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TOWARDS THE DEVELOPMENT OF ASH VARIETIES RESISTANT TO EMERALD ASH BORER

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TOWARDS THE DEVELOPMENT OF ASH VARIETIES RESISTANT TO EMERALD ASH BORER

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ABSTRACT

Kolich, Lindsay Ann. M. S., Purdue University, May 2014. Towards the development of ash varieties resistant to emerald ash borer. Major Professor: Matthew D. Ginzel.

In Chapter 1, I review the literature regarding the host colonization behavior of the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera; Buprestidae). I explore the details of the natural history of EAB and the aspects of its physiology which cause it to be such a pervasive pest in North America. I also discuss characteristics of the host of EAB, *Fraxinus spp.,* including foliage and bark-emitted volatile organic compounds, host plant resistance, and the role of roots in herbivory defense. Finally, I discuss the various management practices currently employed to combat EAB.

In Chapter 2, I determine the extent to which grafting susceptible North American ash scions onto resistant Asian ash rootstocks confers resistance to the scion through root-shoot connectivity. The mean leaf area consumed on grafts with green ash scions and Asian ash rootstocks received significantly less feeding than the reverse. Grafting of North American ash species to Asian ash rootstock quantitatively affected the volatile profile of chimeras, often producing an intermediate profile between the conspecific grafts of each species.

CHAPTER 1. HOST COLONIZATION BEHAVIOR OF THE EMERALD ASH BORER, *AGRILUS PLANIPENNIS*

1.1. Introduction

The emerald ash borer, *Agrilus planipennis* Fairmaire (EAB; Coleoptera: Buprestidae), is among the most destructive insect pests threatening North American forests. Since its arrival in 2002, EAB has been found in 22 states as well as two Canadian provinces, and its range is expected to continue expanding (USDA 2014). EAB is particularly difficult to control because larvae develop concealed beneath the bark of the host tree where they are protected from sprayed insecticides. The larvae feed on the vascular tissue and disrupt the flow of nutrients throughout the tree. Infestations often go undetected until the trees begin to die and adult beetles have emerged, leaving characteristic D-shaped exit holes. There are at least 16 endemic *Fraxinus* species at risk in North America and millions of trees have already been lost (USDA 2014). All North American species are vulnerable to EAB but susceptibility varies between species (Cappaert et al. 2005; Poland and McCullough 2006; Anulewicz et al. 2008). For example, *Fraxinus* species of Asian origin that coevolved with the beetle are most resistant to attack, and only stressed trees are generally affected (Rebek et al. 2008). EAB is capable of killing otherwise healthy North American ash and colonizes trees as small as saplings – most affected trees die within four years of colonization (Cappaert et al. 2005; Poland and McCullough 2006).

EAB locate suitable hosts via volatile organic compounds (VOCs) released by the leaves and shoots of their host (Rodriguez-Saona et al. 2006; Crook et al. 2008b; Crook and Mastro 2010). The variation in susceptibility of *Fraxinus* may be due to quantitative and qualitative differences in volatile emission (Pureswaran and Poland 2009). These subtle differences in volatile chemistry are recognized by EAB and also the parasitoids being imported to the US fromtheir native range for use in classical biological control efforts (Wang et al. 2010a). Another proposed explanation for the variation in susceptibility is differences in phloem chemistry between *Fraxinus* species (Eyles et al. 2007; Cipollini et al. 2011). For example, one of the most resistant ash species to EAB, Manchurian ash, *Fraxinus mandschurica* Ruprecht, has higher concentrations of lignins and hydroxycoumarins than those of the North American species (Eyles et al. 2007; Whitehill et al. 2012). Due to their structure, lignins are difficult for insects to digest and may also cause mandibular wear (Feeny 1975; Rhoades and Cates 1976; Peeters 2002). Hydroxycoumarins have been found to deter herbivorous insects, and may function in trehalase inhibition and have anti-microbial activity (Leszczynski et al. 1995; Silva et al. 2006; Rodriguez et al. 2000; Cipollini et al. 2011). If these properties of resistance could be conferred to North American species, EAB resistance could be achieved.

Grafting has historically been used to confer certain properties of a rootstock to a scion (Mascall 1569; Jensen et al. 2003). Roots synthesize secondary metabolites that are involved in defending plants against herbivory (Erb et al. 2009), and are integral in the production of constitutive and induced defenses. Prior to the arrival of EAB, green ash (*F. pennsylvanica* Marshall) was the universal donor of rootstock for a wide variety of ornamental ash cultivars (Ball 2004). The use of susceptible green ash as a rootstock donor may have unfortunate consequences for relatively resistant scions grafted on to this rootstock due to the effects of root-shoot connectivity. For example, autumn purple ash, a white ash scion grafted on to green ash, is more susceptible to EAB than white ash (*F. americana* Linnaeus) (Rebek et al. 2008). If a species with more resistance such as Manchurian or blue ash (*F. quadrangulata* Michaux) were used as donor rootstock instead, resistant properties might be translocated from the root to the shoot.

Ash is not only an important forest tree and street tree in urban forests, but also historically important in the production of baseball bats, tool handles, and other wood products (Schlesinger 1990). Unfortunately, the spread of EAB will continue to devastate North American ash. It is estimated that from 2009 to 2019, $10.7 billion could be spent on ash treatment, removal, and replacement of trees if current EAB range expansion continues (Kovacs et al. 2010). Grafting of ash to produce resistant trees and the elucidation of the resistance mechanisms would ensure that *Fraxinus* is not lost from North America. This work will also uncover the ways in which grafted trees attract EAB parasitoids and further protect them from EAB related mortality.

In this chapter, I will review the natural history of EAB and the characteristics of ash which affect host colonization including foliage and bark-emitted VOCs, host plant resistance, the role of roots in herbivory defense, and the current management methods employed to control EAB.

1.2 *Agrilus planipennis:* Natural history

The genus *Agrilus* consists of almost 3,000 known species worldwide, 171 of which are found in North America, making it among the most diverse genera of Buprestidae (Buck and Frappier 2011). They are easily distinguished from other buprestids by their elongate and cylindrical shape, the lobe protruding at the base of the prothorax (Parsons 2008) and a metallic and iridescent elytra (Bellamy and Nelson 2002). *A. planipennis* adult beetles are green in color, with coppery red iridescence on the dorsal surface of their abdomen.

Female EAB lay their eggs in crevices and vertical slits in the bark of suitable ash trees. They may lay one or more in the same area, or other females may also oviposit in the same location (Wang et al. 2010b). Eggs are oblate in shape, roughly 1.0 x 0.6 mm (length x width) and are initially pale in color, turning a darker shade as they near hatching (Yu 1992). The eggs may be yellowish-orange to orange-brown in color, with a flat cover that resembles a plastic film. A sticky substance is present on the underside of the egg which allows it to attach to the bark (Chamorro et al. 2012). The egg stage lasts between 12-19 days, with higher temperatures leading to a shorter development time (Wang et al. 2010b).

The EAB larval stage consists of four instars characterized by differences in length and width of various body parts, including urogomphi, peristoma, and prothoracic plates (Wang et al. 2005; Wang et al. 2010b). All larval instars are whitish in color and their body is comprised of a brownish retracted head with visible mouthparts, swollen prothorax with narrow meso and metathorax, and a 10-segmented abdomen with urogomphi at the end (Yu 1992). The first larval instar is roughly 6 mm long, with poorly sclerotized mandibles, the shortest terminal processes of any instar, and indistinct microspinulae on the antennae. The second larval instar is 10-12 mm in length, with darker, more sclerotized mandibles, microspinulae surrounding the antennae and on the thorax, and longer terminal processes with excretory ducts present. The third instar is 16-26 mm in length and similar to the second instar except with almost fully sclerotized mandibles and longer terminal processes with grooves forming. The fourth instar may reach 30-36 mm in length and develops into the prepupa, in which the body segments are compacted (Chamorro et al. 2012).

The complete EAB life cycle may take one to two years to complete in North America (Cappaert et al. 2005). Late instar larvae or prepupae overwinter in sapwood galleries, requiring the accumulation of approximately 230 to 260 degree days for emergence using a baseline of 10 ºC (Brown-Rytlewski and Wilson 2004; Cappaert et al. 2005). During the prepupal stage, EAB bodies are J-shaped within their pupal chamber. When temperatures begin to rise in the spring, their bodies straighten out and they enter the pupal stage, which lasts 2-3 weeks. Pupae resemble adults, but lack coloration. Adults usually emerge in late spring or early summer (Wang et al. 2010b). Adult EAB are generally 10-13 mm in length, and are larger than most North American *Agrilus* species.

EAB feed on the foliage of the host tree throughout their adult lives. Upon emergence, both male and female EAB require a period of maturation feeding before mating can occur, usually lasting 5 to 7 days. Mated females then feed for another 5 to 7 days before laying eggs (Poland and McCullough 2006; Pureswaran and Poland 2009; Wang et al. 2010b). EAB have been found to prefer mature leaves over young, and those which have grown in the sunlight as opposed to those in the shade (Chen and Poland 2009). Feeding occurs on the leaf margins, which can lead to a tattered appearance. This feeding is an aesthetic concern, likely not causing the tree any serious damage (Wang et al. 2010b; Poland and McCullough 2006).

Adult EAB are active on warm, sunny days and fly about the canopy of their host and those nearby (Wang et al. 2010b). Male EAB have been found to locate females on the host visually in this manner. Once a male detects a female beetle he flies in a circle around the foliage, dives and lands precisely on the female's back, and then begins to probe her with his aedeagus. This behavior has been deemed 'paratrooper copulation', and precedes the detection of contact pheromones, which are used by the beetles in sex discrimination (Lelito et al. 2007, 2009). The contact pheromone, 9-methyl-pentacosane, is present only in the cuticle of mature female EAB and elicits copulation attempts in males (Silk et al. 2009).

Following mating, female beetles disperse to an oviposition site on a suitable host. In 2001, an infested load of wood was unintentionally transported into an EAB-free area in Tipton, MI. Adult beetles emerged the following summer and the natural dispersal from the woodpile into the surrounding area was observed. Over 70% of the galleries found the subsequent year were within 100 m from the woodpile and gallery density decreased with distance. One gallery was found 750 m away from the initial point, and to prevent the infestation from spreading, all ash trees within 800 m of the woodpile were destroyed prior to emergence of adults the following year (Mercader et al. 2009). Flight mill experiments using tethered EAB found that mated female EAB flew 2.5 times further than unmated females, and of the 177 beetles tested, over 50% flew >750 m in a 24 hour period (Taylor et al. 2010) suggesting that mated female EAB are capable of dispersing great distances from their point of origin to locate suitable hosts.

EAB locate host trees through the detection of VOCs released from the bark and foliage of *Fraxinus* (Rodriguez-Saona et al. 2006; Crook et al. 2008b; Crook and Mastro 2010). Although females will occasionally make an error and lay eggs on a non-ash tree, larvae are not able to develop unless able to feed on ash phloem (Anulewicz et al. 2008). EAB host preference and the use of VOCs to locate hosts will be further discussed in Section 1.3 of this chapter.

EAB has many physiological characteristics which make it such a pervasive pest in North America. EAB are able to survive a temperature range of approximately -30° C (Crosthwaite et al. 2011) to 53° C (Sobek et al. 2011), allowing them to spread in North America unrestricted by temperature. Characteristics of EAB which contribute to their cold-hardiness include the ability to depress their supercooling point, protective waxes on the cuticle, and high concentrations of the cryoprotectant glycerol and other antifreeze factors in the hemolymph (Crosthwaite et al. 2011). While many cold-hardy insects rely on dehydration as a means of surviving, EAB only loses 6% of its water mass, suggesting that it relies more heavily on the cryoprotectants and cuticular waxes for protection (Crosthwaite et al. 2011).

EAB is also equipped with antioxidant genes (Rajarapu et al. 2011) and unique gut microbiota (Vasanthakumar et al. 2008) which aid in the consumption of plant material. EAB possess antioxidant defense genes including superoxide dismutase, catalase, ascorbate peroxidase and glutathione peroxidase. These allow for the consumption of reactive oxygen species such as H_2O_2, the release of which is likely one of the primary defense responses of ash against herbivory (Rajarapu et al. 2011). Along with these genes, EAB also harbor a microbial community in their gut which aids in the digestion of wood as a larvae and foliage as an adult (Vasanthakumar et al. 2008). There are between 44 and 71 operational taxonomic units within the gut of EAB, suggesting that it may be beneficial to consider the gut microbiota when developing control measures (Vasanthakumar et al. 2008).

Physiological and behavioral characteristics contribute to the invasive nature of EAB and warrant further research in order to develop better management techniques. EAB also lacks natural enemies in North America and the *Fraxinus* species which it uses as a host lack resistance to these foreign invaders.

1.3 Foliage and bark-emitted VOCs

The ability to perceive and respond to VOCs released by host plants plays an important role in the host selection and reproduction of many insects, and different genotypes of the same plant emit different volatiles (Dicke and Baldwin 2010). EAB locate suitable hosts via the constitutive and herbivore-induced volatiles released by ash (Rodriguez-Saona et al. 2006; Crook et al. 2008b; Crook and Mastro 2010). Ash bark (Crook et al. 2008a, 2008b) and foliage (Rodriguez-Saona et al. 2006) release volatiles which are attractive to adult EAB and are used in the detection of the host. Even small amounts of green leaf volatiles (GLVs) are antennally active to EAB in electroantennogram experiments (Rodriguez-Saona et al. 2006).

All North American ash species are vulnerable to EAB but susceptibility is variable, with blue ash exhibiting more resistance than the more susceptible green and white ash (Agius et al. 2005). EAB utilizes Manchurian and Chinese Ash (*F. chinensis* Roxburgh) in its native range, but only infests stressed trees and prefers the North American species such as green and velvet ash (*F. velutina* Torrey) that have been introduced to the region (Yu 1992; Duan et al. 2012). The differences in VOC emission may play a role in the differential attraction of EAB to the various *Fraxinus* species. For example, Manchurain ash has been found to release higher levels of volatiles than green ash, suggesting that the release of lower amounts of VOCs may result in less resistance to EAB (Pureswaran and Poland 2009; Crook and Mastro 2010).

The differences among unique volatile compounds released by various ash species may also contribute to differential attraction. At least 16 antennally active compounds have been identified from Manchurain ash foliage, including hexanal, (*E*)-2-hexenal, (*Z*)-

3-hexen-1-ol, 3-methyl-butylaldoxime, 2-methyl-butylaldoxime, (*Z*)-3-hexen-1-yl acetate, hexyl acetate, (*E*)-β-ocimene, linalool, 4,8-dimethyl-1,3,7-nonatriene, and (*E*,*E*)-α-farnesene, with Z-3-hexenol eliciting the greatest response in both male and female EAB (Rodriguez-Saona et al. 2006). In contrast, green and white ash have been found to emit only eight different volatile compounds, with (*Z*)-3-hexenol and (*Z*)-3-hexenyl acetate representing over 80% of the volatile profile (de Groot et al. 2008). In field experiments using traps baited with different combinations of the GLV compounds found previously, (*Z*)-3-hexenol was the only GLV consistently attractive to male EAB and holds promise for the development of a lure for detection of beetles (de Groot et al. 2008).

Bark-emitted sesquiterpenes from ash also hold promise for use in lures for EAB. The practice of girdling, or removing a ring of phloem around the trunk of the tree, has been used to produce 'trap trees' which attract EAB more readily than unstressed trees (McCullough et al. 2009). Six antennally active compounds were identified from girdled green ash; α-cubebene, α-copaene, 7-epi-sesquithujene, trans-β-caryophyllene, and α-humulene were found by Crook et al. (2008b), and eremophilene found by Cossé et al. (2008). Due to the high cost of synthesizing sesquiterpenes, manuka oil (containing all antennally active compounds except 7-epi-sesquithujene) (Crook et al. 2006, 2007, 2008a, 2008b) and phoebe oil (containing all six compounds) (Cossé et al. 2008) have been used as lures to test attraction of EAB (Crook and Mastro, 2010). Both manuka and phoebe oil yield higher trap catches than unbaited traps and are used by the USDA to detect EAB infestations (Crook et al. 2008b; Crook et al. 2009).

