This, in addition to the lack of natural predators, can lead to devastating outbreaks (Hajek and Bauer, 2007).

Currently, three management practices are being utilized in the United States to control EAB and ALB populations, they are: containment/eradication, bio-control, and chemical control.

7.a. Containment/Eradication:

The current strategy of USDA-APHIS for ALB is complete eradication. Eradication efforts can be effective when populations are discovered and acted upon immediately and/or before human assisted dispersal have taken place at a large scale. Thus far, ALB populations have been discovered relatively quickly and have been kept somewhat isolated. Chicago has not removed an ALB infested tree since 2003 (Antipin and Dilley, 2004). New York City and Jersey City however, are still infested (Richmond, 2008).

In contrast, initial attempts to isolate and eradicate EAB infestations were often unsuccessful. This practice would remove and destroy non-symptomatic trees that may be current or future hosts to EAB larvae (McCullough and Seigert 2007). Currently, eradication practices are used only in a few unique situations. Eradication of established EAB infestations is no longer a viable option.

7. b. Bio-Control:

In Michigan, bio-control efforts are being explored as a method of controlling EAB populations particularly in forests. In 2007, *Spathius agrili* Yang (Hymenoptera: Braconidae) was released by USDA-APHIS and *Oobius agrili* Zhang and Huang

(Hymenoptera: Encyrtidae) and *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) were released by the USDA Forest Service to aid in the control of EAB (Federal Register, 2007, Bauer et al., 2008). These insects are parasitoid wasps that were discovered in China (Yang et al., 2005, Strazanac and Zhong-qi, 2005, Gould et al., 2005). In their native range, these three wasps may parasitize 50-90% of the EAB present (Gould et al., 2005). After their discovery, the wasps were brought to the US, reared in laboratories and tested extensively to determine host preference, ability to disperse, and survivability in North American EAB infestations (Gould et al., 2005). In addition, various political hurdles were cleared before the insect was released in July 2007 (Bauer et al., 2008).

Microbial control agents like entomopathogenic fungi and nematodes may also a means of bio-control for ALB and EAB in forested areas where chemical application and containment/eradication efforts are not physically or economically feasible (Hajek and Bauer, 2007). Strains of *Beauveria brongniartii*, Sacc. applied via fungal bands have shown to be effective against ALB for up to 31 days. The bands specifically target cerambycid beetles because of their post-emergent wandering habits. The beetles travel across the bands, become infected with the fungus, and die (Dubois et al., 2004a, 2004b). They might also spread the fungi to other beetles during the mating process (Hajek and Bauer, 2007). For control of EAB, *B. bassiana* strain GHA labeled as BotaniGard® was highly virulent against adults when compared to other bacterial agents. Fungal treatment is applied as a pre -emergent broadcast spray and is ingested by EAB adults as they chew their way out of the tree (Liu and Bauer, 2006). Short persistence of the fungicide limits its effectiveness.

7. c. Chemical Control:

The most common treatment for landscape trees with wood- or phloem-borer infestations is chemical control (Frank and Mizell, III., 2006). The threat of ALB infestations continues to loom in the US and chemical treatments have been field-tested in its native range so that researchers will be prepared in case beetle populations re-emerge. It is currently recommended that all susceptible trees within 0.2 km (1/8 mile) of known infested trees be either treated or removed. In general, healthy tree removal is very unpopular with residents. As an alternative, trees are chemically treated as a precaution (Antipin and Dilley, 2004).

Chemical control is used to treat infested high-value ash trees, primarily in urban areas. Since the discovery of EAB in 2002, researchers have tested several methods of chemical control including: broadcast sprays, soil drenches, trunk applications, and trunk injections (Cappaert et al., 2005a, Cregg et al., 2006, Harrell, 2006, McCullough et al., 2003, 2004, 2005a, 2005b, Poland and McCullough, 2006, Smitley et al, 2004, 2005).

One of the most common and effective active ingredients in these chemical control products is imidacloprid, a highly effective, neo-nicotinoid insecticide. It can be applied as a foliar spray, soil injection or drench, or as a trunk injection (Nauen et al, 2001, Tatter et al., 1998). It is used to control a wide variety of landscape insect pests including leaf feeding beetles, lace bugs, aphids, scales, psyllids, adelgids leaf-miners and wood-borers (Szczepaniec and Raupp, 2007). Imidacloprid may affect both larval and adult stages of ALB and EAB (Poland et al., 2006a, McCullough et al., 2005b).

Imidacloprid is most effective when it is ingested by the insect (Gill et al., 1999). Neo-nicotinoid insecticides act on insects by blocking their nicotinic acetylcholine

receptors. When ingested, imidacloprid is bound to the receptor site, but is only slowly degraded, so death usually results (Gill et al., 1999). It has a chemical structure and mode of action similar to nicotine, but mammalian toxicity is approximately 700 times less (Sur and Stork, 2003). Imidacloprid may also act as an anti-feedant. In less than lethal doses, it causes insects to stop feeding leading to death by starvation (Lawson and Dahlsten, 2003, Castle et al., 2004, Wang et al., 2005). However it is possible that if sustained levels of insecticide are not available, larvae may eventually resume feeding (Poland et al., 2006a).

When applied as a trunk injection, imidacloprid moves rapidly up the tree and into the canopy leaves, in some cases in as little as two days (Cregg et al., 2006). When applied as a soil drench, it may take three to four weeks to reach the leaves (Tatter et al., 1998).

8. Trunk Injection Application:

Imidacloprid applied as a trunk-injection has several advantages over traditional applications including: efficiency, avoiding excess environmental contamination, few non-target impacts, and acceptance by the public (Sanchez-Zamora and Escobar, 2000, Sur and Stork, 2003). During application, chemical treatments using crown and/or trunk sprays in high traffic areas can pose both perceived and real hazards. Drift is of particular concern (Sclar and Cranshaw, 1996). When applied as a trunk injection, imidacloprid enters the tree directly, which eliminates potential drift and over application. In addition, trunk injection applications can reduce insecticide rate, number of applications necessary (Poland et al., 2006a), and provide a high level of efficacy for a longer period of time than externally applied pesticide (Sanchez-Zamora and Escobar,

2000). Insecticide is wholly contained within the tree and not merely on the surface of leaves or trunk where rain or irrigation water might wash it off or dilute it. Systemically injected insecticide treatments are also safer for applicators than other conventional application methods (Poland et al., 2006a) and have fewer negative effects on populations of naturally occurring insect predators (Cowles et al., 2006).

One consequence of the trunk injection process is that the tree is physically wounded in the process of insecticide application. Trunk injections scar the conductive tissues (Lawon and Dahlsten, 2003) and in many cases wounding takes place on an annual basis (Cappaert et al., 2005a). The drilling process can also create entry ports for bacterial and fungal pathogens (Lawon and Dahlsten, 2003). For example, horse-chestnut (*Aesculus hippocastaneum* L.) trees being treated for horse-chestnut leaf-miner (*Cameraria ohridella*, Deschka and Dimic, Lepidoptera: Gracillariidae), may receive as many as 25 injection holes. Unfortunately, horse chestnut is limited in its ability to compartmentalize wounds and weakened trees are even more limited. The wounds often become infected with bacteria and trees produce an abundance of sap to flush the bacteria from its vascular system. Wound response may discolor 30-50% of the white wood in the first two years, resulting in tree depreciation (Oszako et al., 2007). Although serious wounding takes place in some species, trees such as avocado seem to overcome drill lesions by growing new tissue over the top. However, even in trees that are able to heal relatively quickly after injection, recommendations still caution against performing “insurance” trunk injections, as these excess events could lead to permanent damage caused by the injection process (Robbertse and Duvenhage, 1999). In addition to the

wounds caused from drilling, bark cracking, splitting and separation can also result from the trunk injection process (Lewis et al., 2004).

9. Efficacy Variability:

Trunk injection success can depend on many environmental and physical variables (Sanchez-Zamora and Escobar, 2000). With the possible exception of a new product containing emamectin benzoate (McCullough et al., 2008) there are no insecticides that are 100% effective against ALB or EAB (Cappaert et al., 2005b, Poland, 2007). However, 100% insect control may not be necessary because of the tree's resilience to low level infestation (McCullough et al., 2004, Poland et al., 2006a). One potential cause for variability in efficacy is that the uptake of trunk-injected insecticides can be affected by a wide range of physical parameters including tree health, previous injury, (Smith and Lewis, 2005) and size (McCullough et al., 2005a,b). It can also be affected by environmental factors such as temperature and water availability or by cultural practices like timing of application and delivery method (Smith and Lewis, 2005). Variability also occurs among and between tree species (Tatter et al., 1998). Variability between trees of the same species suggests that root and crown structure, crowding, and nutrient or water stress could all affect homogeneity of trunk-injected insecticides (Young, 2002).

Efficacy variability could also be affected by the length of time between treatments. In China, efficacy of imidacloprid against ALB in *Populus* spp. trees was highest in the weeks immediately following trunk injection but was greatly reduced over time (Poland et al., 2006b). The same was found in pin oak (*Quercus palustris*, Muenchh.) and eastern hemlock (*Tsuga canadensis*, L.) where imidacloprid concentration

in sap began to decrease after approximately eight weeks (Tatter et al., 1998). Imidacloprid provided 70% control against avocado thrips (*Scirtothrips perseae* Nakahara) for the first 14 weeks in all leaves present at the time of application but efficacy steadily decreased thereafter and levels were reduced in newly formed leaves (Byrne et al., 2007). Additional studies on red gum eucalyptus (*Eucalyptus camaldulensis*, Schlecht.) (Young, 2002) and green ash trees also showed reduced imidacloprid residue levels one year after treatment (Lewis et al., 2004).

Most researchers agree that one year of control can be expected from trunk-injected imidacloprid, but there are conflicting reports of efficacy beyond one year, both among and between species. Laboratory bioassays performed to test the efficacy of imidacloprid against elm leaf beetle (*Xanthogaleruca luteola*, Müller) found the insecticide to be ineffective 382 days after application in one study (Lawon and Dalhsten, 2003) but another, effective control of the same insect persisted for more than one year (Sclar and Cranshaw, 1996). Differences in this instance could be a result of different insecticide rates. Similarly, a 5% imidacloprid trunk-injection in green ash was successful against EAB for two years in one study (Doccole et al., 2006), but in another, a 10% imidacloprid trunk injection was successful for only one year (Cregg et al., 2006). Poland et al. (2006b) suggest that as imidacloprid concentration increases, translocation capability decreases. Examination of commercial formulations of imidacloprid (Merit[®], Pointer[®], and Imicide[®]) insecticide labels contain only the percentages of active and non-active ingredients, therefore no comparisons of adjuvants could be made.

One possible explanation for lack of extended efficacy in most cases is that imidacloprid is not translocated from leaves back into the tree before leaf abscission. In

fact, a large concentration of insecticide is lost from the tree after litterfall (Cregg et al., 2006). And so the question remains, is there enough insecticide left in the tree after litterfall to provide a second season of effective control? Ash are resilient trees, a trait that helps them to recover from the trunk injection process, but if two years of control can be gained from a single injection, damage to the tree that occurs during the injection process and overall cost of treatment could be reduced. Better understanding of the fate of insecticides in plants could also reduce the number of “insurance” treatments that are used in cases where efficacy is questionable (Castle et al., 2004).

Another factor influencing trunk-injected insecticide efficacy is the timing of the injection process. Environmental conditions such as temperature, humidity level, and cloud cover can all play an important part in ensuring that the maximum amount of insecticide is moving from the injection site to the leaves (Smith and Lewis, 2005). The timing of injection in relation to tree phenology is critical in order to attain and maintain effective insecticide levels (Giblin et al., 2007). Trunk injections in the tree maintenance trade usually take place in either the spring or fall of the year. Spring injections are advantageous because the insecticide is being injected into the tree during a period of maximum translocation. Fall injections are popular among arborists because there is often a lag in business opportunities during this time. Dye studies performed by Tatter and Tatter (1999) suggest that injected materials move downward as well as upward within trees. This could be a result of pressure from the injection process or wounding events which interrupt xylem flow. With the lack of a water potential gradient which normally moves liquid upward, downward movement into the roots can take place.

Tatter and Tatter (1999) hypothesized that this process could provide the mechanism that makes fall injected materials efficacious the following spring.

10. Sectoral Flow:

Another possible explanation for efficacy variability might be that trunk-injected insecticide is not distributed evenly within the tree (Cregg et al., 2006) which suggests that the xylem anatomy of the tree is a highly influential factor on insecticide movement. In addition to resource availability gradients, the xylem architecture of a tree can have a direct effect on disease and insect control because many systemic insecticides move along the same xylem pathways as water. (Kozlowski and Winget, 1963). If this is the case, insecticide efficacy could be influenced by the location on the tree trunk where the insecticide is injected. Efficacy variability could result because the insecticide is being translocated within the tree in such a way that it never reaches insect feeding sites (Poland et al., 2006b).

Sectoriality is a trait most often found in trees and dicotyledonous herbs. Since it was first identified, the theory of sectoriality has been used to describe differences in resource allocation, plant growth, and plant-herbivore interactions (Vuorisalo and Hutchings, 1996). Resource movement in plants is accomplished and limited by the vascular system, which is either integrated or sectoral to varying degrees. Integrated vascular systems have highly interconnected networks of xylem cells. Sectoral vascular systems have highly specific xylem cell pathways. Sectoral cell paths restrict the transport of nutrients and water to specific parts of the plant. Orians et al., (2004) demonstrated that sap flow in trees may be sectoral or integrated depending upon tree species. Sectoriality might be specific to different parts of a plant, branches of a tree, or

even leaves on a branch (Orians et al, 2005), the term “integrated physiological units” (IPUs) is used to describe these distinct vascular sections (Orians et al., 2002).

The ability to isolate stresses and increase exploitation efficiency (example, light) are two advantages of sectoriality (Orians and Jones, 2001). In fact, sectored species may perform best in variable environments (Zanne et al., 2006), which might explain the ability of green ash to tolerate ever-changing urban environments. In sectored trees, stress to a single branch seldom affects the other branches. Different sectors with very different chemical and physical characteristics may co-exist on the same plant for a very long time. Sectored vascular systems create spatial variability of resources that could result in non-uniform plant growth and plant chemistry (Orians et al., 2004); conditions that might effect herbivory (Orians et al., 2002).

Some patterns of sectoriality can be visually observed such as when a branch of a tree exhibits bright fall foliage while the rest of the tree is still lush and green. The brightly colored branch is likely being supplied by its own IPU with resources that differ from the rest of the tree. Another visual indication might be patchy dieback patterns in the tree crown (Ellmore et al., 2006). The degree of sectoriality probably changes continuously within a plant over time. New connections form as new leaves, roots and shoots are produced (Orians and Jones, 2001). But even though new connections are formed, the patterns of sectoriality are probably consistent between saplings and mature trees (Ellmore et al., 2006).

Sectoriality differs within and among species (Orians et al., 2004) because of differences in anatomical traits (Zanne et al., 2006). It is generally accepted that ring porous species (e.g. ash) are more highly sectored than diffuse porous species (e.g. maple)

(Zanne et al., 2006, Ellmore et al., 2006, Farrar, 1995). This could be a result of differences in vessel diameter size throughout early and late wood. Ring porous species have variable vessel diameters while diffuse porous species have smaller, more constant vessel diameters (Shigo, 1988). Although these differences can provide some indication as to whether a tree will be sectorial or integrated, they are not absolute. Some diffuse porous hardwood species, even some conifers that lack vessels, exhibit varying degrees of sectoriality (Zanne et al., 2006). In some cases, sectoriality could be considered a comparative anatomical trait (Ellmore et al., 2006). Despite some variation between species, closely related species often have similar IPU patterns (Orians et al., 2004). Sectorial IPU patterns may exhibit spiraling patterns. Helical ascent patterns are common in both hard- and soft-wood trees (Erdesi, 1957). The pattern may turn either to the left or right depending on tree species, age, or height. Ascent may begin vertically near the base of the tree, spiral with increasing height and then finally become integrated in the tree crown (Kozlowski and Winget, 1963). Ash trees may exhibit this tendency to spiral (Erdesi, 1957). Maple trees, on the other hand, have ascent patterns that vary between straight, highly irregular, or spiraling. The marked differences in ascent patterns between and within species are correlated to xylem structure (Kozlowski and Winget, 1963), which could be a product of phylogeny or current environment (Orians et al., 2004).

Several anatomical traits combine to determine levels of integration and sectoriality including, vessel arrangement and size, pit structure, density, and resistance. Zanne et al. (2006) suggest that this is the most important trait when determining the degree of sectoriality for tree species. Pit densities might also be an integration indicator but Ellmore et al. (2006) determined that pit densities alone are not an absolute predictor

of sectoriality. Percentage of vessel wall area as inter-vessel pits could also effect levels of integration. For example, *Betula papyrifera* Marsh., a highly integrated species, had 26% vessel wall as pits when compared to *A. saccharum* Marsh., an intermediate species, which had 9%. Tree species that have a high percentage of pits in the vessel walls could have decreased pit resistance. In vertical pathways, this could lead to increases in lateral movement and as a result, greater degrees of integration (Orians et al., 2005, Ellmore et al., 2006). Kitin et al. (2004) suggest that *Fraxinus* trees have individual vessels that deviate from straight longitudinal along a more tangential direction and that these vessels are part of an interconnected group that touches and interacts with one another. This interaction could result in a helical ascent pattern as shown in some dye experiments (Chaney and Kozlowski, 1977). In general, species that have large early-wood areas, low vessel density, high variation of vessel area, and greater distance between neighboring vessels have greater degrees of sectoriality (Zanne, et al., 2006).

In a previous study at Michigan State University, researchers used ^{14}C labeled imidacloprid to track trunk-injected insecticide movement in ash trees. The study demonstrated that imidacloprid moves slowly but steadily through the tree over time and that it accumulates in the leaves. However, the sampling protocol did not permit a thorough examination of the spatial variability of imidacloprid distribution in the trees (Cregg et al., 2006). We hypothesized *a priori* that ash trees have sectoried patterns that might cause uneven distribution of insecticide in the tree crown leading to variable efficacy of trunk-injected imidacloprid. We further hypothesized that maple trees have more integrated insecticide ascent patterns. Depending on injection location and spacing, there could be portions of the tree that receive little if any insecticide.

Overall, the efficacy of trunk-injected insecticides might be influenced by how often trunk injections take place, amount of insecticide injected, time of year that trees are injected, and tree sectoriality. Understanding the spatial and temporal flow of insecticide through *Fraxinus* spp. and *Acer* spp. trees will enable us to improve the efficacy and persistence of treatments and better understand the potential for extended EAB and ALB control.

11. Radio-labeled Tracers:

To track insecticide movement within the tree, we incorporated the use of ^{14}C Carbon (^{14}C) labeled imidacloprid. The use of radioactive isotopes as tracers is not a recent development. In 1923, Hevesy used a naturally occurring lead isotope to study mineral transport in plants. Radioactive isotopes are more accurate and take less time than plant dyes (Jenkins, 1957) and are often used by scientists to follow the chemical and biological pathways of plants. To use ^{14}C as a tracer, a carbon (C) molecule of the compound being traced must be replaced by a ^{14}C isotope. The replacement of C by ^{14}C does not alter the formula of the compound. Radio-labeled compounds are often applied systemically through injection (Domir, 1978), by applying radio-labeled solutions to plant parts (Weichel and Nauen, 2003) or by placing the radio-labeled compound in solution and allowing the plant to translocate it through vascular tissues (Nir and Lavee, 1981).

An advantage of using ^{14}C is that it is readily detected by Geiger counter, methanol extraction, or by processing samples through a biological tissue oxidizer and then a liquid scintillation counter (LSC). However, in some cases methanol extraction

can be inconclusive. In a study by Domir, (1978), American elm trees (*Ulmus americana* L.) were injected with ^{14}C daminozide to determine translocation patterns. The researchers used a methanol extraction technique and after repeated attempts, they observed that significant amounts of labeled compound were un-extractable from plant stem tissues. Results from biological tissue oxidizers are more accurate because the entire tissue sample is burned and resulting $^{14}\text{CO}_2$ is trapped in a liquid scintillation cocktail (Thomson, 2002). After oxidation, the resulting cocktail is processed through a LSC which converts the radioactivity present in the cocktail to light. The light events are recorded as counts per minute which can then be extrapolated backward to determine the amount of labeled compound in the tissue (Packard Tri Carb LSC homepage, 2006). Use of the biological tissue oxidizers eliminates the need for extraction techniques, enabling researchers to trap nearly the entire amount of tracer compound.

Sectoriality patterns in plants can be determined using isotope labels. Peppermint (*Mentha pepperita* L.) sectoriality was determined by applying ^{32}P to two different sections of root. Results showed that the radio-labeled compound was confined to leaves with the same vascular pathway as labeled roots, indicating that peppermint xylem anatomy is sectorial in nature (Rinne and Langston, 1960). Another study using ^{15}N supplied as a fertilizer to lateral roots of *Acer* spp. and *Populus* spp. saplings showed greater degrees of sectoriality in *Acer* spp. than *Populus tremuloides* Michx. (Orians et al., 2004). In a study designed to understand the fate of ^{14}C maleic hydrazide, a plant growth inhibitor, a radio-labeled isotope was used to track the compound through maple (*A. saccharinum* L.) and sycamore (*Platanus occidentalis* L.) trees. Samples of plant

tissues were taken over time to determine the rate of chemical uptake. The presence of the ^{14}C tracer was tracked and it was concluded that the compound was translocated to all parts of the plant within 24 hours. In addition, the radio-labeled tracer enabled the researcher to determine differences in the concentration of ^{14}C maleic hydrazide in various plant parts. It was also observed that there was a difference in the rate of tracer movement between the two tree species (Domir, 1978).

Use of radio-labeled tracers can also aid in the study of the metabolites that form after trunk injection (Domir, 1978). To determine which metabolites are formed, scientists examine plant tissues for “new” compounds that contain radio-labeled molecules. It is well established that imidacloprid is transformed into at least three metabolites after injection into various plants (Sur and Stork, 2003). Upon discovery and identification, metabolites as well as pure compounds, can be tested for efficacy against insect pests. In ash trees, metabolites can be as toxic to EAB as pure imidacloprid (Mota-Sanchez, 2007).

