The National Program for Long Term Seed Storage for Ash Germplasm Preservation[1]

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Abstract

The U.S. Department of Agriculture Forest Service (USDA FS) began ash (*Fraxinus*) germplasm preservation in 2005, through seed collections for long term seed storage. The work was coordinated with the Agricultural Research Service (ARS). Collections have been accomplished through many cooperators. Various methods of outreach were deployed to solicit cooperators. No method stood out as better than another. However, the level of interest in genetic conservation held by the cooperator seemed much more important in determining if they chose to participate rather than how they were contacted. The most effective collectors were conservation professionals. Collections were made according to a plan written jointly by ARS, USDA FS, and Bureau of Land Management. About 4000 seed lots have been collected by the ARS and the USDA FS. Seed samples have been supplied to USDA FS research and to the Animal and Plant Health Emerald Ash Borer parasitoid rearing program.

Introduction

The Emerald ash borer (*Agrilus planipennis*) was first discovered in the United States in 2002 in the Detroit Michigan area. Initial response to the EAB focused on eradication and containment by the state of Michigan and federal agencies. In the course of a few years it became apparent that these efforts were not effective at containing the EAB. With the loss of ash (*Fraxinus*) in infested areas at almost 100 percent, it became imperative that conservation efforts be initiated to preserve genetic resources for any future restoration or resistance breeding work. Of the options available for conservation, seed collections seemed a good choice. Ash species are regular and abundant seed producers and there was good evidence that the seed would store for long periods in freezers. The storability of a seed is directly related to its desiccation tolerance and degree of dormancy. Nursery experience had demonstrated ash seeds to have moderate to strong dormancy, requiring at least 30 days of cold moist treatment and many cases 60 or 90 days of such treatment for germination to occur. Barton (1945) reported good storability of ash seeds when they were dried to 7 to 10 percent moisture content and kept at 5 ºC. All available evidence pointed to ash being a good candidate for long term seed storage.

Maintaining seed collections is also a much less expensive conservation option than methods such as a clone bank that require long term access to suitable land and regular annual maintenance such as controlling competing vegetation and protecting the trees from the borer. Seeds on the other hand are stored in existing seed storage freezers, and do not require annual attention. A viability test is necessary at some interval to know that the seed resource is able to produce seedlings. If decreases in viability are detected it becomes necessary to produce a replacement seed lot. The frequency of viability testing and when to regenerate the seed lot are decisions made by the curator of the seed collection.

Initially three federal agencies took an interest in the seed collections: the Natural Resource Conservation Service (NRCS) at Rose Lake Plant Material Center, the Agricultural Research Service (ARS) both at the Northern Woody Ornamental Collection at Ames. Iowa and the National Arboretum in Washington, DC, and the Forest Service (FS) National Seed Laboratory. Long term seed storage for genetic conservation was added to the National Seed Laboratory (NSL) mission in 2005. The three agencies had different initial approaches and resources for the collections, but within a few years a common protocol was adopted. When it became apparent that the seed collections would require range

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wide efforts and capacity to store seeds for decades the NRCS transferred their collections to the FS NSL and discontinued direct efforts to make collections. The Bureau of Land Management (BLM) also joined the effort in 2009 as they had opportunity to make collections through the Seeds of Success program as they collected other native plants from BLM lands.