1.4 Host plant resistance

Host plant resistance is the term given to the suite of adaptations that plants have evolved to protect themselves from herbivory and attack. For example, Manchurian and Chinese ash are able to cause EAB mortality through the formation of a callous which encapsulates and kills the burrowing larva (Duan et al. 2010b). This type of herbivore-induced defense is common in plants that have coevolved with their pests. VOCs are often included in the category of induced defenses due to their tendency to be elicited following herbivory. The VOCs released by plants can act as both repellants and attractants to herbivores (Unsicker et al. 2009). For example, the volatiles released by *Nicotiana attenuata* attract predators to the plant which feed on the herbivores that attack it. However, GLVs and terpenoids also attract flea beetles, which damage the plant (Halitschke et al. 2001). In Y-tube olfactometer experiments, *Spathius agrili* Yang, a parasitoid of EAB, is attracted to volatiles of ash and not attracted to odors associated with its insect host, such as larval EAB frass and hemolymph (Wang et al. 2010a). These volatile cues could be incorporated into the current methods used to control EAB, which includes the use of parasitoids as biological control.

Ash also has a complement of constitutive defense systems which aid in deterring herbivory. These defenses include phenolics (Eyles et al. 2007) and protease inhibitors (Chen and Poland 2010) which are present throughout the tree and reduce feeding. Phenolic compounds generally reduce the nutritive value of plants and increase resistance to herbivory by causing oxidative stress in the insect (Summers and Felton 1994). Protease inhibitors also defend plants against herbivory by inhibiting the digestion of plant proteins (Broadway et al. 1986). These proteins act on the gut of insects and may be found constitutively throughout the plant or induced in response to herbivory (Jongsma and Bolter 1997). Phenolics present in the phloem of ash could negatively affect the development of EAB larvae and thus deter females from ovipositing on ash species with higher concentrations. Unlike green and white ash, Manchurian ash phloem has been found to contain hydroxycoumarins which are correlated with insect feeding deterrence (Eyles et al. 2007; Leszczynski et al. 1995). The lignans pinoresinol glucoside and pinoresinol dihexoside were found in green, white, and Manchurian ash, but significantly higher amounts were present in the Manchurian ash. Pinoresinol is an antifeedant and hinders larval growth and molting in some Hemipterans (Eyles et al. 2007; Cabral et al. 1999; Garcia et al. 2000).

The nutritive content of ash foliage may also play a role in the preference of adult EAB to some ash species over others. In dual-choice feeding experiments, green and white ash are preferred over black ash by adult beetles. EAB that fed exclusively on black ash experienced decreased longevity compared to those that fed on green and white ash (Chen and Poland 2010). In six-choice feeding assays, EAB concentrated and fed preferentially on green, black, and white ash compared with blue, European (*F. excelsior* Linneaus), and Manchurian ash (Pureswaran and Poland 2009). The results of these studies indicate that there may be fitness advantages for EAB to feed on non-native *Fraxinus.*

1.5 Role of roots in herbivory defense

Roots are a vital part of the "shoot-root-shoot loop" in the herbivory defense systems of plants. For example, the roots of tobacco produce much more nicotine than the shoot (Solt 1957), and nicotine is transported via the xylem for storage in leaf vacuoles to defend the plant from herbivory (Morita et al. 2009; Shoji et al. 2000). Trees also exhibit this root to shoot defense network. For example, simulated herbivory on hybrid poplar leaves (*Populus trichocarpa* Torr. & A. Gray x *Populus deltoides* Bartr. ex Marsh) induced production of a trypsin inhibitor and increased transcription of herbivory defense genes in the roots (Major and Constabel 2007). In reciprocal grafting studies, the genotype of the rootstock, not the scion, of poplars was found to be responsible for flood tolerance (Peng et al. 2013). Grafting could hold promise for the propagation of ash trees more resistant to EAB if Asian ash species were used as rootstock.

Examples of successful grafting systems can be found in many plant groups. When *Citrus clementina* Hort. ex. Tan. is grafted onto differing citrus rootstocks, it appears that the rootstock induces physiochemical changes to the scion that influence resistance of the chimera to herbivory by the citrus leafminer, *Phyllocnistis citrella* Stainton (Muñoz et al. 2008). In several solanaceous plants, including nightshade, tropane alkaloids are produced in the roots and transported to the leaves (Ziegler and Facchini 2008). In reciprocal grafts with other species, the alkaloid patterns of chimeras mirror those of the rootstock, suggesting the rootstock confers secondary metabolites to the grafted shoots (Bais et al. 2001).

1.6 Current management methods

Trees in general increase property values, provide shade and cooling which decreases energy costs, absorb storm water, and increase air quality. These functions are especially important in urban forest systems, where ash trees often line the streets (McPherson et al. 2007). Several methods are currently employed to preserve North American ash and slow the spread of EAB. EAB is managed in the US through a combination of biological control, pesticide use, and various cultural control methods.

When EAB is first found in an area, quarantines are established to keep infested material from being transported into new areas. Quarantines can slow the spread and growth of EAB populations and delay the need for action in an area (McCullough and Mercader 2012). Once EAB is established, insecticides may be used to kill the pest. Due to their cryptic larval stage, young EAB are hard to kill with topical sprays. Systemic insecticides in the form of trunk injections and soil drenches are often used to overcome this issue. The active ingredient in these treatments is often imidacloprid, which can be mixed with water and applied directly to the soil for uptake or injected into the trunk (Herms et al. 2009). The systemic use of imidacloprid significantly decreases EAB populations within a treated tree and sublethal doses reduce EAB fitness as well (Mota-Sanchez et al. 2009). However, insecticides must be reapplied annually to be effective. The use of emamectin benzoate in trunk injections may be the most cost effective method of treatment, as it lasts multiple years and provides nearly 100% control of EAB larvae in treated trees up to 45 cm dbh for 2 to 3 years (Smitley et al. 2010).

Noninvasive bark sprays and protective cover sprays are also an option to manage EAB. Dinotefuran is a soluble systemic bark spray, allowing for penetration of the bark and movement throughout the tree. This method is a good alternative to trunk injections because it does not require special equipment and does not damage the tree. Protective cover sprays kill adult EAB feeding on the foliage and emerging through the bark of treated ash trees. Active ingredients may include permethrin, bifenthrin, cyfluthrin, and carbaryl. These sprays must be timed to coincide with adult emergence and egg laying for maximum efficiency (Herms et al. 2009).

Insecticide treatment can be costly and must be reapplied at regular intervals to provide maximum protection. A more environmentally-friendly method of combating EAB is the release of biological control agents. Volatiles induced by herbivory act as attractants for a variety of predators and parasitoids (Dicke and Baldwin 2010). With regard to EAB, a suite of parasitoid wasps help to regulate EAB populations in its native range. *Spathius agrili* is a gregarious idiobiont ectoparasitoid, capable of reaching levels of parasitism as high as 60% in some areas of China (Wang 2005). *Oobius agrili* Zhang and Huang, a more recently discovered egg parasitoid and biological control agent, has proven to be more difficult in terms of assessment of its parasitism rates. Researchers have developed a method of placing egg-sentinel logs (bolts of ash with known numbers of EAB eggs on them) in the field to assess the success of parasitism and establishment of the species in an area (Duan et al. 2012a). *Tetrastichus planipennisi* Yang, a koinobiont endoparasitoid, can paralyze up to 40% of EAB larvae. Together with *O. agrili*, a 73.6% reduction in EAB populations has been achieved in green ash in parts of China (Liu et al. 2007).

These natural enemies have been released in the US with some success, but difficulties in establishment have occurred. The most effective strategy for releasing parasitoids is to release them when EAB populations are low to moderate in size and parasitoids have ample time to establish before EAB populations crash and all ash in the area are killed (Duan et al. 2012a). For this reason, a reservoir of surviving ash is needed for the maintenance of parasitoid populations. These reservoirs are most effective when they include a mixture of susceptible and resistant trees. Green and Manchurian ash plantings in China are believed to aid in the parasitism rate of *T. plannipennisi* and *O. agrili* enough to allow both ash species to survive (Liu et al. 2003). Sustainable control of EAB may be possible if the proper forest composition is present. However, it is unclear which ash volatiles the parasitoids respond to most strongly and how grafting might affect VOC production.

1.7 Summary

If the ash resource in North America is to be preserved, the answer may lie in the production of EAB resistant trees through traditional breeding, creating hybrids, and grafting. It seems likely then that the Asian ash species which exhibit host plant resistance would make good candidates for breeding efforts. Hybrid trees are created through cross-pollination and offspring may exhibit characteristics of both parent plants. Success thus far in creating *Fraxinus* hybrids has been limited, but horticultural cultivars of a hybridization of *F. nigra* and *F. mandshurica* have been produced (Davidson 1999). Traditional breeding may be effective at creating resistant ash varieties if those which have survived on their own are used. The last standing ash trees in area that has experienced a heavy infestation may possess a less preferred phenotype or other resistant characteristics that could be incorporated into resistant varieties (Gould et al. 2012). Resistant ash phenotypes would also make ideal rootstock donors for grafting efforts and could lead to the propagation of resistant grafted trees.

Urban forests in the eastern US are predominantly comprised of both *Acer* and *Fraxinus* (Raupp et al. 2006). Low genetic diversity and a predominance of green and white ash, which are also the most susceptible to EAB, could have serious consequences on these urban areas (MacFarlane and Meyer 2005). Ash is also a minor component of many different forest types throughout North America (MacFarlane and Meyer 2005) and the loss of these trees will impact the ecosystems in which they are found. Current methods for the control of EAB are costly and time consuming, and a long-term plan for maintaining the ash resource is needed. Grafting of ash to produce resistant trees and the elucidation of the resistance mechanisms would ensure that *Fraxinus* is not lost from North America. It is also necessary to uncover the ways in which grafted trees will attract EAB parasitoids and further protect them from EAB-related mortality.

CHAPTER 2. CONSTITUTIVE AND EAB-INDUCED VOC PRODUCTION IN GRAFTED *FRAXINUS* spp.

2.1 Abstract

The emerald ash borer (EAB; *Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae) is a destructive invasive beetle from Asia which has killed millions of ash trees (*Fraxinus* spp.) throughout North America. This phloem-feeding borer is capable of killing otherwise healthy trees and colonizes trees as small as saplings – most affected trees die within four years of colonization. Asian ash species (i.e., Manchurian and Chinese) appear to be more resistant to EAB than those native to North America (e.g., green, white, and black ash). In this project we use reciprocal and conspecific grafts of these five ash species to determine the extent to which resistance to EAB can be conferred to a scion when grafted onto resistant rootstock. Specifically, I addressed this question through the analysis of leaf associated volatile organic compounds (VOCs) of each grafted tree to determine the extent to which: 1) rootstocks influence the composition of VOCs of a grafted scion; and 2) herbivory by adult female beetles induces changes in the VOCs released by the reciprocal grafts. Volatile profiles of the ash species differed quantitatively and adult EAB feeding induced systemic changes in VOC profiles of grafted ash. This work may pave the way toward developing systems for propagating

Fraxinus scions that are resistant to EAB and exploit potential phytochemical connectivity between roots and shoots.

2.2 Introduction

The emerald ash borer, *Agrilus planipennis* Fairmaire (EAB; Coleoptera: Buprestidae) is an invasive wood-boring beetle that has decimated the *Fraxinus* resource in eastern North America and continues to spread across the continent with devastating effect. First detected in southeastern Michigan in 2002, EAB has caused over 99% mortality of green (*F. pennsylvanica)*, white (*F. americana*), and black (*F. nigra*) ash trees ≥2.5 cm in diameter in sites near the epicenter of the invasion (Klooster et al. 2013). In its native range, EAB attacks only stressed trees, but all North American ash species are susceptible to EAB, and even apparently healthy trees succumb to this pest (Liu et al. 2003; Cappaert et al. 2005). The resistance of Asian ash species is attributed to foliar and phloem chemistry that differs from the North American ash species (Rodriguez-Saona et al. 2006; Eyles et al. 2007; Chen et al. 2011; Cipollini et al. 2011).

EAB locate suitable hosts for colonization by detecting volatile organic compounds (VOCs) emitted constitutively from the bark and foliage of *Fraxinus* (Rodriguez-Saona et al. 2006; Crook et al. 2008b; Crook and Mastro 2010). Many herbivorous insects use olfaction to detect plant-emitted compounds to guide them to suitable oviposition sites, food resources, and conspecific mates (de Bruyne and Baker 2008). Plants release unique blends of VOCs, and insects respond to the blend associated with their host to locate them, often failing to respond to the compounds when presented with them individually (Bruce and Pickett 2011). Some of the VOCs released by ash produce an electrophysiological response in the antennae of EAB with (*Z*)-3-hexenol eliciting the greatest response in both male and female EAB (Rodriguez-Saona et al.

2006). Ash emits many volatile compounds (see Table 2.1) and there are qualitative and quantitative differences in the VOCs produced between ash species. These differences in VOC emission may play a role in the differential attraction of EAB to the various *Fraxinus* species and the mechanisms of volatile production could be exploited to yield resistant grafted or hybridized trees.

As the ash resource continues to decline due to EAB-related mortality, research efforts have intensified to identify possible solutions to maintaining North American ash species. The loss of the American chestnut (*Castanea dentata*) to chestnut blight and the American elm (*Ulmus americana)* to Dutch elm disease have inspired researchers to propagate tolerant cultivars, create resistant transgenic trees, and to breed these species with related resistant species with the goal of restoring these "heritage trees" back into North American forests (Merkle et al. 2007). Similar restoration efforts could be applied to ash to preserve this important forest resource. One method of conferring resistance to a tree is through the use of grafting. Grafting involves the joining of the vascular tissues of two plants and is a common practice in horticulture and agriculture (Hottes 1925; Atkinson et al. 2003). The rootstock of plants with resistant properties, such as resistance to a common herbivorous pest, is often attached to the scion of more susceptible species to create a more resistant chimera (Bais et al. 2001; Major and Constabel 2007; Muñoz et al. 2008). Roots are a vital part of the "shoot-root-shoot loop" in the herbivory defense systems of plants, and produce secondary metabolites which are transported throughout the plant (Erb et al. 2009).

In the current study, I use reciprocal grafts of five ash species (i.e., green, white, black, Manchurian and Chinese) to determine the extent to which resistance to EAB can be conferred to a scion when grafted onto resistant rootstock. I test the hypothesis that when grafted onto resistant rootstock, the VOCs emitted by the leaves and branches of the susceptible scions will be altered due to root-shoot connectivity to reflect those of the donor. I also determine the extent to which herbivory by adult female beetles induces local and systemic changes in the volatiles released by these reciprocal grafts. This work may pave the way toward developing grafting strategies for propagating *Fraxinus* scions that are resistant to EAB and exploit potential phytochemical connectivity between roots and shoots.

2.3 Materials and Methods

2.3.1 Field Site and Experimental Design

Saplings of five different ash species (Table 2) were purchased from Lawyer Nursery (Plains, MT); Bailey Nurseries (Newport, MN); and Musser Forests, Inc. (Indiana, PA) or harvested from the Purdue University Department of Forestry and Natural Resources ash plantation. Reciprocal and conspecific scion and rootstock grafts were performed in April 2010 and maintained under drip fertigation in a shade frame at the Purdue University John S. Wright Forestry Center (Tippecanoe Co., Indiana) and then transferred to a cold dome until planted. In May 2011, the ash planting was arranged at the Purdue University Harrold Woodland (Whitley Co., IN). A completely randomized block design with four replicates (blocks) and all combinations of chimeras (n=20) and conspecific grafts (n=5) were planted in a 5x5 arrangement in each block. Five ash saplings of each species (propagated from rootstock) were randomized between blocks and serve as buffer rows. The trees were spaced 6 m apart within each row and the distance between each row was 3 m.

2.3.2 Source of beetles

EAB used in this study were obtained from the USDA-APHIS-PPQ Biological Control Production Facility located in Brighton, MI. Male and female beetles were separated and placed in 300 cm^3 screen cages and sustained on fresh foliage from blue ash (*Fraxinus quadrangulata* Michx.) and 10% sucrose solution (replaced every 2-3 days). Cages containing beetles were stored at 25 ºC in an environmental chamber (~70% relative humidity and photoperiod of 14:10 h L:D) until used in feeding assays. Female beetles used in bioassays were vigorous and active.

