Influence of rootstock on herbivory by emerald ash borer (*Agrilus planipennis* Fairmaire) on grafted native and Asian ash species

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Introduction

The emerald ash borer (EAB; Coleoptera: Buprestidae) is a problematic insect pest, which is causing ecological and economic damage across North America. EAB is an invasive species that was likely transported to North America via wood packing material from its native range in eastern Russia, northern China, Korea, and Japan (Cappaert et al. 2005). In 2002, EAB was first detected in North America in Michigan and since then has been detected in 22 states and two Canadian provinces (USDA 2014). The larvae of EAB kill the ash due to heavy phloem feeding which creates S-shaped galleries in the sapwood and causes the disruption of water and nutrient flow to the crown of the tree (Cappaert et al. 2005). The larvae feed under the bark, making direct management with sprayed insecticides difficult and causing infestations to go undetected until trees begin to die. Unlike many native borers, EAB does not only attack weakened trees, but also healthy trees, which makes management of infestations more difficult as tree vigor cannot be used to identify risk of EAB (USDA 2010). All North American ash 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).

Chinese and Manchurian ash grow in the native range of EAB, and studies show that these species are only attacked when stressed by factors such as drought (OSU 2013). Green ash is the most susceptible North American ash species and is a major concern because it is the primary ash species planted in many urban areas (Herms et al. 2007). If the properties of resistance in Asian ash could be conferred to North American species, EAB resistance could be achieved. Grafting is a process by which a rootstock with desired traits is chosen and attached to a scion (the top part of a tree) of a different tree species, which creates a vascular connection (University of Minnesota, 2013). It may be possible to use a resistant ash rootstock, such as Chinese or Manchurian, and graft them to a susceptible North American ash scion to create a more resistant tree. In this study, I determined the extent to which Asian rootstock conferred resistance to North American ash scions in terms of adult EAB feeding on the foliage. Grafting of ash to produce resistant trees would ensure that *Fraxinus* is not lost from North America.

Materials and Methods

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) was used in a 2x2 arrangement 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 inter-row spacing is 6 m and distance between each row is 3 m.

Table 2: *Fraxinus* spp. that served as scion and rootstock donors in reciprocal grafts of ash.

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

Feeding assays were performed using ten 8-10 day old adult female EAB enclosed in an aluminum window screen bag surrounding 5-8 leaflets. This was repeated on three trees from each of the twenty-five unique graft combinations. The beetles were allowed to feed for 48 hours before being removed. The leaves with feeding damage were collected, placed on ice, and taken back to the lab where they were scanned for use with ImageJ software.

ImageJ software, an image processing and analyzing software, was used to determine the total leaf area consumed (cm^2) by the beetles. This data was recorded into Excel and organized based on the graft combination. The mean and standard error were calculated for the each of the graft combinations. This data was then used to make graphs for side by side comparisons. After all the data was recorded, the data were analyzed using ANOVA (Statsoft 2013) to determine the extent to which grafting influenced leaf consumption by the adult beetles.

Results

Similar patterns were found with both Asian ash species when grafted with green ash (see Figures 1 and 2). When a green rootstock was grafted to an Asian ash scion, the Asian ash seemed to become more susceptible to EAB and experienced significantly more herbivory than the conspecific Asian ash grafts (ANOVA, $F_{3,7} = 3.41$, $P=0.082$). Another pattern worth noting is that when green ash scions were grafted on to Asian ash rootstock, there was less feeding on the green scion when compared to the green conspecific graft. This suggests that the properties of a rootstock may be conferred to the grafted scion. There were no significant differences in herbivory for grafts combinations containing black and white ash with Chinese and Manchurian.