Methods and Materials

Collection Locations/Numbers of Trees

Collections were focused initially on the areas being infested as this is where the resource was fast disappearing. Five ash species were in the immediate path of EAB: white ash (*Fraxinus americana*), green ash (*F. pennsylvnica*), black ash (*F. nigra*), blue ash (*F. quadrangulata*), and pumpkin ash (*F. profunda*). These five species of ash have very broad ranges, with white and green ash ranges covering much of the eastern United States. Making collections of every local population was not practical nor even necessary to capture most of the genetic resource. Therefore, a systematic and scientifically sound method was needed to divide the full range of a species into smaller areas, seed zones, from which seed collections would be made. A seed source trial had been conducted for both white and green ash, but these only gave general overviews of the genetic variation among populations and had not been developed into seed zones. As surrogates to seed zones, Omernik level III ecoregions were adopted. Seed was to be taken from 50 trees evenly spread over the ecoregion. These trees could either be at 50 individual locations or 10 locations with five trees each. This was a minimum and more trees could be included if convenient. A minimum of about 1.9 to 2.8 liters (2 to 3 quarts) of seeds was to be taken from each tree. White and green occur well dispersed over the landscape while the occurrence of blue, pumpkin, and black is more clustered. Therefore, white and green were more likely to be collected from 50 individual collection sites and seeds of the other three species were collected in small populations of five trees. The protocol stipulated that when seeds were collected from clusters of five trees, the five trees needed to be at least 30.5 m (100 ft) apart (to avoid collecting from trees too closely related), and not over 1.6 km (1 mile) apart (to put an upper limit on the size of a local population). These procedures were expected to provide seed collections containing at least 90 percent of the species' genetic variation. Trees were all to be naturally occurring trees and not planted landscape trees. A tree near a residence was acceptable as long as it could be determined that the tree was part of the natural vegetation. Ash, a pioneer species, is a frequent and successful invader of road sides and property boundaries. The full collection plan is found at http://nsl.fs.fed.us/Fraxinus_Germplasm_Preservation_Plan_March_2010.pdf.

Translating the plan into action required maps to indicate where to collect seeds. Maps were assembled using ArcGIS with layers for geography, ecoregions, and species ranges. Fifty dots were then manually placed evenly across each ecoregion that contained part of the species range. Each dot was then to be used as a general guide to a potential collection site. Not uncommonly, potential collections sites landed in the middle of a large agricultural field which meant the site had to be moved at least to the edge of the field. Because there was no information as to where an ash tree might actually be growing, the distance from the potential collection site to an ash tree was, in some cases, several km. However, there was enough distance among potential sites that collecting several km away from a potential collection site was easily accommodated. The GPS coordinates for each potential site were transferred to a spreadsheet from which collectors could identify proposed sites. Neither the ArcGIS maps nor the spreadsheet of coordinates were effect tools to communicate with cooperating seed collectors. The most easily understood and most universally applicable method of directing collectors to potential collection sites was to provide a list of towns that corresponded to GPS coordinates and instruct collectors to find a tree as close to that town as possible. Then either a paper road map or automobile GPS could be used to find the collection sites.

Access to Trees

Most land in the eastern United States is privately owned, which meant that permission to access trees had to be considered. Simply speaking to the property owner proved sufficient to gain approval to make a collection. No land owner ever denied access once the program was briefly explained. Most collection sites were located along road ways which meant they were in a public right of way where the public is accustomed to seeing workers trimming vegetation or performing other tasks. Therefore, by wearing a safety vest the seed collector was just another road side worker and collections were made without inquiring with a landowner. Should a tree occur in front of a residence or back from the road by more than 0.3 or 0.6 m (10 or 20 ft), collections were not made unless the land owner could be contacted. Road side trees were easy to access and 10 to 20 trees could be collected each day. Access to trees in natural areas required greater effort resulting in collections of four or five trees per day. When trees were found on public lands the local manager was contacted to grant permission. No managers denied permission to collect and all were enthusiastic to participate in the program.

Collection Procedure

The first steps were to determine the species identity and open a few seeds to determine if the embryos were matured adequately and the seeds free of insects. If the seed was good, the data sheet (fig. 1) was completed and the paper collection sack marked clearly with the accession number. To speed the data recording, a check system was used. Next a healthy twig section 15.2 to 22.9 cm (6 to 9 inches) long and including a terminal bud was taken and placed into the collection sack. Photos of the trunk and the whole tree were taken next. Finally the seeds were collected and the bag stapled shut. The twig, seeds, and the two photos were used to make up the voucher specimen for documenting the species identity. Detailed instructions to the procedure are available at http://nsl.fs.fed.us/GeneticConservation_Ash.html. These instructions were also used to train collectors.