Radio-labeled compounds can also be used to determine insect lethal doses in insect bioassays. Knockdown and mortality rates of male and female EAB were determined by using leaves from ^{14}C imidacloprid trunk-injected *Fraxinus* trees. Radio-labeled leaves were placed in enclosed containers with EAB adults. The insects fed on the leaves, weakened, and finally died. The leaves were removed at various stages and scanned with a digital scanner to determine the amount of tissue consumed by the insect. The weight of tissue consumed was then translated into the amount of insecticide consumed based on the amount of radioactivity within the leaf. In other words, the

amount of insecticide necessary to cause insect death can be discovered by using radio-labeled compounds (Mota-Sanchez et al, 2005).

Understanding where insecticides are within a plant at a given time is important when making recommendations for insecticide application. Radio-labeled compounds can be used to do this because they allow researchers to see where, when, and how insecticides travel within the plant and what concentrations are necessary within leaves to cause insect death. Use of ^{14}C labeled imidacloprid in various plants has shown that it moves primarily to shoots and leaves via the xylem (Bromilow and Chamberlain, 1989, Sur and Stork, 2003, Tanis et al., 2007). Concentrations in leaves are usually higher than concentrations in stems, trunk, or roots (Botts et al., 2006, Mota-Sanchez et al., 2005, Tanis et al., 2007) and highest concentrations are expected in older leaves (Sur and Stork, 2003). Imidacloprid generally increases in leaves and stems during the first growing season after trunk injection. At the end of the growing season, concentrations in leaves at the time of abscission are as high as or higher than concentrations 60 days after injection, which suggests that imidacloprid is not translocated back into the plant (Cregg et al., 2006). In addition, a high amount of compound often remains around the injection site (Arron et al., 1992, Tanis et al., 2008) which may serve as the source of compound that could provide a second year of insect control (Tanis et al., 2007).

Summary:

In summary, ALB and EAB are invasive exotic beetles. Imidacloprid, a neonicotinoid insecticide applied as a soil drench or trunk injection, is an effective but variable means of control for both insects. Efficacy of trunk-injected insecticides could be affected by one or more of the following factors including: (1.) length of time that the

insecticide has been inside the plant; (2.) time of year that trunk injections take place and (3.) location of the injection site. All of these factors might further be influenced by tree internal structure, health, and phenology. The xylem architecture of trees can play a major role in insecticide translocation and efficacy depending on whether or not the tree is sectored or integrated. We hypothesize that ash trees have sectored xylem structure and that maple trees have more integrated xylem anatomy. This study will use ^{14}C labeled imidacloprid to determine which of the above is influential on insecticide translocation. We will use the results to develop recommendations for arborists that will enable them to provide the best possible means for EAB and ALB control.

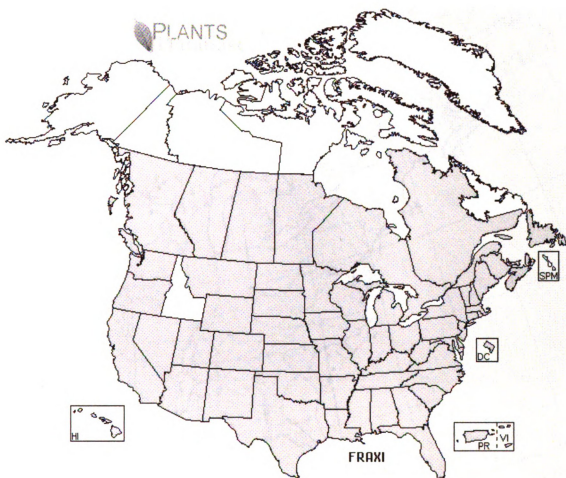


Figure 1.2: Distribution of ash trees (*Fraxinus* spp., Oleaceae) in North America (USDA NRCS, 2008).

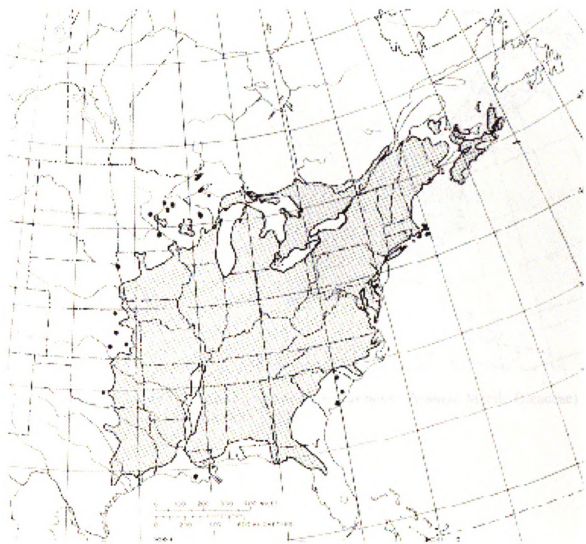


Figure 1.3: Distribution of native white ash (*Fraxinus americana* L., Oleaceae) in North America (Burns and Honkala, 1990).

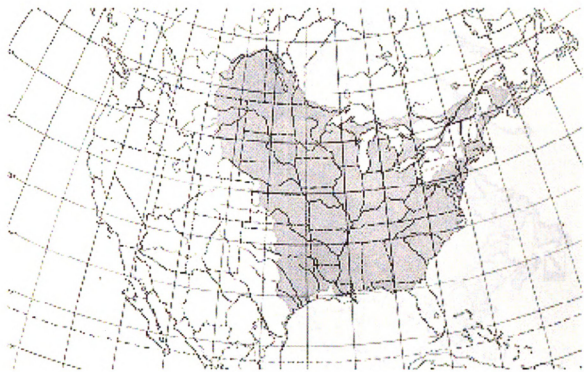


Figure 1.4: Distribution of native green ash (*Fraxinus pennsylvanica*, Marsh. Oleaceae) in North America (Burns and Honkala, 2008).

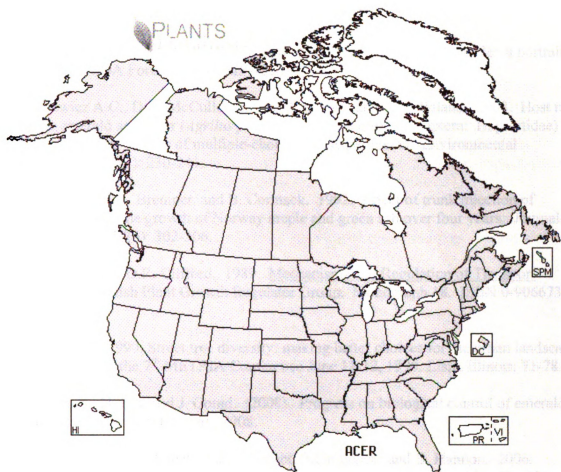


Figure 1.5: Distribution of maple trees (*Acer* spp., Aceraceae) in North America (USDA NRCS, 2008).

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Distribution of Trunk-Injected ^{14}C Imidacloprid in *Fraxinus* Trees: A Test of the Sectored-flow Hypothesis.

ABSTRACT

The emerald ash borer (*Agrilus planipennis* Fairmore, Coleoptera: Buprestidae) is an invasive exotic insect pest that has destroyed millions of Ash (*Fraxinus spp.*) trees in Michigan, surrounding states, and Canada. Imidacloprid applied as a trunk injection is often used as a means of control, but effectiveness can be variable. The objective of this study was to determine the extent to which movement of imidacloprid is sectored within ash trees. Highly sectored flow could result in uneven distribution of compound within trees and result in variable efficacy of pesticide. One set of 32 trees (16 *Fraxinus americana*, 16 *F. pennsylvanica*) was injected during the spring; a second set of 8 trees (4 *Fraxinus americana*, 4 *F. pennsylvanica*) was injected during the fall. This allowed for comparison between two injection times. We injected the trees with ^{14}C labeled imidacloprid either directly below a branch in the first whorl or at a right angle to branches of the first whorl. Each branch of the first three whorls was labeled $0^\circ/180^\circ$, or $L90^\circ/R90^\circ$ depending on the location of the branch in relation to the injection point. Leaves and fine roots were sampled through two growing seasons. Trunk cores were sampled during 2006. Tree stems were sampled 60 days after treatment (DAT) (spring-injected trees only) and final destructive harvest. Litterfall was collected at the end of both growing seasons. Imidacloprid equivalent concentration (IEC) in leaves varied with time, and orientation to the injection point. Results are consistent with the sectored flow hypothesis. Imidacloprid equivalent concentrations were higher in leaves of branches in the plane of the injection point (0°) as opposed to leaves of branches on the opposite side of the injection point (180°). Leaves from $R90^\circ$ branches had higher IEC than leaves

from L90° branches. We conclude that ash trees have a zigzag xylem architecture pattern which could lead to variable IEC in the tree crown. Leaves from trees injected in the spring had higher IEC than trees injected during the fall 21 DAT. During 2007, the year after injection, IEC in leaves from both sets of trees were basically the same, some patterns of sectoriality were still present, and IEC in leaves from spring-injected trees was greatly reduced. Imidacloprid equivalent concentrations in leaves, stems, and litterfall were higher than IEC in roots, trunk cores, and stems, indicating that imidacloprid moves primarily through the xylem.

CHAPTER 2

Distribution of Trunk-Injected ^{14}C -Imidacloprid in *Fraxinus* spp. Trees:

A Test of the Sectorial Flow Hypothesis.

INTRODUCTION:

Emerald ash borer (EAB, *Agilus planipennis* Fairmaire) (Coleoptera: Buprestidae) is a phloem-boring insect native to Asia. The invasive metallic phloem-borer arrived in the United States near Detroit, Michigan in the early 1990's and was first identified in 2002 after reports from homeowners of dead and dying ash (*Fraxinus* spp.) trees (Siegert et al., 2007). Since its arrival, it has killed over 25 million ash trees in Michigan and is now found in seven states in the US and Ontario, Canada. Infested trees exhibit a variety of symptoms including epicormic shoots, blistered and discolored bark, D-shaped adult exit holes, wood pecker holes and crown death (Cappaert et al., 2005b). Economic loss of urban ash trees in the United States was estimated at \$20-60 billion, a figure that includes tree removal but not tree replacement. Economic loss of forest ash trees in the United States is estimated at \$282.3 billion (Federal Register, 2003). Emerald ash borer is expected to attack all North American ash species (*Fraxinus* spp.) which have not evolved resistance against it (Herms et al., 2004). If left unchecked, EAB could be responsible for virtually eliminating native ash in urban and forest environments in North America.

In Michigan, EAB usually has a one year life cycle, although in some outlier sites, larvae have been known to develop over two years (McCullough et al., 2005b). The larvae are phloem feeders and develop through four instars; it is these larval stages that cause the most tree damage. Larvae feed on nutrient rich phloem and create serpentine galleries throughout the cambium of trees. After three to five years, multiple galleries in a tree interrupt the translocation of nutrients and water and eventually kill it. Adults feed on leaves but rarely cause more than noticeable damage. (Cappaert et al., 2005b).

Since the discovery of EAB in 2002, researchers have tested several methods of chemical control including broadcast sprays, soil drenches, trunk applications, and trunk injections ((Cappaert et al., 2005a,b, Cregg et al., 2006, Harrell, 2006, McCullough et al., 2003, 2004, 2005a, 2005b, Smitley et al, 2004, 2005, Poland and McCullough, 2006). One of the most common active ingredients in these applications is the neo-nicotinoid compound imidacloprid. Imidacloprid acts on the acetylcholine receptor sites of the emerald ash borer (Gill et al., 1999). It has very low mammalian toxicity and can be applied systemically as a soil drench or trunk injection, which makes it acceptable in urban settings where drift is both a real and perceived concern (Sur and Stork, 2003). Systemic insecticides reduce the effects of drift on humans, wildlife, and non target insects because all of the insecticide applied is directly taken up by the plant. In addition, trunk-injected insecticides like imidacloprid are applied at lower rates, applied less often, and are safer for applicators than other forms of insecticide application (Poland et al., 2006a).

With the possible exception of a new product containing emamectin benzoate (McCullough et al., 2008), there are no insecticides that are 100% effective against EAB

(Poland, 2007). Although researchers have determined that imidacloprid is lethal to EAB adults, efficacy is highly variable (McCullough et al., 2005a and Harrell, 2006).

Variability of trunk-injected insecticides could be due to a variety of parameters including air temperature, water availability, tree health, tree injury, and tree anatomy (Smith and Lewis, 2005). Poland et al. (2006a) suggest that the point of trunk injection also influences insecticide efficacy and that insecticides might be translocated within trees in such a way that they would have no effect on target insects.

Sectoriality might also affect the translocation of insecticides within trees. Orians et al. (2004) demonstrated that sap flow in trees may be sectorized or integrated depending upon tree species. Trees with greater degrees of sectoriality have specific translocation paths that move resources primarily through a longitudinal plane. Trees with greater degrees of integration have broader translocation paths that move resources in both longitudinal and radial planes (Orians et al., 2005). Degrees of sectoriality are influenced by the extent of vessel to vessel contact within xylem tissue and the ratio of vessel walls that contain bordered pits. Though not an absolute rule, ring porous trees (e.g. ash) are generally considered sectorized while diffuse porous trees (e.g. maple) are considered more integrated. Increased vessel to vessel contact and higher pit ratio in integrated species usually equates to higher degrees of integration, in contrast, sectorized trees have less contact between vessels and lower bordered pit ratios (Zanne et al., 2006). Highly sectorized xylem anatomy could result in uneven distribution and variable efficacy of trunk-injected insecticide.

In a previous study at Michigan State University, researchers used ^{14}C labeled imidacloprid to track trunk-injected insecticide movement in ash trees. The study

demonstrated that imidacloprid moves slowly and steadily through trees over time and accumulates in leaves. However, the sampling protocol did not permit a thorough examination of spatial variability of imidacloprid distribution (Cregg et al., 2006). If ash trees have straight sectorial xylem anatomy, trunk-injected insecticides may move in a straight line up to the canopy. Therefore, depending on injection location and spacing, there could be areas of the crown that have reduced insecticide concentrations. If we better understand the translocation path within trees, we can make recommendations to arborists that might improve efficacy of trunk-injected insecticides.

Efficacy variability could also be affected by the length of time that imidacloprid has been in the tree. Currently there are conflicting opinions about the persistence of imidacloprid after trunk injection. Some research suggests that products can achieve two years of insect control from a single injection (Docola et al., 2006), but other studies have shown that only one season of control can be achieved (McCullough et al., 2005a,b, Poland et al., 2006a,b, Tatter et al., 1998). The differences in these reports could be a result of this percentage of active ingredient, application rates, or other differences in trunk-injection procedures. Poland et al. (2006a,b) found that efficacy of imidacloprid against Asian longhorned beetle (*Anoplophora glabripennis* Motschulsky) in *Populus* spp. was highest in weeks immediately following trunk injection and then was diminished over time. Tatter et al. (1998), also found that imidacloprid concentration increased in eastern hemlock (*Tsuga canadensis*, L.) in weeks immediately following injection but began to decrease after approximately eight weeks. Cregg et al. (2006) concluded that imidacloprid is not translocated from leaves back into the tree before abscission and that a large concentration of imidacloprid is lost through litterfall. The question remains – is

there enough compound left in the tree after litterfall to provide a second season of effective EAB control and if so, where is the compound stored? Ash are resilient trees, a trait that helps them to recover from the trunk injection process; but if two years of control can be gained from a single injection, damage to trees that occurs during the trunk injection process could be reduced as well as the cost of treatment.

Another factor affecting insecticide efficacy could be the timing of imidacloprid trunk injections. Trunk injections in the tree maintenance trade typically occur in either spring or fall. Spring injections are advantageous because insecticide is being injected into trees during a period of maximum translocation. Fall injections are popular because there is often a lag in business opportunities during this time. Whether one application time is more effective against EAB than the other is a question that has not been answered.

Overall, the efficacy of trunk-injected imidacloprid might be influenced by tree sectoriality, how often trunk injections take place, and the time of year that trees are injected. Understanding the spatial and temporal flow of insecticide through *Fraxinus* spp. trees will enable us to optimize trunk injection treatments and better understand the persistence and potential for extended EAB control.

The objectives of this study are to determine the (1) spatial movement of imidacloprid in *Fraxinus* spp. trees; (2) concentration of imidacloprid in *Fraxinus* spp. trees over two growing seasons and (3) variability between spring and fall imidacloprid trunk injections in *Fraxinus* spp. trees.

MATERIALS AND METHODS

This experiment consisted of 40 total trees, 20 *F. americana* ('Autumn Applause' or 'Junginger') and 20 *F. pennsylvanica* ('Patmore') (J. Frank Schmidt and Son Company, Boring, Oregon). Trees were 4.0 to 5.0 cm (1.5- 2.0") caliper bare root liners planted in pure sand in 95.0 L (25 gallon) plastic containers at the Horticulture Teaching and Research Center, Michigan State University, Holt, Ingham County, Michigan.. Containers were spaced 2.5 m (8 ft) on center. After planting, pots were placed inside 1.2 m plastic wading pools and elevated on a single layer of brick pavers. This allowed for drainage but kept leachate from entering the environment.

The experiment included eight replicates for the spring injection and two replicates for the fall injection. Each replicate consisted of four randomly placed trees, two of each species. Trees were fertilized with Osmocote[®] Plus (The Scott's Company, Marysville, Ohio) controlled release fertilizer at a rate of 314 grams per pot and watered to runoff with drip irrigation two to three times per week. Trees for this study were individually selected and/or pruned to ensure that the first three branch whorls were oriented at 90° angles to one another.

Spring Injection:

On 28 June 2006, 32 trees (16 *F. americana*, 16 *F. pennsylvanica*) were trunk-injected at a single injection point. It is important to note that this date is approximately three weeks later than we had originally planned due to complications related to importing the radio-labeled imidacloprid into the United States. Each tree was injected with 25 μCi of ^{14}C labeled imidacloprid mixed with 3.1 ml Imicide[®] (Mauget[®], Arcadia,

California, 10% active ingredient) in a ratio of 1:1300 labeled to unlabeled compound. *Fraxinus americana* trees were injected at 0° to the first whorl of branches (i.e. injection port was directly below one of the branches in the first whorl, Figure 2.1a.). *Fraxinus pennsylvanica* trees were injected at either 0° or 90° to the first whorl of branches (Figure 2.2a.). Injection holes were drilled 10.0 cm above the graft union with a cordless drill and an 8.0 mm ($\frac{5}{16}$ ") drill bit. Holes were drilled to a depth of approximately 1.5 cm and systemic tree injection tubes (STIT) were inserted and gently tapped in with a hammer (Helson et al., 2001). After tubes were inserted, 3.1 ml of imidacloprid mixture was added through the top of the lower half of the STIT and 2.0 bar pressure was applied using a hand operated bicycle pump. All lower components of the STIT were securely wrapped with black electrical tape to ensure that leakage was contained and to provide stability. The upper half of the STITs were secured to trees with tape. The STITs were monitored for pressure, re-inflated as needed, and kept in position 2-48 hours until all liquid was taken up by the tree. After all liquid was translocated into the tree, tape and STIT were carefully removed. The area of the tree that contained the drill hole was double-wrapped with electrical tape to prevent leakage or leaching of radioactive material. Tape remained in place throughout the experiment. Three trees were removed from the experiment due to excessive leakage during the injection process. All handling of radioactive material complied with Michigan State University's Office of Radiation, Chemical, and Biological Safety (ORCBS) standards. All areas were surveyed periodically using a Geiger counter (Ludlums Measurement Inc., Survey Meter Model 44-9, Sweetwater, TX) to ensure that no radioactive material leached into the environment.

Fall Injection:

On 5 September 2006 eight additional trees, four *F. americana*, four *F. pennsylvanica* were injected with 2.95 ml of ^{14}C labeled imidacloprid mixture in the same manner as previously described for the spring injection. The tree canopies were fully flushed but not yet senescing. Trees were fertilized immediately after planting in the spring and were kept well watered throughout the summer prior to injection.

Sampling:

Branches from the first three branch whorls of each tree were labeled either 0°, 180°, Right 90° (R90°) or Left 90° (L90°) depending on the position of the branch in relation to the injection point (Figure 2.2b). Leaf samples were obtained by pinching off individual leaflets from proximal, middle and distal portions of each of the six branches. Leaf samples from the terminal leader were removed with a pole pruner. Trunk cores were taken 1 m above the injection point at 0° and 180° (in relation to the injection point) using a #1 cork borer. The borer was pushed into the tree to remove approximately 2 mm depth of tissue (bark, phloem, and sapwood). Root samples were obtained by digging into the sand until a portion of the root system was exposed. Approximately 1 g dry weight of fine roots were removed from coarse roots and rinsed with tap water. Whole stem samples approximately 2 cm in length were taken from branch tips of the first three whorls using hand pruners. All samples were placed in small paper bags, labeled and placed in a 70° C drying oven for at least 72 hr. After drying, all leaf samples were removed from the oven, ground to a fine powder using a mortar and pestle and placed into individually labeled paper envelopes. Root, core, and stem samples were left whole.

A 150.0 – 180.0 mg sub-sample of tissue was processed in a biological tissue oxidizer (OX300 R. J. Harvey Instrument Corporation, Tappan, NY, USA). Stem samples were burned on a four minute cycle, leaf samples on a three minute cycle, and root and core samples on a two minute cycle. Resulting $^{14}\text{CO}_2$ was trapped in scintillation cocktails and processed through a Packard Tri Carb liquid scintillation counter (LSC, Packard Bioscience, Currently PerkinElmer Life and Analytical Science, Waltham, Massachusetts, USA) on a one and one half minute cycle. Resulting counts per minute (CPM) were recorded for each sample and converted to imidacloprid equivalents after accounting for background activity and oxidizer and LSC efficiencies. Imidacloprid injected into trunks of *Fraxinus* trees is broken-down into a series of metabolites (Mota-Sanchez et al., 2007a), many of which exhibit insecticidal properties; therefore we refer to the concentration of insecticide in the tree in terms of imidacloprid equivalents. All results are presented in μg of imidacloprid equivalents per g dry weight of sample.