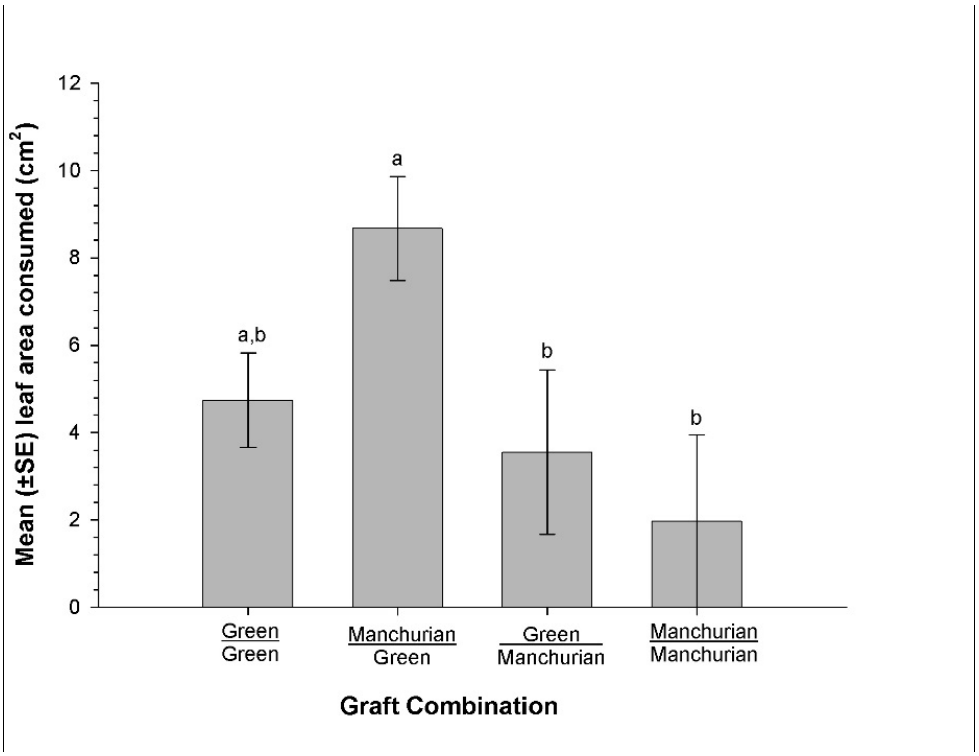

Figure 1: Mean (±SE) leaf area consumed (cm²) by adult female EAB on grafts containing green and Manchurian ash.

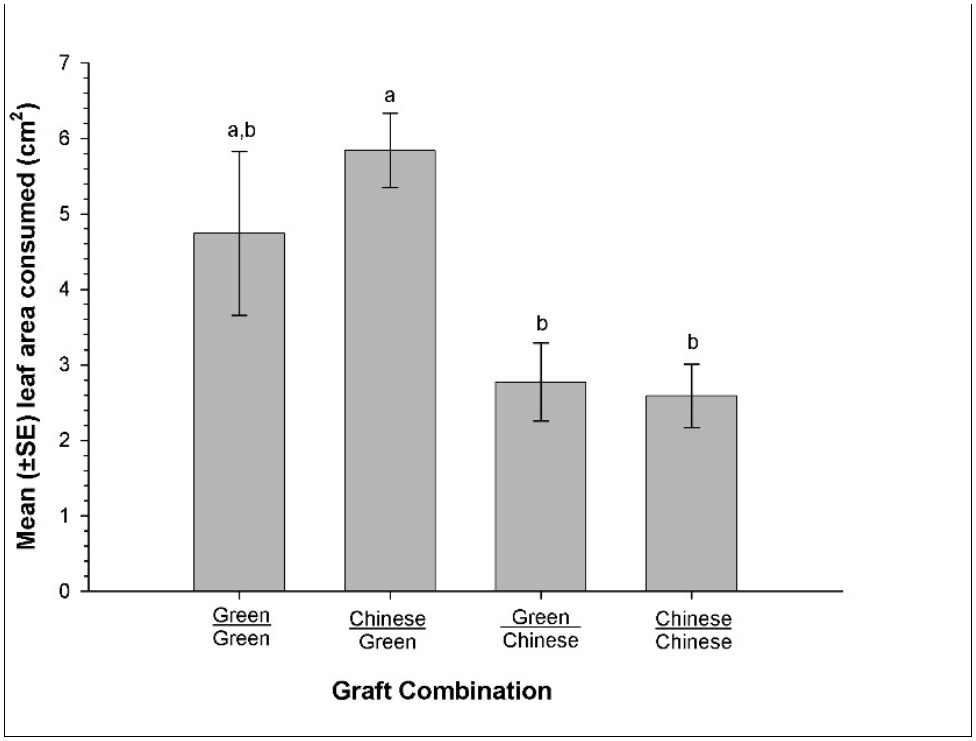

Figure 2: Mean (±SE) leaf area consumed (cm²) by adult female EAB on grafts containing green and Chinese ash.

Discussion

The results from graft combinations containing black ash could have been affected by the site conditions; black ash normally thrives in colder environments and wet soil (USDA 2001), whereas this study took place during a high drought and hot summer. This environment would have put great stress on the black ash, resulting in poor growth and possibly affecting adult feeding. The stress issue could have affected all the grafted ash trees used in this study, because due to the heat and lack of water, all trees were dealing with stress. Even Chinese and Manchurian ash trees are not fully resistant to EAB under times of stress and drought.

The results showed that the rootstock did affect the amount of feeding on the scion of the grafted trees. However, my objective was to determine the extent to which Asian ash rootstock influenced adult EAB herbivory on North American scions. The results of this study suggest that the amount of EAB herbivory is more highly affected when Asian scions are placed on green ash rootstock, making the grafted tree more susceptible. However, although not statistically significant, Asian ash rootstock did lead to generally less feeding on green ash scions when compared to green conspecific grafts.

Future directions may include the determination of the specific compounds that are transported from the rootstock to the scion that are present in the leaves and influence feeding. It may be important to identify what specifically influences EAB behavior to inform strategies for propagating resistant ash varieties in the future.

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