STRATEGIES FOR SELECTING AND BREEDING EAB-RESISTANT ASH

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ABSTRACT

Breeding for pest resistance in forest trees is a proven approach for managing both native and nonnative insects and diseases. A recent study by the Food and Agriculture Organization of the United Nations reports 255 forest tree breeding programs for insect or disease resistance in 33 different countries (http://www.fao.org/forestry/26445/en/). Advantages to incorporating breeding as a management approach to nonnative insects such as the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire include: (1) wide public acceptance; (2) a proven successful approach (many examples of success); (3) not dependent on prior knowledge of mechanisms of resistance; and (4) not dependent on the number of genes involved (qualitative or quantitative traits are equally successful), or the mode of inheritance of resistance (can select for dominant or recessive traits).

We are employing two different strategies to breeding for resistance: hybrid and traditional breeding. In hybrid breeding, the desired resistance trait is found in a different species, often an exotic species. The exotic species is crossed with the native species to create hybrids. The hybrids go through subsequent rounds of testing, selection, and backcrossing to the native species to carry only the resistance genes from the exotic parent into the native population while retaining all of the traits of the native species, as is being done in the American Chestnut Foundation's breeding program (Hebard 2006). In traditional breeding, the parents are both from the same species, generally the native species, and either one or both of them have the resistance trait of interest. This approach has been used successfully in other species (see FAO forestry reports link above for detailed examples).

Hybrid Breeding

Initial reports after discovery of the EAB infestation indicated that all native ash species were highly susceptible. The theory that ash species that coevolved with EAB developed mechanisms of resistance that allowed them to coexist indicates that Asian species of ash would be the most likely source of genetically heritable resistance (Rebek et al. 2008). The first step in initiating a hybrid breeding program is to identify and confirm genetic control of such EAB-resistant species for selection as breeding parents. A common garden study demonstrated that a cultivar of *F. mandshurica*, 'Mancana', was resistant to EAB relative to North American species, and anecdotal evidence indicates the same may be true of *F. chinensis* (Liu et al.,2007, Rebek et al. 2008). To identify additional EAB-resistant species, we have been accessioning Asian ash species across a wide geographical and ecological range. Each accession is confirmed for the proper species identity through the use of DNA-based technologies including ITS sequencing and AFLPs. This step is important because 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 of exotic ash, including 11 Asian species and 2 European species. In the process of confirming species identities, we uncovered seven different Asian ash accessions from reputable botanical gardens and arboreta that were incorrect, illustrating the difficulty of distinguishing *Fraxinus* species, even by experts. Additionally, six different seed lots (representing both Asian and North American species) from two different commercial seed companies were found to be incorrectly identified. Once the species identity is confirmed, the individual

accessions are replicated through grafting and eventually assessed for their EAB resistance through bioassays and field plantings.

Inter-specific hybridization of ash has not been widely reported. Hybridization of *F. nigra* and *F. mandshurica* resulted in the release of two horticultural cultivars (Davidson 1999). To determine what other species combinations may readily hybridize, we pollinated as many combinations as possible (dependent on availability of viable male/female flowers) under controlled conditions. In 2010, 42 different species combinations were crossed and 15 combinations successfully produced seed which was collected and put into stratification for germination. Resulting seeding families will be screened for EAB resistance. A total of 971 hybrid seed were collected representing full-sib family sizes up to 450 seeds. The hybrid parentage of one small family has been confirmed with DNA markers, and as tissue becomes available, the parentage of all full-sib families will be confirmed. Such large family sizes will be very useful for segregation analysis to confirm the genetic basis of resistance, linkage mapping, marker development, and the identification of proteins and metabolites that segregate with the resistance phenotype. Association of a protein or metabolite of interest that segregates with only resistant progeny in a full-sib family allows the identification of truly functional differences from among the many differences due only to the species differences between parents and the novel combination of these species.

Breeding work in 2010 also included the backcrossing of an interspecies hybrid cultivar (Davidson 1999) to both parental species. The seed from these crosses, should they successfully germinate, will be the most advanced *Fraxinus* interspecies pedigree, and a demonstration that at least some F1 hybrids are reproductively competent providing support for the potential of the hybrid/backcross breeding approach.

Traditional Breeding

Initial EAB infestations were in urban areas where a few ash cultivars were planted in high numbers. Although a large number of trees were killed, these represented a small number of genotypes. As EAB moved into natural stands and monitoring plots were established, it became apparent that there are trees that are still alive and rare trees that support a healthy crown even when over 99 percent of ash trees in the area are dead. These "lingering ash" trees may simply be the last to die. Alternatively, they may be trees with rare phenotypes that are less preferred, tolerant or resistant to EAB infestation. We have been identifying, grafting to preserve and replicate, and testing these trees. We believe it is important to investigate these trees and to verify if the lingering ash phenotype has an underlying physiological or biochemical basis that can be utilized in a breeding program.