For the 2006 growing season, spring injected trees were sampled at 0, 2, 7, 21, 60, and 98 days after treatment (DAT). Fall injected trees were sampled at 0, 2, 7, and 21 DAT. The sampling protocol included leaves from the first three branch whorls, terminal leader leaves, trunk cores, and fine roots. Stem samples were taken from spring injected trees only at 60 DAT to prevent excessive tree damage. In late September, all trees were netted with bird netting to collect litterfall. After abscission, all leaves were removed from nets, placed in paper bags and oven dried. Sub-samples of five to seven leaves from each tree were ground, weighed, oxidized, and processed through the scintillation counter. After nets and leaves were removed, trees were lifted with a sling and skid steer and

placed pot to pot in a holding area. A double layer of hay bales was placed around the holding area to protect the roots from winter frost. In April 2007, trees were lifted with the sling and skid steer and placed back in their original configuration. Fertilizer and irrigation rates in 2007 were the same as used in 2006.

For the 2007 growing season, both spring- and fall-injected trees were sampled to collect whorl and leader leaves, and fine roots on 29 May. Additional leaf and root samples were taken on 12 June (the week roughly corresponding to peak EAB flight in Michigan, Anulewicz et al., 2008b), and again on 31 August. Trunk cores were not taken in 2007 to prevent excessive damage to the trees. At the end of the 2007 growing season, all leaves were removed from the trees by hand. Leaves from the first three whorl branches were kept separate from the rest of the canopy leaves. Stem samples were removed during destructive harvest.

Fifteen trees (five *F. americana*/0° injection, five *F. pennsylvanica*/0° injection, and five *F. pennsylvanica*/90 ° injection) from spring injected trees and one replication of fall injected trees were destructively harvested in October and November, 2007. Trees were lifted out of pots using a skid steer and chain. Sand was rinsed off roots using a garden hose and spray nozzle. Roots were removed from the trunk using hand pruners and loppers and classified as either coarse or fine. Roots that were smaller than 5.0 mm diameter were considered fine roots, all others were considered coarse roots. Branches from whorls one through six (where applicable) were labeled (0°, 180°, R90° or L90°, based on orientation to the injection point) and removed from the bole of the tree with loppers. Stem sections were removed from tips of branches and separated into 2006 and 2007 growth sections as determined by the position of the axillary leaf scars. A sub-

sample from each tree of five to seven leaves each from terminal leader and whorl branches, approximately 3 g each of fine and coarse roots, and stems from harvested trees were dried, ground (leaves only), weighed, oxidized and processed through the LSC. The bole of the tree was cut into sections at 10.0 cm, 1.0 m and 2.0 m above the center of the injection scar, and a 2.0 cm thick cross-section was removed. Cross sections were split into quarters using a hammer and chisel. One of these sections was further divided into three pieces and a single piece was dried, weighed, oxidized, and processed through the LSC. The cross sections cut at 10.0 cm above the injection scar contained portions of xylem that were stained by the initial imidacloprid injection; a portion of this stained area was also chiseled out, dried, weighed, oxidized, and processed through the LSC. All branches, fine and coarse roots, and the tree bole were cut into manageable sections using hand pruners, loppers, and a pruning saw, dried, and weighed.

Statistical Analysis:

Data were tested for normality using residual plots. Variables were not normal. Imidacloprid equivalent concentrations for spring-injected ash trees were transformed using a $\log(x+2)$ transformation. Imidacloprid equivalent concentration for fall-injected ash trees was transformed using the $\log(x+2)$ transformation. Data were analyzed using the Mixed procedure for mixed models in SAS statistical software (SAS Institute, Inc. 1989) with whorl nested in tree, branch position nested in whorl, tree species and day as fixed effects and tree number as a random effect. The models for repeated measures were examined using CS covariance structure. Analysis of variance (ANOVA) was used to determine which effects and interactions were significant ($p < 0.05$) for whorl, branch position, tree species, and day. Different ANOVAs were produced for each injection date

(spring vs. fall) by injection position (0 or 90). If effects were significant, LSMeans procedures were used for mean separation (Littell et al., 1996).

RESULTS

Spring Injection:

Imidacloprid equivalent concentration (IEC) in leaves varied ($p < 0.05$) with time and orientation to the injection point. Species or branch whorl position did not affect IEC ($p < 0.05$). Imidacloprid equivalent concentration increased rapidly following trunk injection throughout the 2006 growing season until 21 days after treatment (DAT). For a given whorl, IEC was greater ($p < 0.05$) in leaves from branches in the plane of injection (0°) than leaves from branches opposite the plane of injection (180°) throughout the 2006 growing season. However, the difference was less pronounced in whorl three, suggesting that flow becomes more integrated with plant height. In general, branch position did not affect ($p > 0.05$) IEC in leaves from 0° or 180° branches in 2007 (Figure 2.2).

Leaves of $L90^\circ$ branches had lower IEC ($p < 0.05$) than leaves on $R90^\circ$ branches throughout the 2006 growing season and were different only in leaves from whorl three in 2007. Similar to leaves on 0° and 180° branches, IEC in 2007 leaves from $R90^\circ$ and $L90^\circ$ branches was reduced by 20-fold when compared to IEC in leaves sampled in 2006 (Figure 2.2).

Imidacloprid equivalent concentration in leaves collected from the terminal leader increased ($p < 0.05$) through 60 DAT in 2006, but IEC levels were greatly reduced in 2007. Imidacloprid equivalent concentration increased ($p < 0.05$) in trees injected at 0° to the first whorl of branches the year after injection (Figure 2.2).

Roots sampled in 2006 and 2007 and trunk cores (0° and 180°) collected in 2006 did not have detectable IEC. Root and trunk core samples collected 60 DAT had lower

($p < 0.05$) IEC than leaves collected from whorl and stems from 0° and $R90^\circ$ branches. Roots, trunk cores, and 180° stems were not different at 60 DAT. For samples collected 60 DAT, stems had lower ($p < 0.05$) IEC than leaves from corresponding branches and whorls (Figure 2.3a). Stems from 0° and $R90^\circ$ branches had higher ($p < 0.05$) IEC than stems from 180° and $L90^\circ$ branches respectively. Overall, IEC in stems from 0° and $R90^\circ$ branches were not different ($p < 0.05$) and stems from 180° and $L90^\circ$ branches were not different ($p < 0.05$) (Figure 2.3b).

Leaves from 0° , $R90^\circ$ and 180° branches had higher IEC values than stems of corresponding branches. Stems from branches in whorl one had higher ($p < 0.05$) IEC than either fine or coarse roots. Fine root IEC was not different ($p < 0.05$) from coarse root IEC (Figure 2.4). Stem samples taken from 2006 growth from 0° and $R90^\circ$ branches had higher ($p < 0.05$) IEC than stems from 180° and $L90^\circ$ branches consistently through whorl four. Beyond whorl four IECs were similar between branches of the same whorl. Stem samples taken from 2007 growth had higher ($p < 0.05$) IEC in leaves from 0° branches than leaves from 180° branches in whorls one and three but no other differences occurred between branches of the same whorl. There was no difference ($p > 0.05$) in IEC between 2006 and 2007 stem samples for any whorl (Figure 2.5).

Wood samples taken from the stained area at 10.0 cm of the trunk cross-sections had IEC approximately 75 times higher than all other wood samples taken from the bole of the tree. Imidacloprid equivalent concentrations were the same for wood cross sections taken from 10.0 cm (no stain) and 1.0 m and samples taken from 1.0 m and 2.0 m for 0 injected trees (Figure 2.6). Samples taken from 90° injected trees had the same

IEC in 0.10 m (no stain) and 2.0 m sections, but sections taken from 1.0 m were higher than both 0.10 m (no stain) and 2.0 m sections (Figure 2.6 inset).

Fall Injection:

For trees that were trunk-injected at 0° and 90° to the first whorl of branches in the fall, IEC did not differ between leaves from 0° and 180° degree branches in whorl one. Imidacloprid equivalent concentration in leaves from 0° branches did not increase beyond 7 DAT and IEC in leaves from 180° branches did not increase over time. The year after injection, leaves from 0° branches had higher ($p < 0.05$) IEC than leaves from 180° branches. In addition, leaves from 0° branches had similar ($p > 0.05$) IEC as leaves from the corresponding branch sampled 21 DAT in 2006. Imidacloprid equivalent concentration in leaves from 180° branches did not increase over time during 2007 (Figure 2.7).

The general trend of IEC in R90° branches indicates some increase in the year after injection. However, in most cases the increase was not statistically different among sampling dates or branches. No IEC increase occurred in leaves from L90° branches (Figure 2.6). Imidacloprid equivalent concentration was lower ($p < 0.05$) in stems than leaves. Roots from 2006 and 2007 and trunk cores from 2006 contained no measurable IECs (Figure 2.8).

Wood samples taken from the 10.0 cm stained area of trunk cross sections had IEC approximately 136 times higher than all other wood samples taken from the bole of the tree. Imidacloprid equivalent concentrations were the same for wood cross sections taken from 10.0 cm (no stain) and 1.0 m which were higher than IEC in 2.0 m trunk cross sections (Figure 2.9).

Spring vs Fall Injected Leaves:

Leaves harvested 21 DAT (19 July 2006 – spring injection, 26 September 2006 – fall injection) from 0°, R90° and L90° branches on trees injected in spring had higher IEC than leaves taken from corresponding branches of trees injected in fall (Figure 2.10). In the year following injection, leaves from 0° branches of fall injected trees had higher ($p < 0.05$) IEC than leaves from 0° branches of spring injected trees. Leaves from 180°, R90° and L90° branches did not differ among corresponding branches of trees injected in spring or fall (Figure 2.11).

Observations:

On June 18, 2007 EAB larval galleries were positively identified in several trees used in this study. Although not part of our original objectives, the presence of larvae on ¹⁴C-imidacloprid trunk-injected trees was noteworthy. Un-infested trees were purchased from a nursery which is well beyond the known EAB infestation. Therefore EAB larvae present on trees were from eggs deposited in summer 2006 by local Michigan beetles. After initial discovery, all trees were examined carefully and the presence of visible galleries was recorded. Trees were not treated with additional insecticide. In some trees, canopy dieback and branch damage did occur; one trunk-injected tree was completely girdled and did not leaf out in 2007.

On trees where galleries were exposed, frass samples were removed, dried, oxidized, and processed through the LSC. No detectable imidacloprid equivalents were present (data not shown). In addition, 16 dead beetles found in 12 of the pots containing injected trees were dried, oxidized and processed through the LSC. Two beetles contained detectable IEC (data not shown).

At the conclusion of the 2007 growing season, 15 trees (see Materials and Methods) were destructively harvested. During that time, number of galleries per tree was recorded and galleries per m² of tree bark was calculated (Table 2.1). In addition 12 additional trees were examined for galleries during spring, 2008. There did not appear to be any patterns in the position of the galleries in relation to the injection point, in fact, galleries appeared in both the plane of and the opposite plane of the injection site. In this particular case, imidacloprid trunk-injection did not prevent larval development.

DISCUSSION

Variability of trunk-injected insecticide translocation can be affected by a variety of parameters including xylem anatomy, time of injection, and time after injection. Although literature does exist pertaining to translocation of imidacloprid in hemlock, avocado, and citrus (Castle et al., 2004, Cowles et al., 2006, Lawson et al., 2003, Sclar and Cranshaw, 1996)) trees, information on the specific translocation paths of imidacloprid in ash trees is not available. Efficacy of imidacloprid in several trials has been variable (McCullough et al., 2005a and Harrel, 2006). This study attempts to explain some of that variability. Our primary objectives were to determine the (1) spatial movement of imidacloprid in ash trees, (2) imidacloprid equivalent concentration in ash trees over two growing seasons and (3) variability of imidacloprid equivalent concentration between spring and fall trunk-injected ash trees.

Objective One-

We found that imidacloprid equivalent concentration varied in leaves from spring and fall injected trees and orientation of branches in relation to the injection point. During 2006, IEC increased in leaves from all six branches of both 0° and 90° spring-injected trees. Leaves from branches in the plane of injection (0°) had greater IEC than leaves on the opposite side of the injection site (180°) in both the 0° and 90° injected trees. In addition, leaves from R90° branches had higher IEC than L90° branches. Movement of labeled compound was very ordered during the first growing season. Insecticide moved quickly in the longitudinal direction (as seen in leaves from branches in the plane of injection (0°)) but very slowly in the radial plane (as seen in leaves from branches

opposite the injection point (180°)). This is consistent with other studies that have shown that *F. americana* has longitudinal movement six orders of magnitude higher than radial movement (Zwieniecki et al., 2001). No deviations from the pattern of ascent occurred in any of the whorls in either injection over time. The consistent, ordered pattern of IEC in leaves from specific branches suggests that *Fraxinus* spp. trees have sectoried xylem pathways. The sectoried patterns of the xylem architecture create uneven distribution of insecticide within the tree crown. Although the trees used for this study were small (due to containment restraints), we believe that the patterns of sectoriality would be similar in larger trees (Orians et al., 2004). As seen here, unbalanced injection sites could lead to uneven insecticide distribution and variable insecticide efficacy.

Ash is a ring porous species and is generally described as straight grained. Ring porous and straight grained species usually have sectoried patterns of xylem architecture. However, patterns of sap ascent in trees normally deviate from purely straight and are often described as helical (Tyree and Zimmerman, 2002). If translocation patterns in ash xylem were purely helical, we would expect to see continued spiral ascent and higher IEC in leaves from third whorl 180° branches when compared to leaves from 0° branches, which was not the case in this study. If the xylem architecture was straight sectoried, leaves from second whorl branches would have intermediate IECs to those of both whorls one and three, this did not occur either. Instead we determined that ash xylem architecture has a zigzag pattern of ascent. Starting at whorl one, the highest concentration of insecticide appears in branches in the plane of injection (0°). Moving up the tree to whorl two, the highest levels occur in leaves from R90° branches. Finally, in whorl three, the highest IEC is in leaves from branches within the plane of injection (0°). A similar

zigzag pattern occurs on the opposite branches of the tree. Again beginning at whorl one, insecticide levels in leaves on the opposite side of the injection site (180°) are low compared to leaves from 0° branches. In whorl two, only small amounts of insecticide are present in leaves from L90° branches. Finally in whorl three, leaves from branches opposite the injection point have low levels of insecticide. High variability in 90° injected trees could be attributed to low sample size. Kiten et al. (2004), propose that sap ascent patterns of *F. lanuginosa* Koidz. are influenced by vessel to vessel contact, and even though ash is considered a straight grained wood, tangential drift in vessel position does occur. Burggraaff (1972) describes a zigzag pattern in the xylem architecture of *F. excelsior* L. which has vessels that slant toward the right. In addition, he states that vessels are in contact with one another in both the tangential and radial directions but that no perforations exist that allow open contact between vessels.

In addition to leaves, imidacloprid equivalents were also present in stems. We did not sample stems more than once per year to prevent excessive tree damage. Stem sections harvested from 0° injected trees 60 DAT (2006) had IEC patterns similar to leaves. Concentrations were highest in stems from 0° and R90° branches when compared to stems from 180° and L90° branches. The IEC pattern in stems also supports the theory of zigzag ascent. Imidacloprid equivalent concentrations in stems from 90° injected trees were more similar between branches, but the same pattern was still present.

Patterns of sectoriality were much less pronounced in trees that were trunk-injected during the fall. In fact, no difference in IEC in leaves between opposing branches occurred during 2006 for trees that were injected at 0° to the first whorl of branches or trees injected at 90° to the first whorl of branches. In 2007, however, IEC in

leaves from 0° branches were higher than leaves from 180° branches of 0° injected trees and leaves from R90° branches had higher IEC than leaves from L90° branches of 90° injected trees. Even though these patterns were not as obvious in fall-injected trees because of low IEC, patterns of sectoriality were consistent with those of spring injected trees and support the zigzag ascent theory.

Several studies have demonstrated that imidacloprid movement in plants occurs via the xylem (Bromilow and Chamberlain, 1989, Sur and Stork, 2003, Tanis et al., 2007a). To determine whether this was the case in ash, we harvested fine roots and trunk cores (2006 only to prevent excess tree damage) and performed leachate tests. As previously noted, imidacloprid was present in measurable concentrations in leaves and stems. In contrast, no measurable IEC was present in roots or trunk cores. If imidacloprid movement was occurring in the phloem, these tissues would have $IEC > 0$. To further investigate fine roots, we also performed pour through leachate tests to determine if fine root turnover from trunk-injected trees could contribute to imidacloprid residues in soil. No detectable imidacloprid equivalents were present in leachate collected in pour through samples (Tanis et al., 2007b).

In a previous study that used ^{14}C to examine imidacloprid movement in ash trees, Cregg et al. (2006) demonstrated that canopy leaves collected after abscission contained IEC as high as those sampled before abscission. In other words, imidacloprid was not translocated from leaves back into the trunk before litterfall. Post harvest analysis of trunk segments of trees used in that study had very high IEC in stained areas around the injection site (Tanis et al., 2007b). We hypothesize that trunk tissue in this area serves as a reservoir that supplies imidacloprid to leaves during subsequent growing seasons. To

further investigate, trees from the present study were destructively harvested at the conclusion of the 2007 growing season. Cross sections of wood were taken from the bole of trees at different heights above the injection point to determine if imidacloprid was present in woody tissue. Similar to the cross sections taken from trees in the previous study, cross sections taken 0.10 m above the injection point had stained areas (from the initial imidacloprid injection) that contained extremely high IECs when compared to all other plant parts. Samples of non-stained wood taken from the same area and points higher on the tree had lower IEC concentrations than stained wood. These results support the reservoir hypothesis that imidacloprid is pooled in woody tissue around the injection point and gradually dissipates as the tree gets taller. High IEC in woody tissue and zero IEC in roots and trunk cores support previous evidence that states that imidacloprid moves primarily through the xylem.

Objective Two:

Our second objective was to determine how much imidacloprid was present in trees after two growing seasons. Previous studies have shown that high levels of efficacy can be obtained from a single injection over two growing seasons, but others have shown that levels of insecticide are greatly reduced during the second year after injection.

In this study, spring-injected insecticide would be providing a second season of EAB control in 2007 while fall-injected insecticide would be providing its first year of control. In leaves from 0° and 90° spring-injected trees, IEC on 11 June 2007 was less than 12% of levels found at the end of the growing season in 2006. In a parallel study, bioassays conducted on adult beetles using leaves from these trees indicated that 50% of

A. planipennis beetles were affected, down from 80% the previous year (Mota-Sanchez et al., 2007b).

Imidacloprid equivalent concentration in leaves from fall injected trees were generally not different between growing seasons even though percent increase was over 75% in all branches of 0 injected trees. The question remains, is there enough compound in the leaves to be efficacious?

Objective Three:

Our third objective was to determine differences in IEC in spring-injected trees compared to fall-injected trees. Here we compare trees from each injection at two sampling dates. First, we compare trees from spring and fall injections at 21 DAT (19 July 2006 and 26 September 2006, respectively). We chose this date because it was the last date that fall-injected trees were sampled in 2006. Leaves from spring-injected trees had higher concentrations 21 DAT than leaves from fall-injected trees in all branch positions. This is probably due to decreases in temperature during the fall, the length of time before abscission, and decreased transpiration rates.

We also compared the two injections on 11 June 2007. This sampling date roughly corresponds to 2007 EAB peak flight in Michigan (Anulewicz et al., 2008b). Imidacloprid equivalent concentrations in leaves from spring-injected trees were lower in 2007 than 2006. Imidacloprid equivalent concentrations in leaves from fall-injected trees were lower initially and remained relatively constant. Since less imidacloprid was present in leaves from fall-injected trees in 2006 than spring-injected trees, less insecticide was lost during litterfall. This would suggest that there would be more ¹⁴C-labeled compound available for translocation in fall injected trees than spring-injected

trees in 2007. However, on 11 June 2007, only leaves from 0° branches from fall-injected trees had higher IEC than leaves from corresponding branches of spring-injected trees. Therefore, even though larger reservoirs of imidacloprid are available to fall-injected trees than spring-injected trees, higher levels of imidacloprid are not translocated to leaves of 180°, R90°, or L90° branches. These patterns of ascent could affect the efficacy of imidacloprid against EAB. If only one third of the branches receive adequate quantities of imidacloprid during the second season (spring-injected), or following fall injection, many EAB could feed on trees and not be affected. Results from efficacy trials for fall-injected trees are not available at this time but if levels from spring-injected trees were <50% efficacious and spring and fall injected trees only differed in two branches, one can assume that fall-injected insecticide might be <50% efficacious as well.

In summary, we have shown that imidacloprid equivalent concentration varies in leaves of ash trees based on the position of branches in relation to injection points and time that trees were injected. Our results indicate that efficacy of trunk-injected imidacloprid could be reduced in trees injected in fall when compared to trees injected during spring. In addition, after over-wintering, new leaves from spring-injected trees do not have adequate levels of insecticide to provide effective EAB control based on the rates applied. We also observed that larval galleries were present in the injected trees, and damage from galleries in some cases was quite severe. Finally we conclude that xylem anatomy in ash trees is such that trunk-injected imidacloprid travels through the tree in a zigzag xylem ascent pattern. This zigzag pattern results in variable levels of insecticide in the tree crown.

Table 2.1: Total number of emerald ash borer (*Agilus planipennis*) larval galleries in ash trees (*Fraxinus americana* and *F.*

pennsylvanica) and gallery density (number of larvae galleries per meter² trunk surface) in trees trunk-injected in either spring or fall with ¹⁴C-imidacloprid.

