

Breeding Strategies for the Development of Emerald Ash Borer - Resistant North American Ash

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Introduction

The emerald ash borer (*Agrilus plannipennis*; EAB) is a phloem-feeding beetle that is endemic to Asia. It was discovered in North America in 2002, found almost simultaneously near Detroit, Michigan and Windsor, Ontario, Canada. Adult beetles feed on ash (*Fraxinus* spp.) foliage, but larval feeding on phloem, cambium, and outer xylem is far more detrimental because it effectively girdles the tree, disrupting nutrient and water transport and ultimately killing the tree. As of 2007, it was estimated that 53 million ash trees had been killed by EAB in North America (Kovacs et al. 2010). Potentially, more than 8 billion ash trees could eventually be affected, or about 2.6 percent of the timber trees in the United States (Sydnor et al. 2007).

Breeding for pest resistance in forest trees is a proven approach for managing both native and non-native insects and diseases (FAO UN 2011). We initiated a breeding program that employs two strategies to incorporate EAB resistance into North American ash species: hybrid and traditional breeding. In the hybrid breeding approach, we are looking for EAB resistance in Asian species of ash from EAB's region of origin (sympatric resistance). Asian ashes resistant to EAB will be crossed with native North American species to create hybrids. The hybrids subsequently will be subjected to rounds of testing, selection, and backcrossing to the native species that will be repeated until only the resistance genes from the exotic species are carried into the native population while all of the traits of the native species are retained, similar to the breeding program of the American Chestnut Foundation (Hebard 2006). For a more traditional breeding approach, we are searching for rare native individuals with resistance or tolerance to EAB (allopatric resistance). The following is an overview of the current status of our program.

Hybrid Breeding

Contrary to the situation in North America, severe EAB outbreaks on ash species native to Asia are a rarity. These tree species, having co-evolved with EAB, are likely to have developed mechanisms of resistance. This hypothesis is supported by a recent common garden study that reports significantly less EAB-induced mortality in Manchurian ash (*F. mandshurica* Rupr.) compared to green (*F. pennsylvanica* Marsh.) and white (*F. americana* L.) ash (Rebek et al. 2008). Evidence for the EAB resistance of Korean ash (*F. chinensis* ssp. *rhynchophylla* (Hance) Hemsley) is described by Liu et al. (2007), who reported a mixed plantation of Korean ash and green ash where 95 percent of all green ash was found to be EAB infested while the Korean ash of similar size and in close proximity remained free of EAB. More recently, a study by Dian et al. (2010) reported that in both Manchurian and Korean ash, more than 75 percent of EAB larvae dissected from the trees had been killed through "host plant resistance," or the formation of callous by the host trees that encapsulated the larvae.

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To identify additional EAB-resistant species that may be used as parents in a hybrid breeding program, we have been accessioning Asian ash species across a wide geographical and ecological range. A summary of our current collections is outlined in table 1.

Table 1: Exotic ash (*Fraxinus*) collection, U.S. Department of Agriculture, Forest Service, NRS, Delaware, Ohio

Species	Origin	Unique accessions
<i>F. asperisquamifera</i>	Japan	1
<i>F. angustifolia</i>	Syria, Europe	2
<i>F. chinensis</i>	China, Korea	26*
<i>F. excelsior</i>	Europe	3
<i>F. insularis</i>	China	6*
<i>F. lanuginosa</i>	Japan	4*
<i>F. longicuspis</i>	China	1*
<i>F. mandshurica</i>	China, Korea, Russia, Japan	25*
<i>F. ornus</i>	Europe	3
<i>F. paxiana</i>	China	5*
<i>F. sieboldiana</i>	China, Korea, Japan	3
<i>F. sogdiana</i>	China	3
<i>F. spaethiana</i>	Japan	1
<i>F. stylosa</i>	China	4*

*Includes accessions from the Morton Arboretum including those from a recent North American Chinese Plant Exploration Consortium (NACPEC) collection.

