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Propagation in Ash for Emerald Ash Borer Resistance

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Summary

The Great Lakes Basin Forest Health Collaborative (GLB-FHC) is a partnership network supported by the United States Forest Service and Holden Forests & Gardens, an NGO near Cleveland, Ohio. I am coordinating the GLB-FHC network of partners (state, fed, tribal, NGO, private landowners, citizens, etc.), that are interested in coordinating and discussing various parts of participatory pest/disease resistance breeding activities that support tree health. Our current goals involve increased communication across organizations with activities involving ash (*Fraxinus pennsylvanica*, *F. americana),* American elm, (*Ulmus americana*) American beech (*Fagus grandiflora*), and eastern hemlock species, (*Tsuga canadensis*). Our collaborative works towards assisting each other in activities that improve and increase our ability to breed pest/disease resistant tree species such as: forest monitoring, tree reporting, tree selection, sample or seed collection, tree propagation and planting/orchard development. In this position I also serve as a technology transfer liaison, providing training for partners wanting to be involved in tree breeding activities for pest/disease resistance, sharing knowledge and answering questions.

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INTRODUCTION

The EAB resistant ash tree breeding (*Fraxinus sp*.) program work flows breakes it down into 6 steps (**Fig. 1**). These steps are important in propagating EAB resistant ash because the order ensures that the best candidate trees are tested, tested trees let us know specific resistance levels as we progress into producing seed orchards. Planting trees into seed orchards allows for preservation of the individuals while producing seeds important for future plantings in the forest.

Figure 1. The 6 simplified steps needed in breeding EAB resistant ash.

One of the areas that slowed progress in EAB resistance work was a lack of available grafters. With the addition of some employees and grant funding we now have more locations that can assist with lingering ash grafting. Let me share some specifics on how the Forest Service grafts lingering ash clones or ramets. It can be dificult to retreive scion from some trees, but there are multiple tools that can help gather scion for grafting, for example, a slingshot, ropesaw, pole pruner extensions, and tree climbers. Trees that are lingering are not always in the best condition and it is important to check that you collect branches that have healthy buds on them. Research has found that hot-callus grafting gives the highest success rate (Carey et al. 2013). This means we will either top graft or side venir graft and add heat to the join area afterwards for around 6 weeks during the coldest time of the year. Before placing it near heat we coat the top of the graft in parafin wax to help it retain moisture. At the Forest Service Northern Research station trees are placed standing up in pots with a heating cable applied to 2 x 4 inch beams in order to keep the heat close to the join. Then surrounding the join there is insulation typically used for window sealer, it's thin size and short width allows for easy application to the beams. Openings are cut in the insulation to allow for placement of the tree and then the opening is taped over.

Temperatures are monitored to keep the heated area no warmer than 75°F (24°C). The room remains cold to keep buds from opening early. Future cloning for green ash can now include rooted cuttings, likely from the grafts, to increase ramets(clonal selections) of individuals (Aletta Doran, Holden Forests & Gardens, "personal communications" 10/15/24).

After grafted trees have grown into saplings individuals are tested for resistance via an EAB egg application bioassay, where those proving to show resistance successfully kill EAB larvae; however variations in resistance indicates only some are able to kill enough larvae to prevent their own mortality (Stanley et al. 2023). Other ramets of resistant individuals are planted out in a test orchard to examine over time how individuals cope with EAB in a more natural setting. Currently, green and white ash(Fraxinus pennsylvanica, Fraxinus americana respectively) from the southern Michigan and Ohio regions are set up in test orchards in hopes of converting them to seed orchards in the future. It's been between 8-15 years since they were planted and they have started to make seed. Plans are now underway to plant these open pollinated seeds out and assess how well they perform in a test orchard. In order to do this we have to collect and propagate ash from seed.

Collection of seed is done in September and sorting is required to remove empty seed or those infested with ash seed weevil larvae, a naturally occurring issue. Setting seed into cold storage for up to four weeks induces the larvae to remove themselves from the seed and leave a clear exit hole. If not propagating that year ash seed can be dried to 30% relative humidity and stored well for up to 5 years and longer if dried and frozen. Stratification for ash takes 3-4 months of cold, moist and dark conditions (Burns et al. 1990). We place our seeds in bags of moist sphagnum moss in cold storage for this timing and then place the bag in the greenhouse to start germination. Emerging seeds can then be potted, but other more direct sowing is an option.

Future work includes research plantings of open pollinated seed from EAB resistant ash plantings, which will likely begin in a year or two. GLB-FHC also has a goal of assisting areas across the Great Lakes Region with adding new lingering clones to new plantings. This will help preserve lingering trees as original trees may die in the forest before EAB resistance testing can be done. Preserving these trees locally allows for a greater remaining gene pool for resistance breeding programs.

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