Tree	Total Trees	% of Trees Infested	Total Galleries	% of Total Infested Trees	Surface Area of Bark (m ²)	Galleries per meter ²
<i>Fraxinus americana</i>	11	22.22%	14	33.33%	1.88	7.50
<i>Fraxinus pennsylvanica</i>	16	37.04%	28	66.67%	3.09	9.06
Total	27	59.26%	42	-	4.97	8.45
Spring Injected <i>F.americana</i>	3	11.11%	8	19.05%	0.73	10.95
Spring Injected <i>F.pennsylvanica</i>	8	29.63%	19	45.24%	0.90	21.1
Fall Injected <i>F.americana</i>	3	11.11%	6	14.29%	1.16	5.17
Fall Injected <i>F.pennsylvanica</i>	2	7.41%	9	21.43%	2.18	4.13

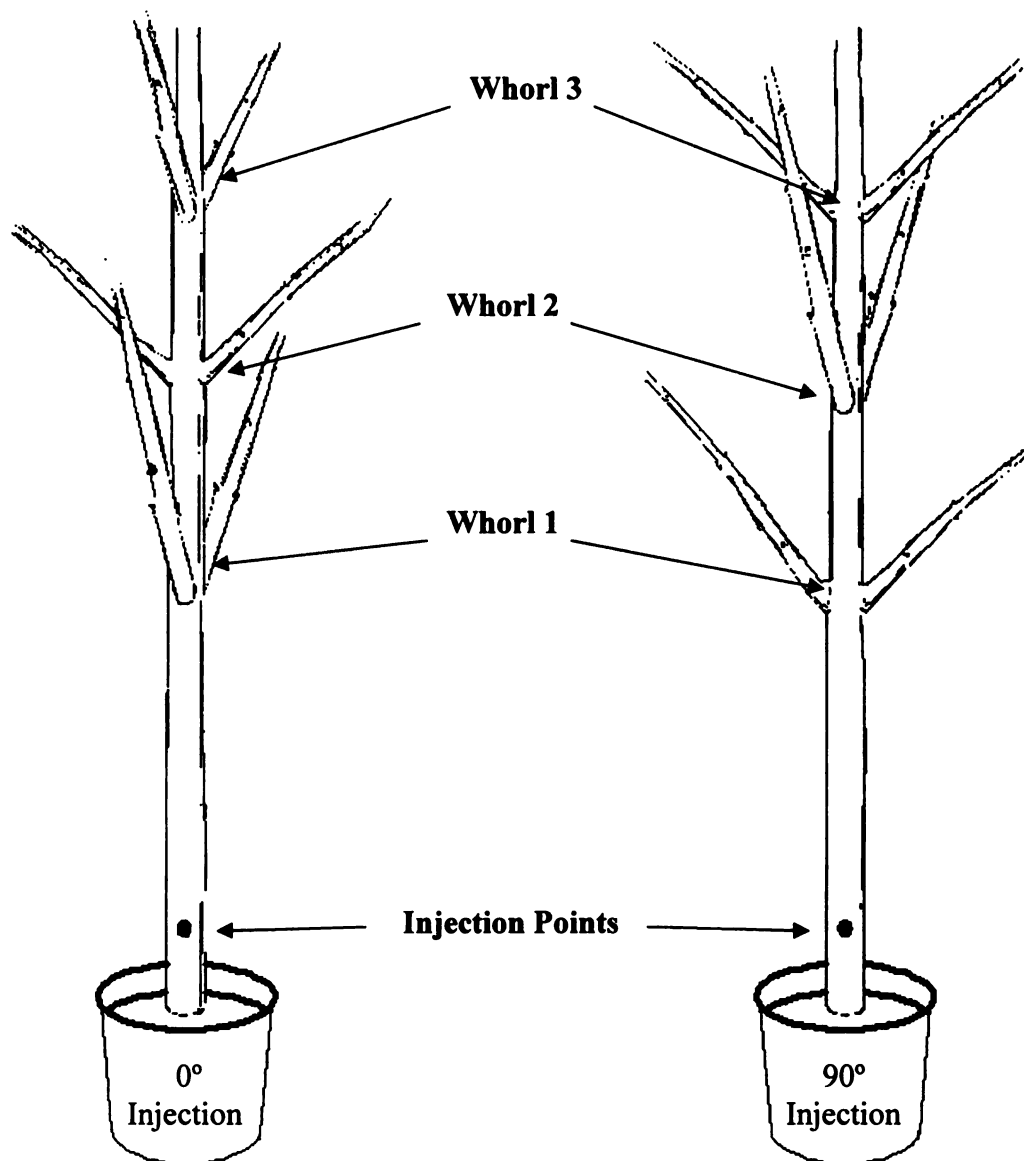


Figure 2.1a: Treatment schematic, trees were injected at either 0° or 90° to the first whorl of branches. Mean distance from injection point to the first whorl = 1.28 m. Mean distance between whorls = 0.18 m.

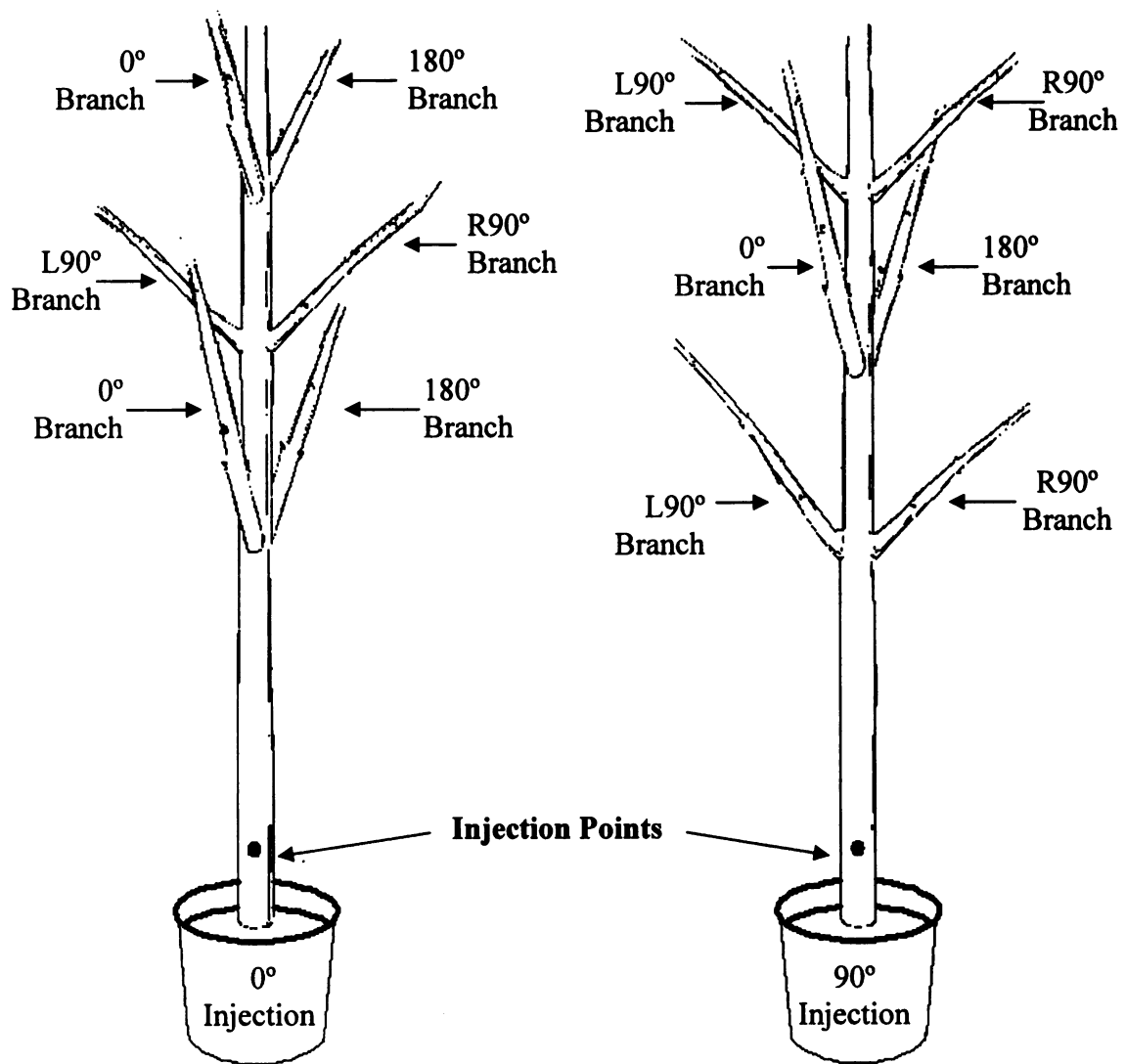
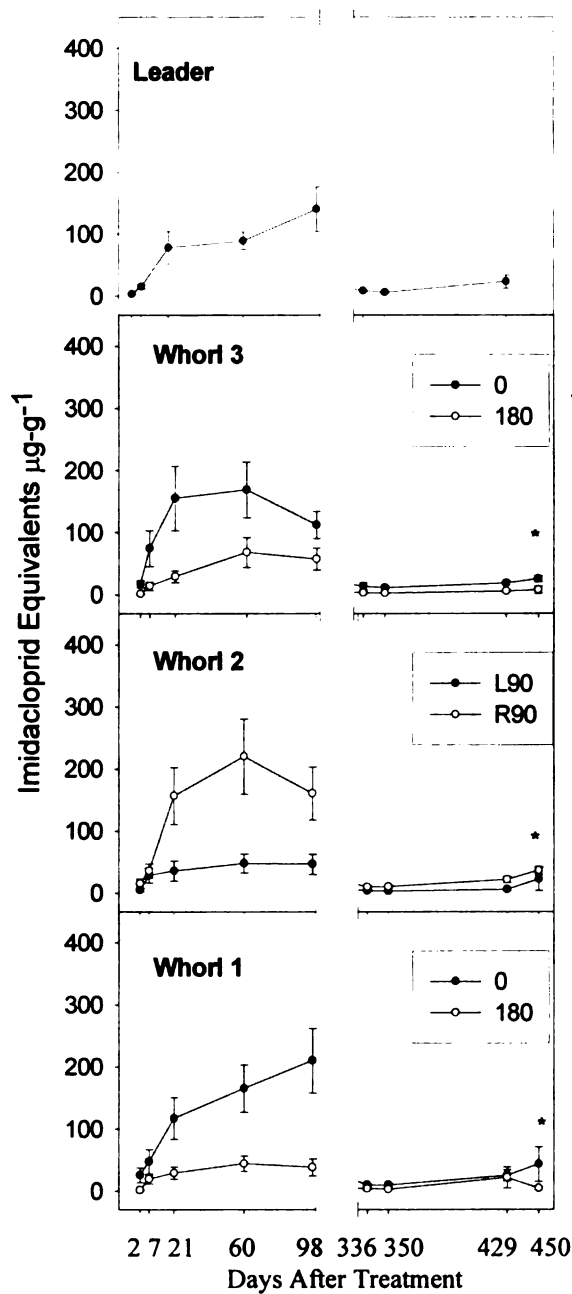


Figure 2.1b: Sampling schematic of trees injected at either 0° or 90° to the first whorl of branches. Branches of the first three whorls were labeled 0°, 180°, L90° or R90° in relation to the injection point. Mean distance from injection point to the first whorl = 1.28 m. Mean distance between whorls = 0.18 m.

Figure 2.2: Mean ^{14}C -Imidacloprid equivalent concentration $\pm$ S.E. in whorl and terminal leader leaves from 0° or 90° (in relation to the first whorl of branches) spring-trunk-injected *Fraxinus americana* and *F. pennsylvanica* trees. Branches were labeled 0° , 180° , L 90° or R 90° (in relation to the injection point. An * denotes leaves collected after abscission.

Fraxinus spp. Trunk injection at 0° to the first whorl of branches



Fraxinus spp. trunk-injected at 90° to the first whorl of branches

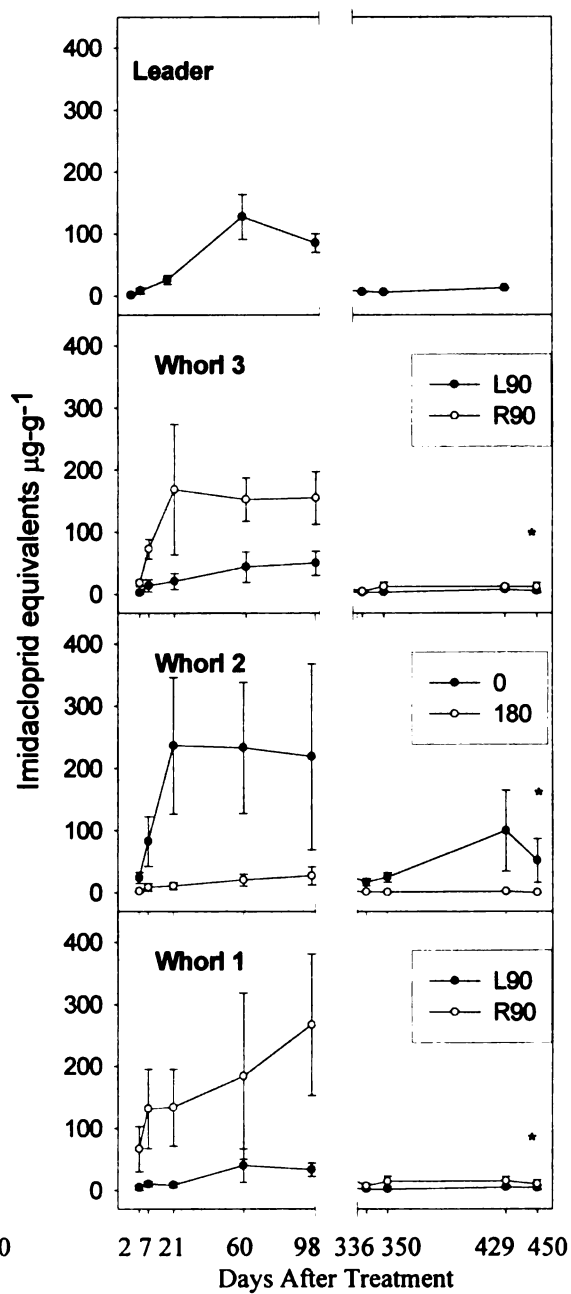


Figure 2.3a: Mean ^{14}C -Imidacloprid equivalent concentration $\pm$ S.E. in whorl one stems and leaves (0° , 180° , $L90^\circ$ or $R90^\circ$ -in relation to the injection point), trunk cores (0° or 180° in relation to the injection point) and roots of 0° and 90° (in relation to the first whorl of branches) spring-trunk-injected *Fraxinus americana* and *F. pennsylvanica* trees, samples taken 60 days after treatment. Inset graphs have leaf data bars removed.

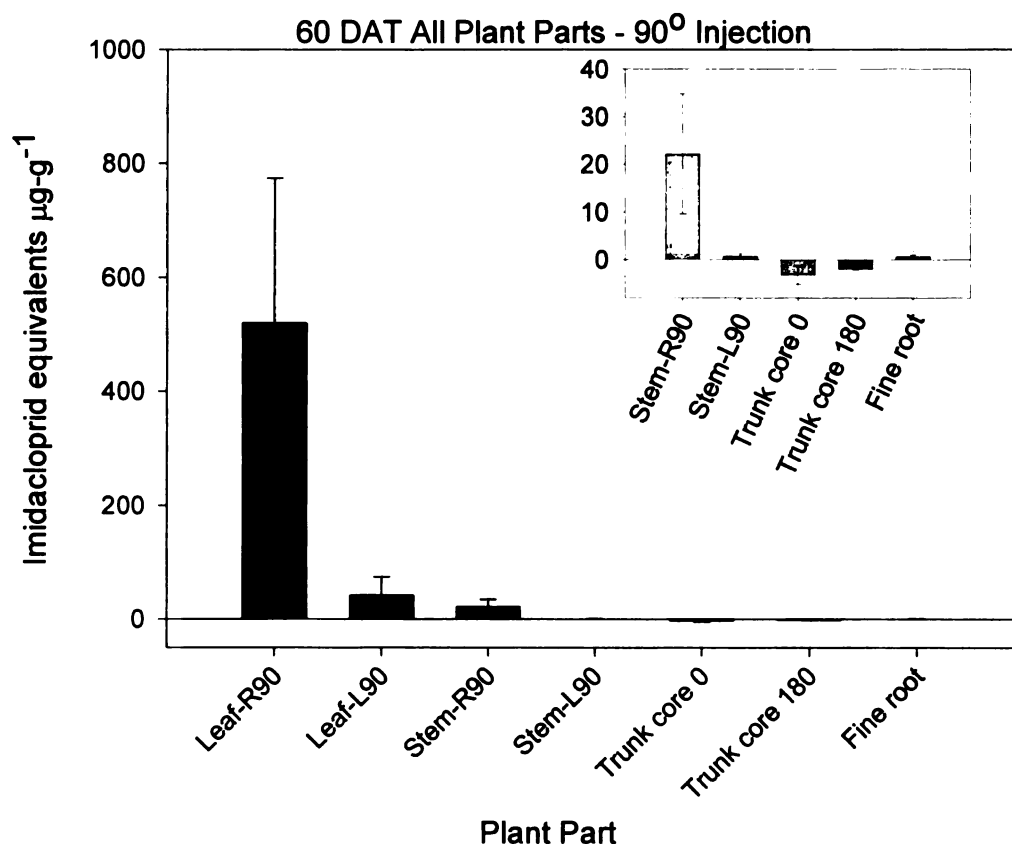
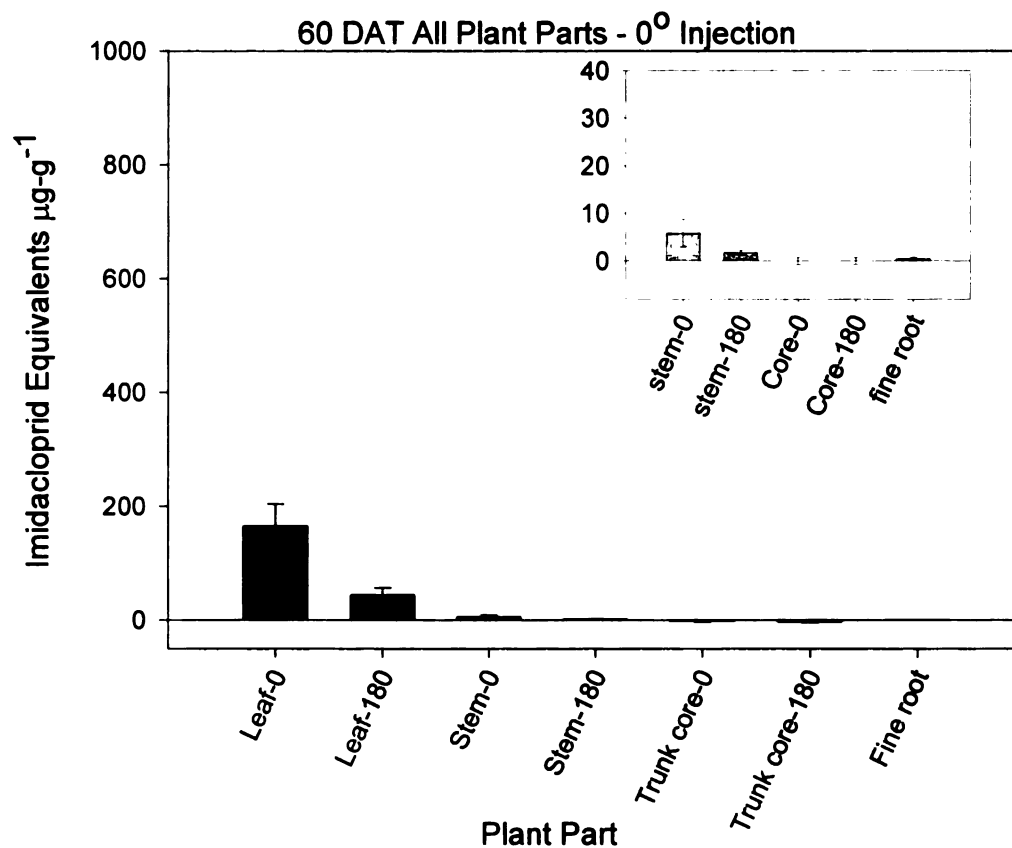
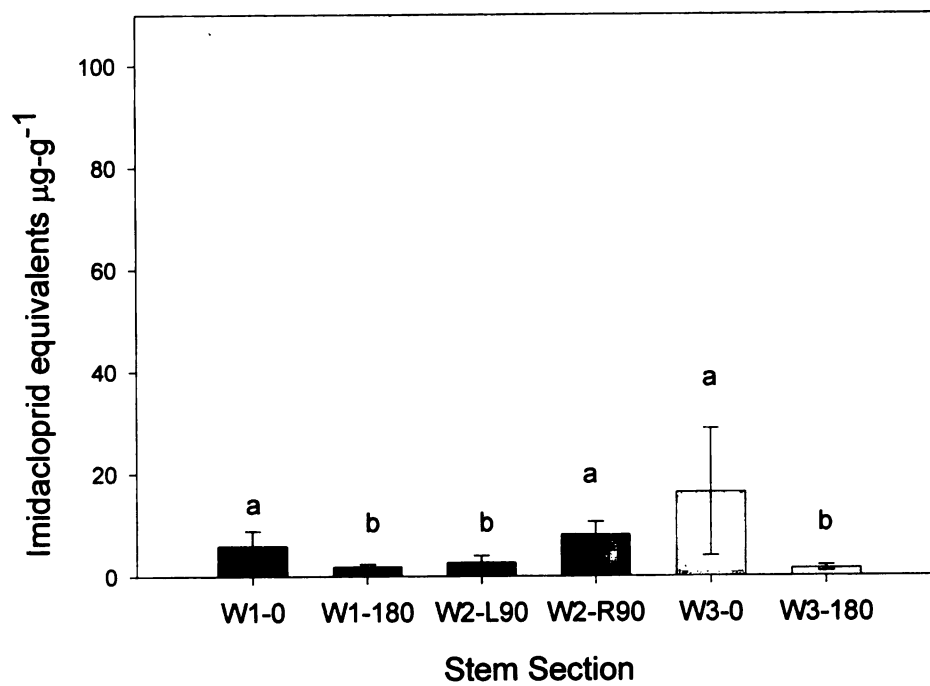


Figure 2.3b: Mean ^{14}C -Imidacloprid equivalent concentration $\pm$ S.E. in stem samples taken from the first three branch whorls of 0° and 90° (in relation to the first whorl of branches) spring-trunk-injected *Fraxinus americana* and *F. pennsylvanica* trees 60 days after treatment. Stem samples were labeled 0° , 180° , L 90° or R 90° in relation to the injection point.

