SOMATIC EMBRYOGENESIS AND CRYOSTORAGE FOR RESTORATION OF ASH FORESTS DEVASTATED BY EMERALD ASH BORER

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Emerald ash borer (EAB), *Agrilus planipennis* has devastated populations of ash trees in at least 20 U.S. States and Canada over the past decade. To date, control measures have had minimal impact on halting the infestation. However, there is some 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 and growth of scattered individual trees for several years following infestations that killed over 99 percent of the ash trees in infested populations. These "lingering" or "surviving" ash individuals may form the basis for reforestation programs in EAB-impacted areas, if these genotypes or their progeny can be mass-propagated. We initiated cultures from seeds collected from several surviving white ash trees in southeastern Michigan. Cultures were initiated by dissecting immature seeds and culturing the developing zygotic embryos on a semisolid modified Woody Plant Medium (induction-maintenance medium; IMM) with 2 mg/l 2,4-D. Multiple highly productive embryogenic culture lines representing six different lingering ash parents were obtained. Embryogenic cultures were grown in suspension culture in liquid IMM, followed by size fractionation on stainless steel sieves and plating on nylon mesh overlaid on semisolid basal medium to produce populations of somatic embryos. Somatic embryo germination and conversion were improved by a combination of pre-germination cold treatment for eight weeks and addition of gibberellic acid to the germination medium. Ash somatic seedlings grew rapidly following transfer to potting mix and almost 100 trees representing 6 white ash clones have been acclimatized and grown in the greenhouse in preparation for clonal screening. Future research will focus on expanding the embryogenic work on other surviving ash populations, cryostoring copies of all ash embryogenic cultures and planting and testing of the clonal ash saplings for EAB resistance in the field.

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