Brown Ash Seed Collection Manual:

Preserving Brown Ash (*Fraxinus nigra*, wikp/wiskoq) through the Ash Protection Collaboration Across Wabanakik, APCAW

Ash Protection Collaboration Across Wabanakik (APCAW)
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Introduction

EAB Invasion Threats Ash Resource

Discovered in 2002 in North America, the emerald ash borer (*Agrilus planipennis,* EAB, Figure 1), has spread across much of the eastern United States and southeastern Canada to the detriment of all native species of ash trees, EAB's primary host species. The damage this invasive forest pest has caused across the continent in undeniable. The insect eats the phloem, a layer under the bark that transports food and nutrients to all parts of the tree. If enough EAB are attacking the tree it cannot survive and will die. Between 2002 and 2006, it is estimated EAB had killed 15 million ash trees in the United States, and as of 2023, many more millions of ash trees have died; it has now been detected in 36 states and five Canadian provinces (Emerald Ash Borer Network 2023; Poland & McCullough 2006). Established populations of EAB were first detected in Maine in 2018 in York Co. and Aroostook Co., the southernmost and northernmost counties in the state, respectively, and the infestation is now spreading toward the center of the state (Siegert 2019).

Figure 1. Example of an EAB feeding gallery on an ash tree, examples of adult and larval EAB specimen (Siegert 2023).

Loss of Culturally Significant Brown Ash

There are three species of naturally occurring native ash found in Maine: green ash (*Fraxinus pennsylvanica*), black ash or brown ash (*Fraxinus nigra*), and white ash (*Fraxinus americana*). Non-native, ornamental ash are also susceptible to EAB. While all native ash trees are important and worth saving, the brown ash tree, is a cultural keystone species to the Wabanaki people who are deeply tied to the tree species and use it almost exclusively for the art of basket making (Costanza et al. 2017). Brown ash is significant in the creation story of each Tribe within the Wabanaki Confederacy. There is no replacement for brown ash in Wabanaki culture. Brown ash is important to all Tribal Nations throughout the species range including but not limited to: the Wabanaki Confederacy, the Haudenosaunee Confederacy and the Anishinaabe Peoples' on both sides of the political boundary between Canada and the United States. Seed collecting is a long-term strategy for protecting ash. Collected seeds may be used for research and conservation of the species, in partnership with the Tribal Nations of the Wabanaki Confederacy, the University of Maine School of Forest Resources, and natural resources agencies for the state of Maine, as well as the United States Forest Service, land trusts and private landowners.

Preserving Brown Ash through Seed Collection

Why harvest seeds? This is an excellent question. There are several research directions and conservation methods with which ash seeds can be used: genetic studies, future plantings and possible regeneration of trees in seed banks. Looking to the Great Lakes region, there are numerous concerned on what the Dawnland or present-day Maine will look like after EAB has spread through the entire state. In many states ash trees have been used for municipal and

residential street trees and with the infestation of EAB, these trees must be removed which has changed the landscape of many cities and towns across the country. Maine is covered in mixed forest and white ash is typically a timber tree. Therefore, loss of ash in Maine will change not only what many of the towns will look like, but forests too. Maine's total forest is only a small percentage of ash, but brown ash is highly important to localized ecology of wetter areas within the forest. While all ash contribute important functions to their environment, brown ash is often essential to the water table in the areas it is found and there has not been a species of tree identified to regulate water in the same way. Brown ash leaves are the last the come out in the spring and some of the first to fall in autumn, making its leaf litter on the forest floor very important to many animals and plants.

Maine is the last state to be impacted in the northeast for brown ash. Therefore, the time is now to collect seed before overstory seed trees are lost. EAB is not likely to go away, even after it has covered the ash range, so it is highly important to collect seed while the forest still has naturally occurring brown ash. A further discussion of research possibilities is presented later in this document in the section titled, "Future Uses of Seeds".

How to Use this Document

This document is to be used as a guideline for collecting ash seed on potentially an annual basis. Ash trees do not produce seed every year, so it is important to at least scout for seed each year, so if trees are producing seed can be collected in the fall. The goal of this document is the provide those interested in protecting brown ash a way to be involved with the effort, regardless of experience or skill in forestry or research. We will be walking through the methods for how to prepare for seed collecting, identifying ash trees, returning to collect seed,

and the post-collection processing. The future uses of seed and an example of a well-stocked seed collection kit developed by APCAW is also included. The final section of this document outlines the collection of ash leaves for a research project that we have partnered with Dr. Jill Hamilton at Pennsylvania State University. Please consider this opportunity to assist fellow ash researchers in collecting these materials.

Steps for Seed Collection

Where do you start? Prioritizing areas for collection

While all ash is important, the genetic diversity of wild ash or non-nursery ash is of utmost importance to those wishing to protect the species for the future. That is why this document is meant to focus on collecting ash in the woods, rather than ornamental or street trees, like what someone might plant in their front yard. For this reason, we recommend reading and practicing the methods outlined in "An Ash Resources Inventory Field Manual" (Everett 2019), a guide for finding ash stands for potential seed collection. The inventory field manual is highly technical and has helpful information regarding the background of ash, EAB, treatments to protect against EAB, and how to find ash stands on your property. While green ash and white ash may be mentioned in comparison, the focus of this manual is on brown ash seed identification, collection, and storage options.

Identification of ash trees and seasonal observations

A helpful guide for all trees in Maine is the "Forest Trees of Maine: Centennial Edition 1908 – 2008" produced by the Maine Forest Service, which provides excellent information on identifying ash. The "Ash Identification Table" in the appendix comes from the Forest Trees of Maine text and will be referenced throughout this seed collection manual. We will now

highlight the differences between the three species of ash that you can find in the northeast: brown ash, green ash, and white ash.

Ash trees are deciduous, meaning that leaves grown in the spring will mature and die in the fall. Generally, the three ash species in Maine will grow between 50-70 feet in height, but are often found in different areas of the forest. Mixed ash stands are not uncommon, but knowing where ash species are typically found is helpful in identifying individual trees. White ash are typically in upland areas, whereas green ash are found in low hills with moist soil, but brown ash is found where 'it can keep its feet wet'. Brown ash is often in swamps and along riverbanks where the water table is higher, which makes it less desirable for timber harvesting, along with it generally not being valued as a timber species. White ash is first and foremost an important timber species, as well as used in residential areas for ornamental or street trees. Green ash is least commonly seen in the wild, mostly found in central Maine. It is however a very common street tree.