2.3.3 VOC collection and bioassays

Volatiles from internal leaf pools were collected from trees in our study to identify; 1) the extent to which rootstock influences the composition of leaf volatiles released by the scion; 2) the extent to which EAB adult feeding induces changes in volatile production; and 3) the extent to which rootstock confers resistance to the scion.

This objective was accomplished through the four step experiment outlined below:

1) Leaf tissue (approximately 2 grams fresh weight) was harvested from three trees from each of the 25 unique grafts. The leaves were immediately frozen on dry-ice and transported back to the laboratory for volatile extraction (see methods below). Collection of volatiles took place in early June, 2013.
2) The following day, ten 8-10 day old adult female EAB were enclosed in a screen sleeve surrounding one intact compound leaf on one of the grafted trees. The beetles were allowed to feed for 48 hours before being removed.
3) The leaves experiencing herbivory were harvested upon removal of the beetles, placed on dry ice and transported to the laboratory where volatiles were extracted to determine if feeding induced changes in the internal leaf pools as a plant defense. Prior to extraction of volatiles, the leaves were scanned using a flatbed scanner for use in Image J software and total leaf area consumed by the beetles was estimated. This allowed us to quantify the feeding damage and make correlations between amount of feeding and change in volatiles. Defoliation was

recorded as area of leaf consumed (mm^3) for each of the replicates. Effects of reciprocal grafts on leaf consumption by adult beetles were analyzed using ANOVA (StatSoft 2013).

4) 24 hours later, volatiles were collected from leaf material found elsewhere on the tree to see if a delayed systemic response was present.

2.3.4 Internal leaf pool volatile extraction and GC-MS analysis

Approximately 2 g of leaf material (one large compound leaf) was harvested from the trees using pruning shears to make a clean cut. These leaves were placed immediately into individual plastic bags and frozen using dry-ice to halt enzymatic activity. Frozen leaves were transported to the lab where volatile extractions were performed. The frozen leaf material was pulverized to a powder in liquid nitrogen using a mortar and pestle, and then transferred to an Erlenmeyer flask (125 mL) to which 10 mL of hexane was added. The flasks were capped and placed on a C24 Incubator Shaker and were allowed to extract for 24 h (@ approximately 25 °C). A pipette was used to collect the liquid from the flasks and transfer it to a clean flask. Extracts were dehydrated using 0.5 g of DrieRite (Xenia, OH) on the shaker for 6 h. The dehydrated extract was then further concentrated using a gentle stream of nitrogen gas to evaporate the excess hexane. Samples were concentrated to 1.5 mL and placed in 4 mL vials. The extracts were analyzed by coupled gas chromatography-mass spectrometry (GC-MS) with electron impact ionization (EI, 70 eV) using an Agilent Technologies 6890N GC (Agilent Technologies, Santa Clara, CA) equipped with a DB-5MS capillary column (30 m x 0.25 mm x 0.25 μm film; J&W Scientific, Folsom, CA) in splitless mode, interfaced to an Agilent Technologies 5975B mass selective detector, with helium carrier gas. The oven temperature was programmed from 40 °C/1 min, ramped at 10 °C/min to 250 °C, and held for 5 min at 250 °C. Injector temperature was 100 °C and transfer line temperature held at 280º C. Compounds were identified by mass spectral fragments after comparing their retention times to those of authentic standards. The percentage that each peak contributed to the total was calculated by integrating the areas under peaks of all compounds that were consistently present in the total ion chromatograms (Chemstation, Version D.05.01; Hewlett Packard Corp.). Quantitative data presented in Figs. 3 through 8 were produced by these analyses. Differences in mean relative abundance of compounds from the graft combinations (area under peak/total area of all peaks) and phase (i.e., constitutive, induced and systemic) were compared by MANOVA (StatSoft, Inc., 2013) following by Tukey HSD test. To meet the equal variance assumption of MANOVA, data were arcsine square-root transformed prior to analysis.

2.4 Results

2.4.1 Leaf area consumed by EAB

In my study, rootstock affected herbivory on Manchurian and Chinese scions. Namely, these resistant scions experienced more herbivory when grafted onto susceptible green rootstock than when grafted onto a conspecific rootstock. Manchurian ash grafted onto green rootstock experienced more herbivory than either green or Manchurian ash grafted onto Manchurian rootstock (ANOVA, $F_{3,7} = 3.41$, $P=0.082$, Figure 2.1). Moreover, a similar pattern of herbivory was observed among all combinations of Chinese and green ash (ANOVA, $F_{3,8} = 5.31$, $P<0.05$, Figure 2), suggesting that properties of the rootstock may be conferred to grafted scions. In each case, however, resistant scions were made more susceptible to herbivory through grafting, rather than increasing the resistance of susceptible scions.

It also appears that with regard to herbivory, this root-shoot connectivity may be more pronounced with green ash than other North American ash species. For example, there were no differences in the mean leaf area consumed of grafted trees containing all combinations of black and Chinese (ANOVA, $F_{3,7} = 1.45$, $P = 0.307$) or black and Manchurian ash (ANOVA, $F_{3,6} = 0.67$, $P = 0.603$). Similarly, there were no differences in the mean leaf area consumed of grafted trees containing all combinations of white and Chinese (ANOVA, $F_{3,8} = 1.22$, $P = 0.364$) or white and Manchurian (ANOVA, $F_{3,7} = 0.86$, $P = 0.502$).

2.4.2 Volatile profiles

Nine VOCs were consistently represented in the internal pools of leaves in this study (Table 2.3). Hexanal, (*E*)-2-hexenal, (*Z*)-3-hexanol, (*Z*)-3-hexenyl acetate, nonanal, methylsalicylate, and farnesene have been previously identified in headspace volatile collections of green, white, black, and Manchurian ash (Table 2.1; Rodriguez-Saona et al. 2006; de Groot et al. 2008) and (*Z*)-3-hexenal, (*E,E*)-2,4-hexadienal, and methylsalicylate are commonly induced through herbivore feeding (e.g., Vuorinen et al. 2007; Karl et al. 2008; Turlings et al. 1998; Shuai et al. 2012). Grafting affected the relative proportions of these compounds in all combinations of North American and Asian ash species except for green and Chinese (see Table 4). There were also quantitative differences in the constitutive, induced and systemic (i.e., phase) volatile profiles among all graft combinations. Moreover, there was a significant interaction of graft and phase for all graft combination other than those involving green and Chinese ash (see Table 2.4).

In grafts with green ash, Manchurian conspecific grafts produced significantly more hexanal, (*Z*)-3-hexanol, and methylsalicylate constitutively and less (*E*)-2-hexenal across all phases, suggesting that grafting did not affect the proportion of these volatiles, but rather that they reflect those of the scion (see Figs. 2.9, 2.11, and 2.13). Also, levels of methylsalicylate were more abundant in systemic collections of conspecific Manchurian grafts than those of constitutive samples, and induced samples were intermediate.

Black ash is phylogenetically more closely related to the Asian species and, interestingly, conspecific grafts produced a volatile profile similar to that of Manchurian conspecific grafts (Fig. 2.11). However, the proportion of (*Z*)-3-hexenal was smaller in constitutive samples of conspecific Manchurian grafts than those of black ash and the levels in black ash grafted onto Manchurian rootstocks were intermediate, suggesting that grafting affected the amount of this compound in the chimeras (see Fig. 2.11). Once again there was also a systemic increase in methylsalicylate in the conspecific Manchurian grafts. With regard to (*Z*)-3-hexanal, black scions with Chinese rootstocks also displayed an intermediate volatile profile to that of black and Chinese conspecific trees (Fig. 2.12), and there was an increase in methylsalicylate across all phases relative to the other graft combinations.

Grafting white ash onto Manuchurian rootstock also affected the proportion of (*E*)-2-hexanal, with constitutive and induced levels being highest in white ash, lower in the Asian ash and intermediate in the chimera (Fig. 2.13). This reduction suggests that rootstock affects the amount of (*E*)-2-hexenal in the chimera. If there was no rootstock effect, levels of chimera should reflect those of the donor scion (i.e., conspecific grafts of white ash). Finally, grafting appears to affect the systemic release of (*Z*)-3-hexenyl acetate in the chimeras of white ash scion and Chinese rootstock, with a systemic increase relative to the proportions in induced and constitutive samples (Fig. 2.14).

2.5 Discussion

Grafted trees containing resistant Asian rootstock and North American scions emitted volatile profiles which differed quantitatively from their conspecific counterparts, often displaying an intermediate profile. EAB are capable of detecting and responding to very low concentrations of VOCs and these intermediate volatile profiles may prove to reduce the susceptibility of grafted trees. In GC-EAD experiments, many of the compounds found in the current study elicit an antennal response in both male and female EAB, including hexanal, (*E*)-2-hexenal, (*Z*)-3-hexenol, (*Z*)-3-hexenyl acetate, nonanal, and farnesene (Rodriguez-Saona et al. 2006). Although methylsalicylate, (*Z*)-3-hexenal, and (*E,E*)-2,4-hexadienal have not been found to elicit an antennal response in EAB, they are induced through herbivory by some insects and mechanical leaf damage (e.g. Vuorinen et al. 2007; Karl et al. 2008; Turlings et al. 1998, Shuai et al. 2012).

Herbivory induces changes in the volatiles emitted from foliage as a result of the hormone jasmonic acid, which signals the activation of defense genes to regulate the formation of terpenoid compounds (Ament et al. 2004; van Schie et al. 2007). Extraction of ILPs is effective for quantifying such compounds, because these oxygenated monoterpenes are present in high concentrations within the aqueous portion of leaves (Loreto et al. 1998). Induced VOCs act as tritrophic signals, attracting natural enemies to the affected plant to prey on pests (Dicke et al., 2009), however they can also act on the plant itself to signal undamaged portions to up-regulate defense (Frost et al. 2007). For example, (*Z*)-3-hexenyl acetate released by herbivore-damaged *Populus deltoides* x *nigra* saplings increased jasmonic acid and linolenic acid in undamaged leaves located elsewhere on the plant (Frost et al. 2007, 2008). In the present study, white ash grafted onto Chinese ash rootstock and black ash grafted onto Manchurian ash rootstock had significantly higher amounts of (*Z*)-3-hexenyl acetate in the systemic phase suggesting that both Asian rootstocks influence the production of this compound. The release of (*Z*)-3-hexenyl acetate systemically allows the plant to defend against further herbivory (Frost et al. 2007) and North American ash species grafted onto Asian ash rootstock may benefit from this signaling.

Many predators and parasitoids use compounds such as those found in this study to locate their prey. *Spathius agrili*, one parasitoid currently being used in EAB biological control efforts, is attracted to the VOCs of both green and velvet ash (*F. velutina*) (Wang et al. 2010a). The coevolution of EAB parasitoids with Manchurian and Chinese ash indicates that the volatile profiles of these species may be more attractive than those of the North American ash species. The intermediate volatile profiles produced through grafting Asian rootstock to native ash species could prove attractive to EAB parasitoids, and warrants further research. For example, (*Z*)-3-hexenal, hexanal, and (*Z*)-3-hexenyl acetate elicit strong electroantennogram responses in the parasitoids *Microplitis croceipes* and *Cotesia marginiventris*, suggesting that they may play a role in the facilitation of prey location in these species (Chen and Fadamiro 2007). No GC-EAD experiments have been performed to identify which volatiles released by ash elicit antennal responses in *S. agrili* and other EAB parasitoids used for biological control, and it is possible that VOCs induced through EAB feeding may also be attractive.

The results of my feeding experiments revealed a clear pattern in the grafts containing green ash and the resistant Manchurian and Chinese ash. Grafts with resistant rootstock had less feeding than the reciprocal grafts with green rootstock. This indicates that the Asian rootstocks are an important part of the root-shoot-root loop and may produce compounds related to resistance. However, Chinese rootstock did not affect the volatile profile of green ash scions when grafted together. Prior to this study, Chinese ash had not been used in studies focusing on EAB resistance and in this study I found that the volatile profile of Chinese ash varied little from that of green ash, yielding a significant graft effect when paired with white or black ash. Due to the resistance of Chinese ash and the susceptibility of green ash, this similarity in volatile profile suggests that there may be other factors influencing resistance besides VOC emissions. Although Chinese rootstock does not alter the volatile profile of green ash scions, white ash grafted onto Chinese rootstock systemically increased (*Z*)-3-hexenyl acetate, which suggests that the pairing may lead to a more resistant chimera. Manchurian ash rootstock was also effective at producing higher amounts of systemic (*Z*)-3-hexenyl acetate when grafted with black and white ash scions. Asian ash rootstock grafted to white or black ash scions may hold promise for propagation of resistant chimeras.

Future work towards the development of ash varieties resistant to EAB should focus on the attraction of EAB parasitoids to the volatiles produced systemically from grafted ash and also the phloem chemistry of chimeras and how grafting can alter the resistant traits of the scion. EAB spend the majority of their life cycle in the larval stage feeding beneath the bark of the tree; the larval stage lasts for approximately 300 days (Wang et al. 2005, 2010b). Manchurian ash possesses phenylethanoids and several hydroxycoumarins which are not present in green or white ash. These phenolic compounds are hypothesized to deter insect feeding and increase host plant resistance (Eyles et al. 2007; Leszczynski et al. 1995). Green ash phloem contains high phenolic concentrations yet it is highly susceptible, leading to the theory that the compounds unique in Manchurian ash may contribute to resistance (Cipollini et al. 2011). However, the analysis of highly susceptible European (*F. excelsior*) and black ash phloem found a suite of hydroxycoumarins comparable to those of Manchurian ash and also higher phenylethanoid concentrations, suggesting that these compounds are not responsible for resistance (Whitehill et al. 2012). Pinoresinol dihexoside is currently the only compound found to be unique to Manchurian ash phloem, and is present in high concentrations in dormant tissue (Whitehill et al. 2012; Eyles et al. 2007). The aglycone pinoresinol has been found to deter feeding and inhibit growth and molting in many insect species and further research could reveal its role in the resistance of ash to EAB (Miyazawa et al., 1994; Cabral et al., 1999; Garcia et al., 2000).

Table 2.1 Volatile organic compounds emitted from the foliage of Manchurian, green, white,

Compound	**Manchurian Ash** ***F. mandschurica***	**Green Ash** ***F. pennsylvanica***	**White Ash** ***F. americana***	**Black Ash** ***F. nigra***
(*Z*)-3-hexenal[b]	nd	+	+	nd
Hexanal[a,b,c]	+	+	+	+
Hexanol[b]	nd	+	+	nd
(*E*)-2-hexenol	nd	+	+	nd
(*E*)-2-hexenal[a,b,c]	+	+	+	+
(*Z*)-3-hexenol[a,b]	+	+	+	nd
(*Z*)-3-methyl-butylaldoxime[a]	+	nd	nd	nd
(*E*)-2-methyl-butylaldoxime[a]	+	nd	nd	nd
(*E*)-3-methyl-butylaldoxime[a]	+	nd	nd	nd
2-Butoxy ethanol[a]	+	nd	nd	nd
(*Z*)-3-hexenyl acetate[a,b,c,d]	+	+	+	+
Hexyl acetate[a,b]	+	+	+	nd
(*Z*)-ocimene[a]	+	nd	nd	nd
(*E*)-β-ocimene[a,c]	+	nd	nd	+
trans-ocimene[d]	nd	+	nd	nd
Linalool oxide[a]	+	nd	nd	nd
Linalool[a,d]	+	+	nd	nd
Nonanal[a,c]	+	nd	nd	+
Benzene acetonitrile[a]	+	nd	nd	nd
4,8-Dimethyl-1,3,7-nonatriene[a,d]	+	+	nd	nd
Indolizine[a]	+	nd	nd	nd
Unidentified, Indole-like[a]	+	nd	nd	nd
(*E*)-3-hexenyl butyrate[a]	+	nd	nd	nd
Methyl salicylate[a,c,d]	+	+	nd	+
(*E*)-3-hexenyl-2-methyl butyrate[a]	+	nd	nd	nd
Indole[a,c]	+	nd	nd	+
(*Z*)-jasmone[a]	+	nd	nd	nd
β-caryophyllene[a,c]	+	nd	nd	+
Z,*E*-α-farnesene[a,c]	+	nd	nd	+
E,*E*-α-farnesene[a]	+	nd	nd	nd
α-farnesene[d]	nd	+	nd	nd
(*E*)-nerolidol[a,d]	+	+	nd	nd
Benzyl cyanide[c]	nd	nd	nd	+
α-humulene[c]	nd	nd	nd	+
α-cubenene[c]	nd	nd	nd	+

and black ash identified in published research.