We have identified, collected, and grafted 33 lingering ash trees (primarily green and white ash) from 2008-2010. Clonal ramets of each of these select trees will be generated and outplanted for field testing and bioassays to confirm their EAB phenotype. Initial bioassays have been conducted for a few lingering ash selections that had been replicated enough to provide an adequate quantity of tissue for experimentation. Adult choice feeding bioassays were conducted in two separate experiments with seven different green ash individuals selected as lingering ash. Unselected green ash seedlings (pe-H880), the EAB-susceptible green ash cultivar 'Summit'(pe-sum), and a selection of *F. mandshurica* (man-19) a known host species for EAB in Asia and a known EAB-resistant control were included in both experiments. Adult feeding, expressed as the proportion of the leaf area fed over 2 days, was analyzed by combining the data from both experiments and fitting a Generalized Linear Model. Genotype was found to be a significant effect, so least square means were estimated for each genotype and compared to the seedling control, pe-H880 (Fig. 1). Four trees had significantly different least square means from the control seedlings. Both *F. mandshurica* (man-19) and 'Summit' (pe-sum) had higher proportions fed, and two of the lingering ash selections (pe-24 and pe-22) had significantly lower proportions fed relative to the seedling control.

The feeding preference data from the choice bioassay was further supported by a natural EAB infestation

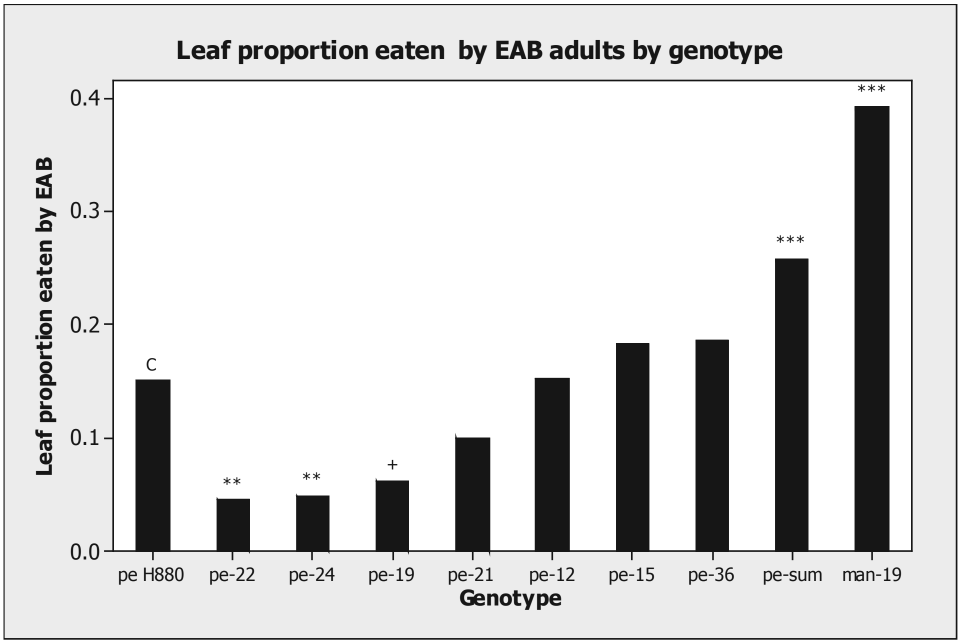

Figure 1.—Leaf proportion eaten by EAB adults by genotype. Least squares means for each genotype were calculated and compared to the seedling control, pe-H880. Four trees were significantly different from the control seedlings and a fifth very nearly different (***highly significant, p<0.003; **significant, p<0.02; + nearly significant, p=0.0052).

in the nursery growing area at the U.S. Forest Service laboratory in Delaware, OH where some lingering ash selections (including the significantly less-preferred lingering ash pe-22 from the feeding bioassay) remained uninfested while known susceptible selections in the same nursery bed were infested. Taken together with the feeding bioassay, this suggests that the lingering ash phenotype is real, and one component of this phenotype may be the reduced feeding preference to EAB adults of these trees.

Future work will focus on attempting to further enrich the lingering ash phenotype through breeding with other lingering ash or with exotic EAB-resistant trees in a combination of traditional and hybrid breeding. Two lingering ash grafts flowered and were cross-pollinated in 2010, successfully producing seed. The resulting F1 families of 'lingering x lingering' seedlings will be grown and assessed for their EAB phenotype so that patterns of inheritance can be discerned. Segregation of phenotype and potential underlying physiological or biochemical features will be analyzed for indications of the possible mechanisms involved.

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