Stems 60 DAT - 0° Spring Injection



Stems 60 DAT - 90° Spring Injection

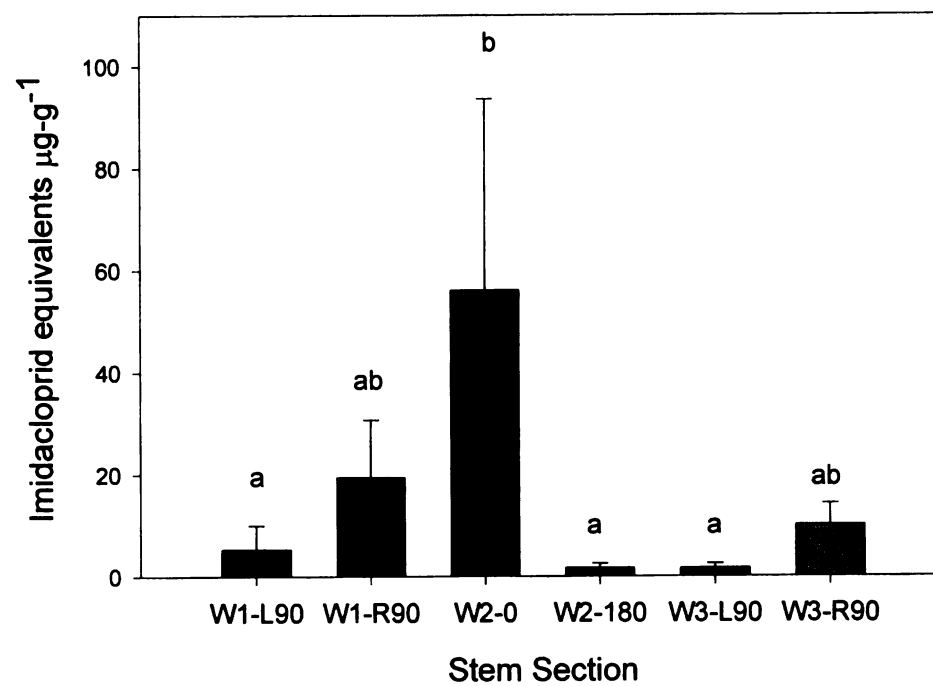
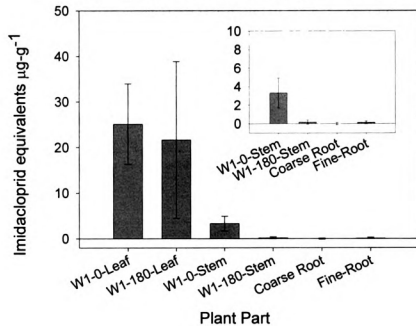


Figure 2.4: Mean ^{14}C -imidacloprid equivalent concentration $\pm$ S.E. in leaves and stems from first whorl branches (0° , 180° , L 90° or R 90° in relation to the injection point), coarse and fine roots. Samples taken from 0° or 90° (in relation to the first whorl of branches) spring-trunk-injected *Fraxinus americana* and *F. pennsylvanica* trees 60 days after treatment. Inset graphs have leaf data bars removed.

Final Harvest - All Parts - Spring injection - 0°



Final Harvest - All Parts - Spring injection - 90°

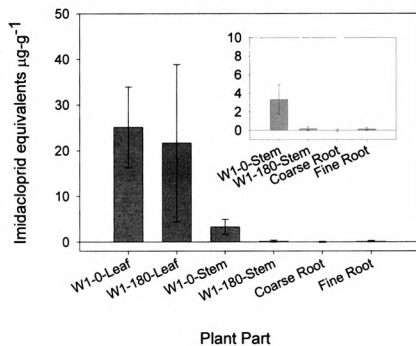


Figure 2.5: Mean ^{14}C -Imidacloprid equivalent concentration $\pm$ S.E. in 2006 and 2007 stem sections from 0° and 90° (in relation to the first whorl of branches) spring-trunk-injected *Fraxinus americana* and *F. pennsylvanica* trees. Stems were harvested in October 2007 from the first six branch whorls (where available) and labeled 0°, 180°, L90° or R90° in relation to the injection point.

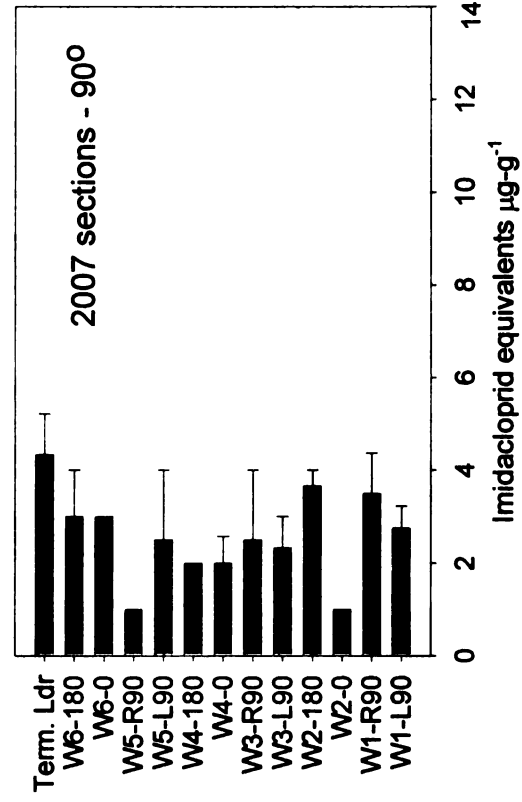
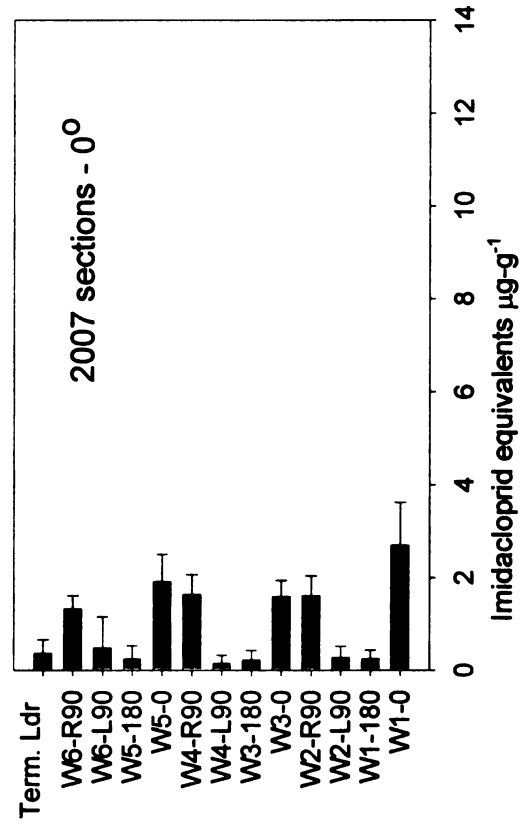
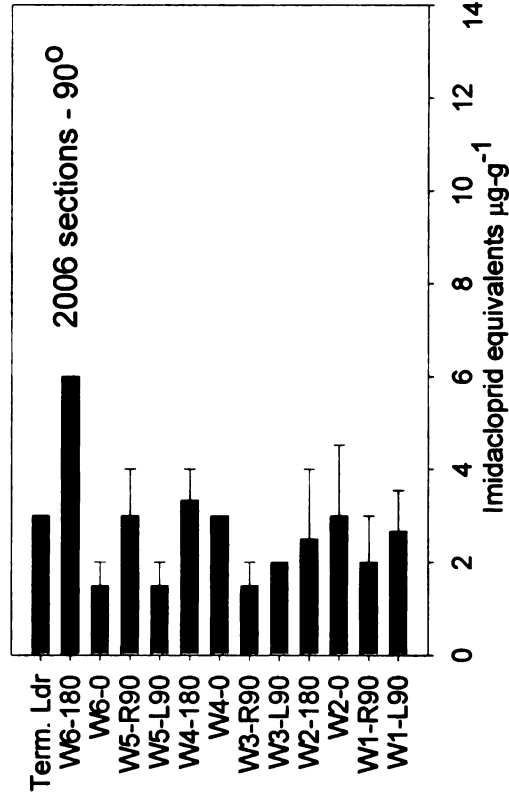
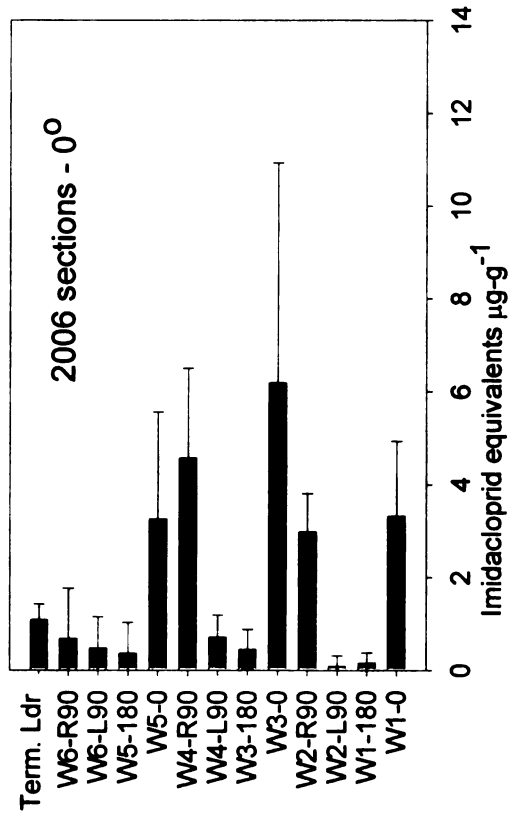
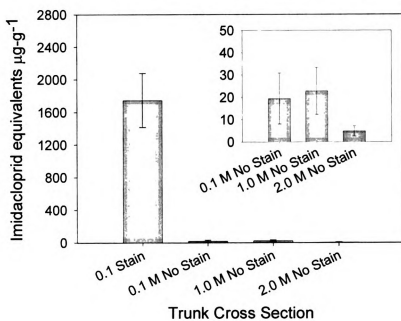


Figure 2.6: Mean ^{14}C -imidacloprid equivalent concentration $\pm$ S.E. in stained and unstained trunk tissue taken at varying heights above the center of the injection scar from 0° or 90° (in relation to the first whorl of branches) spring-trunk-injected *Fraxinus americana* and *F. pennsylvanica* trees harvested in October, 2007. Inset graphs have the stained cross section data bars removed.

Trunk Cross Sections 0° Injections



Trunk Cross Sections 90° Injection

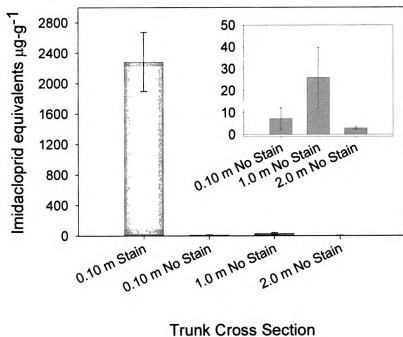
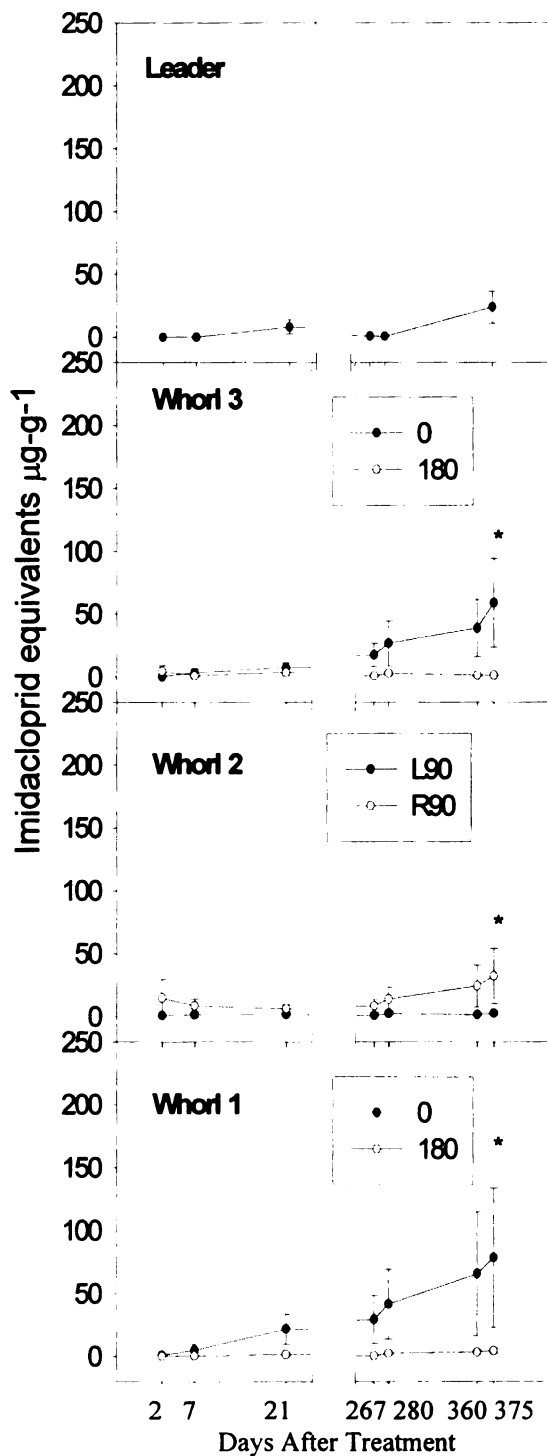


Figure 2.7: Mean ^{14}C -imidacloprid equivalent concentration $\pm$ S.E. in whorl and terminal leader leaves from 0° or 90° (in relation to the first whorl of branches) fall-trunk-injected *Fraxinus americana* and *F. pennsylvanica* trees. Branches were labeled 0°, 180°, L90° or R90° (in relation to the injection point). An * denotes leaves collected after abscission.

Fraxinus spp. fall-trunk-injected at 0°
to the first whorl of branches



Fraxinus spp. fall-trunk-injected at 90°
to the first whorl of branches.

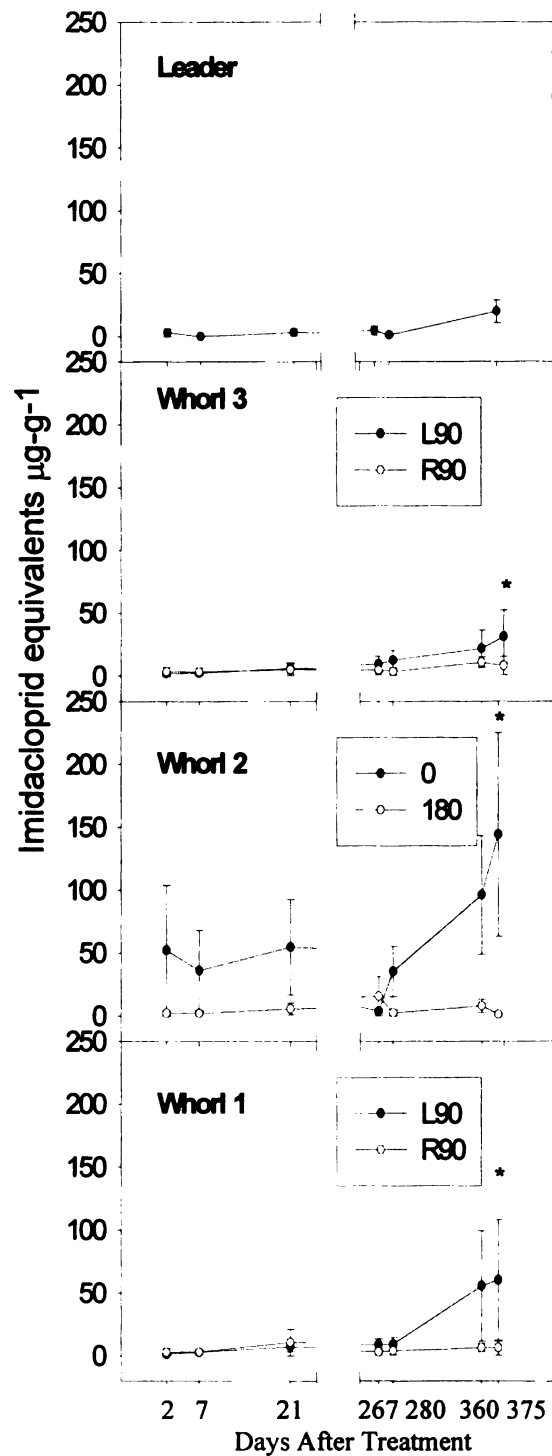
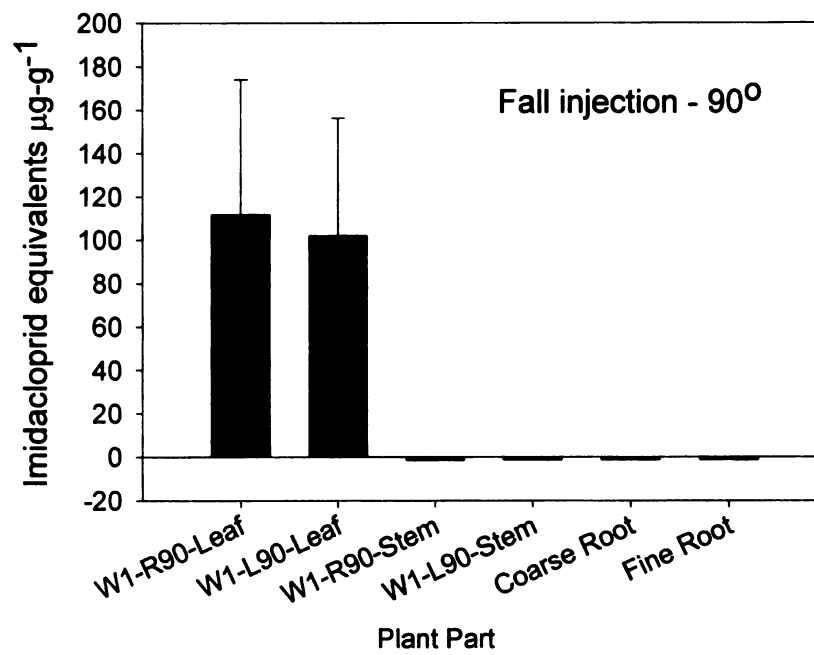
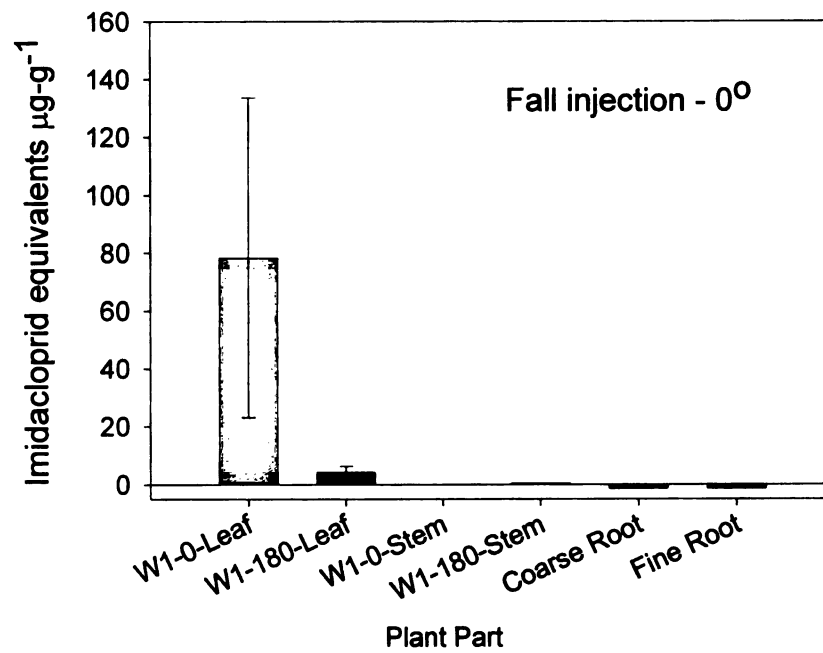


Figure 2.8: Mean ^{14}C -imidacloprid equivalent concentration $\pm$ S.E. in leaves and stems from first whorl branches (0° , 180° , $L90^\circ$, or $R90^\circ$ in relation to the injection point) and coarse and fine roots. Samples were taken during destructive harvest from 0° or 90° (in relation to the first whorl of branches) fall-trunk-injected *Fraxinus americana* or *F. pennsylvanica*.