+ indicates the detection of a compound in head-space collected VOC samples analyzed using GC-MS.

nd indicates that the compound was not detected in the samples.

[a] Compounds identified by Rodriguez-Saona et al. 2006 study on *F. mandschurica*.

[b] Compounds identified by de Groot et al. 2008 study on *F. pennsylvanica* and *F. americana*.

[c] Compounds identified by Chen et al. 2011 study on *F. nigra, F.pennsylvanica,* and *F. americana.*

[d] Compounds identified by Markovic et al. 1996 study on *F. pennsylvanica.*

Table 2.2 *Fraxinus* species that served as scion and rootstock donors in reciprocal and conspecific graftsplanted at the Purdue University Harrold Woodland in Whitley Co. IN.

Common Name	**Species Latin Name**	**Species Group**	**Susceptible to EAB***
White Ash	*Fraxinus americana*	North American	Yes
Black Ash	*F. nigra*	North American	Yes
Green Ash	*F. pennsylvanica*	North American	Yes
Chinese Ash	*F. chinensis*	Asian	No
Manchurian Ash	*F. mandshurica*	Asian	No

*See Rebek et al. 2008

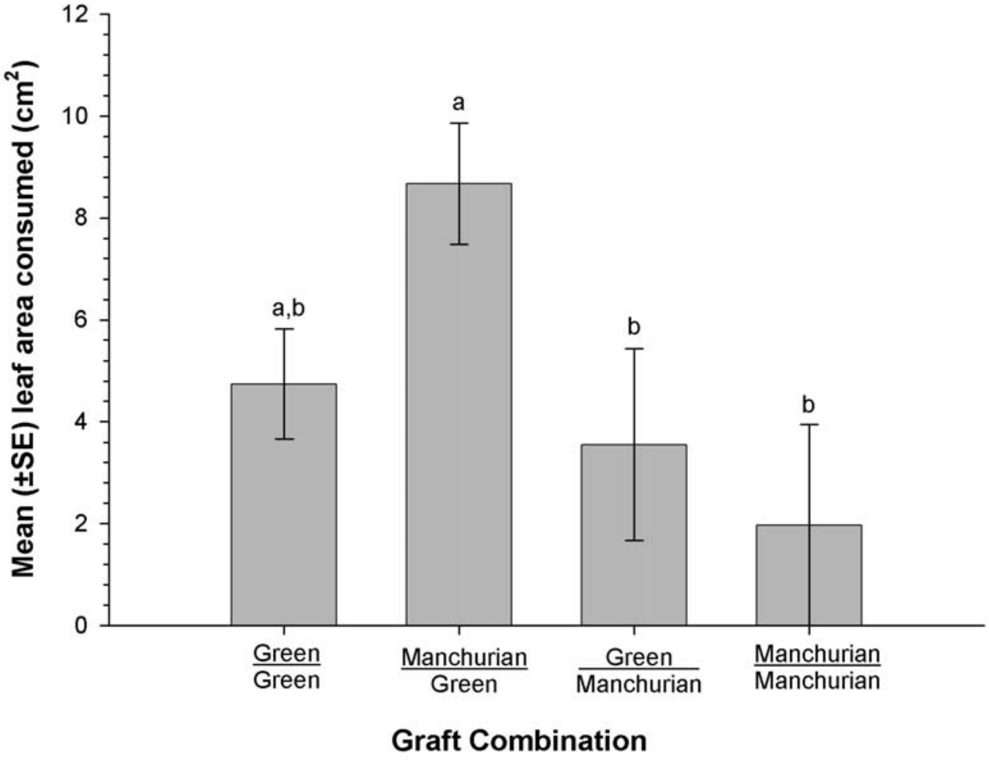

Figure 2.1 Mean (±SE) leaf area consumed (cm²) by adult female EAB on grafts containing green and Manchurian ash. Ten female beetles were placed on each tree in screen bags and allowed to feed for 48 hours. In reference to the x-axid labels, the ash species used for the scion is listed above the line and the rootstock below. Bars with different letters are significantly different (ANOVA, Fisher LSD *P*<0.05).

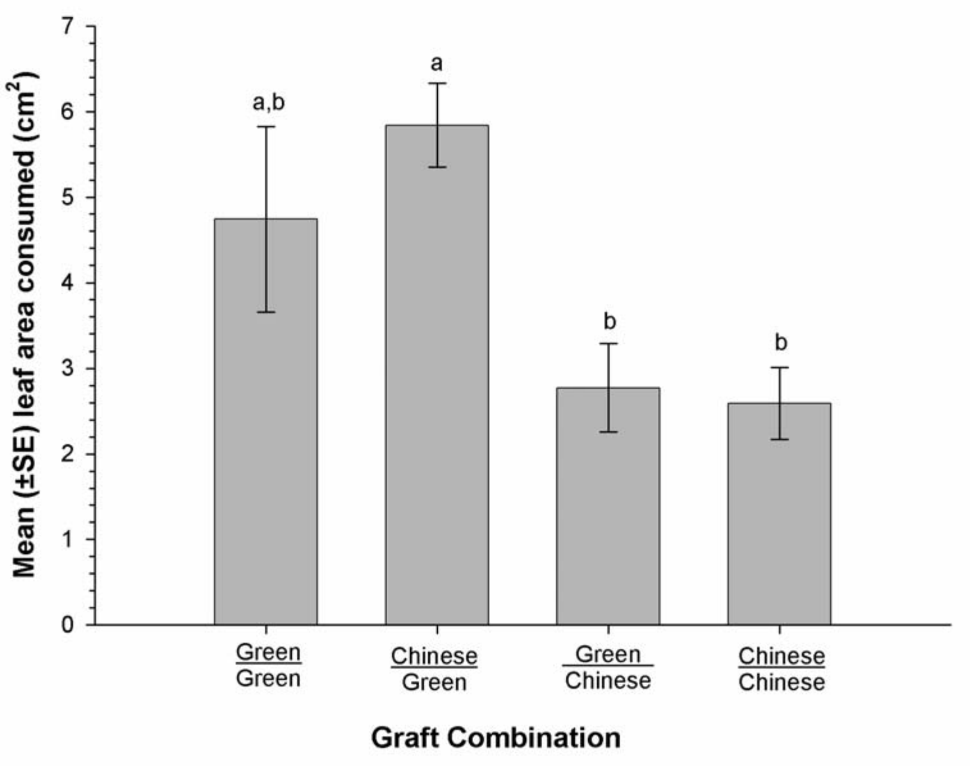

Figure 2.2 Mean (±SE) leaf area consumed (cm²) by adult female EAB on grafts containing green and Chinese ash. Ten female beetles were placed on each tree in screen bags and allowed to feed for 48 hours. In reference to the x-axis labels, the ash species used for the scion is listed above the line and the rootstock below. Bars with different letters are significantly different (ANOVA, Fisher LSD *P*<0.05).

Table 2.3 Volatile organic compounds identified by GC-MS from grafted ash containing combinations of green, white, black, Chinese, and Manchurian ash.

Peak #	**Retention Time (min)**	**Compound***
1	5.40	Z-3-Hexenal
2	5.50	**Hexanal**
3	7.80	**E-2-Hexenal**
4	8.00	**Z-3-Hexenol**
5	9.90	E,E-2,4-Hexadienal
6	12.40	**Z-3-Hexenyl Acetate**
7	14.35	**Nonanal**
8	15.90	Methylsalicylate
9	20.15	**Farnesene****

*Compounds in bold elicit antennal responses in EAG experiments (see Rodriguez-Saona et al. 2006; de Groot et al. 2008).

**Farnesene was not present in any of the constitutive volatile profiles.

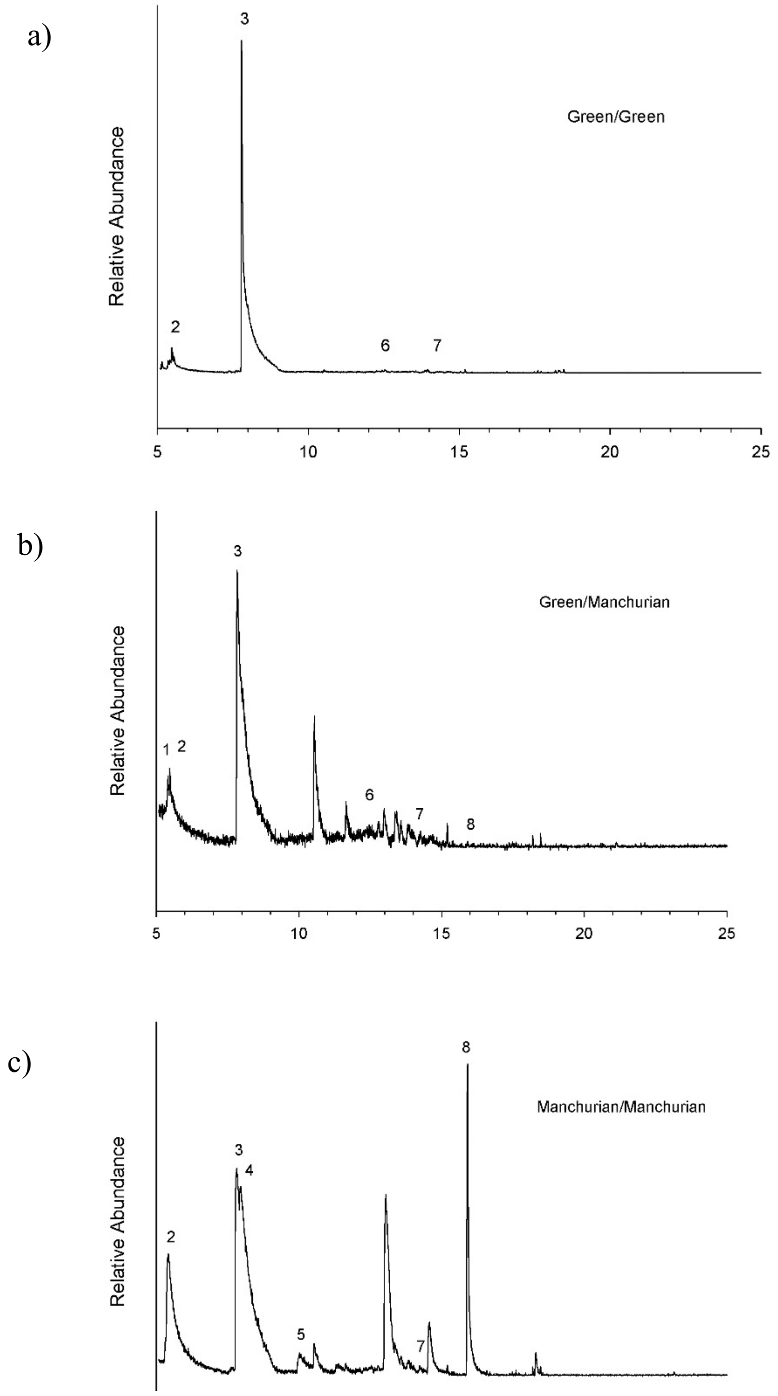

Figure 2.3 Representative GC-MS chromatograms of volatile extracts from a) green ash conspecific grafts, b) green ash scion on Manchurian ash rootstock, and c) Manchurian ash conspecific grafts.

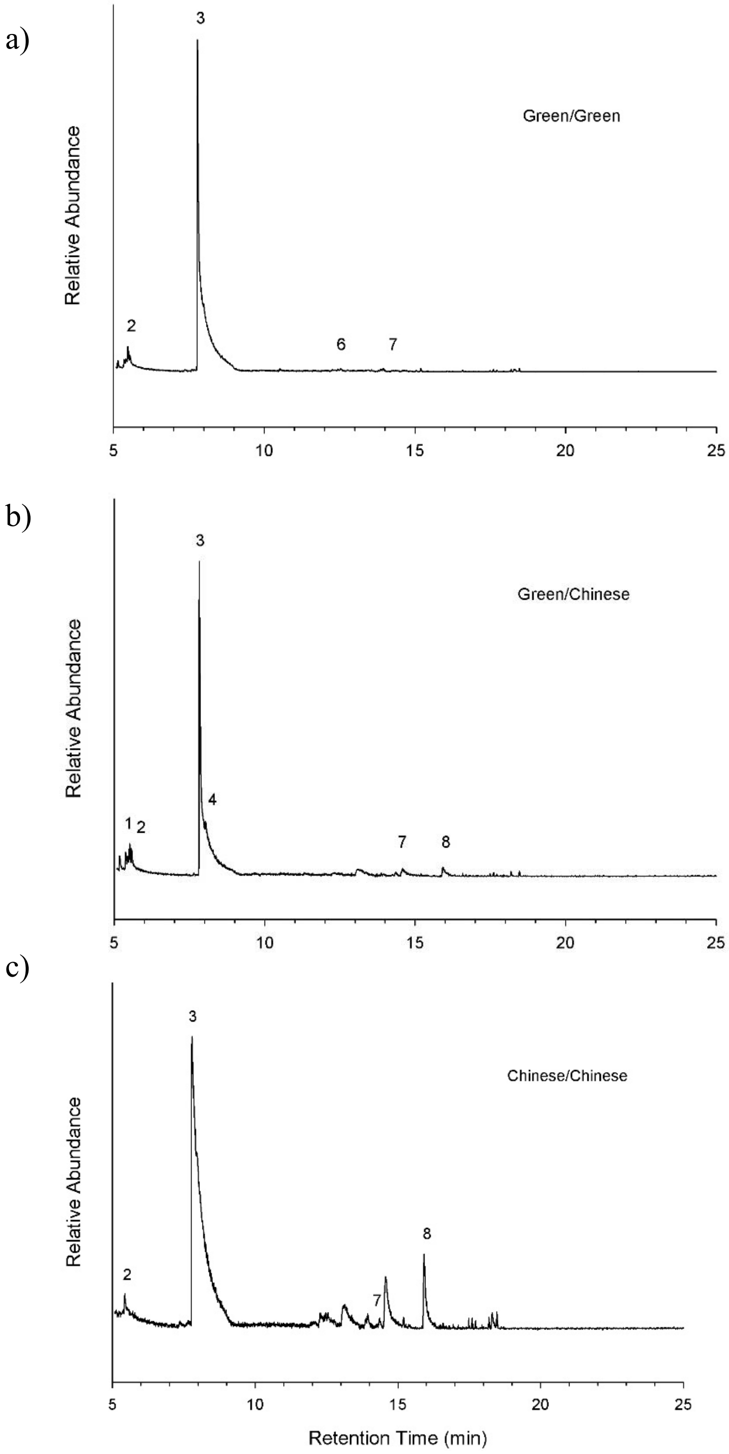

Figure 2.4 Representative GC-MS chromatograms of volatile extracts from a) green ash conspecific grafts, b) green ash scion on Chinese ash rootstock, and c) Chinese ash conspecific grafts.

