COMBINING BREEDING, SOMATIC EMBRYOGENESIS AND CRYOSTORAGE TO CREATE EMERALD ASH BORER-RESISTANT ASH VARIETALS

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Emerald ash borer (*Agrilus planipennis*; EAB) has devastated populations of ash trees in at least 20 U.S. states and Canada over the past few decades. To date, control measures have had minimal impact on halting the infestation. However, there is evidence of genetic resistance or tolerance to EAB in natural populations of white ash (*Fraxinus americana*) and green ash (*Fraxinus pennsylvanica*) trees, as demonstrated by the continued survival of scattered trees for more than five years following infestations that killed over 99% of the trees. These "lingering" or "surviving" ash individuals may form the basis for reforestation programs in EAB-impacted areas, if these genotypes or the best of their progeny can be clonally mass-propagated. Over the past five years, we have initiated embryogenic cultures by culturing immature zygotic embryos from open-pollinated (OP) seeds collected from several surviving white ash and green ash trees in Michigan, Minnesota, Pennsylvania, Virginia and North Carolina. In addition, in 2018, we initiated cultures from crosses made between lingering green ash parents by USDA Forest Service personnel in Delaware, Ohio. Somatic embryos were produced by growing cultures in liquid suspension, followed by fractionation and plating on semisolid medium. Somatic embryo germination and conversion were enhanced by a combination of pre-germination cold treatment and addition of gibberellic acid to the germination medium. Ash somatic seedlings derived from OP explants grew rapidly following transfer to potting mix and 42 somatic seedlings representing 10 ash clones were acclimatized, grown in the greenhouse and planted in a preliminary field test, along with EAB-resistant (*Fraxinus mandshurica*) and EAB-susceptible control seedlings. Somatic seedling production is underway from two cultures that originated from seeds derived from a cross between lingering ash parents. An ex vitro germination protocol to accelerate plantlet production and a cryostorage protocol for the ash embryogenic cultures are under development.