Each accession is confirmed for proper species identity through the use of DNA-based technologies, including internal transcribed spacer (ITS) sequencing and amplified fragment length polymorphism (AFLPs). The ITS sequences were compared to published sequences for each species (Wallander 2008). The AFLP data was assessed for its utility in verifying species identities relative to control reference species previously confirmed with ITS data. Species confirmation is a critical step because currently there is no comprehensive global taxonomic key, and few traits are diagnostic between closely related but geographically isolated species. Our current exotic ash collection includes 85 independent accessions, including 11 Asian species and 3 European species. While confirming species identities, we uncovered seven different Asian ash accessions from reputable gardens and arboreta that were incorrectly identified, illustrating the difficulty of distinguishing *Fraxinus* species (table 2). Most of these errors were native North American green ash mislabeled as Asian species, but in a few cases, Asian species were misidentified as other Asian species, European species, and North American black ash. In addition, six different seed lots (representing both Asian and North American species) from two different commercial seed companies were found to be incorrectly identified as shown in table 3. To date, about 70 percent of all of the commercially obtained *Fraxinus* seed sources have been incorrectly identified at the species level and 15 percent of the Asian accessions.

After species identity is confirmed, the individual accessions are replicated through grafting and will be installed in a common garden study at the Ohio Agricultural Research and Development Center in Wooster, Ohio along with North American EAB-susceptible species. The long-term goal of this study is to assess EAB resistance as well other desired characteristics such as growth rate, form, color, tolerance to native pests and diseases, and site suitability.

Inter-specific hybridization of *Fraxinus* species has not been widely reported, with the exception of hybridization of *F. nigra* and *F. mandshurica* that resulted in the release of two horticultural cultivars, Northern Gem and Northern Treasure (Davidson 1999). To determine what species combinations may produce successful F1 hybrids, we performed controlled cross-pollinations using as many combinations as possible (dependent on the availability of viable male and female flowers). In 2010, 42 different species combinations were crossed and 15 successfully produced seed. Five of the species combinations have now produced seedlings, with family sizes ranging from 11 to 104.

Backcrosses were also performed between the commercially available F1 hybrid Northern Gem and both *F. mandshurica* and *F. nigra*. These crosses successfully produced seed, and the seed is beginning to germinate. These new germinants represent the most advanced *Fraxinus* interspecies pedigree, and demonstrate that F1 hybrids are reproductively competent providing evidence that the hybrid/backcross breeding approach has potential to be successful in ash. All resulting F1 and backcross seedlings will be screened for EAB resistance and their hybrid parentage confirmed using molecular techniques.

Table 2—Misidentified *Fraxinus* accessions from arboreta and botanical gardens

Species labeled as:	Number of incorrect accessions	Correct species IDs (based on ITS and/or AFLP)	Location of arboretum	Origin of accessions
<i>F. chinensis</i>	3	<i>F. pennsylvanica</i>	United States	Chinese Botanical Garden, Chinese Forest Preserve
<i>F. chinensis</i> var. <i>accuminata</i>	2	<i>F. pennsylvanica</i>	Europe	Chinese Botanical Garden
<i>F. chinensis</i> var. <i>rhynchophylla</i>	1	<i>F. mandshurica</i>	United States	China seed collection
<i>F. mandshurica</i>	6	<i>F. pennsylvanica</i> , <i>F. excelsior</i> , <i>F. nigra</i> , and <i>F. chinensis</i>	United States	China seed collection, US nursery, US university
<i>F. sogdiana</i>	1	<i>F. pennsylvanica</i>	United States	unknown

Table 3—Commercially obtained *Fraxinus* seed lots that were incorrectly identified at the species level

Distributed as:	Number of incorrect seed lots	Correct species ID (ITS and/or AFLP)	Number of commercial sources
<i>F. bungeana</i>	3	<i>F. pennsylvanica</i>	3
<i>F. chinensis</i>	3	<i>F. pennsylvanica</i>	2
<i>F. chinensis</i> var. <i>rhynchophylla</i>	1	<i>F. pennsylvanica</i>	1
<i>F. nigra</i>	2	<i>F. excelsior</i>	2

Traditional Breeding

Initial EAB infestations were in urban areas where a few ash cultivars were planted in high numbers. Although a large number of these trees were killed, they represented a small number of genotypes. As EAB moved into natural stands with greater genetic diversity, small numbers of surviving ash trees have been identified that persist in areas where more than 98 percent of the ash trees have been killed by EAB (Knight et al., these proceedings; Marshall et al. 2010). These “lingering ash” may be simply the last trees to die, or they may be trees with rare phenotypes that are less preferred by EAB or are tolerant or resistant to EAB infestation.