Distinguishing between species of ash is not always easy and can be challenging even for those used to working with trees. Please view the appendix section titled, "Ash Identification Table" for a complete description, as well as Figure 2 below. It is suggested to have a field guide that highlights all three species when in the field to help properly identify trees. Brown ash has yellow-green leaflets, brighter than other ash, which are long and thin with 7 to 11 on one leaf. The leaflets are no more than 5 inches long and are "lance-shaped," (Maine Forest Service, 2008, p. 133). Green and white ash will generally have fewer leaflets, between 7 and 9, and 5 and 9, respectively. White ash is the only one whose leaves will turn purple in the fall, green ash and brown ash will turn yellow.

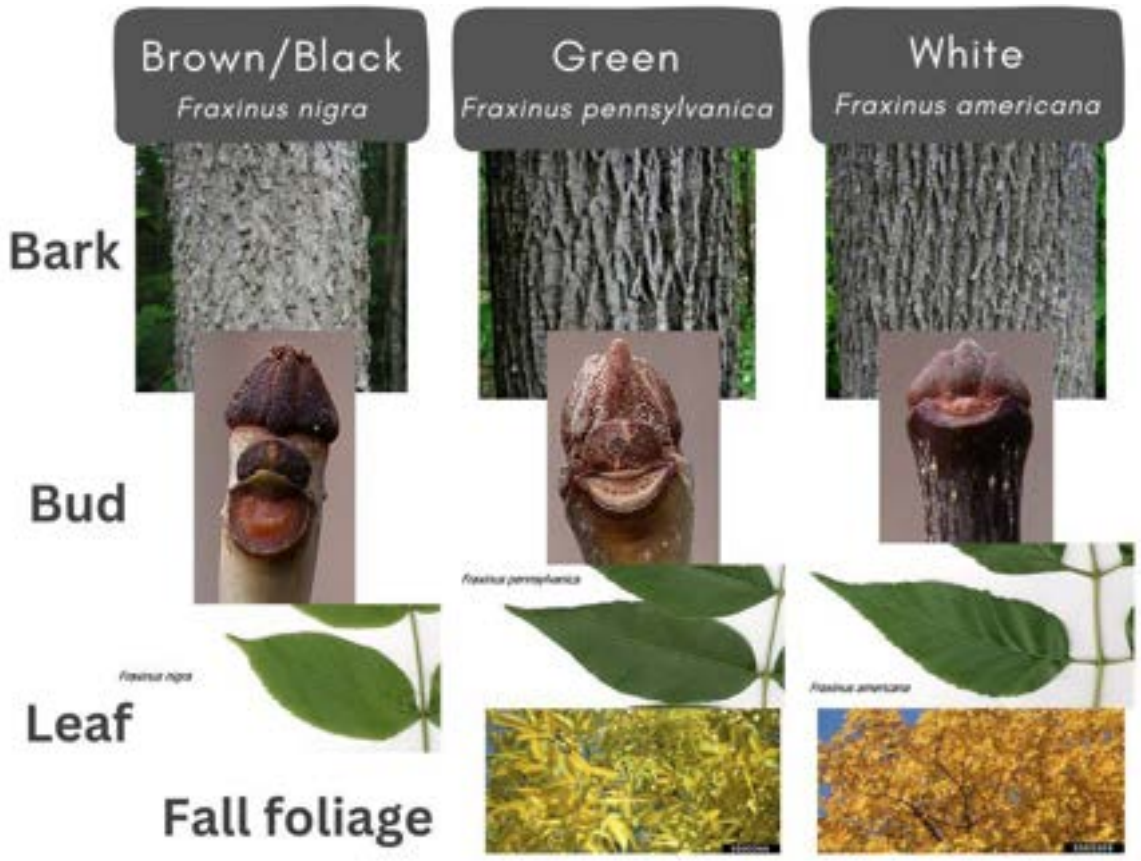


Figure 2. Photo representation of the bark, buds and leaves of each ash species in Maine. Bark photos (Maine Forest Service 2020), bud photos (Dzuik 2014), leaf photos (Chayka 2014), and fall foliage (Syndor 2013a & 2013b).

The bark of the trees is another indicator of which species you are trying to identify. Brown ash is known for its spongy bark which is corky, scaly, flakey or furrowed. The outer layer of bark can sometimes rub off easily when touched and is often gray to dark gray in color. Green ash should feel firmer and is gray to brown in color, often with deep channels in the bark. White ash, as described by the "Forest Trees of Maine", "resembles a woven basket," (2008 p.128) which is confusing language because this tree is not used in traditional Wabanaki basket making. This texture comes from ridges in the bark. White ash is usually a dark gray or brown color.

The buds of the trees are another way to differentiate ash species, if you are able to reach the limbs. Between the three subspecies the buds are ¼ of an inch or less in size. Brown ash buds are dark in color and “sharply-pointed”, green ash are brown and “cone-shaped” and white ash are brown and “blunt-pointed” (Maine Forest Service 2008 p. 127). Being able to tell the difference between buds is important depending on the time of the year you are surveying ash trees. Surveying before the leaves are present in the spring means you must be able to tell the difference in the trees with the bark and maybe buds.

Finally, the differences between the fruit of the ash trees. The fruit is the container in which the seeds are kept, which is the goal of this document, finding and collecting ash seeds. The fruit of the ash tree is called a samara, and each of the ash trees have similarly sized and shaped samaras. The samara is what we will be collecting. Brown ash samara wings are the long almost oval-shaped housing, like a canoe paddle blade, which contains the flat seed. When it is growing on the tree, they are green in color and yellow when dried. These are what are often seen on the ground under a brown ash tree after the growing season. Green ash has similar samaras, although they are described as “funnel-shaped” (Maine Forest Service 2008 p. 131), and white ash samaras as, “cigar-shaped” (p. 128). Figure 3 shows the seeds of the three ash species next to each other for comparison.

Figure 3. An example of samaras from each type of ash species found in Maine: green ash, white ash, and brown ash.

Identifying the sex of a brown ash tree

Brown ash trees have three possible sexes: female, male or polygamous, meaning that the tree can produce both female and male flowers. Identifying the sex of a tree is important as it will indicate which trees can produce seeds. Finding the female trees is essential to seed collection and a very important reason for scouting your ash stand in spring, which will be reviewed in the next section. The female trees are often described as wispy or "feathery, yellow panicles, 1 to 2 inches long," as seen in Figure 4a and 4c (MinnesotaWildflowers.info n.d.). Male flowers are rounded or globular, and dark in color (Figure 4b). These can often be seen after spring and into the summer for a very short window of time, and can be hard to distinguish on particularly tall trees if still visible after the trees have bud out. Remnants of the flowers can sometimes be seen throughout the rest of the year from the ground with binoculars. Male

flowers often remain on the tree throughout the year due to the stocky stature. They are most visually recognizable when they have been impacted by the ash flower gall mite. This causes the male ash flowers to have very dark growths that can be seen across the trees canopy well into the fall and winter months. The female flowers leave behind the stems, making them look like long bristles at the end of the tree branches, and male flowers are dark blobs in the same place, but on male trees. Polygamous trees can be difficult to distinguish unless both parts of flowers are left behind. The female and male flowers bloom at different times to prevent self-pollination, and therefore it is possible to mark a tree as one sex and later see it present as another. Therefore, it is important to keep records of the trees you collect from over time and can consult which trees did produce seed. It is also important to remember that all seed producing trees are important to collect from, as many factors go into seed production. As ash trees do not produce seed every year, it is essential to collect from as many seed producers as possible to collect the broadest range of genetic variation.