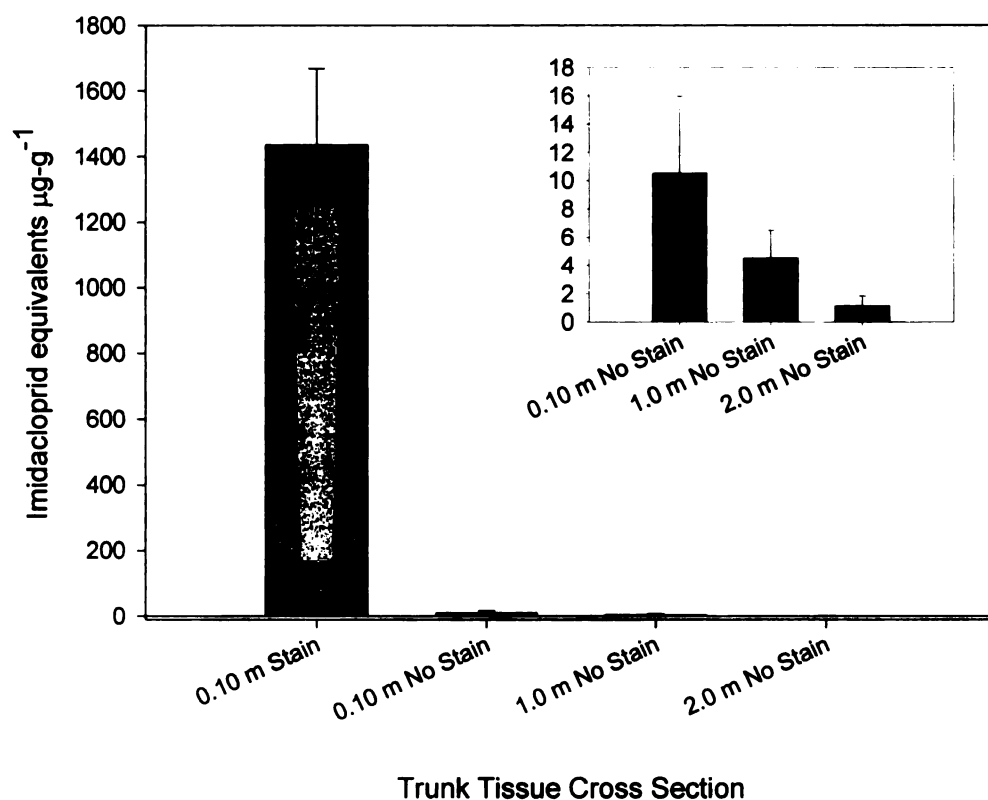


Figure 2.9: Mean ¹⁴C-imidacloprid equivalent concentration $\pm$ S.E. in stained and unstained trunk tissue taken at varying heights above the center for the injection scar from 0° and 90° (in relation to the first whorl of branches) fall-trunk-injected *Fraxinus americana* and *F. pennsylvanica* trees harvested in October, 2007. Inset graph has the stained data bar removed.

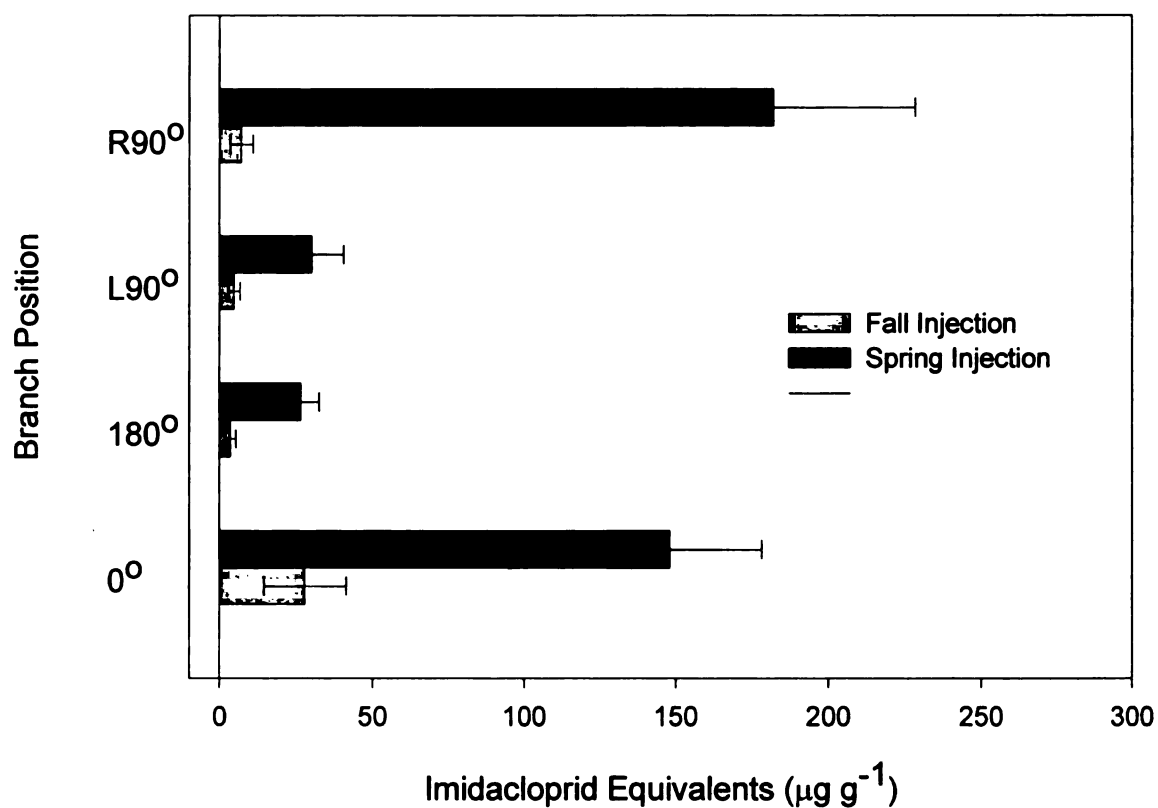


Figure 2.10: Mean ¹⁴C-imidacloprid equivalent concentration $\pm$ S.E. in leaves from the first three branch whorls of 0° and 90° (in relation to the first whorl of branches) of spring- and fall-trunk-injected *Fraxinus americana* and *F. pennsylvanica* trees. Values were averaged across whorls. Branches labeled 0°, 180°, L90° or R90° in relation to the injection point. Samples were taken 21 days after treatment (19 July 2006 and 26 September 2006).

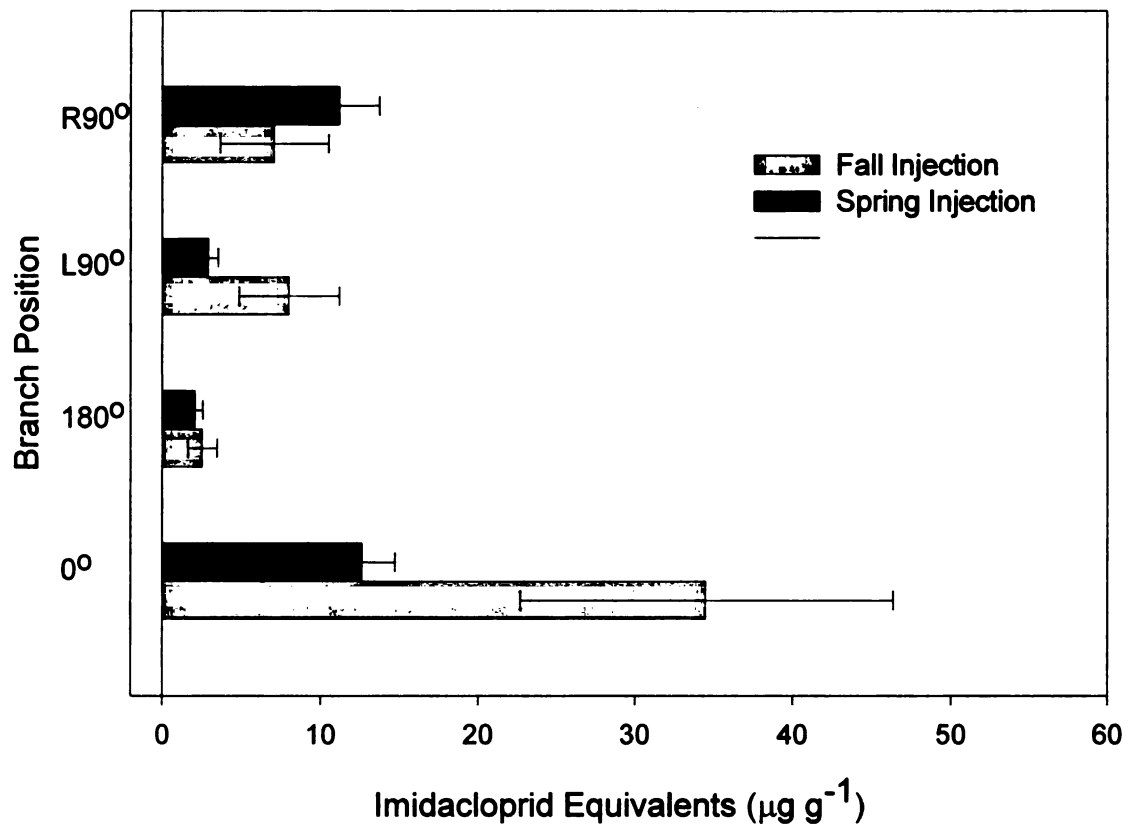


Figure 2.11: Mean ¹⁴C-imidacloprid equivalent concentration $\pm$ S.E. in leaves from the first three branch whorls of 0° and 90° (in relation to the first whorl of branches) of spring- and fall-trunk-injected *Fraxinus americana* and *F. pennsylvanica* trees. Values were averaged across whorls. Branches labeled 0°, 180°, L90° or R90° in relation to the injection point. Samples were harvested on 11 June 2007.

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Distribution of Trunk-Injected ^{14}C Imidacloprid in *Acer* Trees: A Test of the Integrated-flow Hypothesis.

ABSTRACT

The Asian Longhorned beetle (ALB, *Anoplophora glabripennis* Motschulsky, Coleoptera, Cerambycidae) is an invasive exotic insect pest. In the United States, ALB prefers host trees in the genus *Acer*. Imidacloprid applied as a trunk injection is often used as a means of control, but effectiveness can be variable. The objective of this study was to determine the extent to which movement of imidacloprid is sectorized within the trunk of maple trees. Highly sectorized flow could result in uneven distribution of the compound within the tree and result in variable efficacy of pesticide. One set of eight trees (four *Acer x freemanii*, four *A. platanoides*) was trunk-injected during the fall with ^{14}C labeled imidacloprid either directly below a branch in the first whorl or at a right angle to branches of the first whorl. Each branch of the first three whorls was labeled 0° /180°, or L90° / R90° depending on the location of the branch in relation to the injection point. Leaves and fine roots were sampled through two growing seasons. Trunk cores were sampled during 2006. Tree stems were sampled during destructive harvest, fall 2007. Litterfall was collected at the end of both growing seasons. Imidacloprid equivalent concentration (IEC) was generally constant over time and between leaves from opposing branches of the same whorl. As opposed to ash, a sectorized species, branch position did not affect IEC in maples. We conclude that maples trees have an integrated xylem architecture pattern which should allow for even distribution of trunk-injected imidacloprid in the tree crown. During 2007, the year after injection, IEC in leaves from corresponding branches were similar to levels observed in 2006.

Imidacloprid equivalent concentrations in leaves, stems and litterfall were higher than IEC in roots, trunk cores, and stems, indicating that imidacloprid moves primarily through the xylem.

CHAPTER 3

Distribution of Trunk-Injected ^{14}C -Imidacloprid in *Acer* spp. Trees:

A Test of the Sectorial Flow Hypothesis.

INTRODUCTION

The Asian longhorned beetle (ALB, *Anoplophora glabripennis* Motschulsky Family: Cerambycidae) is an invasive wood-boring exotic insect from Asia. It was first identified in the United States in New York City, New York in 1996 (Haack et al., 1997) and later in Chicago, Illinois in 1998 (Poland et al., 1998). Since that time ALB has been identified in other major cities including Jersey City, New Jersey and Toronto, Ontario, Canada (Wang et al., 2005). Additional beetles have been found in 26 warehouses in several states across the United States (Nowak et al., 2001). The ALB is native to Southeast Asia and China where it is one of the top ten economic pests due to the damage it causes to *Populus* spp. plantations (MacLeod et al., 2002).

Asian Longhorned Beetle has a wide host range including *Acer*, *Populus*, *Prunus*, *Malus*, *Salix*, *Ulmus* and at least 21 other tree species (MacLeod et al., 2002). In urban landscapes in the United States, ALB prefers host trees in the genus *Acer* (Nowak et al., 2001)). Susceptible trees make up as much as 62% of urban tree landscapes in some cities (Chicago, Illinois) and canopy loss in urban areas may reach as high as 68% (Jersey City, New Jersey) if all susceptible trees were to become infested. Chicago and New York City alone cost of removing infested trees was \$25 million (Nowak et al., 2002).

Economically, ALB could represent \$669 billion in compensatory damage throughout the 48 contiguous states. Fruit tree, maple syrup, lumber, and seed production industries could also be severely impacted (Ludwig et al., 2002).

Human assisted dispersal has led to ALB establishment in the United States, Canada, and Europe (Haack, 2003). Normally, beetles stay on trees from which they emerge or travel short distances to find suitable host trees (Wang et al., 2005). Adult beetles feed on bark, young stems and occasionally leaves, but larvae cause the majority of structural tree damage. First, second, and third instar larvae feed on nutrient-rich phloem just beneath the bark surface and create galleries that disrupt nutrient and water translocation paths within the tree. In areas with high ALB populations, this can lead to tree mortality within two to four years. As larvae grow and develop, they tunnel deeper into the xylem heartwood of trees and create weak spots in branches or trunks. Weakened branches may succumb to wind and snow load and cause injury to pedestrians or damage vehicles, houses, and other property (MacLeod et al., 2002).

Because ALB larvae bore deep into the xylem, it is difficult to kill them using chemical control (Nowak et al, 2001). Imidacloprid, a neo-nicotinoid insecticide that targets acetylcholine receptor sites in insects, is currently being applied as a trunk injection to kill adults and larvae (Poland et al., 2006a). Imidacloprid is a systemic insecticide that has a high translocation rate (Wang et al., 2005). Thus far, ALB infestations in the US are in urban areas. Imidacloprid applied as a systemic trunk-injected insecticide has a higher degree of public acceptance than conventionally applied cover sprays (Smith and Lewis, 2005) because it has very low mammalian toxicity and few non-target affects (Poland et al., 2006b, Sur and Stork, 2003).

Imidacloprid acts initially as an anti-feedant. It is crucial to deliver a strong and sustained dose of imidacloprid to successfully kill larvae and adults. Poland et al (2006a) showed that larvae can survive and pupate under sub-lethal concentrations. Adults could exhibit the same responses, or they might fly to an untreated tree and resume feeding (Poland et al., 2006a).

To ensure that the maximum amount of insecticide is arriving at the feeding sites of the larvae and adult beetles it is important to understand that translocation (and therefore efficacy) of trunk-injected compounds is affected by a variety of factors including temperature, tree species, tree injury, and water availability (Poland et al., 2006a). Efficacy of trunk-injected imidacloprid is variable against ALB (Wang et al., 2005). The amount of time that elapses between injection events might also affect efficacy. Several studies have shown that imidacloprid concentrations increase during the first year after treatment and then gradually decline (Tanis, this volume, chapter two). The point on trees where injection takes place might also influence efficacy. Poland et al. (2006b) suggest that xylem architecture influences translocation patterns and that insecticides might be injected and translocated in such a way that it would have no effect on developing larvae tunneling within branches. In a previous study at Michigan State University, researchers used ^{14}C labeled imidacloprid to track trunk-injected insecticide movement in *Fraxinus* spp. trees. The study demonstrated that imidacloprid moves slowly but steadily through the tree over time and accumulates in the leaves (Cregg et al., 2006). Variability in efficacy in ash could be due to uneven distribution of insecticide in the canopy due to tree anatomy. Orians et al. (2004), reported that trees can have varying degrees of sectorial or integrated flow. Sectorial trees have highly ordered, specific paths

of translocation, while integrated trees have less specific paths of translocation. In general, diffuse porous trees (e.g. maple) are considered more integrated than ring porous trees (e.g. ash) (Zanne et al., 2006). Diffuse porous trees have evenly sized vessels. Ring porous trees have variable sized vessels (Shigo, 1998). The difference results in increased vessel to vessel contact in diffuse porous trees that allow resource movement to occur in both radial and tangential planes (Zanne et al., 2006). This results in even distribution of resources within the plant. Information about tree xylem anatomy would be helpful to ensure that injection events are taking place on the tree in such a way that would maximize the translocation of insecticide. Spatial variability in trunk-injected integrated tree species should be less than in sectored species. We hypothesize that maple trees have integrated xylem anatomy.

Currently there are no insecticides that are 100% effective against ALB (Poland 2007). But if we can better understand the translocation of insecticide within trees we might increase the efficacy of trunk-injected imidacloprid. Increased efficacy would enhance ALB eradication efforts by ensuring that maximum amounts of insecticide are reaching ALB feeding sites. Overall, understanding spatial and temporal flow of insecticide through *Acer* spp. trees will enable us to improve efficacy of trunk-injection treatments and better understand the persistence and potential for ALB control.

The objectives of this study were to determine the spatial movement of trunk-injected imidacloprid in *Acer* spp. (maple) trees and concentration of imidacloprid in maple trees the spring after fall-trunk-injection.

MATERIALS AND METHODS

This experiment was performed at the Horticulture Teaching and Research Center, Michigan State University, Holt, Ingham County, Michigan. Trees were purchased from Weigand's Nursery and Garden Center, Macomb, Michigan.

The eight maple trees, four *Acer x freemanii* 'Jeffersred' Autumn Blaze[®] and four *A. platanoides* 'Princeton Gold' used for this study were 5 cm (2") caliper, container-grown specimens planted in a light weight pine bark media in 75 L (15 gallon) (*A. x freemanii*) or 95 L (25 gallon) (*A. platanoides*) containers. They were individually selected to ensure that the first three branch whorls were oriented at 90° angles to one another. Containers were spaced 2.5 m (8 ft) on center, placed inside 1.2 m plastic wading pools and elevated on a single layer of brick pavers. This allowed for drainage and kept leachate from entering into the environment. Trees were staked to prevent wind-throw. Trees were fertilized with Osmocote[®] Plus (The Scott's Company, Marysville, Ohio) controlled release fertilizer at a rate of 314 grams per pot and watered to runoff with drip irrigation two to three times per week.

Trunk Injection:

On 5 September 2006, trees were trunk-injected at a single injection point with 2.94 ml of 25 µCi of ¹⁴C labeled imidacloprid mixed with Imicide[®] (Mauget[®], Arcadia, California, 10% active ingredient) in a ratio of 1:1300 labeled to unlabeled compound. Half of the trees were injected at 0° to the first whorl of branches (i.e. the injection port was directly below one of the branches on the first whorl) the other half was injected at 90° to the first whorl of branches (Figure 3.1a.). Injection positions were assigned

randomly within species. Injection holes were drilled 10.0 cm above the graft union with a cordless drill and an 8 mm (5/16") drill bit. Holes were drilled to a depth of approximately 1.5 cm and systemic tree injection tubes (STIT) were inserted and gently tapped in with a hammer (Helson et al., 2001). After tubes were inserted, 2.94 ml of imidacloprid mixture was added through the top of the lower half of the STIT and 2.0 bar pressure was applied using a hand operated bicycle pump. All lower components of the STIT were securely wrapped with black electrical tape to ensure that leakage was contained and to provide stability. The upper halves of the STITs were also secured to trees with tape. The STITs were monitored for pressure, re-inflated as needed and kept in position 2-48 hours until all liquid was taken up by the tree. After all liquid was translocated into the tree, tape and STIT were carefully removed. The area of the tree that contained the drill hole was double-wrapped with electrical tape to prevent leakage or leaching of radioactive material. Tape remained in place throughout the experiment. Two trees were removed from the experiment due to excessive leakage and bark cracking during the injection process. All handling of radioactive material complied with Michigan State University's Office of Radiation, Chemical, and Biological Safety (ORCBS) standards. All areas were surveyed periodically using a Geiger counter (Ludlums Measurement Inc., Survey Meter Model 44-9, Sweetwater, TX) to ensure that no radioactive material leached into the environment.

Sampling:

Branches from the first three branch whorls of each tree were labeled either 0°, 180°, Right 90° (R90°) or Left 90° (L90°) depending on the position of the branch in relation to the injection point (Figure 3.1b). Leaf samples were obtained by pinching off

individual leaves from proximal, middle and distal portions of each of the six branches. Terminal leader leaf samples were removed with a pole pruner. Trunk cores were taken 1 m above the injection point at 0° and 180° (in relation to the injection point) using a #1 cork borer. The borer was pushed into the tree to remove approximately 2 mm depth of tissue (bark, phloem, and sapwood). Root samples were obtained by digging into the soil media until a portion of the root system was exposed, and then approximately 1 g dry weight of fine roots were removed from coarse roots and rinsed with tap water. Stem samples approximately 3 cm in length were taken from branch tips of the first three whorls using hand pruners. All samples were placed in small paper bags, labeled and placed in a 70° C drying oven for at least 72 hours. After drying, all leaf samples were removed from the oven, ground to a fine powder using a mortar and pestle and placed into individually labeled paper envelopes. Root, trunk core, and stem samples were left whole.

A 150.0 - 180.0 mg sub-sample of tissue was burned in a R.J. Harvey OX300 biological tissue oxidizer (R. J. Harvey Instrument Corporation, Tappan, NY, USA). Stem samples were burned on a four minute cycle, leaf samples on a three minute cycle, and root and trunk core samples on a two minute cycle. The resultant $^{14}\text{CO}_2$ was trapped in scintillation cocktail and processed through a Packard Tri Carb scintillation counter (LSC, Packard Bioscience, Currently PerkinElmer Life and Analytical Science, Waltham, Massachusetts, USA) on a one and one half minute cycle. Resulting counts per minute (CPM) were recorded for each sample and converted to imidacloprid equivalents after accounting for background activity and oxidizer and LSC efficiencies. Imidacloprid trunk-injected into plants is broken-down into a series of metabolites many of which

exhibit insecticidal properties (Sur and Stork, 2003); therefore we refer to the concentration of insecticide in trees in terms of imidacloprid equivalents. All results are presented in μg of imidacloprid equivalents per g dry weight of sample.

For the 2006 growing season, trees were sampled at 0, 2, 7, and 21 days after treatment. The sampling protocol included leaves from the first three branch whorls, terminal leader leaves, trunk cores, and fine roots. In late September, all trees were netted with bird netting to collect litterfall. After abscission, all leaves were removed from nets, placed in paper bags, and oven dried. A sub-sample of five to seven leaves were ground, weighed, oxidized, and processed through the LSC. After nets and leaves were removed, trees were lifted with a sling and skid steer and placed pot to pot in a holding area. A double layer of hay bales was placed around the holding area to protect roots from freezing temperatures over winter. In April 2007, trees were transferred into new 95 L (25 gallon) pots, and topped off with pure sand to add weight to help prevent wind-throw. After transplanting, they were lifted with the sling and skid steer and placed back in their original configuration. Trees were fertilized and watered at the same rates in 2007 as in 2006.