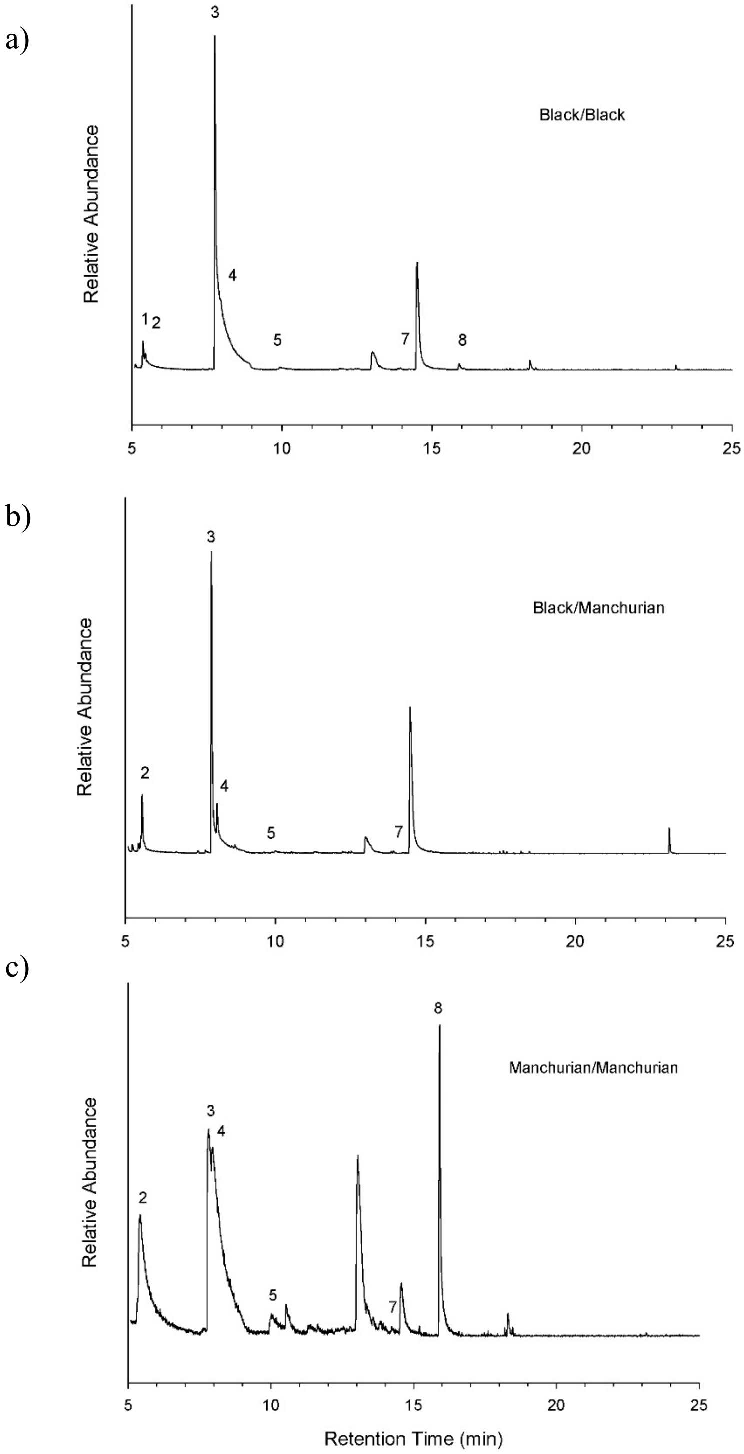

Figure 2.5 Representative GC-MS chromatograms of volatile extracts from a) black ash conspecific grafts, b) black ash scion on Manchurian ash rootstock, and c) Manchurian ash conspecific grafts.

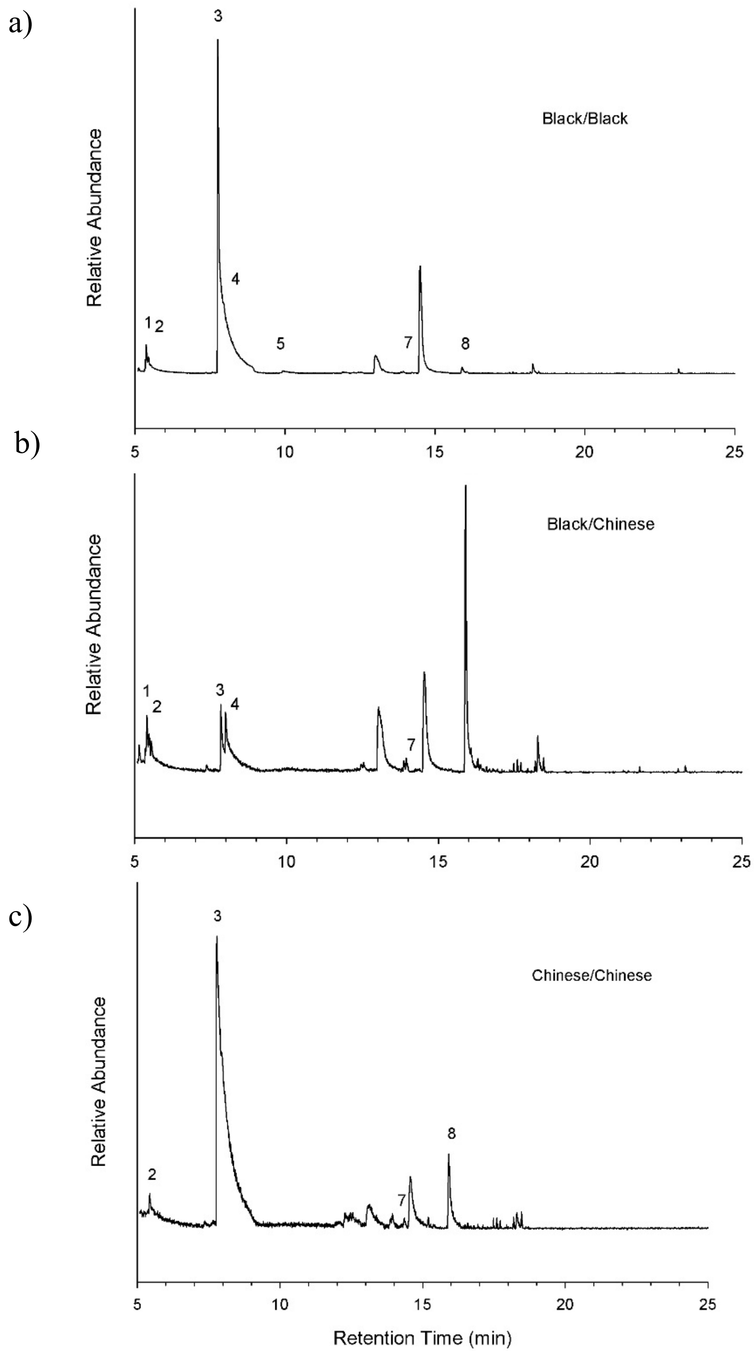

Figure 2.6 Representative GC-MS chromatograms of volatile extracts from a) black ash conspecific grafts, b) black ash scion on Chinese ash rootstock, and c) Chinese ash conspecific grafts.

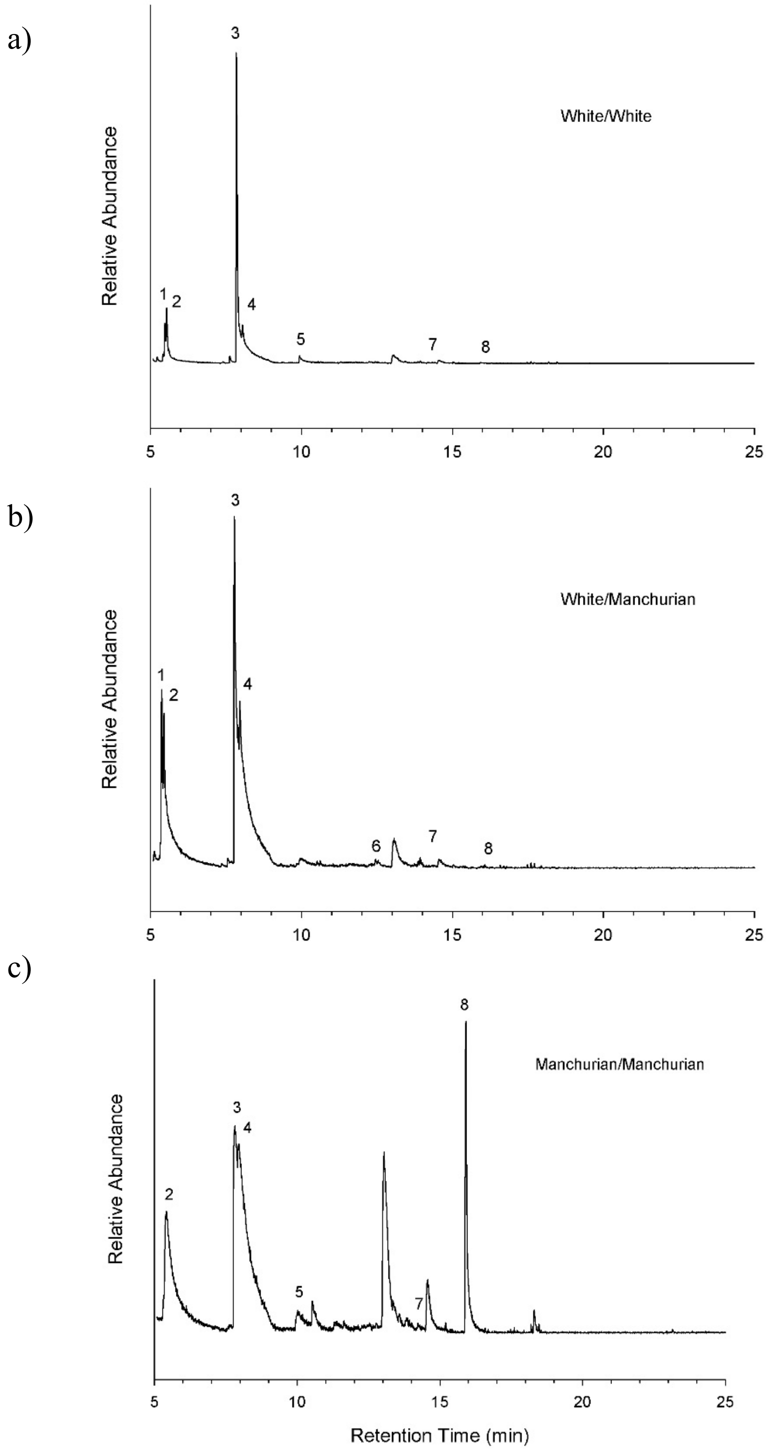

Figure 2.7 Representative GC-MS chromatograms of volatile extracts from a) white ash conspecific grafts, b) white ash scion on Manchurian ash rootstock, and c) Manchurian ash conspecific grafts.

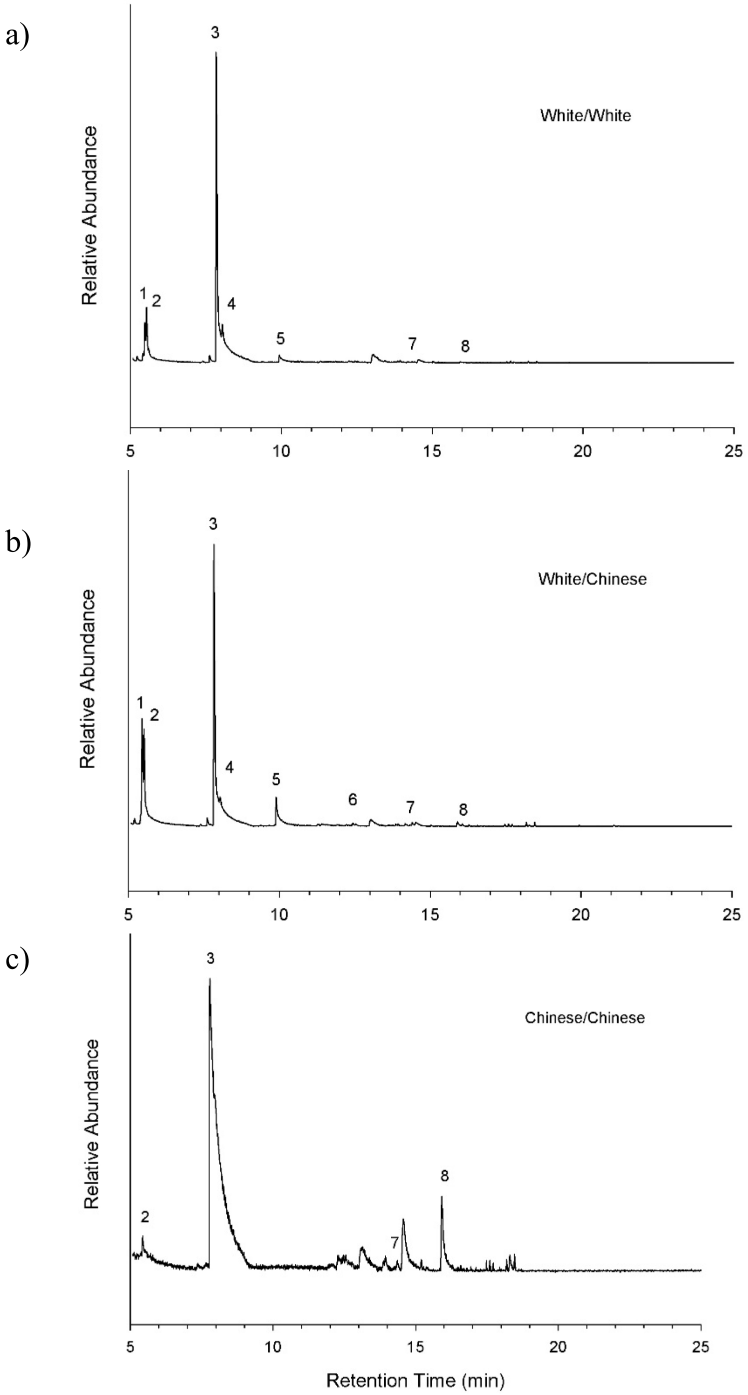

Figure 2.8 Representative GC-MS chromatograms of volatile extracts from a) white ash conspecific grafts, b) white ash scion on Chinese ash rootstock, and c) Chinese ash conspecific grafts.

Table 2.4 Results of MANOVA for effects of graft combination (species of the rootstocks and scions), phase of the trees (constitutive, induced, systemic), and their interaction on

Ash Species Combination	Source of Variance (F values)		
	Graft	Phase	Graft x Phase
Green and Manchurian	10.2247**	3.3841**	1.5222*
Green and Chinese	0.58837$^{n.s.}$	3.76231**	1.06716$^{n.s.}$
Black and Manchurian	7.82397**	2.19167*	1.53518*
Black and Chinese	10.20799**	2.39001**	1.84358**
White and Manchurian	9.3157**	3.9099**	1.8266**
White and Chinese	4.2997**	3.3574**	1.2763$^{n.s.}$

the volatile emissions of compounds from grafted ash.