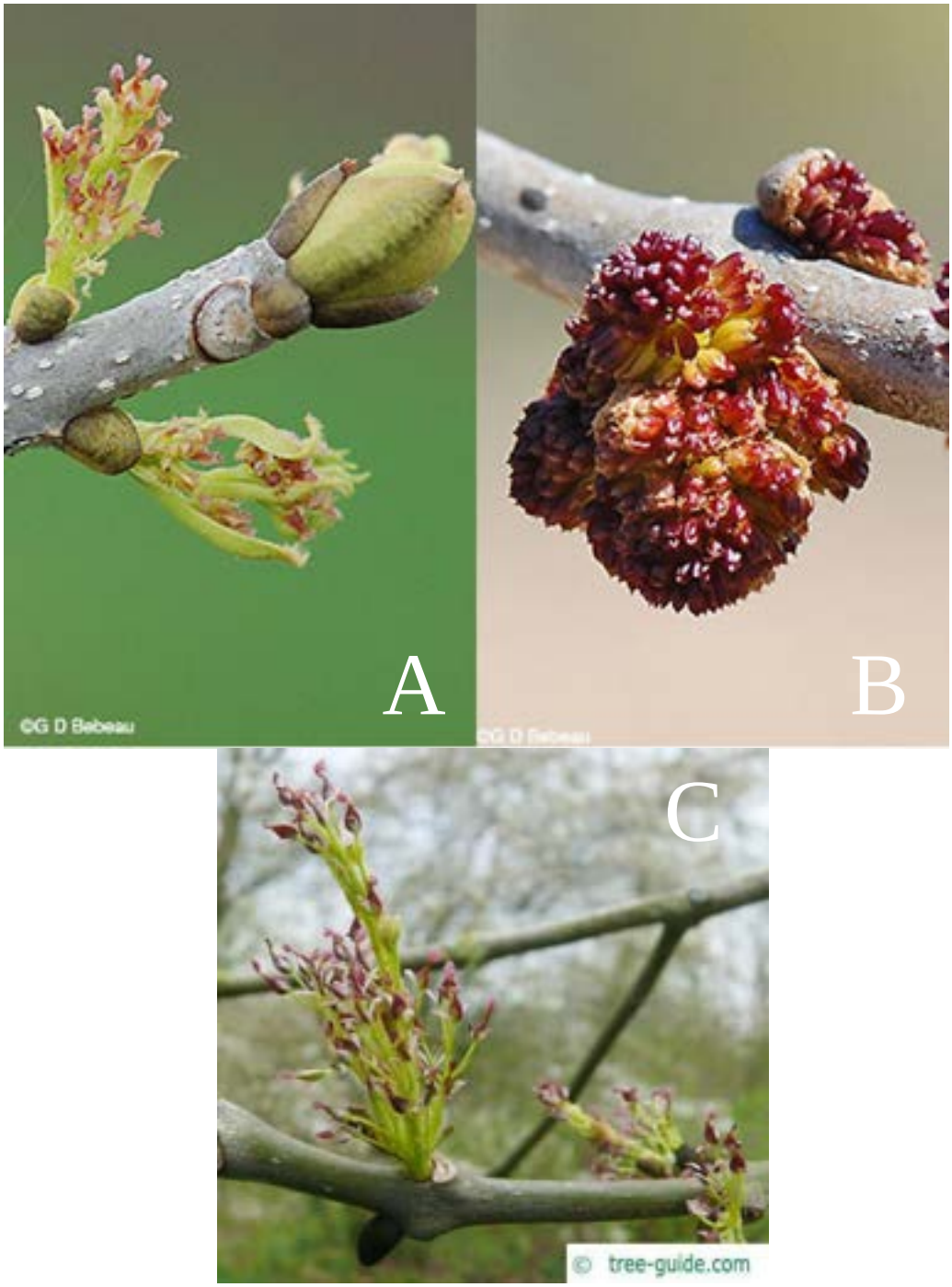


Figure 4. Image A represents developing female brown ash flowers (Bebeau 2013a). Image B represents a male brown ash flower (Bebeau 2013b). Image C represents female brown ash flowers (Benning n.d.).

Timing of seed collection

Ash has a broad range in North America, and different subspecies differ in when they have harvestable seeds. According to partners and seed collection experts with the Akwesasne Saint Regis Mohawk Tribe, as well as from basket makers, ash harvesters, and foresters in Maine, it is suggested to begin scouting trees in early spring. Brown ash trees will start producing flowers slightly before they "leaf out", or when you will start to see leaves. Scouting is recommended to occur in early to late spring, between April and May, see Table 1. If seed is detected in the spring, you may want to monitor the trees during the summer months of June, July and August. Seed collecting takes place between September and the first week of October with the processing and storage immediately after collection. The end of October through December is time to evaluate the collection cycle, complete records, and share outcomes with partners. Late winter, January to March, can be a time for resupplying and updating protocol for the next round of spring tree scouting, and the whole cycle starts again.

ACTIVITY	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Evaluate previous year protocol and update, if needed. Resupply seed collection kits.	■	■	■									
Spring tree scouting: identifying trees, sex, evidence of EAB activity, and marking for potential collection.				■	■							
Monitoring of seed growth and development. Leaf collection research – see Ash Foliage Collection section.						■	■	■				
Seed collection time. Sort, clean and dry seeds. Must be packaged and stored very soon after collection.									■	■		
Evaluate completeness of collections and make plans for the next collection cycle. Complete records of collection, and share outcomes with seed collection partners.										■	■	■

Table 1. Annual seed collection activities divided by month.

Things to consider before collecting

The suggested and recommended items for ash seed scouting and collecting can be found in the "Ash Seed Collection Kit: Recommended Checklist" in the appendix. This list of items was curated by discussing seed collecting with experts with the Akwesasne St. Regis Mohawk Tribe's Akwesasne Task Force on the Environment (ATFE), forestry professionals, as well as harvesters, to find what works best for collecting ash seed with the pruning pole method in mind. The "Seed Collection Techniques" section will provide other options for collecting seed, but the list provided here highlights what is recommended when using pruning poles to cut branches for ash seed. The scouting trees section of the checklist should be universal to all seed collecting methods. Having the proper items for ash seed scouting and collecting is very important.