Ash Seed Collection Data Sheet

Date of collection: ________________________

Collector's name: ________________________

Species (check one): __ Black ___ Blue

__ Green ___ Pumpkin ___ White

Seed Lot Identification

Collector's ID number _________

Seed lot number ______________

State ________________________ County______________________________

GPS Coordinates: lat ___° ___' ___" long ___ ° ___' ___" elevation ______m

Accuracy ± ______ ft

Number of ash trees within 20 to 40 feet of this tree: ___ 0, ___ 1, ___ 2 to 4, __ 5 or more

Number of other trees that are not ash within 100 feet this tree: ___ 0, __ 1, __ 2, __ 4 or more

Distance between this tree and nearest other ash tree from which seeds were collected.
____ 100 feet (minimum), ___ 200 feet, ___ more than 200 feet

Soil: ___ Rocky ___ Gravel ___ Sand ___ Loam ___ Clay

Site type: ___ upland __ wetland __aquatic.

Complete only for upland sites
Topography: ___ Flat ___ Slope (Aspect: ___ N ___ S ___ E ___ W)

Directions to the site if not using GPS: ____

____

____

Twig sample has been put in bag ___ Trunk and whole tree photos have been taken ___________

Leaf sample has been put in this envelope ___

Figure 1—The data collection sheet used in the USDA Forest Service ash seed collection program.

Harvesting the seeds was accomplished in several ways. The easiest was simply to hand pick the seeds directly from the branch when the seeds were low to the ground. Generally this was not possible. A pole pruner gave access to the seeds up to a height of about 6.1 or 7.6 m (20 or 25 ft), and occasionally to 9.1 m (30 ft). These techniques worked well for road side trees and black ash which often had seed bearing branches within reach of the pruner. Seeds above 9.1 m (30 ft) required different techniques. The technique most easily deployed and most affordable was to use a large sling shot to throw a rope over seed bearing branches and then to shake them to knock the seeds down onto a tarp spread below the tree (Knight et al. 2010). This technique required great patience as even the slightest breeze would cause the seeds to miss the tarp and fall into the ground litter, but it did prove very effective in natural areas were access was primarily by foot. Climbing and bucket trucks were also very successful for collecting seeds high in the crown but not used extensively because of the skills and cost required.

Collectors

Collections were largely made by volunteers and persons who could work the collection activities into their regular work schedules. Outreach to collectors was made through direct email to colleagues in and outside government agencies, through presentations at meetings, and via the NSL website. Several workshops were presented with cooperators who in turn recruited additional cooperators. The most successful collectors were persons who had plant experience of some sort, including ecologists, foresters, nursery personnel, and the like.