We have been working to identify such “lingering ash” and preserve and replicate them through grafting. Currently, we have 33 individual grafted lingering ash tree genotypes grafted (primarily green and white). Two of these genotypes flowered and controlled cross-pollinations were performed resulting in two small seedling families. In addition, 2011 was a good seed year for ash and we were able to collect open-pollinated seed from nine lingering ash located in the southeast Michigan survey site and seven lingering ash trees in the northwest Ohio survey site described by Knight et al. (these proceedings). Because of the high degree of ash mortality in these areas, the pollen donors are assumed to also be lingering ash, so these open-pollinated seed are likely the resulting progeny of two lingering ash parents. These families will be analyzed for segregation of EAB tolerance/resistance phenotypes and will give an indication of the potential for enhancing native resistance through breeding.

Currently, our work focuses on developing a screen to distinguish EAB-resistant and EAB-susceptible phenotypes in grafted lingering ash ramets and seedling families. Adult EABs are being reared at the East Lansing, Michigan, Northern Research Station laboratory. Adult EAB that emerge from naturally infested logs are placed along with ash foliage in plastic containers covered by a paper coffee filter. Groups of approximately 10 females and 4 males are placed in each container; adults mate and the females lay eggs on the coffee filters. Small sections of coffee filter with eggs attached are cut and then placed on each tree and wrapped with cheesecloth to prevent scavenging by predators (fig. 1A).

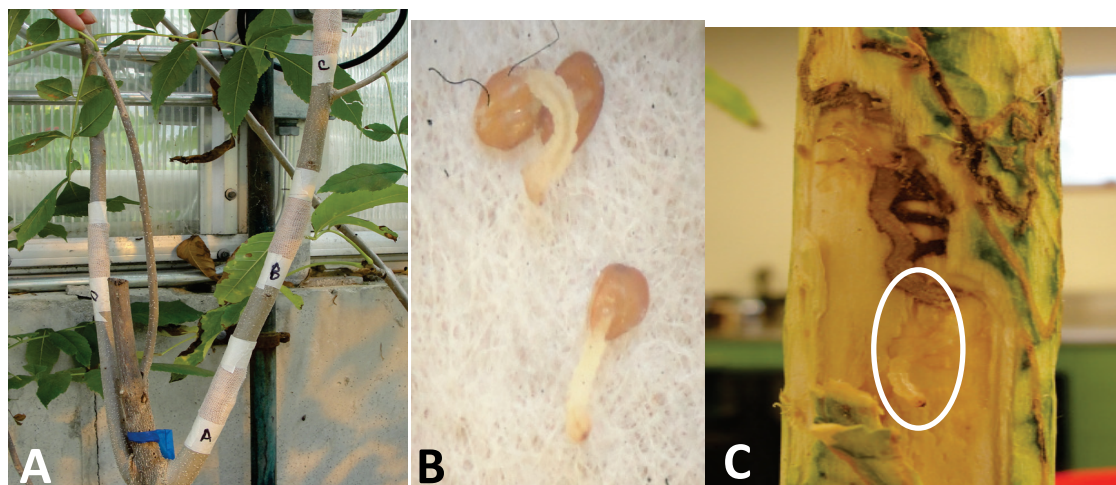


Figure 1—Development of emerald ash borer (EAB)-resistance screen. A. Coffee filters with EAB eggs are placed against the tree and covered with cheesecloth. B. Neonate larvae emerging from their eggs on the coffee filter. C. 3rd instar larva and gallery 6 weeks after egg hatch.

Approximately 8 to 10 weeks after the expected egg hatch date, the coffee filters will be removed and inspected to determine the number of eggs that successfully hatched. At each egg placement site, the bark will carefully be peeled back to dissect out larvae, recording the number of larvae, instar

development, larval weight, and any evidence for host defense response such as callous formation. Data will be analyzed to discern any differences between lingering ash and controls regarding successful larval infestation.

Lingering ash grafted ramets and seedling families will also be installed in replicated field trials to assess differences in response to natural EAB infestation. Interestingly, the nursery growing area at the Delaware, Ohio, USDA Forest Service Laboratory was plagued by a naturally occurring EAB infestation in the summer of 2010. One of the infested nursery beds contained grafted lingering ash selections that remained uninfested while known susceptible grafted selections immediately adjacent to the lingering ash were infested, an observation that supports the hypothesis that lingering ash are less preferred by EAB.

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