Brown ash can be difficult to access due to the wet areas it grows and may not be located close to your vehicle. Ash trees can also have hard to reach branches, depending on where a tree is located. This is something to consider when looking for ash trees to collect from: can you safely reach the branches that have seed? If not, look for roadside or forest edge trees that may be more accessible to reach, rather than the taller canopy trees. It is important to have everything you might need before heading into the field, so you will be prepared during both scouting and collecting trips. While it is not listed in the checklist, there are other items you might want to bring anytime you are heading into the woods in Maine: a good pair of boots, bug spray and/or tick spray, sunscreen, a hat, a cell phone with full power, a camera (if your phone does not have one) and drinking water. We also suggest working in 2 or 3 person teams for safety and always letting someone know your plans before heading out. The

complete checklist of items can be found in the appendix under: Ash Seed Collection Kit: Recommended Checklist.

Documentation of trees and seeds

Documentation of trees and seeds collected from these trees are an essential part of the seed collection process. Without it, there will be no data to locate trees, their geographic location, and all the information associated with the collection. Depending on where you will be sending your seed will depend on which of two data collection sheets you will need to fill out. We have included two forms and how to fill them out in this document. It is important to decide where you plan to send your seeds for storage prior to collection so you can follow the correct guidelines and requirements for each location.

The three locations described in this manual include:

- United States Department of Agriculture (USDA) Agricultural Research Service National Plant Germplasm Facility in Ames, Iowa;
- USDA Agricultural Research Service's National Plant Germplasm Facility in Fort Collins, Colorado, and
- University of Maine, School of Forest Resources in Orono, Maine.

The distinction between the two National Plant Germplasm Facilities will be discussed in the "Post-harvest Seed Handling and Processing" section of this document. We refer to the National Seed Laboratory as NSL and the National Plant Germplasm Facilities as NPGF while the forms for each are overviewed.

If you are collected for one of the Wabanaki Tribal Nations, please use the address of the Nation's headquarters (Table 2) and share the longitude and latitude of the collection

location separately with the Tribe. This can also be shared with APCAW and the information can be forwarded to the respective Tribe. This allows the location of the ash seed trees to be protected by the Tribe and not publicly available online when the seed is documented by the USDA.

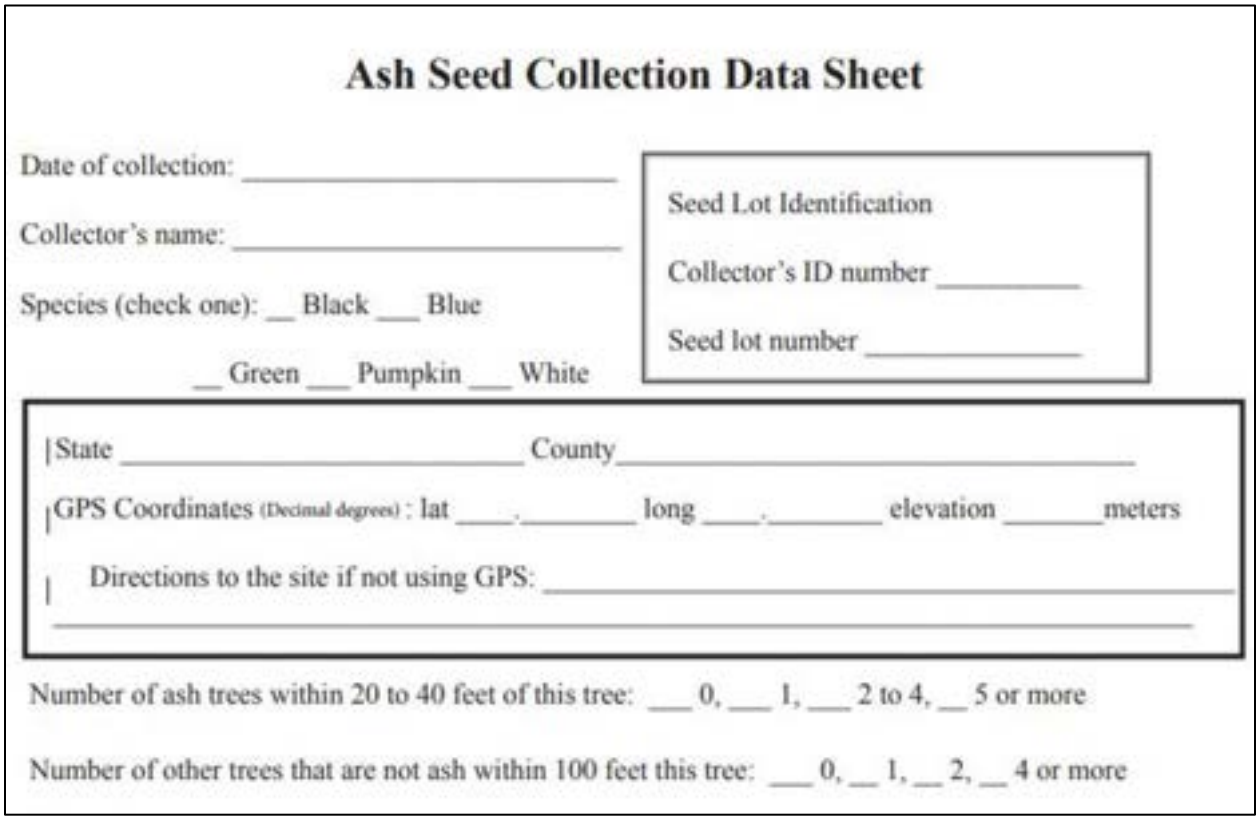
Houlton Band of Maliseet Indians	**Mi'kmaq Nation**	**Penobscot Nation**	**Passamaquoddy Tribe – Indian Township**	**Passamaquoddy Tribe – Sipayik**
88 Bell Rd, Littleton, ME 04730	7 Northern Road Presque Isle, Maine 04736	27 Wabanaki Way, Indian Island, ME, 04468	8 Kennebasis Rd, Indian Twp, ME 04668	9 Sakom Drive, Perry, ME 04667

Table 2. Addresses for Wabanaki Tribal headquarters to be used when submitting seed collection.

National Seed Laboratory Data Sheet

Please use the USDA Ash Seed Collection Data Sheet as a reference, found in the Appendix B, as we walk through the steps to filling it out. One sheet should be completed for each tree sampled, each time it is sampled and will correspond with one bag a seed. You will need a clipboard, datasheets, and a pen or pencil. Starting at the top of the sheet, make sure to document the date of collection and the name of the collector, or team of collectors. As this effort is focusing on brown ash, once the species is identified, this should be marked. If working in a seed lot, you may have a Collector's ID and seed lot information and can be applied to the sheet, see Figure 5. Please work with whoever is leading your collection to identify Collector's ID numbers or codes, as well as identifying the seed lot numerical identification you will be using. If you are working as an individual, your initials can be used for a Collector's ID with a number corresponding with sample number for your own records. This information will be written on the seed collection paper bags, so it is essential these numbers be correctly documented on the corresponding data sheet. The large, black outlined box in the next section,

contains the geospatial information for the state, county and GPS, Geospatial Positioning System coordinates in decimal degrees. The latitude, longitude, elevation, and meters should be included there. If a GPS unit is not being used for this collection, directions of how to relocate the tree should be noted.



