**Ash Genetic Conservation Plan**

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**Introduction**

The emerald ash borer (EAB) is killing all native ashes (*Fraxinus spp.)* in Michigan, Indiana, and Ohio, and is beginning to spread into surrounding states and provinces. The loss of these species has cultural, ecological, and economic implications that warrant preserving the genetic resources before too much is lost to the insect. Trees, as do all plants, must be adapted to their environment to thrive. Natural ash populations have adapted to their environments, and preserving a significant number of these populations is required for reintroduction of these species once adequate environmental control measures for EAB are developed or trees resistant to the insect are bred and introduced. Breeding resistant ash trees for reintroduction will ultimately require an array of adapted parental populations. The projected degree of EAB destruction to native stands is so great that, only an adequate ex situ germplasm collection will be able to provide the needed material for breeding and reintroduction.

**The Threatened Values**

*Fraxinus* is a genus that is significant in many ways to many people. Indians have used the black ash for centuries to make baskets that are central to their tradition way of life. White ash is the preferred species of wood to make baseball bats used at all levels of the game from little league to the majors. When elms died out of the urban environment about 30 years ago, ash became a major replacement species. Ash is a major component of many riparian, wildlife, and recreational areas. Ash is very common along US highways. As these trees are lost it is not clear if the ecological structure and function of the ash component can be preserved by another species or if an undesirable exotic species will fill in the gaps. Losing ash from swamps and riparian areas might lead to a permanent increase in water and soil temperatures which in turn will affect many organisms. These concerns are widely expressed by tribal leaders, homeowners, forest scientists and land managers. Resistance in the native populations is at least rare and possibly nonexistent as tree mortality is extremely high in infested stands of ash. New infestations are occurring at an alarming rate despite strict quarantine efforts on the movement of raw wood over 1 inch in diameter. Therefore, the threat of losing ash germplasm is great.

**Goal of the Plan**:

The preservation of an adequate amount of genetic variation is the primary goal at this point as the EAB is advancing quickly. This will facilitate the long term goal of returning these species to the ecosystem once a workable solution to EAB is identified. The actions needed for restoration will be better defined following the ash conference in 2010.

**The Conservation Strategy: Management Options**:

There appears little that could be done from a management side at the present. Quarantine is the most effective and affordable management option at present. Unfortunately the quarantine is easily breached by careless movers of firewood. High value trees can be protected with insecticide. Therefore, ex situ conservation appears to be the only workable solution at present.

**The Conservation Strategy: Seed Collections**:

Seed collection is the primary focus of the conservation efforts at the present because seeds are a good, fast, and relatively inexpensive way to collect and preserve germplasm ex situ. The easiest way to assemble such a collection is to collect seeds from representative native stands following a systematic plan. The seeds will not transmit EAB to non-infested areas. When properly handled, seeds survive well under medium and long-term storage conditions, and they can be easily distributed to scientists and growers to produce seedlings for EAB research, breeding, other scientific study, and future ecological restoration work. This plan outlines the steps to organize these seed collections. Specific accomplishments will vary from year to year and this plan should be viewed as a multi-year effort. Mark Widrlechner, the Agricultural Research Service, Northern Woody Ornamental curator at Ames Iowa, and Robert Karrfalt, Director USDA Forest Service National Seed Lab prepared the initial version of this seed collection plan in 2007. Coordination with other federal agencies is evolving.

**Deployment of seeds for restoration of ash to the ecosystem**

Seeds collected through this plan will be provided to researchers who are able to conduct research into controlling the EAB and to the geneticists and breeders who will be developing populations resistant to EAB attack. We will seek out these cooperators in order to find and begin deploying solutions as soon as possible. Seeds from the existing wild populations will also be made available for reintroduction of ash into the ecosystem, if an environmental or biological control of the EAB is found and successfully deployed. These distributions will be made via the US Forest Service National Seed Laboratory (FS NSL) for seed samples collected from individual mother trees. Samples will be provided to the US National Plant Germplasm System (NPGS) for long-term conservation and distribution to its users, as well.

**Collection sites/number of trees/number of seeds**

The most critical areas to sample are those where EAB is currently located and destroying the trees. The genetic resources in these areas will be totally or largely lost if immediate efforts are not made to preserve them. Nearly as crucial are those ash populations adjacent to infested areas, because the EAB will be able to migrate into these areas in the near future. This philosophy based on urgency will be used to apply limited resources towards the collection of the most threatened populations.