For the 2007 growing season, trees were sampled for whorl and terminal leader leaves, and fine roots on 29 May, 12 June, and 31 August. Trunk cores were not taken in 2007 to prevent excessive damage to the trees. At the end of the 2007 growing season, all leaves were removed from trees by hand. Leaves from the first three whorl branches were kept separate from the rest of the canopy leaves.

Stem sections from branches in whorls one through three and terminal leaders were removed in late October 2007 from branch tips and separated into 2006 and 2007

growth sections as determined by the position of axillary leaf scars. A sub-sample of five to seven leaves from canopies, terminal leaders, and whorl branches were dried, ground, weighed, oxidized, and processed through the LSC.

Pour through leachate tests were performed on 4 June, 5 July, 15 August, and 15 September 2007. Pot media containing the maple trees described above were saturated with water and allowed to drain for ten minutes. After drainage, 2 L of fresh water was added to the media and allowed to drain. Leachate was captured through drainage holes at the bottom of pots with a Petri dish, poured into 15 ml scintillation vials, sealed, and brought back to the laboratory. Immediately, 300 μ L of leachate was added to 15 ml of scintillation cocktail (Safety-Solv[®] Scintillation Cocktail, Research Products International Corporation, Chicago, IL). Samples were hand shaken and processed through the LSC.

Statistical Analysis:

Data were tested for normality using residual plots. Variables were not normal. Imidacloprid equivalent concentrations were transformed using a log ($x+2$) transformation. Differences were analyzed using the Mixed procedure for mixed models in SAS statistical software (SAS Institute, Inc. 1989) with whorl nested in tree, branch position nested in whorl, tree species, and day as fixed effects and tree number as a random effect. The models for repeated measures were examined using CS covariance structure. Analysis of variance (ANOVA) was used to determine which effects and interactions were significant ($p < 0.05$) for whorl, branch position, tree species and day. Different ANOVAs were produced for each injection position (0° or 90°). If effects were significant, LSMeans procedures were used for mean separation (Littell et al., 1996).

RESULTS

Imidacloprid equivalent concentration in *Acer* spp trees was not affected by whorl height, branch position, days after treatment, or species. In general, leaf IEC from 0° branches did not increase over time with the exception of 2007 litterfall. Leaves from branches opposite the injection point (180°) in the first whorl had increasing IEC through 21 DAT, while leaves from other 180° branches did not increase or decrease. Leaves from 0° and 180° branches in whorl one had similar IEC, although IEC in 2007 litterfall from 0° branches was higher than litterfall from 180° branches. Imidacloprid concentrations did not vary between leaves from R90° or L90° branches during 2006 or 2007. Leaves from terminal leader branches had increasing IEC through 7 DAT but no other variation in IEC occurred in 2006 or 2007 (Figure 3.2).

Roots from 2006 and 2007 and trunk cores from 2006 (Figure 3.3) and leachate samples (data not shown) contained no measurable IECs. Stem samples taken from the first three branch whorls at the end of the 2007 growing season did not exhibit any systematic pattern of variation in IEC among branches although IEC of stems from 0° branches were generally higher ($p < 0.05$) than terminal leader stems (Figures 3.4a and 3.4b). Imidacloprid equivalent concentrations were higher ($p < 0.05$) in leaves than in stems at a given branch position.

DISCUSSION

In the United States, trunk-injected imidacloprid is used to treat several species of trees against Asian longhorned beetle. We chose to use maple trees in this study because they are planted extensively in urban areas, are a preferred ALB host tree in the US, and have opposite branches which aids in determining sectorial xylem patterns. The primary objectives of our study were to determine patterns of imidacloprid movement within maple trees and imidacloprid levels in leaves from fall-injected maple trees the spring after injection.

Objective One:

Patterns of sap ascent in trees normally deviate from purely straight and are often described as helical (Tyree and Zimmerman, 2002). In the case of maples however, we did not see any patterns of helical or straight ascent. Imidacloprid equivalent concentration in leaves did not vary between opposite branches from the same whorl throughout either growing season although some isolated incidences of IEC difference were apparent at 2007 litterfall.

Ring porous trees (e.g. ash) are generally considered sectoried while diffuse porous trees (e.g. maple) have greater integration in their xylem anatomy due to more contact between vessels (Shigo, 1988). Most trees in the *Acer* genus are classified as diffuse porous (Zanne et al., 2006). The results of this study also suggest that *Acer x. freemanii* and *Acer platanoides* have integrated xylem pathways.

Poland et al (2006a) suggest that xylem architecture could affect the amount of insecticide moving through a tree and whether insecticide is translocated to ALB feeding

sites. As seen in a related study on ash trees (Tanis, chapter two, this volume), sectorial xylem anatomy can cause uneven distribution of insecticide in the tree crown. Leaves from branches opposite the injection point (180°) had much lower IEC than leaves from branches in the plane of the injection point (0°). Patterns of sectoriality were also evident in stems. Integrated xylem anatomy on the other hand, may contribute to more even distribution of insecticide within the tree. Integrated xylem pathways are more interconnected than sectorial pathways so injection point and branch position may not affect overall distribution of insecticide within the tree because movement is occurring in both longitudinal and radial planes. This could be a result of the area of vessel walls as bordered pits (Ellmore et al., 2006). In this study, imidacloprid equivalents moved in integrated patterns throughout 2006 and 2007 growing seasons in trees injected at both 0° and 90° degrees. This suggests that insecticide was evenly distributed throughout the tree canopy. In contrast, imidacloprid moved through ash trees in a sectorial zigzag pattern. There were no patterns of sectorial flow in maples during either growing season. As seen here, injection site and branch position might not be as critical when performing trunk injections on maples as they are when trunk-injecting ash trees or other strongly sectorial species.

In general, we found that imidacloprid equivalent concentration appears to increase over time. However, differences in IEC from date to date were not statistically different due to high variation among trees that is probably a result of small sample size.

In addition to leaves, imidacloprid equivalents were also present in stems. We did not sample stems more than once to prevent excess tree damage. Like ash, the leaves and stems of maple trees shared similar integration patterns. The lack of pattern supports our

theory that maple trees have integrated ascent. It is important to understand imidacloprid movement within stems because late instar ALB larvae feed and tunnel in the xylem. In this study we determined that IEC in maple stems is not affected by species, whorl height, or branch position and therefore were evenly distributed throughout the tree. In ash trees, sectoriality patterns were evident in both leaves and stems (Tanis, Chapter two, this volume).

Several studies have demonstrated that imidacloprid movement in plants occurs via the xylem (Bromilow and Chamberlain, 1989, Sur and Stork, 2003, Tanis et al., 2007). Imidacloprid was present in measurable concentrations in leaves and stems but no measurable IEC was present in roots or trunk cores. If imidacloprid movement was occurring in phloem, these tissues would have $IEC > 0$. To further investigate IEC in fine roots, we also performed pour-through leachate tests to determine if fine root turnover from trunk-injected trees could contribute to imidacloprid residues in soil. No detectable imidacloprid equivalents were present in collected leachate samples. The absence of detectable IEC in fine roots, trunk cores, and water collected from leachate tests suggests that imidacloprid equivalents are not moving via the phloem from the injection point down to roots or upward through bark. Instead, the presence of IEC in leaves and stems supports previous studies that indicate imidacloprid moves through trees primarily in the xylem.

In a previous study that used ^{14}C to examine imidacloprid movement in ash trees, Cregg et al. (2006) demonstrated that canopy leaves collected after abscission contained IEC as high as those sampled before abscission. In other words, imidacloprid was not translocated from leaves back into the trunk before litterfall. This trend was also

apparent in maple trees used in this study. Generally, IEC in leaves from 2007 litterfall were not different from IEC in leaves from other sample dates indicating that imidacloprid is not translocated back into the tree before litterfall.

Objective Two:

Our second objective was to determine how much imidacloprid was present in leaves from fall injected trees the year following injection. Some questions remain regarding the timing of imidacloprid trunk-injections. Trunk-injections performed in spring are advantageous because temperatures and transpiration rates are generally higher than in the fall. These parameters promote quick translocation of insecticides to the tree canopy. Fall injections are popular among tree care professionals because there is often a lag in business opportunities during this time. Unfortunately, decreased temperatures and transpiration rates during the injection process might hinder overall imidacloprid uptake into stems and canopy leaves. One might argue that if less insecticide is lost during litterfall, more insecticide might be available for translocation the spring after treatment.

In leaves from 0° and 90° injected trees, IEC on 11 June 2007 was approximately 35% less than levels found at the end of the growing season in 2006 (Figure 3.5). In general, leaf IEC in maple trees was higher than IEC in ash leaves on both the final sample date in the year of injection (26 September 2006), and the sample date that might correspond to periods when both insects are in active adult stages (11 June 2007). Although the focus of our study was not to compare these two trees, it is helpful to create a visual illustration of differences between sectorized species (e.g. ash) and more integrated species (e.g. maple). These differences were particularly evident on 11 June 2006 when patterns of sectoriality were visible in ash trees and clear patterns of integration are

visible in maple trees. Degrees of integration/sectoriality are a result of xylem anatomy, most likely the amount of vessel to vessel contact and/or area of walls as bordered pits. Higher IEC in maple leaves when compared to ash leaves might be due in part to more rapid insecticide movement. Integrated trees might be able to move imidacloprid more efficiently from reservoirs at the base of trees up to canopy leaves when compared to sectoried trees, again probably as a result of differences in xylem architecture. Another possibility is that increased movement of imidacloprid in maple trees might be due to differences in leaf biomass between species. Orians et al., (2004) showed that dye ascent can be affected by the amount of biomass associated with particular branches or sections of trees. He also stated that differences in biomass effect on movement were different between species. Highly integrated species like *Betula papyrifera* Marsh were greatly influenced by biomass partitioning. It is possible that there might be enough difference between maple and ash leaf area to account for some of the differences in IEC but we were unable to make that comparison in this study.

In summary, we have shown that imidacloprid equivalent concentration does not vary in leaves or stems of maple trees based on the position of branches in relation to injection points, species, or length of time after injection. The pattern of integration was consistent through time. We were also able to show that imidacloprid is moving primarily through the xylem and that translocation of insecticide does not occur from leaves back into the trunk before litterfall.

An interesting follow up study might further examine the possible difference in IEC of leaves and stems between spring and fall injections or mortality rates of live larvae or adult beetles using parallel bioassays. Finally, we conclude that xylem

anatomy of *Acer x. freemanii* and *Acer platanoides* allows for insecticide movement in longitudinal, radial, and circumferential planes. This pattern of ascent allows for integrated movement within the plant and even distribution of insecticide in the tree crown at the rates applied. Based on this information, the position of trunk-injection is not as important in integrated species as it is in sectorized species.

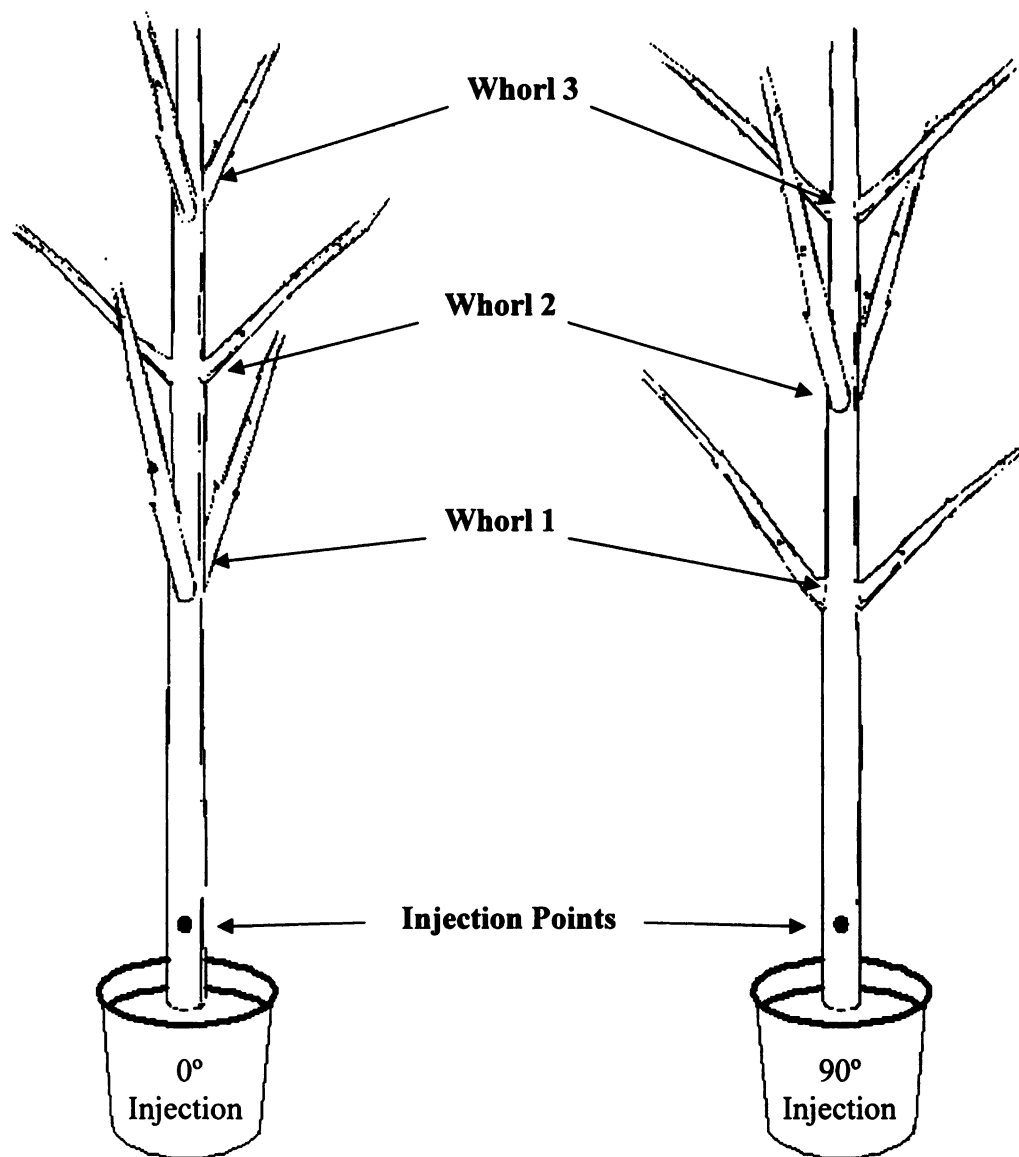


Figure 3.1a: Treatment schematic, trees were injected at either 0° or 90° to the first whorl of branches. Mean distance from injection point to the first whorl = 1.43 m. Mean distance between whorls = 0.18 m.

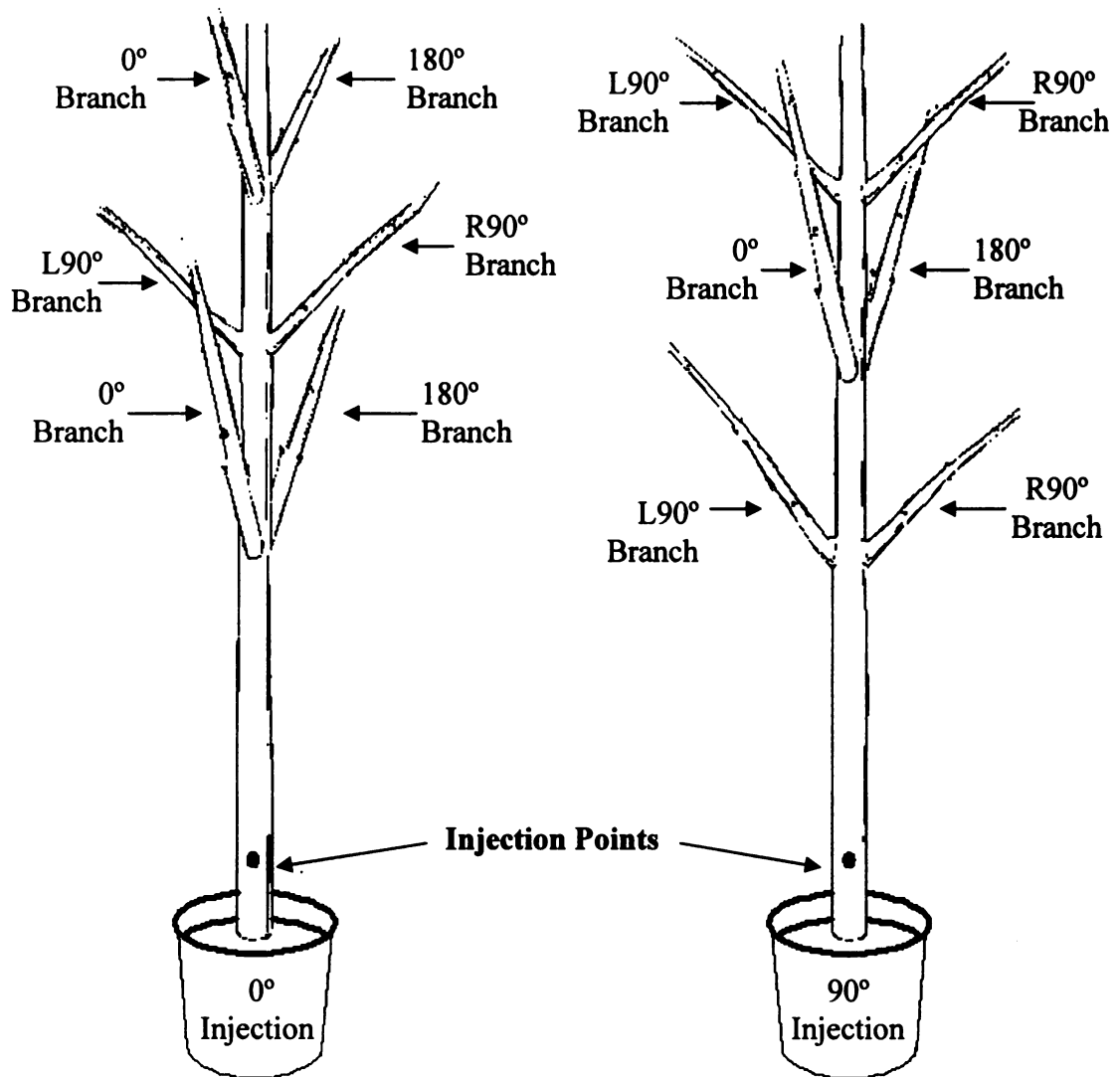
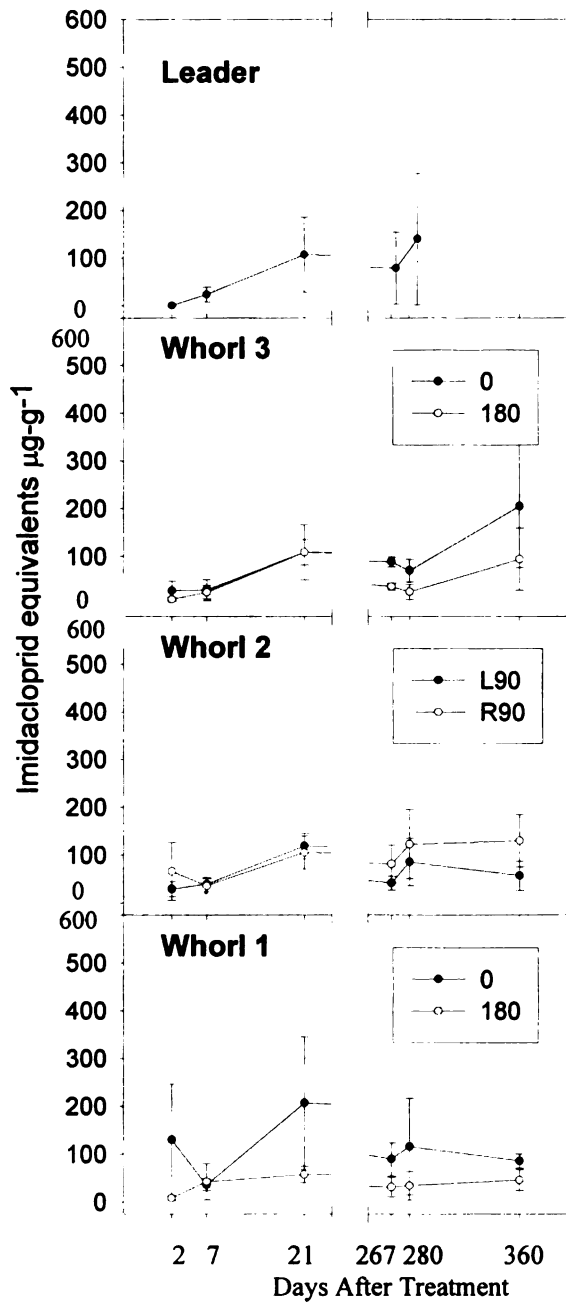


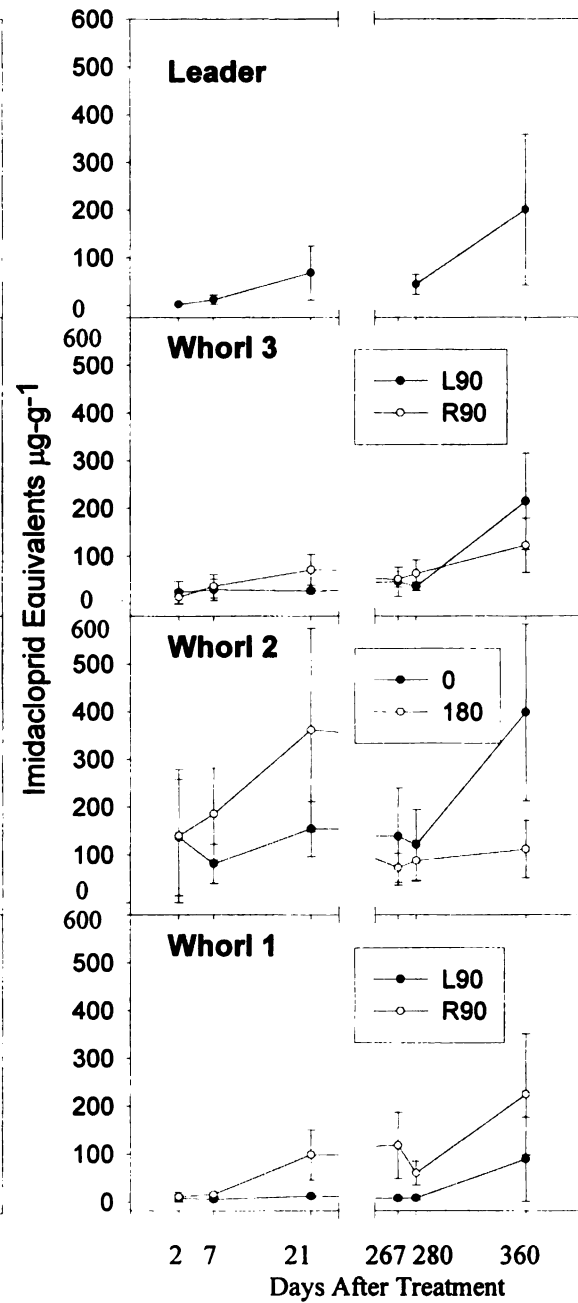
Figure 3.1b: Sampling schematic of trees injected at either 0° or 90° to the first whorl of branches. Branches of the first three whorls were labeled 0°, 180°, L90° or R90° in relation to the injection point. Mean distance from injection point to the first whorl = 1.43 m. Mean distance between whorls = 0.18 m.