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 (Decimal degrees) : lat ____._____ long ____._____ elevation _____meters

Directions to the site if not using GPS: _____

_____

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

Figure 5. Top half of the NSL Ash Seed Collection Data Sheet.

Beneath the GPS box, the following questions to be answered include: the number of ash trees within 20 to 40 feet of the tree being sampled, number of other trees- not ash- within 100 feet of the tree, and distance between the tree and other ash trees that have been or will be collected from. Note that these questions are referring to brown ash trees as the species in question, as this is a brown ash seed collection project. The National Seed Lab, requests a, "minimum of 100 feet between trees… so that related mother trees are not collected. This [is]

especially important for … ash which can root sucker, [that is] put up sprouts from the roots. Several trees growing close to each other might in fact even be the same tree or clone", words added for clarity (Karrfalt 2011, slide 67).

The next two questions (Figure 7) involve EAB evidence and activity: Is it [EAB] present surrounding the tree(s); and are there signs of EAB activity on the sampled tree? There is a list of the four terms: epicormic sprouting, canopy thinning, blonding, and 'D' shaped exit holes. Epicormic sprouting are branches that come out of the main stem of the tree in unusual places. Canopy thinning is when the canopy, the highest branches of the tree, are missing leaves. Blonding is when the bark of the tree is a lighter color than it should be because the top layer of bark has been removed due to woodpecker activity. Finally, 'D' shaped exit holes are what EAB makes when they bore out of the tree as an adult, as seen in Figure 1. Examples of these holes can be found on the Maine Department of Agriculture, Conservation and Forestry web page titled, "Signs and Symptoms of Emerald Ash Borer Infestation" at https://www.maine.gov/dacf/php/caps/EAB/EABsigns.shtml, as well as the reporting web page highlighted in Figure 6.

*****If there is suspected activity from an Emerald Ash Borer in the State of Maine, it must be reported at the following website: https://www.maine.gov/dacf/php/caps/EAB/EABreportFORM.shtml Or search online for "Maine Emerald Ash Borer (EAB) Report Form"*****

Emerald ash borer (EAB) present in surrounding area: Yes_____ No _____

Signs of EAB present on collection tree (check all that apply):

_____ epicormic sprouting _____ canopy thinning _____ blonding _____ 'D' shaped exit holes

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

Site type: ___ upland __ wetland __aquatic.

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

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

Figure 6. Bottom half of the NSL Ash Seed Collection Data Sheet.