A GIS map, the Emerald Ash Borer Viewer, is posted on the Internet at: <http://www.emeraldashborer.info/files/MultiState_EABpos.pdf> showing all identified EAB infestations and quarantine areas. It can serve as a valuable tool for deploying collection efforts.

The EAB Viewer indicates the locations of lakes and rivers and can, therefore, guide collectors to the habitat in which certain species might be found. White ash (*F. americana*) and blue (*F. quadrangulata*) will typically be on upland sites, while green (*F. pennsylvanica*), black (*F. nigra*), and pumpkin (*F. profunda*) ash are more often found in wet-mesic or swampy sites. However, green ash can also be found on upland sites. Other geospacial references can also be used.

A 3-person federal interagency committee from Agricultural Research Service, Bureau of Land Management, and the Forest Service developed an interagency sampling plan for the ash seed collections. It is uses Omernik ecoregions level III as the basic sampling units. These ecoregions can be viewed on this web site <http://nationalatlas.gov/natlas/Natlasstart.asp>. The interagency sampling plan states that 50 trees minimum be sampled per Omernik region level III, and that these trees come from at least 10 locations that are at least 1 mile apart and evenly spread across the ecoregion. There can be 10 locations of 5 trees each, 50 locations of 1 tree each, or any combination in between. This recommendation is in agreement with the recommendation of FAO which recommended an optimal target 25-50 seed trees per population (see FAO Forest Resources Division, 1995). Population in this case would be the ecoregion. Individual seed trees should be spaced preferably at least 300 feet, and not less than 100 feet apart to reduce the likelihood of sampling closely related individuals.

Collections will be made primarily from naturally occurring trees. Acceptable areas for collection include: the edges of farm fields, along rural roads, railroad tracks, utility right-of-ways, abandoned fields, or other such disturbed or open areas. Here the trees will be accessible, likely to have abundant seed crops, and be easy to sample without climbing or using mechanical lifts such as bucket trucks. The seed trees should not be noticeably diseased or attacked by insects. Damage by humans or poor shape of the tree is acceptable for seed collection. The shape of an open-grown ash tree can reflect environmental influences and not necessarily reflect its genetic makeup. Seeds will not be taken from trees in residential yards, developed parks, or in other such developed areas unless their genetic origins and isolation can be positively established. (e.g. A home owner in a rural area transplanted ash trees from the wild near his home into the yard.) Trees in developed areas are generally of uncertain genetic origin or represent selected clones. Some species such as blue, black and pumpkin ash will be primarily found in natural areas.

Urban trees are usually of uncertain genetic origin because landscape trees are often moved great distances in their production and cultivar or source identity is typically lost over time. However, if a tree has obtained a diameter of at least 9 inches at 4.5 feet above the ground and is healthy and vigorous in appearance, it very likely represents material adapted to the particular urban setting it has grown in. Since nearly all ash cultivar selections are staminate, the presence of a significant number of seed-producing ash trees strongly suggests the presence of seedling populations that may be of local origin. Therefore, seed can be collected in these urban settings for use in the same urban setting or in urban areas of the same ecoregion. Each city or town will serve as a separate urban seed collection area

For purposes of harvesting, cleaning, and drying, seeds will be kept separate by mother tree. At least one paper grocery sack (12 x 7 x 17 inches) filled with seeds to a depth of approximately 2 inches will be needed from a tree for it to be counted as one of the minimum number of mother trees. When filled to a depth of 2 inches the sack can be folded closed and stapled. If possible with reasonable effort, the bag should be filled to a depth of up to 12 inches before closing and stapling. This larger quantity of seeds will assure adequate amounts of seeds for research and for restoration work. The seeds can remain in clusters but should be completely removed from the tree branch.

**Species verification**

Species identification of each tree from which seeds are collected will be verified from a twig sample from the tree and two photographs of the tree. The twig sample will be the terminal end of a branch that clearly shows the lateral and terminal buds and leaf scars. It should be 6 to 8 inches in length. One photograph will be a close-up of the trunk for documentation of the bark pattern and color and the second will be of the entire tree. The whole-tree photo will also assist in verifying the site type for the tree. A complete leaf should also be taken and pressed if it is available. Frequently the leaves have shed or are in a degenerated state at seed collection time. If the seed clusters are not shattering a whole cluster should also be taken and put in the seed collection bag. At the time of seed cleaning, the twig, seeds (individually and in a cluster), leaf (if available) will be photographed for use in a digital herbarium.