* = $P \leq 0.05$; ** = $P \leq 0.01$; n.s. = not significant

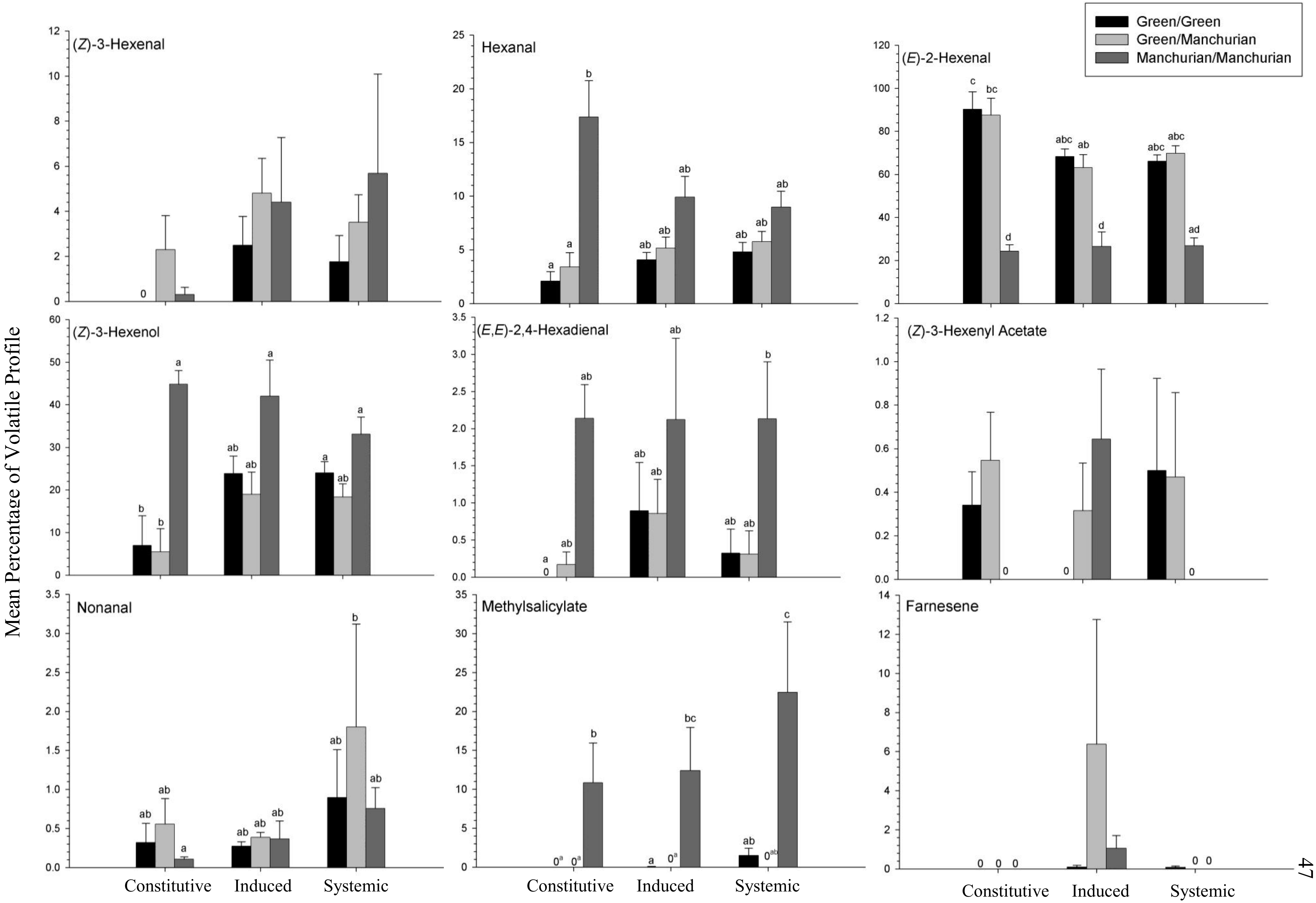

Figure 2.9 Volatile compounds identified from green scions grafted onto green rootstock, green scions grafted onto Manchurian rootstock, and Manchurian scion grafted on Manchurian rootstock. Bars marked with different letters are significantly difference (ANOVA, Tukey HSD, $P<0.05$).

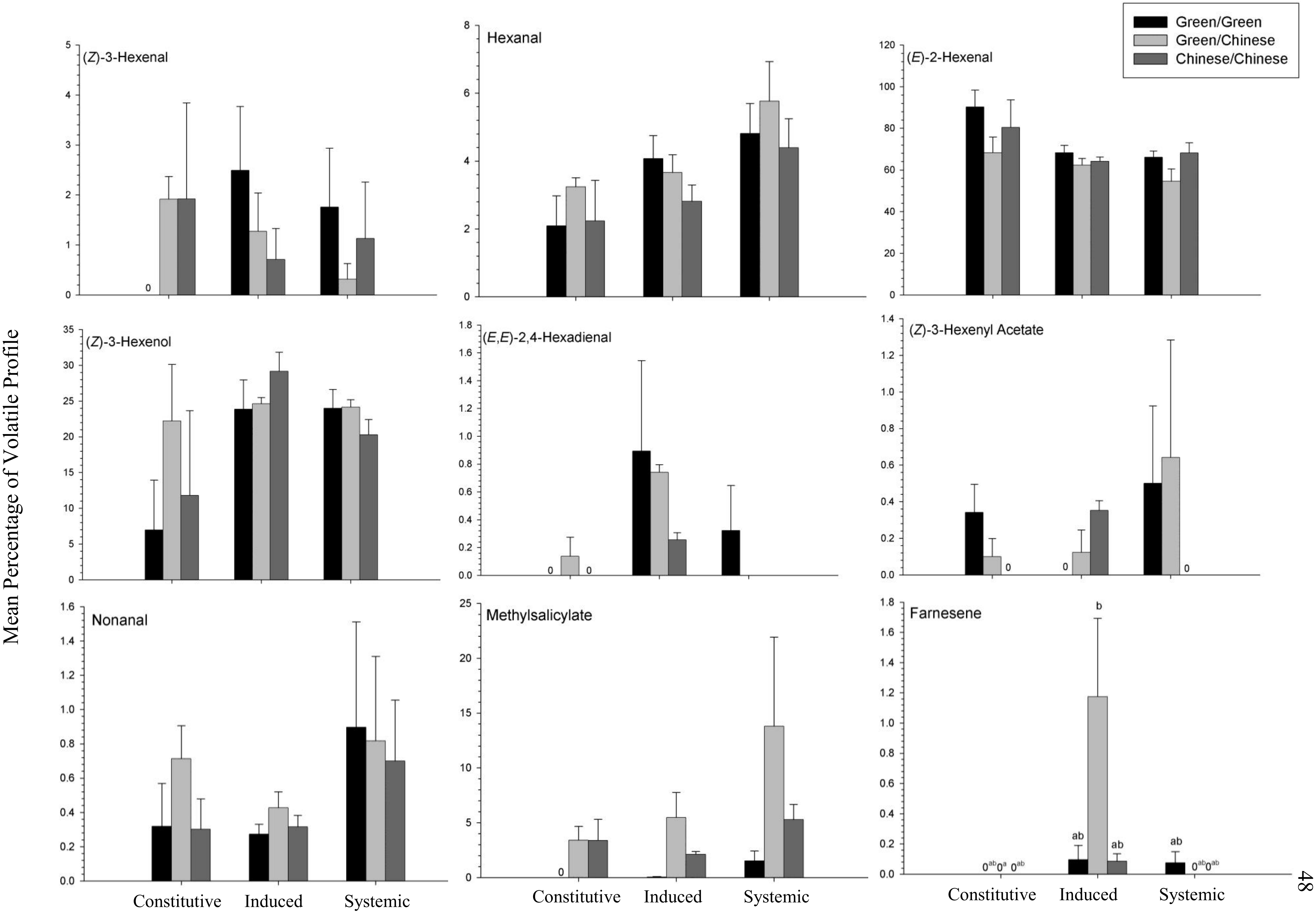

Figure 2.10 Volatile compounds identified from green scions grafted onto green rootstock, green scions grafted onto Chinese rootstock, and Chinese scion grafted on Chinese rootstock. Bars marked with different letters are significantly difference (ANOVA, Tukey HSD, *P*<0.05).

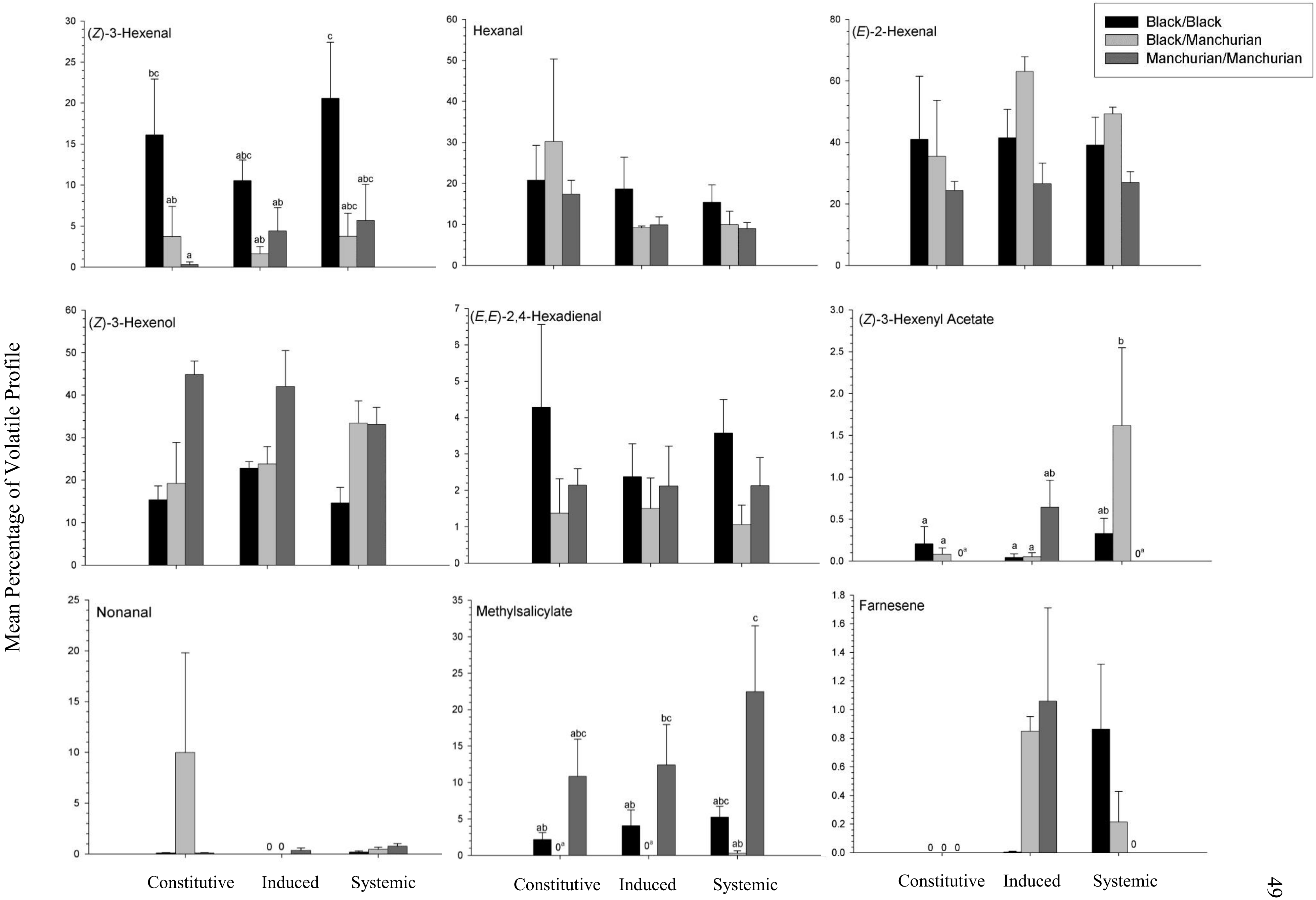

Figure 2.11 Volatile compounds identified from black scions grafted onto black rootstock, black scions grafted onto Manchurian rootstock, and Manchurian scion grafted on Manchurian rootstock. Bars marked with different letters are significantly difference (ANOVA, Tukey HSD, $P<0.05$).

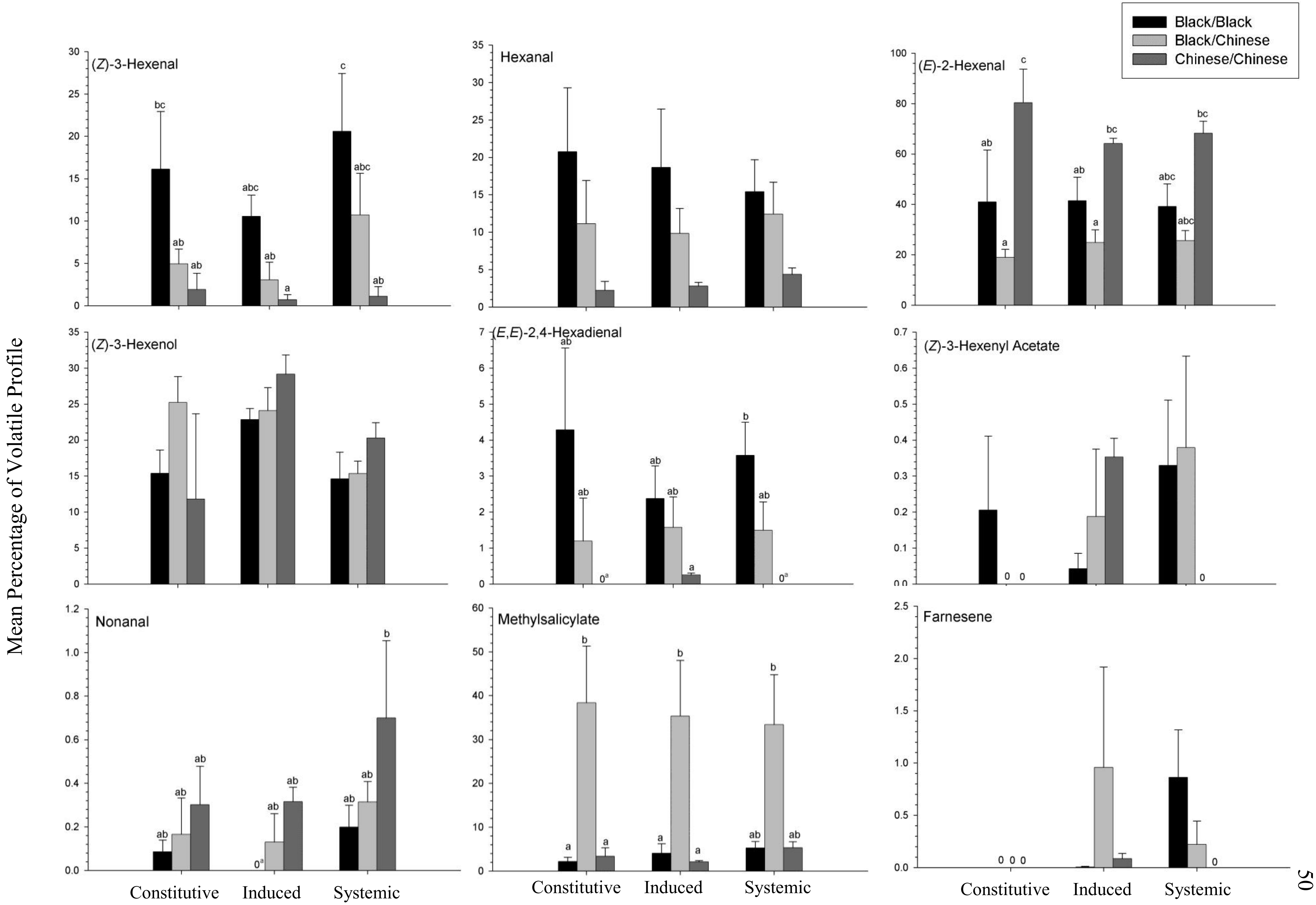

Figure 2.12 Volatile compounds identified from black scions grafted onto black rootstock, black scions grafted onto Chinese rootstock, and Chinese scion grafted on Chinese rootstock. Bars marked with different letters are significantly difference (ANOVA, Tukey HSD, $P<0.05$).