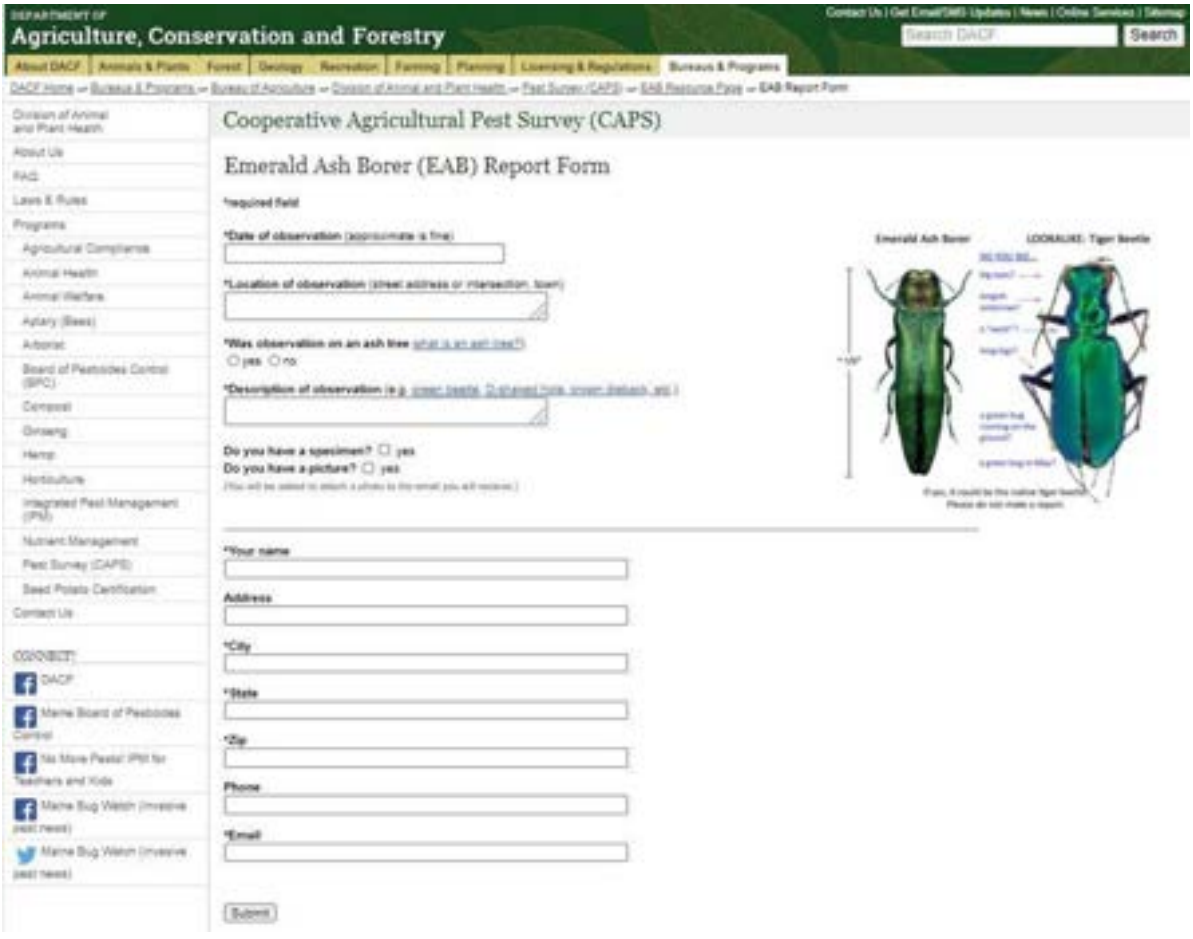


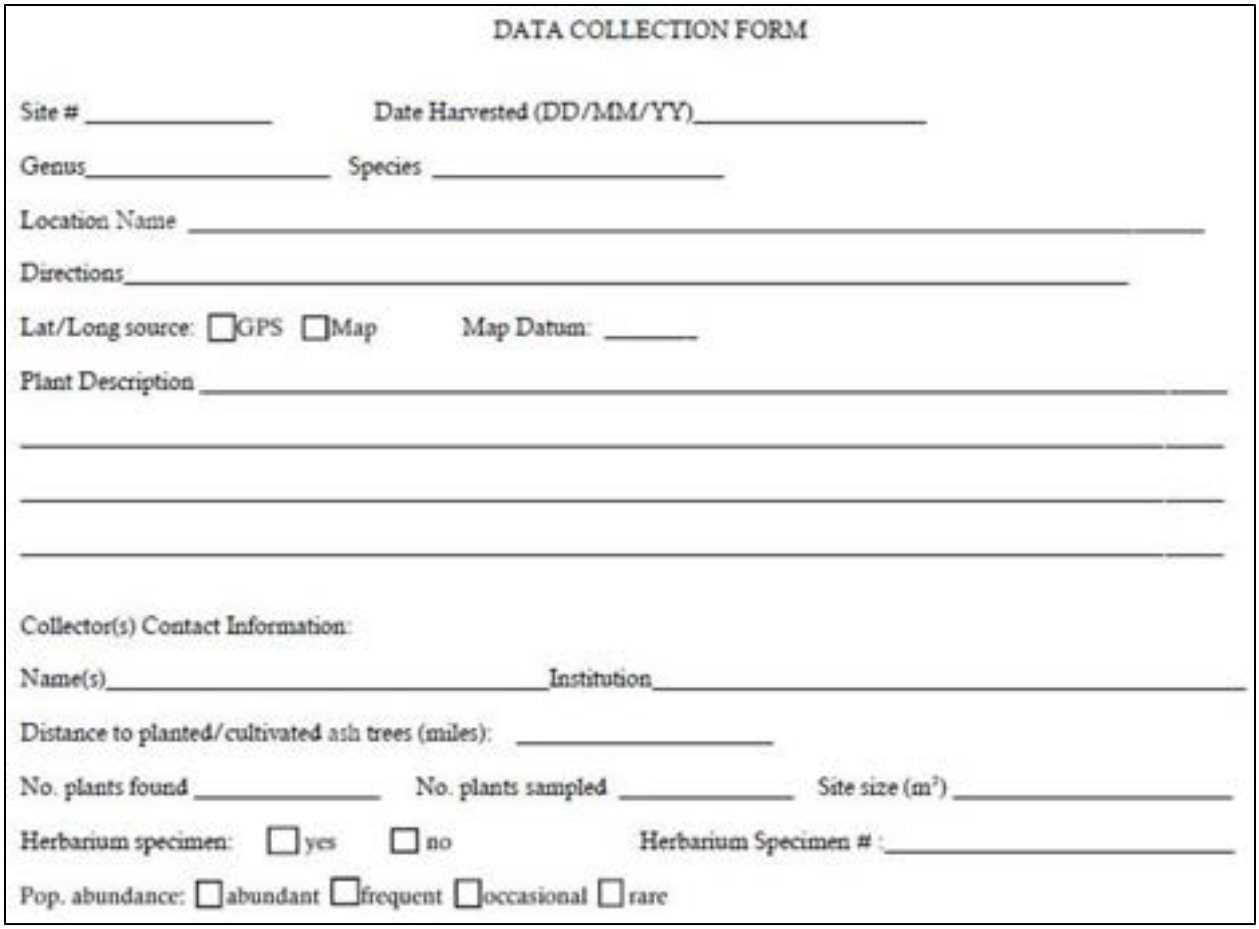
Figure 7. Maine DACF Cooperative Agricultural Pest Survey website for reporting suspected EAB (Maine Department of Agriculture, Conservation and Forestry 2020).

The next three questions on the sheet involve the soil, site type, and topography around the sample tree. Is the soil around the tree: rocky, gravel, sand, loam, or clay? Is the site surrounding the tree: upland, wetland, or aquatic? If the site is upland, is the tree on flat ground or on a slope, and what is the cardinal direction aspect of the slope (North, South, East or West)?

Before leaving the site, make sure to take photographs of the sample tree, including identification in the image that corresponds with the seed sample and collection data sheet. Photographs should include images of the tree, pictures of the crown (the top of the tree), bark, seeds, and leaves. A voucher specimen (e.g., leaf or twig) should be documented in a photograph, if possible. A 6-to-9-inch twig should be included in the collection bag, as well for further tree identification. Mark the data sheet that photographs have been taken and a twig sample has been included in the collection bag.

National Plant Germplasm Facility Data Sheet

Please use the National Plant Germplasm Facility data sheet and Definition sheet, both found in the Appendix B, as a reference while reading this section. **The main difference in the collection sheets is that the NPFG sheet can be used for multiple tree samples, whereas the National Seed Lab sheet must be filled out for each individual sample.** The NPFG data sheet is also less specific to collecting ash, but it is equally important to put as much information as you can. Starting at the top with Site # (Figure 8), this is assigned by yourself as the collector. It is important to have some code that you understand to differentiate where you collect. The date of collection is required in the format of DD/MM/YY.



DATA COLLECTION FORM

Site # _____ Date Harvested (DD/MM/YY)_____

Genus_____ Species _____

Location Name _____

Directions_____

Lat/Long source: ☐GPS ☐Map Map Datum: _____

Plant Description _____

_____

_____

_____

Collector(s) Contact Information:

Name(s)_____Institution_____

Distance to planted/cultivated ash trees (miles): _____

No. plants found _____ No. plants sampled _____ Site size (m^2) _____

Herbarium specimen: ☐yes ☐no Herbarium Specimen # :_____

Pop. abundance: ☐abundant ☐frequent ☐occasional ☐rare

Figure 8. Top half of the NPGF Data Collection Form.