Laboratory Procedures

Seeds were sent by overnight parcel service to the NSL were they went through a series of steps to prepare and evaluate them for long term storage. The first step was to put the seeds into the cold room for at least a few days. This caused any weevil larvae to exit the seeds (fig. 2). Removal of the larvae is important if the seeds were ever to be provided to another country as international phytosanitary requirements would likely require the seeds be insect free. Next seeds were equilibrated to 30 percent equilibrium relative humidity as measured by a hygrometer (Karrfalt 2014) (fig. 3). Thirty percent eRH was found to correspond to approximately 7 percent seed moisture content which was reported by Barton (1945) to be a suitable moisture to store ash seeds. With species of seeds able to remain alive at moisture contents below 10 percent, 25 percent to 30 percent eRH is the point where maximum viability is preserved. Stems and large sticks were removed from the seed lots by hand. Viability of the seeds was determined by both x-ray (fig. 4) and embryo excision test (fig. 5). A seed lot is accepted for long term storage if 80 percent of the seeds were full. A full seed is one that has a good embryo, a fully formed endosperm, and no insect damage. A lower percentage of full seeds was accepted for some collections that were more difficult to obtain. For seed lots meeting the full seed requirement, an excised embryo test was made on 10 undamaged embryos excised from full seeds. Again, an 80 percent germination of the embryos was desired; however, seed lots with as little as 50 percent germinating embryos are sometimes kept. A precise estimate of viability was not obtained in this test, but it did provide complete assurance that the seed lots had a useable number of viable seeds. Viability and eRH are both evaluated at 10 year intervals beginning at year 10. The final storage preparation steps included checking the eRH, drying as needed to put the seeds at an eRH of 30 percent, sealing the seeds in storage bags, and filing in the freezer. Seeds are kept in two separate collections. One is the working collection kept at the NSL and the second is the security backup collection at the National Laboratory for Genetic Resource Preservation. Working collection samples are used to fill requests from persons with a good justification to have some of the seeds. Security backup samples are not used for distribution, but are solely to provide one more assurance that the germplasm is not lost in the event of a catastrophe with the working collection. Working collection samples are stored in 6 mil poly bags; while security backup samples are stored in foil laminate bags. Close to 99 percent of all samples received make it through the evaluation process and are entered into the collections. This is a testament to the quality of the work done by the collectors. All data are recorded into the Germplasm Information Resources Network database called GRIN Global (https://npgsweb.ars-grin.gov/gringlobal/search.aspx). This database is maintained by the ARS for all materials entered into the National Plant Germplasm System. Once accepted into the collection, seed lots are assigned a final accession number. A photo is made of every voucher specimen (fig. 6). The photos will ultimately replace the actual physical voucher specimens as there is insufficient space to store the physical specimens.

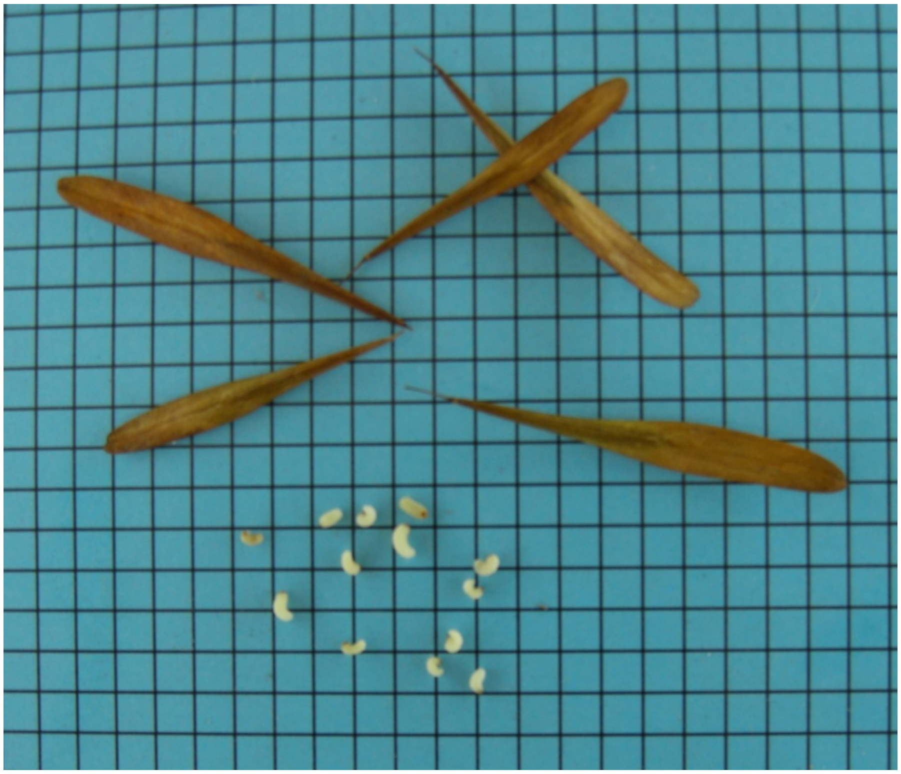

Figure 2—Weevil larvae exit the ash seeds after placing the seeds in a cooler at approximately 3 ºC for a few days. The grid is composed of 5 mm squares.