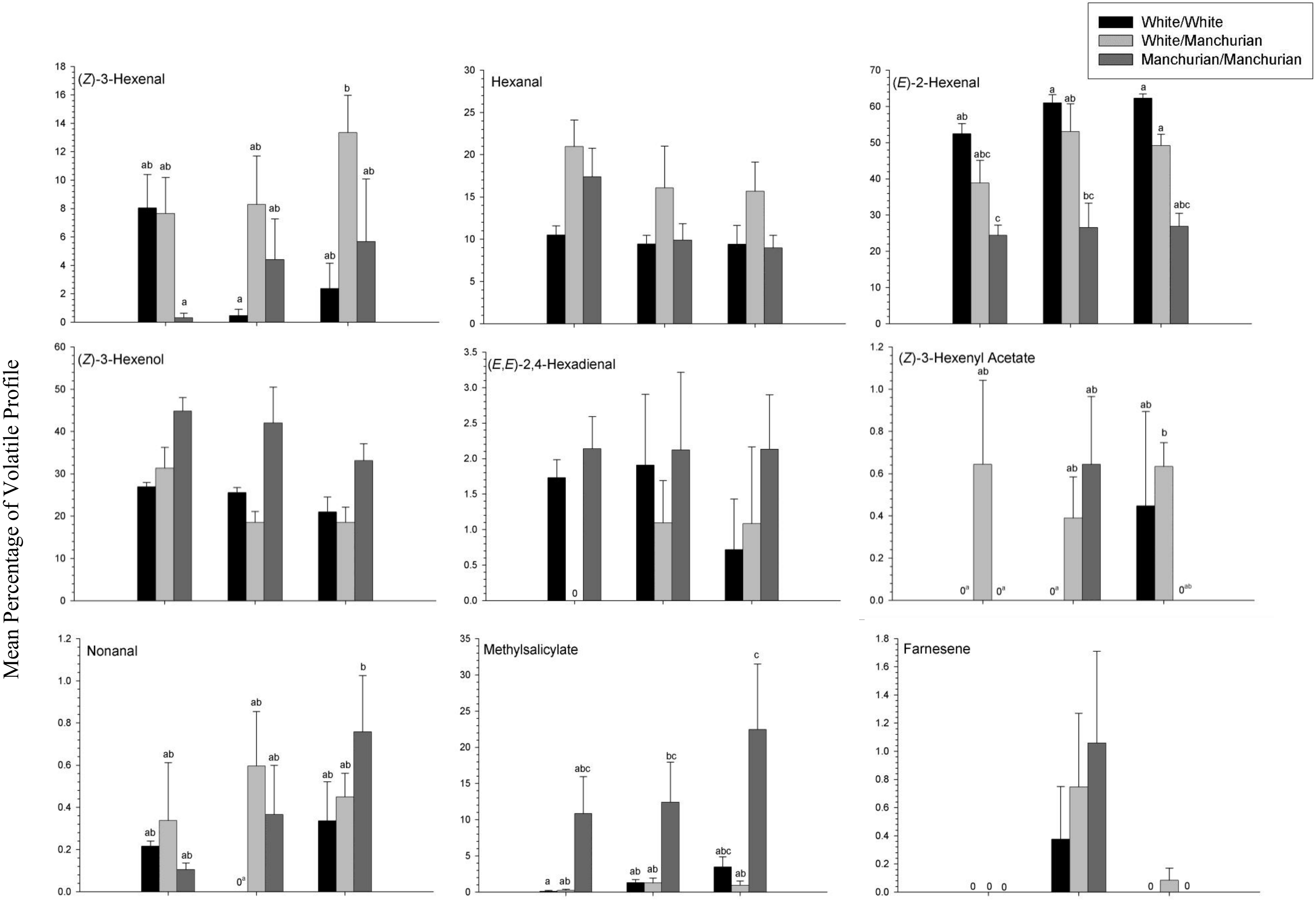

Figure 2.13 Volatile compounds identified from white scions grafted onto white rootstock, white scions grafted onto Manchurian rootstock, and Manchurian scion grafted on Manchurian rootstock. Bars marked with different letters are significantly difference (ANOVA, Tukey HSD, $P<0.05$).

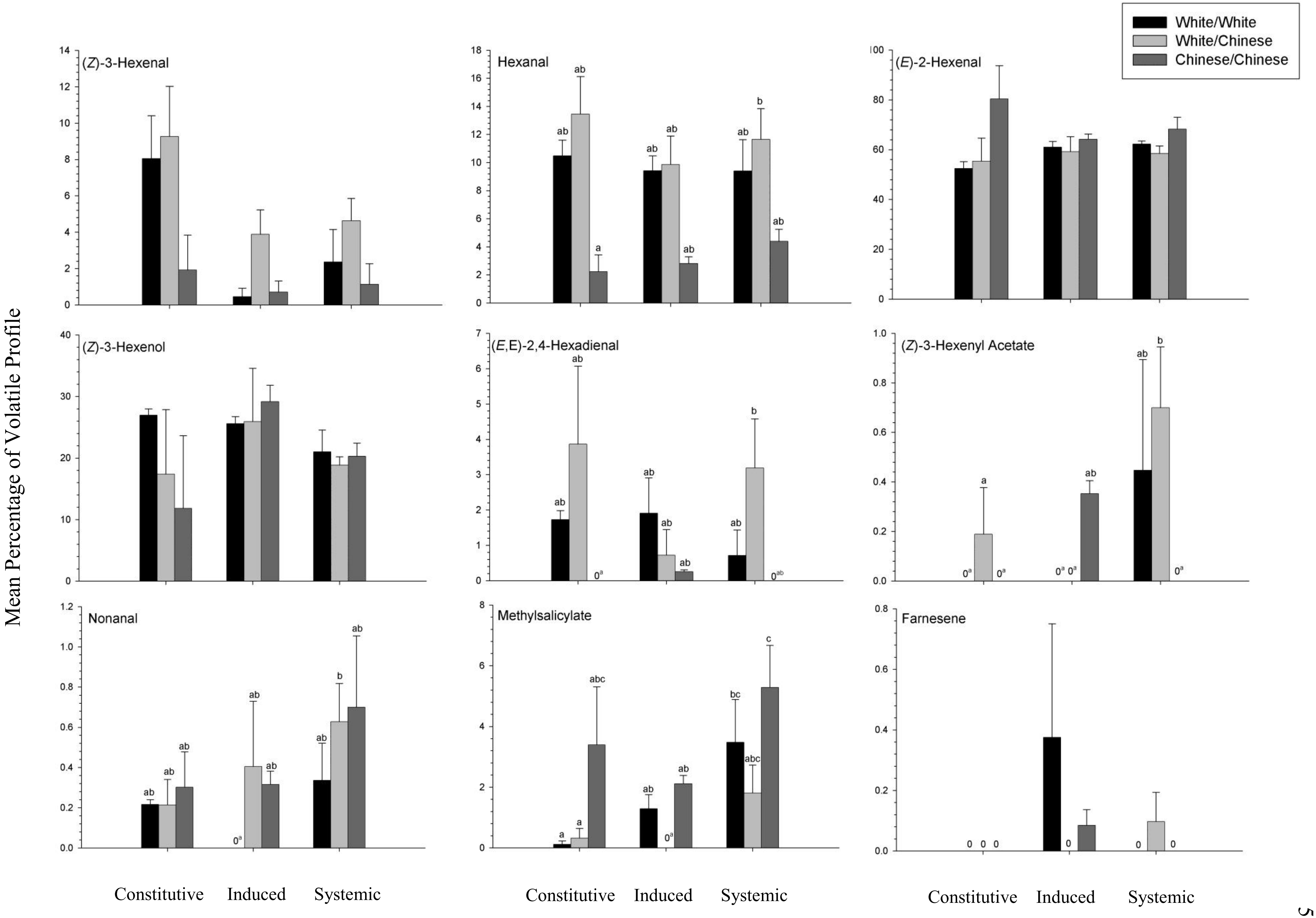

Figure 2.14 Volatile compounds identified from white scions grafted onto white rootstock, white scions grafted onto Chinese rootstock, and Chinese scion grafted on Chinese rootstock. Bars marked with different letters are significantly difference (ANOVA, Tukey HSD, $P<0.05$).

LIST OF REFERENCES

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Agius, A.C., McCullough, D.M., and D. A. Cappaert. (2005). Host range and preference of the emerald ash borer in North America: preliminary results, pp. 28–29. *In* Mastro and Reardon (2005).

Ament, K., Kant, M.R., Sabelis, M.W., Haring, M.A., and R.C. Schuurink. (2004). Jasmonic acid is a key regulator of spider mite-induced volatile terpenoid and methyl salicylate emission in tomato. *Plant Physiology, 135*: 2025–2037.

Anulewicz, A.C., McCullough, D.G., Cappaert, D.L., and T.M. Poland. (2008). Host range of the emerald ash borer (*Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae) in North America: results of multiple-choice field experiments. *Environmental Entomology, 37*: 230–241.

Atkinson, C.J., Else, M.A., and C.J. Dover. (2003). Root and stem hydraulic conductivity as determinants of growth potential in grafted trees of apple (*Malus pumila* Mill.). *Journal of Experimental Botany*, *54*: 1221–1229.

Bais, H.P., Loyola-Vargas, V.M., Flores, H.E., and J.M. Vivanco. (2001). Invited review: rootspecific metabolism: the biology and biochemistry of underground organs. *In Vitro Cellular & Developmental Biology–Plant, 37*: 730–741.

Ball, J. (2004). Arboreal schizophrenia. *Arid Zone Times, 11*: 1–4. http://www.aridzonetrees.com/images/AZTimes/Arboreal%20Schizophrenia.pdf (last accessed 4/4/2013).

Bellamy, C.L. and G.H. Nelson. (2002). Chapter 45. Buprestidae Leach 1815, pp. 98–112. In: Arnett, Jr., R. H. et al. (Eds.). American Beetles, Volume 2, CRC Press.

Broadway, R.M., Duffey, S.S., Pearce, G., and C.A. Ryan. (1986). Plant proteinase-inhibitors – a defense against herbivorous insects? Entomologia Experimentalis et Applicata,41: 33–38.

Brown-Rytlewski, D.E. and M.A. Wilson. (2004). Tracking the emergence of emerald ash borer adults, pp. 13–14. *In* Mastro and Reardon (2005).

Bruce, T.J.A. and J.A. Pickett. (2011). Perception of plant volatile blends by herbivorous insects– Finding the right mix. *Phytochemistry, 72*: 1605–1611.

Buck, J.H. and S. Frappier. (2011). Emerald ash borer program manual, *Agrilus planipennis* (Fairmaire), version 2.0. USDA, APHIS, PPQ, 1–106.

Cabral, M.M.O., Kelecom, A., and E.S. Garcia. (1999). Effects of the lignan, pinoresinol on the moulting cycle of the bloodsucking bug *Rhodnius prolixus* and of the milkweed bug *Oncopeltus fasciatus*. *Fitoterapia, 70*: 561–567.

Cappaert, D., McCullough, D.G., Poland, T.M., and N.W. Siegert. (2005). Emerald Ash Borer in North America: a research and regulatory challenge. *American Entomologist, 51*: 152–165.

Chamorro, M.L., Volkovitsh, M.G., Poland, T.M., Haack, R.A., and S.W. Lingafelter. (2012). Preimaginal stages of the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae): An invasive pest on ash trees (Fraxinus). *Plos One, 7*: 1–12.

Chen, L. and H.Y. Fadamiro. (2007). Differential electroantennogram response of females and males of two parasitoid species to host-related green leaf volatiles and inducible compounds. *Bulletin of Entomological Research, 97*: 515–522.

Chen, Y. and T.M. Poland. (2009). Biotic and abiotic factors affect green ash volatile production and emerald ash borer adult feeding preference. *Environmental Entomology, 38*: 1756–1764.

Chen, Y. and T.M. Poland. (2010). Nutritional and defensive chemistry of three North American ash species: possible roles in the host performance and preference by emerald ash borer adults. *Great Lakes Entomologist, 4*: 20–33.

Chen, Y., Whitehill, J.G.A., Bonello, P., and T.M. Poland. (2011). Differential response in foliar chemistry of three ash species to emerald ash borer adult feeding. *Journal of Chemical Ecology, 37*: 29–39.

Cipollini, D., Wang, Q., Whitehill, J., Powell, J., Bonello, P., and D. Herms. (2011). Distinguishing defensive characteristics in the phloem of ash species resistant and susceptible to emerald ash borer. *Journal of Chemical Ecology, 37*: 450–459.

Cossé, A.A., Bartelt, R.J., Zikowski, B.W., I. Fraser. (2008). Identification and antennal electrophysiology of ash bark volatiles for the emerald ash borer, pp. 81–82, in V. Mastro, D. Lance, R. Reardon and G. Parra (eds.). Proceedings of the Emerald Ash Borer and Asian Longhorned Beetle Research and Technology Development Meeting, Pittsburgh, Pa., 23–24 October 2007. FHTET-2008-07, USDA Forest Service Forest Health Technology Enterprise Team, Morgantown, W. Va.

Crook, D.J., Fraser, I., Francese, J.A., and V.C. Mastro. (2006). Chemical ecology of the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), in relation to tree volatiles, p. 63, in V. Mastro, R. Reardon and G. Parra (eds.). Proceedings of the Emerald Ash Borer Research and Technology Development Meeting, September 26–27, 2005, Pittsburg, PA, United States Department of Agriculture Forest Service, FHTET-2005-16. USDA Forest Service Forest Health Technology Enterprise Team, Morgantown, W. Va.

Crook, D.J., Khrimian, A., Francese, J.A., Fraser, I., Poland, T.M., and V.C. Mastro. (2007). Chemical ecology of emerald ash borer, p. 79, in V. Mastro, D. Lance, R. Reardon and G. Parra (eds.). Proceedings of the Emerald Ash Borer and Asian Longhorned Beetle Research and Technology Development Meeting, Cincinnati, Ohio, 29 October–2 November 2006. FHTET-2007-04, USDA Forest Service Forest Health Technology Enterprise Team, Morgantown, W. Va.

Crook, D., Francese, J., Khrimian, A., Fraser, I., and V. Mastro. (2008a). Field responses of emerald ash borer to multicomponent lures, p. 78, in V. Mastro, D. Lance, R. Reardon and G. Parra (eds.). Proceedings of the Emerald Ash Borer and Asian Longhorned Beetle Research and Technology Development Meeting, Pittsburgh, Pa., 23–24 October 2007. FHTET-2008-07, USDA Forest Service Forest Health Technology Enterprise Team, Morgantown, W. Va.

Crook, D.J., Khrimian, A., Francese, J.A., Fraser, I., Poland, T.M., Sawyer, A.J., and V.C. Mastro. (2008b). Development of a host-based semiochemical lure for trapping emerald ash borer *Agrilus planipennis* (Coleoptera: Buprestidae). *Environtal Entomology, 37*: 356–360.

Crook, D.J., Francese, J.A., Zylstra, K.E., Fraser, I., Sawyer, A.J., Bartels, D.W., Lance, D.R., and V.C. Mastro. (2009). Laboratory and field response of the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), to selected regions of the electromagnetic spectrum. *Journal of Economic Entomology, 102*: 2160–2169.

Crook, D.J. and V.C. Mastro. (2010). Chemical ecology of the emerald ash borer *Agrilus planipennis*. *Journal of Chemical Ecology, 36*: 101–112.

Crosthwaite, J.C., Sobek, S., Lyons, D.B., Bernards, M.A., and B.J. Sinclair. (2011). The overwintering physiology of the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). *Journal of Insect Physiology, 57*: 166–173.

Davidson, C.G. (1999). 'Northern Treasure' and 'Northern Gem' hybrid ash. *HortScience, 34*: 151–152.

de Bruyne, M. and T.C. Baker. (2008). Odor detection in insects: volatile codes. *Journal of Chemical Ecology, 34*: 882–897.

de Groot, P., Grant, G.G., Poland, T.M., Scharbach, R., Buchan, L., Nott, R.W., MacDonald, L., and D. Pitt. (2008). Electrophysiological response and attraction of emerald ash borer to green leaf volatiles (GLVs) emitted by host foliage. *Journal of Chemical Ecology, 34*: 1170–1179.

Dicke, M., Van Loon, J.J.A., and R. Soler. (2009). Chemical complexity of volatiles from plants induced by multiple attack. *Nature Chemical Biology, 5*: 317–324.

Dicke, M. and I.T. Baldwin. (2010). The evolutionary context for herbivore-induced plant volatiles: beyond the 'cry for help'. *Trends in Plant Science*, *15*: 167–175.

Duan, J.J., Ulyshen, M.D., Bauer, L.S., Gould, J., and R. Van Driesche. (2010). Measuring the impact of biotic factors on populations of immature emerald ash borers (Coleoptera: Buprestidae). *Environonmental Entomology, 39*: 1513–1522.

Duan, J.J., Bauer, L.S., Abell, K.J., and R. van Driesche. (2012a). Population responses of hymenopteran parasitoids to the emerald ash borer (Coleoptera: Buprestidae) in recently invaded areas in north central United States. *BioControl, 57*: 199 –209.

Duan, J.J., Yurchenko, G., and R. Fuester. (2012b). Occurrence of emerald ash borer (Coleoptera: Buprestidae) and biotic factors affecting its immature stages in the Russian Far East. *Environmental Entomology, 41*: 245–254.

Erb, M., Lenk, C., Dengenhardt, J., and T.C.J. Turlings. (2009). The underestimated role of roots in defense against leaf attackers. *Trends in Plant Science, 14*: 653–659.