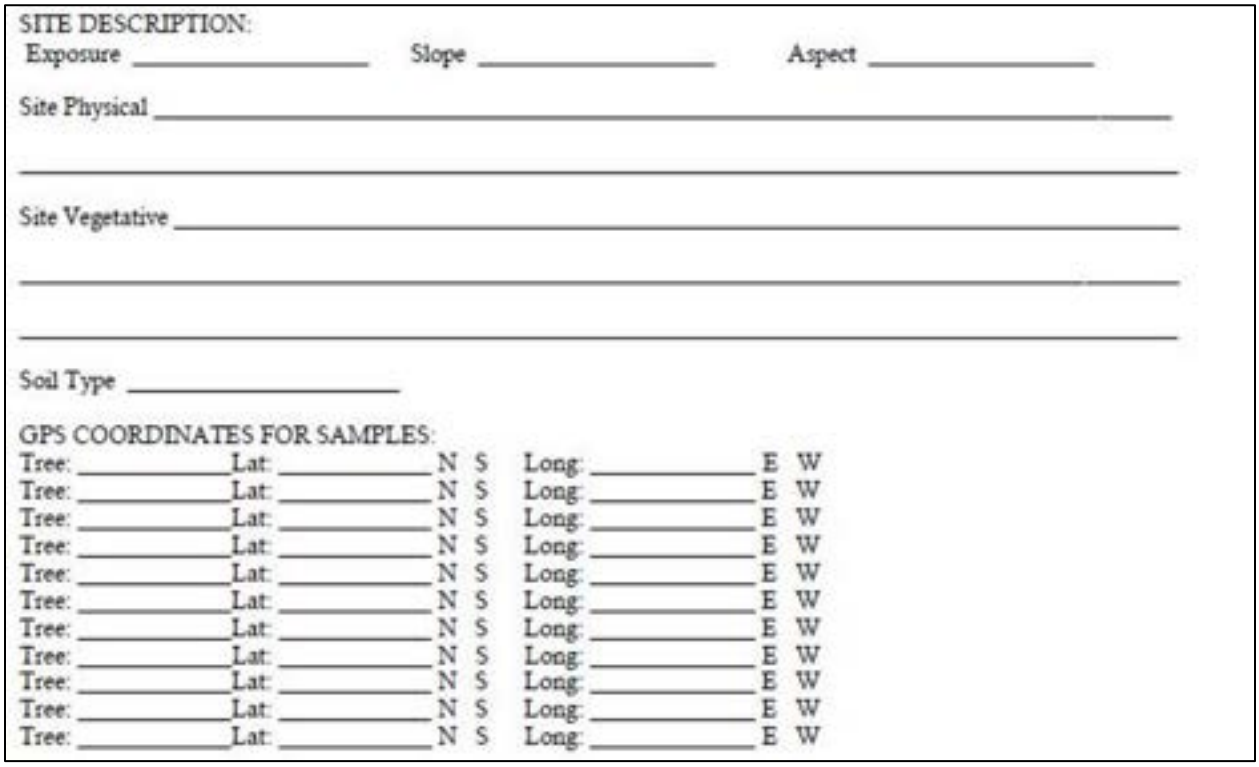
As you are collecting ash the genus will be *Fraxinus* and the species will be *nigra*, if collecting brown ash, *pennsylvanica*, for green ash, and *americana*, for white ash. Location name is where you are collecting from. If it is from your woodlot, you can label this as "private woodlot in (Town name)". Directions can be an address and description of the location, for example, the address to your woodlot and its distance from a landmark like a town or body of water. The seed collection kits recommend a GPS unit to collect the latitude and longitude, but if you are using a map, you will mark the appropriate answer in the Lat/Long Source section. The Map Datum can be found in your GPS unit. If you are using a map, this does not apply. The Plant Description section is used to describe the tree that you have collected from. Are there

any characteristics worth noting? Is there visual dieback in the canopy? Blonding in the bark? This is a place to make notes on the specific tree you are collecting from.

The next section is where you identify yourself as the collection in Collector(s) Contact Information. Please include the names of those collecting and the institution you are associated with. If you do not have an institution, you can list yourself as a private landowner, or whatever applies to you. The “Distance to planted/cultivated ash trees (miles)” is asking how far your sample is to ash trees that are planted or cultivated. As we are looking for wild ash trees, rather than planted trees from nursery, if you know how far your sample is from a planted or cultivated ash tree, please include that information. Number of plants (ash trees) found and number sampled is asking how many ash trees are in the general location you are sampling in and how many did you sample. The site size in meters squared is requested, which can be calculated using your GPS unit. Herbarium specimens are not necessary for collecting, but this would include leaf samples with the seed samples. Population abundance is asking how many ash are in the area. Are they abundant (do you see a lot of them?), frequent (do you see some of them?), occasional (do you have to seek them out), rare (are they hard to find?).

The Site Description section allows you to provide information about the area that you have sampled ash trees (Figure 9). Exposure is asking if the ash trees are in full sun or shade during the day, whereas Slope is the percentage referring to the grade of slope where the tree being collected from is located. The Aspect is the direction that the slope is facing in: north, south, east or west. The Site Physical is a description of the surroundings to the site you are collecting from. This can be the type of habitat the sample is in, like a wetland or an open field. The Site Vegetation is requesting you to list what other plants are growing in the immediate

area of the site. If you can, identify species of trees, shrubs or grasses around the site. Soil Type is also asking for a description of the soil around the sampled tree.


SITE DESCRIPTION:
Exposure _____ Slope _____ Aspect _____

Site Physical _____

_____

Site Vegetative _____

_____

_____

Soil Type _____

GPS COORDINATES FOR SAMPLES:

Tree: _____ Lat: _____ N S Long: _____ E W
Tree: _____ Lat: _____ N S Long: _____ E W
Tree: _____ Lat: _____ N S Long: _____ E W
Tree: _____ Lat: _____ N S Long: _____ E W
Tree: _____ Lat: _____ N S Long: _____ E W
Tree: _____ Lat: _____ N S Long: _____ E W
Tree: _____ Lat: _____ N S Long: _____ E W
Tree: _____ Lat: _____ N S Long: _____ E W
Tree: _____ Lat: _____ N S Long: _____ E W
Tree: _____ Lat: _____ N S Long: _____ E W

Figure 9. Bottom half of the NPGF Data Collection Form.

The last section on the NPGF data sheet is the GPS Coordinates for Samples. This is where your number or code is the individual tree that you have collected from is entered. These numbers are very important because they will be written on the corresponding seed bag in black permanent marker for storage. The latitude and longitude that you have taken from your GPS for each sampled tree must be listed with the tree number or code.

Seed collection techniques

Ash trees can vary in height, branch distance from the ground, and surrounding environment, which can cause different options for collection to be preferable to others. Some ash trees may not be reachable with a pruning pole, the preferred method in this case is

explained in next section or may require more pruning pole extensions than what we have suggested in the Ash Seed Collection Kit found in the appendix. This is important when scouting for ash to consider how you plan to reach seed to harvest and plan accordingly. Anecdotally, trees on roadsides and fields may be easier to reach with pruning poles, than those located in the interior of the forest. This is because trees growing in the interior of the forest structure their canopy to best compete with adjacent forest trees for sunlight, which places their crown high above the forest floor. Making notes in the scouting stage may make it simpler in the collection stage and ensure you have the proper equipment on hand for collections at certain sites.

Pole pruners

Pole pruners are useful for seeded branches up to 30 feet from the ground, see Figure 10 (Knight 2010). Caution should be taken for using pole pruners, as they can be unwieldy when extended from the operator and cut branches can be dangerous falling to the ground. Safety precautions should be taken with safety glasses and hardhats. It is recommended that those using the pole pruners protect themselves and their heads while collecting. Never use pole pruners near power lines.