Collectors will also complete and submit forms documenting site location and some environmental conditions in order for the seed lots to be properly documented in the NPGS’s GRIN database. Standard forms will be used by all individual seed collectors. Cooperating entities may use their own variation of the form, as long as all information on the supplied standard form is included on the cooperator’s form.

**Collectors**

Collectors to carry out this plan will be identified from within state and federal forestry agencies, universities, and NGOs, such as TNC, arboreta, or master gardeners. Choice of organization will depend on the type of land ownership and the activities of that group in the target counties.

The ability of all collectors will be assured by training. The objective of the training is to ensure that ash trees are located and properly identified, that appropriate collection techniques are taught, that high quality seed is collected, that quality is maintained in handling and shipping, and that accurate and complete data and voucher specimens are collected.

**Collection of seeds**

Seeds may be collected once the seeds (fruits) reach full size, the embryos fill the seeds, the seed coats turn from green colored to tan or yellowish green colored and the of the fruit has turned from green to pale yellowish green. Some fruits will have reddish color also. As the season progresses, fruits will become light tan to brown or darker. Fruits reach full size well in advance of the embryos reaching full size. Therefore, seeds must be cut open and examined carefully to be sure they are mature before collecting. Various methods will be used to collect seeds from the trees. These methods will be documented in training materials and updated as improvements are developed.

**Conditioning and storage of seeds**

The seeds will be placed into paper grocery sacks immediately following collection and kept there until drying for storage occurs. The sacks of seeds can be stacked on top of each other and no attempt to dry then should be made. This is also true even when the seeds are wet from dew or rain. The bags need only be kept in a cool place to prevent heating and molding. Some seed drying might occur under the collectors’ supervision as they gather the samples, but this is likely not of any consequence. As soon as practical, the collectors will overnight the seeds to a conditioning facility where the seed bags will be placed in refrigerated storage for a few weeks. This will cause any seed weevils to leave the seeds and facilitate the mechanical removal of these damaged seeds. After cold storage, drying will be completed and the seed cleaned, quality evaluated with x-ray and excised embryo tests, and placed in freezer storage. Seed lots for restoration work will likely be held at forestry agency seed-storage facilities. The FS NSL will store and distribute seeds for research and ecosystem restoration efforts. Back-up security samples from each collection area will be held at the National Center for Genetic Resources Preservation (NCGRP) according to the agreement between the NCGRP and the FS NSL. The FS NSL will integrate its preservation and seed-distribution efforts with the NPGS. The FS NSL will accession incoming samples and enter them into the NPGS’s GRIN database.

**Annual work plan**

Each year a work plan will be developed including a timeline for work activities**.** A work plan is expected to include the following activities and deadlines

Mid June to August 31: Identify collections and collectors to be made in 2007. Update training materials.

September 1 to October 1: Continue training collectors, distribute any needed supplies.

August 15 to September 15: Make seed crop surveys. Collect voucher specimens and begin mounting.

September 15 to early Dec: Make collections, dry and clean seeds.

Dec to February: Finish drying and cleaning, testing, packaging and placing in storage.

April 1: Evaluate completeness of collections and make plans for 2008 collections.

May 1: Report success to cooperators, collectors, program administrators, and the general public.

 Coordinate with other agencies involved ash seed collection efforts.

**Monitoring and communicating progress:** The seed collection areas defined in the seed collection plan will be mapped and identified as complete or incomplete. These maps along with other information on the collections will be posted on the internet. New infestations of the EAB will be compared to the progress of seed collections to ensure collections are progressing fast enough to preserve the germplasm ahead of the EAB.

Training and follow-up will be provided to all collection groups to ensure they will be making useable seed collections. Collection supplies will be given to all collecting groups to help ensure that the needed materials are available to gather all necessary data and a sufficient number of seeds.

Cost in dollars and worker hours per seed lot collected per species will be monitored to predict the needed resources for upcoming seed years.

**Literature cited**

FAO Forest Resources Division. 1995. Collecting woody perennials. Pp. 485-509. Chapter 23. In: Collecting Plant Genetic Diversity: Technical Guidelines. L. Guarino, V. Ramanatha Rao, and R. Reid (eds.). CAB International, Wallingford, Oxon, UK.