Figure 3.2: Mean ^{14}C -Imidacloprid equivalent concentration $\pm$ S.E. in whorl and terminal leader leaves from 0° or 90° (in relation to the first whorl of branches) trunk-injected *Acer x freemanii* and *Acer platanoides* trees. Branches were labeled 0° , 180° , L 90° or R 90° (in relation to the injection point).

Acer spp. fall-trunk-injected at 0°
to the first whorl of branches.



Acer spp. fall-trunk-injected at 90°
to the first whorl of branches.



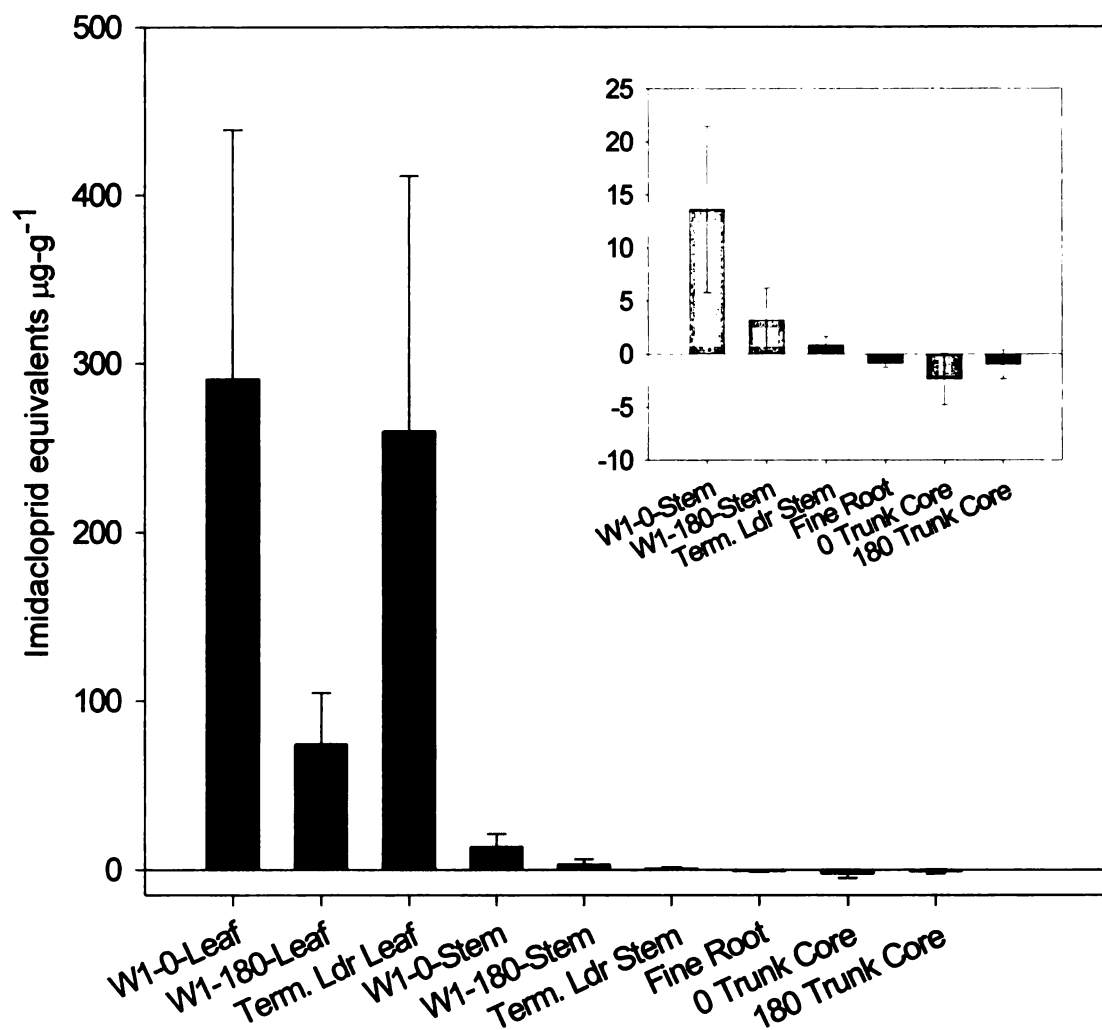


Figure 3.3: Mean ¹⁴C-Imidacloprid equivalent concentration ± S.E. in whorl one leaves and stems (0°, 180°, L90° or R90°, in relation to the injection point), trunk cores (0° or 180°, in relation to the injection point) and roots of 0° and 90° (in relation to the first whorl of branches) trunk-injected *Acer x freemanii* and *Acer platanoides* trees, samples collected during destructive harvest. Inset graph has leaf data bars removed.

Figure 3.4a: Mean ^{14}C -Imidacloprid equivalent concentration $\pm$ S.E. in 2006 and 2007 stem sections from 0° (in relation to the first whorl of branches) trunk-injected *Acer x freemanii* and *Acer platanoides* trees. Stems were harvested in October 2007 from the first three branch whorls and labeled 0°, 180°, L90° or R90° in relation to the injection point.

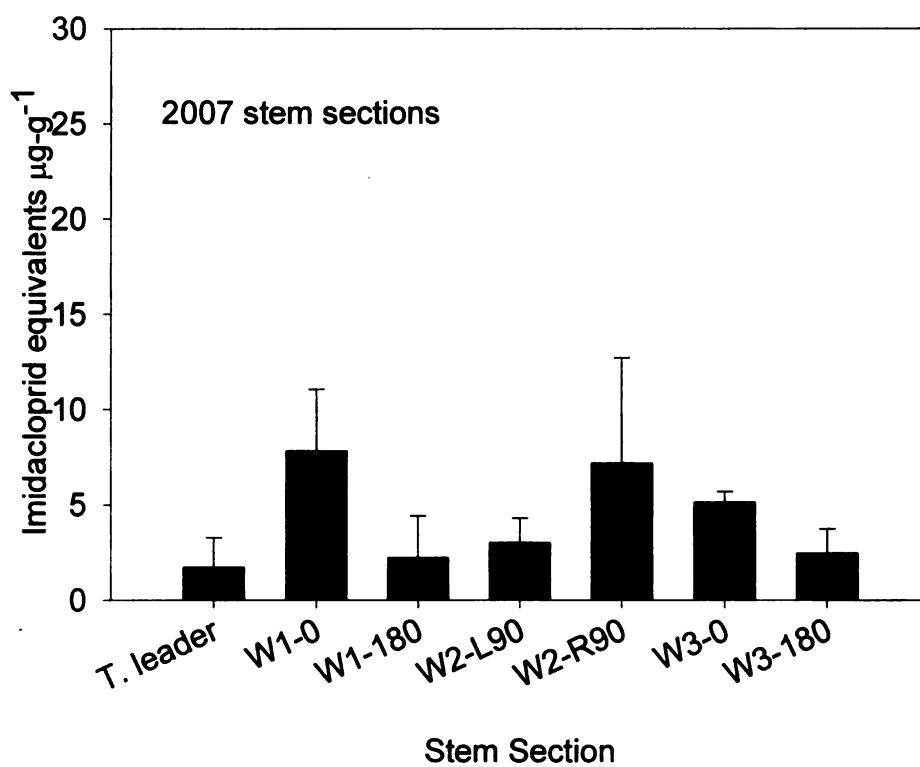
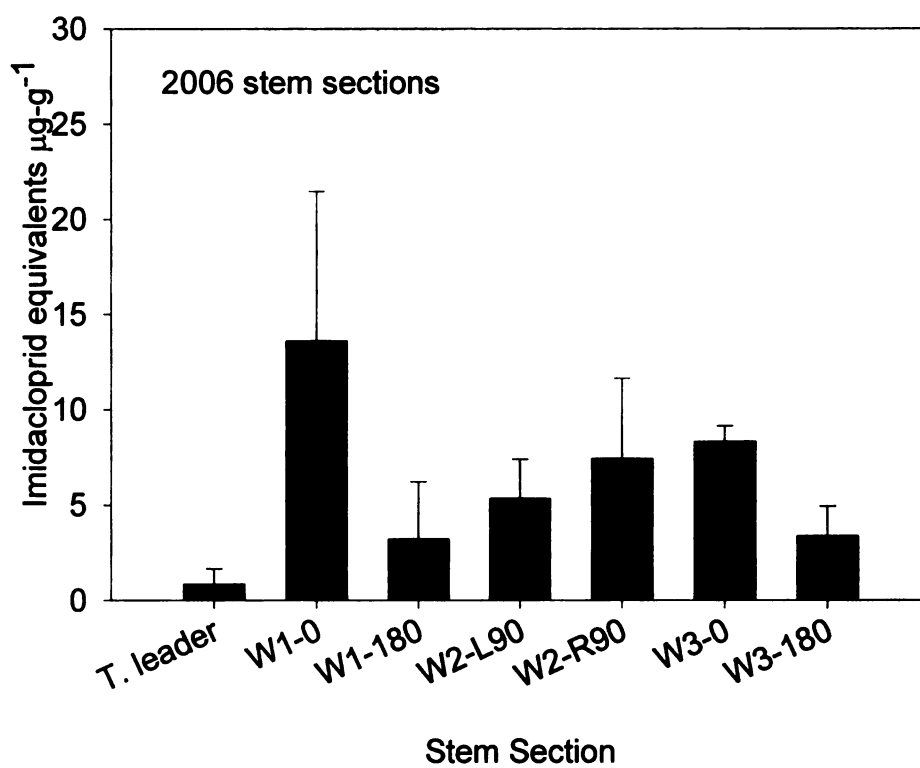


Figure 3.4b: Mean ^{14}C -Imidacloprid equivalent concentration $\pm$ S.E. in 2006 and 2007 stem sections from 90° (in relation to the first whorl of branches) trunk-injected *Acer x freemanii* and *Acer platanoides* trees. Stems were harvested in October 2007 from the first three branch whorls and labeled 0°, 180°, L90° or R90° in relation to the injection point.

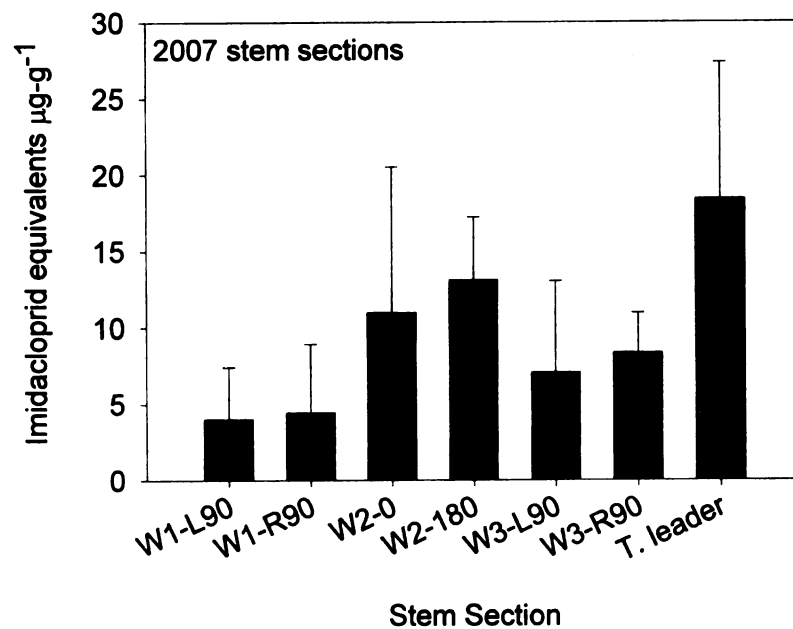
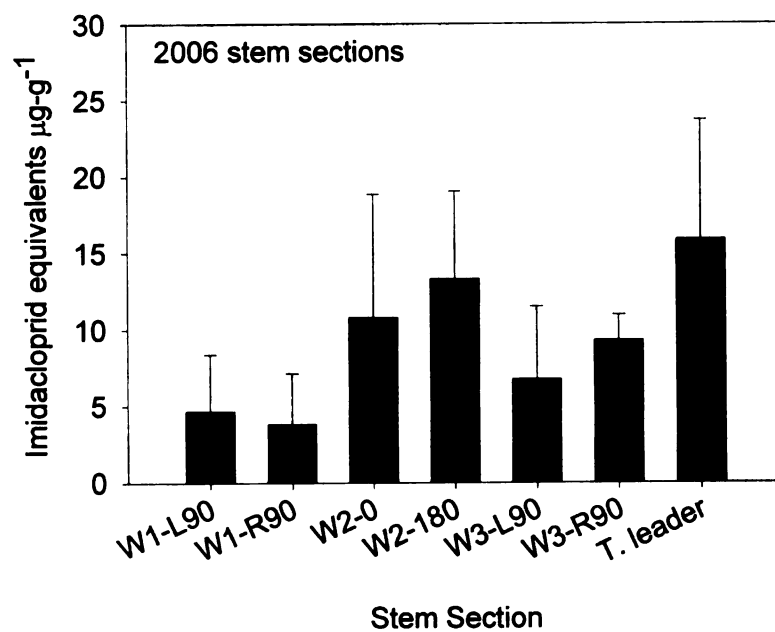
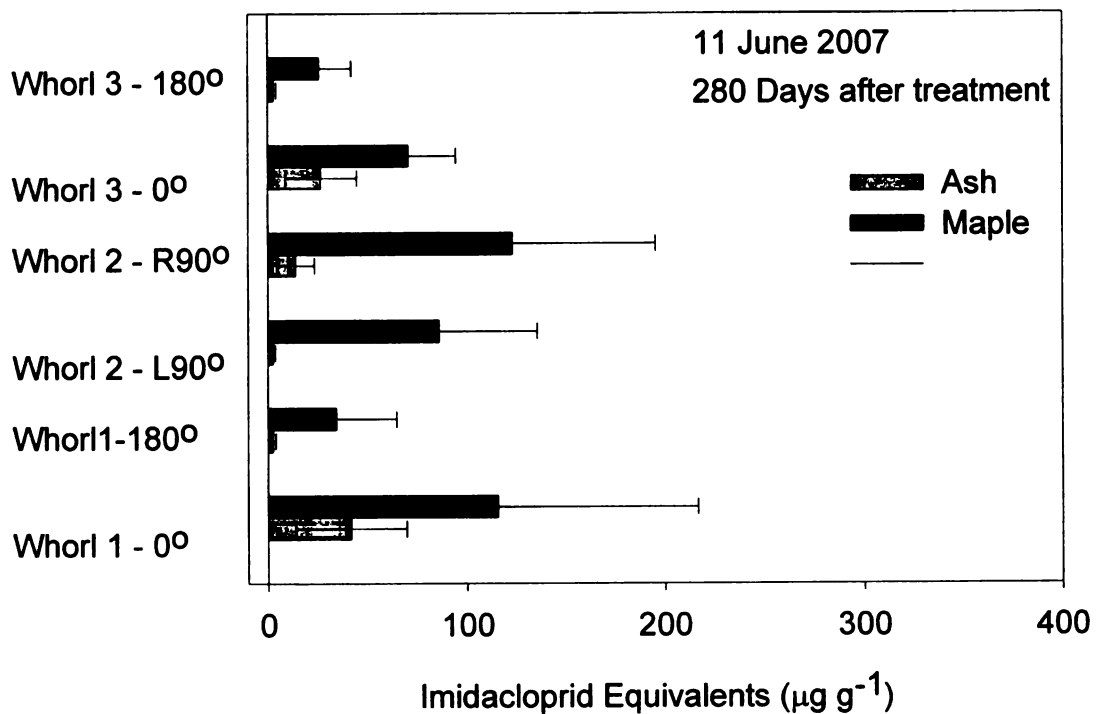
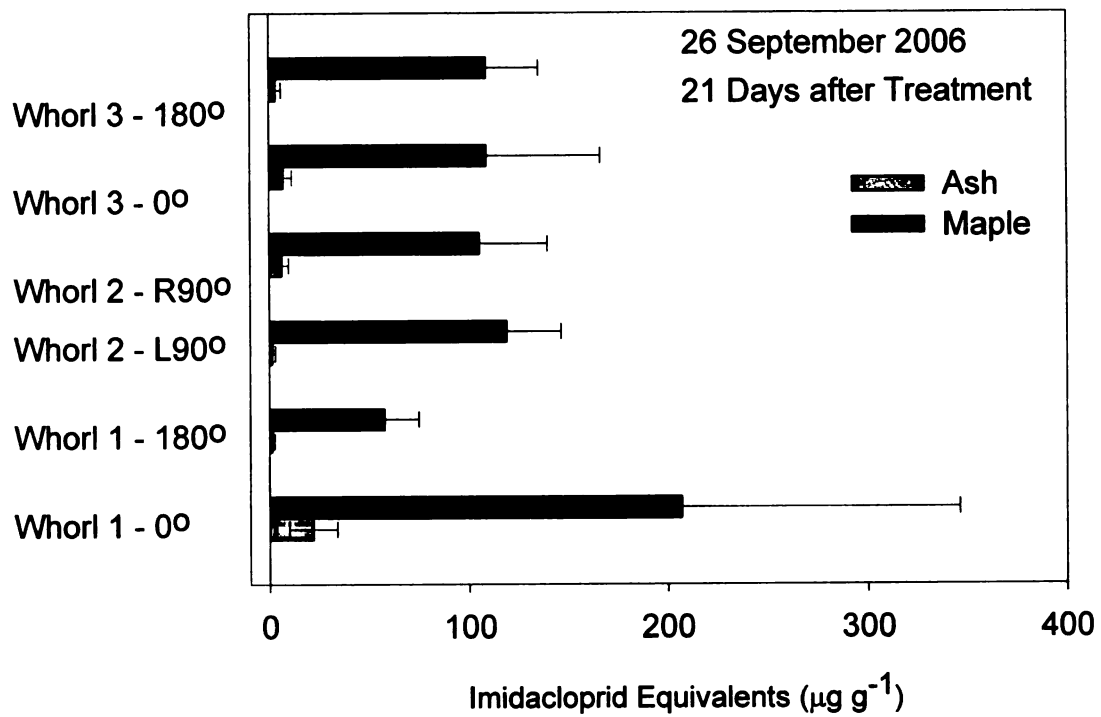


Figure 3.5: Mean ^{14}C -imidacloprid equivalent concentration $\pm$ S.E. in leaves from the first three branch whorls of 0° and 90° (in relation to the first whorl of branches) of trunk-injected *Acer x. freemanii* and *Acer platanoides* trees. Branches were labeled 0° , 180° , L 90° or R 90° in relation to the injection point. Samples were taken 21 and 280 days after treatment.



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Table A.1: Analysis of variance of imidacloprid equivalent concentration in *Fraxinus americana* and *F. pennsylvanica* trees trunk-injected with ^{14}C – imidacloprid 28 June 2006.

Source	Imidacloprid Equivalent Concentration		
	df	F Value	Pr>F
Species	1	0.00	0.9925
Whorl (Tree)	9	0.66	0.7417
Whorl (Tree) X Spp	9	1.40	0.182
Day	8	40.97*	<.0001
Spp X Day	8	1.92	0.0545
Whorl(Tree) X Day	69	0.66	0.9837
Whorl(Tree) X Spp X Day	63	0.70	0.9622
Branch Position (Whorl)	4	35.55*	<.0001
Spp X Bp(Whorl)	4	0.64	0.6329
Bp(Whorl) X Day	24	0.75	0.8028
Spp X Bp(Whorl) X Day	24	0.36	0.9982

* $p \leq 0.05$

Table A.2: Analysis of variance of imidacloprid equivalent concentration in *Fraxinus americana* and *F. pennsylvanica* trees trunk-injected with ^{14}C – imidacloprid 5 September 2006.

Source	Imidacloprid Equivalent Concentration		
	df	F Value	Pr>F
Branch position (Whorl)	6	2.93*	0.0108
Day	6	0.74	0.6186
Bp (Whorl) X Day	30	0.88	0.6521
Whorl (Tree)	8	1.33	0.2362

* $p \leq 0.05$

Table A.3: Analysis of variance of Imidacloprid equivalent concentration in *Acer platanoides* and *A. x. freemanii* trees trunk-injected with ^{14}C – imidacloprid 5 September 2006.

Source	Imidacloprid Equivalent Concentration		
	df	F Value	Pr>F
Branch position (Whorl)	6	2.49*	0.0300
Day	6	5.76*	<0.0001
Bp (Whorl) X Day	30	0.31	0.9997
Whorl (Tree)	8	2.31	0.0655

* $p \leq 0.05$

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