Figure 3—A hygrometer is used test whether the seeds are sufficient dry for long term storage.

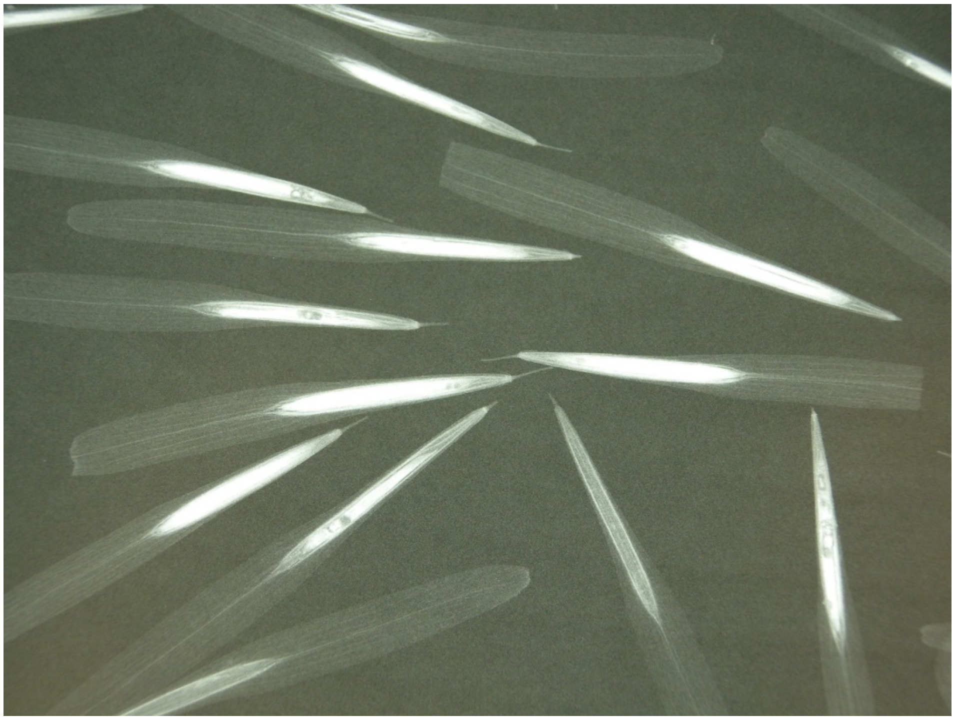

Figure 4—This radiograph quickly and accurately shows the number of good seeds, the number with a weevil larvae, and the number that are empty in a sample of ash seeds.

Figure 5—This excised embryo test is a good way to estimate the viability in ash seeds.

Figure 6—Photo made to electronically document seeds and twig portion of the voucher specimens. The number shown is the permanent accession number assigned to the seed lot. The grid is 5 mm squares.

Results and Discussion

Over 4000 single tree collections of ash species were accomplished among all agencies. Most collections were of white and green ash. Black, blue, and pumpkin ash were also collected. A full listing of collections held by USDA agencies can be viewed at https://npgsweb.ars-grin.gov/gringlobal/search.aspx. The oldest samples in the collection are now 10 years old and viability tests on these samples show that viability is remaining high and the storage is successful. Many collections remain to be made and in no ecoregions are collections completed to the original target of 50 trees evenly spread across the region, although some are very close (Karrfalt et al. 2013). The EAB continues to spread across the country and collection efforts will continue as resources become available and interest is shown by cooperating agencies and individuals.

Conclusions

The genetic resources of ash, despite ongoing loss in the wild, are being successfully preserved through long term seed storage. Mortality caused by EAB continues to erode the germplasm base in new areas, so many more samples remain to be collected.

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