Eyles, A., Jones, W., Riedl, K., Cipollini, D., Schwartz, S., Chan, K., Herms, D.A., and P. Bonello. (2007). Comparative phloem chemistry of Manchurian (*Fraxinus mandshurica*) and two North American ash species (*Fraxinus americana* and *Fraxinus pennsylvanica*). *Journal of Chemical Ecology, 33*: 1430–1448.

Feeny, P.P. (1975). Biochemical coevolution between plants and their insect herbivores. Pages 3-19 in L.E. Gilbert and P.H. Raven (eds.). Coevolution of Animals and Plants. Austin, TX: University of Texas Press.

Frost, C.J., Appel, H.M., Carlson, J.E., De Moraes, C.M., Mescher, M.C., J.C. Schultz. (2007). Within-plant signaling via volatiles overcomes vascular constraints on systemic signaling and primes responses against herbivores. *Ecological Letters, 10*: 490–498.

Frost, C.J., Mescher, M.C., Dervinis, C., Davis, J.M., Carlson, J.E., and C.M. De Moraes. (2008). Priming defense genes and metabolites in hybrid poplar by the green leaf volatile *cis*-3-hexenyl acetate. *New Phytologist, 180*: 722–734.

Garcia, E.S., Cabral, M.M.O., Schaub, G.A., Gottlieb, O.R., and P. Azambuja. (2000). Effects of lignoids on a hematophagous bug, *Rhodnius prolixus*: feeding, ecdysis and diuresis. *Phytochemistry, 55*: 611–616.

Gould, J.S., Bauer, L.S., Lelito, J., and J. Duan. (2012). Emerald ash borer, *Agrilus planipennis* (Fairmaire), biological control release and recovery guidelines. *USDA-APHIS-ARS-FS,* 1-76.

Halitschke, R., Schittko, U., Pohnert, G., Boland, W., and I.T. Baldwin. (2001). Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*: III. Fatty acid-amino acid conjugates in herbivore oral secretions are necessary and sufficient for herbivore-specific plant responses. *Plant Physiology, 125*: 711–717.

Herms, D.A., McCullough, D.G., Smitley, D.R., Sadof, C.S., Williamson, R.C., and P.L. Nixon. (2009). Insecticide options for protecting ash trees from emerald ash borer. *North Central IPM Center Bulletin*, 1–12.

Hottes, A.C. (1925). Practical plant propagation: an exposition of the art and science of increasing plants as practiced by the nurseryman, florist and gardener. New York, NY: A.T. De La Mare Company, Inc.

Jensen, P.J., Rytter, J., Detwiler, E.A., Travis, J.W., and T.W. McNellis. (2003). Rootstock effects on gene expression patterns in apple tree scions. *Plant Molecular Biology, 493*: 493–511.

Jongsma, M.A. and C. Bolter. (1997). The adaptation of insects to protease inhibitors. *Journal of Insect Physiology, 43*: 885–895.

Klooster, W.S., Herms, D.A., Knight, K.S., Herms, C.P., and D.G. McCullough. (2013). Ash (*Fraxinus* spp.) mortality, regeneration, and seed bank dynamics in mixed hardwood forests following invasion by emerald ash borer (*Agrilus planipennis*). *Biological Invasions*, doi:10.1007/s10530-013-0543-7.

Kovacs, K.F., Haight, R.G., McCullough, D.G., Mercader, R.J., Siegert, N.W., and A.M. Liebhold. (2010). Cost of potential emerald ash borer damage to US communities, 2009-2019. *Ecological Economics, 69*: 569–578.

Lelito, J.P., Fraser, I., Mastro, V.C., Tumlinson, J.H., Böröczky, K., and T.C. Baker. (2007). Visually mediated 'paratrooper copulations' in the mating behavior of *Agrilus planipennis* (Coleoptera: Buprestidae), a highly destructive invasive pest of North American ash trees. *Journal of Insect Behavior, 20*: 537–552.

Lelito, J.P., Böröczky, K., Jones, T.H., Fraser, I., Mastro, V.C., Tumlinson, J.H., and T.C. Baker. (2009). Behavioral evidence for a contact sex pheromone component of the emerald ash borer, *Agrilus Planipennis* Fairmaire. *Journal of Chemical Ecology, 35*: 104–110.

Leszczynski, B., Tjallingii, W.F., Dixon, A.F.G., and R. Swiderski. (1995). Effect of methoxyphenols on grain aphid feeding behavior. *Entomologia Experimentalis et Applicata*, *76*: 157–162.

Liu, H., Bauer, L.S., Gao, R-T, Zhao, T-H, Petrice, T.R., and R.A. Haack. (2003). Exploratory survey for the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), and its natural enemies in China. *Great Lakes Entomologist, 36*: 191–204.

Liu, H., Bauer, L.S., Miller, D.L., Zhao, T., Gao, R., Song, L., Luan, Q., Jin, R., and C. Gao. (2007). Seasonal abundance of *Agrilus planipennis* (Coleoptera: Buprestidae) and its natural enemies *Oobius agrili* (Hymenoptera: Encyrtidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in China. *Biological Control, 42*: 61–71.

Loreto, F., Ciccioli, P., Brancaleoni, E., Cecinato, A., Massimiliano, F. (1998). Measurement of isoprenoid content in leaves of Mediterranean *Quercus spp*. by a novel and sensitive method and estimation of the isoprenoid partition between liquid and gas phase inside the leaves. *Plant Science, 136*: 25–30.

MacFarlane, D.W. and S.P. Meyer. (2005). Characteristics and distribution of potential ash tree hosts for emerald ash borer. *Forest Ecology and Management, 213*: 15–24.

Major, I.T. and C.P. Constabel. (2007). Shoot-root defense signaling and activation of root defense by leaf damage in poplar. *Canadian Journal of Botany, 85*: 1171–1181.

Markovic, I., Norris, D.M., Phillips, J.K, and F.X. Webster. (1996). Volatiles involved in the non-host rejection of *Fraxinus pennsylvanica* by *Lymantria dispar* larvae. *Journal of Agricultural and Food Chemistry*, *44*: 929–935.

Mascall, L. (1569). Howe to set your wylde trees come of pepynes, when they be first plucked up. In: A booke of the art and maner, howe to plante and graffe all sortes of trees, H. Bynneman for J. Wight, London, pp. 6–7.

McCullough, D.G. and R.J. Mercader. (2012). Evaluation of potential strategies to slow ash mortality (SLAM) caused by emerald ash borer (*Agrilus planipennis*): SLAM in an urban forest. *International Journal of Pest Management, 58*: 9–23.

McCullough, D.G., Poland, T.M., D. Cappaert. (2009). Attraction of the emerald ash borer to ash trees stressed by girdling, herbicide treatment, or wounding. *Canadian Journal of Forest Research, 39*: 1331–1345.

McPherson, G.E., Simpson, J.R., Peper, P.J., Gardner, S.L., Vargas, K.E., and Q. Xiao. (2007). Northeast community tree guide: benefits, costs, and strategic planting. Gen. Tech. Rep. PSW-GTR-202. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. 106 p.

Mercader, R.J., Siegert, N.W., Liebhold, A.M., and D.G. McCullough. (2009). Dispersal of the emerald ash borer, *Agrilus planipennis*, in newly-colonized sites. *Agricultural and Forest Entomology, 11*: 421–424.

Merkle, S.A., Andrade, G.M., Nairn, C.J., Powell, W.A., and C.A. Maynard. (2007). Restoration of threatened species: a noble cause for transgenic trees. Tree Genetics and Genomes 3: 111–118.

Morita, M., Shitan, N., Sawada, K., Van Montagu, M.C.E., Inze, D., Rischer, H., Goossens, A., Oksman-Caldentey, K-M, Moriyama, Y., and K. Yazaki. (2009). Vacuolar transport of nicotine is mediated by a multidrug and toxic compound extrusion (mate) transporter in *Nicotiana tabacum*. *Proceedings of the National Academy of Science USA, 106*: 2447–2452.

Mota-Sanchez, D., Cregg, B.M., McCullough, D.G., Poland, T.M., and R.M. Hollingworth. (2009). Distribution of trunk-injected ^{14}C-imidacloprid in ash trees and effects on emerald ash borer (Coleoptera: Buprestidae) adults. *Crop Protection, 28*: 655–661.

Muñoz, S., Trapero, G.A., Hervalejo, P.M., Jiménez, B.J.R.,Vela, J.M., E. Martínez-Ferri. (2008). Effects of rootstock and flushing on the incident of three insects on ‘Clementine de Nules’ citrus trees. *Environmental Entomology, 37*: 1531–1537.

Parsons, G.L. (2008). Emerald ash borer; *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae); A guide to identification and comparison to similar species. Retrieved from http://www.emeraldashborer.info/files/eab_id_guide.pdf.

Peeters, P. (2002). Correlations between leaf structural traits and the densities of herbivorous insect guilds. *Biological Journal of the Linnean Society, 77*: 43–65.

Peng, Y.J., Dong, Y.M., Tu, B.K., Zhou, Z.X., Zheng, B., Luo, L.W., Shi, C.M., Du, K.B. (2013). Roots play a vital role in flood-tolerance of poplar demonstrated by reciprocal grafting. *Flora, 208*: 479–487.

Poland, T.M. and D.G. McCullough. (2006). Emerald ash borer; invasion of the urban forest andthe threat to North America’s ash resource. *Journal of Forestry, 104*: 118–124.

Pureswaran, D.S. and T.M. Poland. (2009). Host selection and feeding preference of *Agrilus planipennis* (Coleoptera: Buprestidae) on ash (*Fraxinus* spp.). *Environmental Entomology, 38:* 757–765.

Rajarapu, S.P., Mamidala, P., Herms, D.A., Bonello, P., and O. Mittapalli. (2011). Antioxidant genes of the emerald ash borer (*Agrilus planipennis*): Gene characterization and expression profiles. *Journal of Insect Physiology, 57*: 819–824.

Raupp, M.J., Cumming, A.B., and E.C. Raupp. (2006). Street tree diversity in eastern North Americca and its potential for tree loss to exotic borers. *Arboriculture & Urban Forestry, 32*: 297–304.

Rebek, E.J., Herms, D.A., and D.R. Smitley. (2008). Interspecific variation in resistance to emerald ash borer (Coleoptera: Buprestidae) among North American and Asian Ash (*Fraxinus* spp.). *Environmental Entomology, 37*: 242–246.

Rhoades, D.F. and R.G. Cates. (1976). Toward a general theory of plant anti-herbivore chemistry. Pages 168–213 in J.W. Wallace and R.L. Mansell, (eds.). Biochemical Interactions Between Plants and Insects. New York: Plenum Press.

Rodriguez, M.X., Buschmann, H., Iglesias, C., and J.R. Beeching. (2000). Production of antimicrobial compounds in cassava (*Manihot esculenta* Crantz) roots during post-harvest physiological deterioration. Pages 320–328 in L.J.C.B. Carvalho, A.M. Thro, A.D. Vilarinhos, (eds.). *Cassava Biotechnology. IVth International Scientific Meeting Cassava Biotechnology Network.* Embrapa, Brasilia.

Rodriguez-Saona, C., Poland, T.M., Miller, J.R., Stelinski, L.L., Grant, G.G., de Groot, P., Buchan, L., and L. MacDonald. (2006). Behavioral and electrophysiological responses of the emerald ash borer, *Agrilus planipennis*, to induced plant volatiles of Manchurian ash, *Fraxinus mandshurica*. *Chemoecology, 16*: 75–86.

Schlesinger, R.C. (1990). *Fraxinus americana* L: White Ash. *Silvics of North America, 2*: 333–338.

Shoji, T., Yamada, Y., and T. Hashimoto. (2000). Jasmonate induction of putrescine N-methyltransferase genes in the root of *Nicotiana sylvestris*. *Plant Cell Physiology, 41*: 831–839.

Silk, P.J., Ryall, K., Lyons, D.B., Sweeney, J., and J. Wu. (2009). A contact sex pheromone component of the emerald ash borer *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). *Naturwissenschaften*: 10.1007/s00114-009-0513-1.

Silva, M.C.P., Terra, W.R., C. Ferreia. (2006). Absorption of toxic beta-glucosides produced by plants and their effect on tissue trehalases from insects. *Comparative Biochemistry and Physiology, 143*: 367–373.

Smitley, D.R., Doccola, J.J., and D.L. Cox. (2010). Multiple-year protection of ash trees from emerald ash borer with a single trunk injection of emamectin benzoate, and single-year protection with an imidacloprid basal drench. *Arboriculture and Urban Forestry, 36*: 206–211.

Sobek, S., Rajamohan, A., Dillon, D., Cumming, R.C., and B.J. Sinclair. (2011). High temperature tolerance and thermal plasticity in emerald ash borer *Agrilus planipennis*. *Agricultural and Forest Entomology, 13*: 333–340.

Solt, M.L. (1957). Nicotine production and growth of excised tobacco root cultures. *Plant Physiology, 32*: 480–484.

StatSoft, Inc. (2013). STATISTICA (data analysis software system), version 12. www.statsoft.com.

Summers, C.B. and G.W. Felton. (1994). Prooxidant effects of phenolic-acids on the generalist herbivore *Helicoverpa zea* (Lepidoptera: Noctunidae) – potential mode of action for phenolics-compounds in plant anti-herbivore chemistry. *Insect Biochemistry and Molecular Biology, 24*: 943–953.

Taylor, R.A.J., Bauer, L.S., Poland, T.M., and K.N. Windell. (2010). Flight performance of *Agrilus planipennis* (Coleoptera: Buprestidae) on a flight mill and in free flight. *Journal of Insect Behavior, 23*: 128–148.

Unsicker, S.B., Kunert, G., and J. Gershenzon. (2009). Protective perfumes: the role of vegetative volatiles in plant defense against herbivores. *Current Opinion in Plant Biology, 12*: 479–485.

USDA. (2014). Emerald ash borer. http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/importexport?1dmy&urile=wcm%3apath%3a%2Faphis_content_library%2Fsa_our_focus%2Fsa_plant_health%2Fsa_domestic_pests_and_diseases%2Fsa_pests_and_diseases%2Fsa_insects%2Fsa_emerald_ash%2Fct_emerald_ash_borer (last accessed 2/11/2014).

van Schie, C.C., Haring, M.A., and R.C. Schuurink. (2007). Tomato linalool synthase is induced in trichomes by jasmonic acid. *Plant Molecular Biology, 64*: 251–263.

Vasanthakumar, A., Handelsman, J., Schloss, P.D., Bauer, L.S., K.F. Raffa. (2008). Gut microbiota of an invasive subcortical beetle, *Agrilus planipennis* Fairmaire, across various life stages. *Environmental Entomology, 37*: 1344–1353.

Wang, X.Y., Yang, Z.Q., Liu, G.J., and E.S. Liu. (2005). Larval instars and stadia of the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). *Scientia silvae Sinicae, 41*: 97–102.

Wang, X.Y., Yang, Z.Q., Gould, J.R., Wu, H., and J.H. Ma. (2010a). Host-seeking behavior and parasitism by *Spathius agrili* Yang (Hymenoptera: Braconidae), a parasitoid of the emerald ash borer. *Biological Control, 52*: 24–29.

Wang, X.Y., Yang, Z.Q., Gould, J.R., Zhang, Y.N., Liu, G.J., and E.S. Liu. (2010b). The biology and ecology of the emerald ash borer, *Agrilus planipennis*, in China. *Journal of Insect Science, 10*: 1–23.

Whitehill, J.G.A., Opiyo, S.O., Koch, J., Herm, D.A., Cipollini, D.F., and P. Bonello. (2012). Interspecific comparison of constitutive ash phloem phenolic chemistry reveals compounds unique to Manchurian ash, a species resistant to Emerald ash borer. *Journal of Chemical Ecology, 38*: 499–511.

Yu, C. (1992). *Agrilus marcopoli* Obenberger (Coleoptera: Buprestidae). In Xiao, G. Forest Insects of China (400–401). Publishing House, Beijing, China.

Ziegler, J. and P.J. Facchini. (2008). Alkaloid biosynthesis: metabolism and trafficking. *Annual Review of Plant Biology, 59*: 735–769.