Figure 10. Saint Regis Mohawk Tribal foresters using pruning poles to collect brown ash seeds. Photograph by Emily Francis.

Becoming familiar with individual pole pruners before use is important, as each type of pole pruner has specifications of maximum branch diameter it can cut, maximum height it can reach, etc. Knowledge of these specifications will indicate what is appropriate for cutting. When a branch has been chosen for pruning, a tarp should be placed under where the branch will fall. This is to collect seeds falling during the pruning process and for cleaner sorting after the branch has fallen. It will also collect seeds that may fall from other branches during cutting.

There are different kinds of pruning heads available, and it is important that you are familiar with and understand how the pruning head you are going to use works. We suggest

searching the type of pruning head you have on YouTube for videos giving directions on your specific equipment. Essentially, with the pruning head suggested in our collecting kit, see Figure 11, the cutting head of the pole pruner should be rested on a cross branch while preparing to cut. The rope should be clear of other branches, so as not to get caught while sawing. Depending on the height of the pole pruner, it may be easiest to place the end of the pole in the instep of the operator's boot to guide the cutting without holding the pole above the ground. Cutting may be a two-person job, depending on the height of the branch and the length of the pruning pole. One person should focus on holding the pole and another on using the rope to saw the branch. Care must be taken when the branch dislodges from the tree, as the branch is going to fall straight to the ground, depending on wind, most likely at those operating the pole pruners. Some pole pruners have hooks under the cutting head that will attach and lower the cut branch to the ground. This can be useful to ensure the least number of seeds become loose from the branch, and a greater collection can be made.

Figure 11. Example of the Jameson PH-11 Tree Trimmer Head (Jameson n.d.)

Loppers or handheld branch cutters may be helpful to clear the area around an ash tree for collection, as some areas can be thick with foliage and brush around a tree. It is important to be able to have space for the seeds and branches to fall onto the tarp, which may require clearing up to 8 or more feet around a particular tree. Permission to harvest is especially important if brush needs to be cleared before cutting branches for seed.

Rope and weight - "Tarp shaking"

This method uses ropes and weights to bend branches within reach of a pole pruner or to break a branch. Knight (2010) notes that, "in our experience, black [brown] ash branches are easiest to break," of all the ash tree species (pg. 7). Therefore, this method may be used for branch breaking, rather than to lower a branch for pole pruning.

A large tarp, rope, pole pruner (if branch does not break), and a lightweight throw bag and throw rope. These weights are usually between 12 to 16 ounces. The US Forest Service suggests the Big Shot® slingshot made by Sherrill Tree (Knight 2010 pg. 7). This device can throw a 14 ounce throw bag over 80 foot high branches and does require some practice before use. The US Forest Service has provided a video showing the use of this equipment, titled "Ash Collection Video (action of using the big shot sling shot and more)" at the following web address: https://www.fs.usda.gov/nsl/GeneticConservation_Ash.html.

It is possible to use this method without breaking or cutting a branch, but rather shaking a branch to dislodge seeds. That is why this method is also called the "tarp shaking" method. Throwing or shooting a weight over a branch and shaking for seeds can be difficult, especially on a windy day when the seeds may not fall directly beneath the branch and onto the tarp. Caution must be exercised for this method to avoid trees near power lines and for the

operators to wear glasses and hard hats. As Knight mentions, “a 400-g (14oz) throw bag shot at 24 m (80ft) into the air will hit the ground with a velocity of 22 m/s (49 mph)”, therefore, it is important for all involved to pay attention (p. 9)

Pocket rope saw

This method is particularly useful for smaller branches under ¾ of an inch in diameter (Knight 2010). Like other methods, a tarp should be placed under the chosen branch and a rope with weight or shot with a slingshot is sent over the branch. Once the pocket rope saw, see Figure 12, is positioned over the branch, both sides of the rope are pulled back and forth to cut through the chosen branch. Knight states that, “some ash trees have flexible limbs, [therefore] it is necessary to cut larger branches on these trees. One or two people can operate the saw… Steeply angled branches should be avoided because the saw will slide down the branch. Sharp crotches should also be avoided because the saw or knots can become stuck on them” (pg. 10). The US Forest Service suggests the Pocket Chainsaw® by Supreme Products. Once again, trees and branches near power lines should be avoided. Hard hats and safety glasses should be worn by all participants in the seed collection process in the field. Falling branches are unpredictable and can be dangerous.

Figure 12. Example of a rope saw provided by US Forest Service (Mason 2010).

By hand or tarp basket method

Depending on the height of the branches, it may be possible to collect seeds by hand. As mentioned previously, a sample from one tree should equate to filling the bottom of a paper bag at least 3 inches. This is just a suggestion, as the amount required for submitting seed will depend on where you are sending seeds, see section Post-harvest Seed Handling and Processing for more information. This method can be used to supplement other methods, or by itself, however, it should result in a full sample. A tarp should be placed under the tree where the collection is occurring to collect the maximum number of seeds, which may fall while seeds are being collected. Pruning clippers can be used to cut the lower branches within reach. Depending on the size of the tree, it can also be shaken to dislodge seeds, as well.

It is also possible to take a canvas tarp, making sure it has a drainage hole, and tying the four corners beneath an ash tree. The tarp must not be in the way of someone walking into it, animals getting caught in it, or rainwater to pool in the tarp. Make sure to choose a "good

seed-bearing tree", which will drop seeds into the tarp basket. This should be checked daily to make sure that other kinds of seed are not mixing with the ash seeds (Benedict & David 2003).

Post-harvest Seed Handling and Processing

There are different locations to send seed after processing it, and how much the seed will be processed will affect where it can be sent for storage. Each storage facility also has different potential uses for seed, like who can request it for research or how it is used. Deciding where you want to send what you have collected also depends on how much seed you collect. For that reason, the three locations we suggest seed to be sent will be overviewed by the processing requirements. From there, the requirements for shipping to seed storage facilities will be reviewed. Online locations for access to documents in the appendices can be found in Appendix D.

Deciding which of the three locations that we recommend sending seed to, must be considered early in the collection process, as the type of data sheet used in the field during collection will be dictated by this decision. The flowchart, "Deciding where to send ash seed", breaks down each section in the decision-making process: level of sorting, locations for storage, data sheet type, and required seed quantities (Figure 13). First on the flowchart, level of cleaning, highlights two options: simple cleaning of seeds and complete sorting and cleaning of seeds. Each of these processes will be reviewed in the next section. The simple cleaning of seeds can be submitted to the University of Maine and the USDA Agricultural Research Station (ARS) in Iowa. A complete sorting and cleaning of seeds is required to submit seed to the USDA ARS facility in Colorado, which is also called the "Black Box". The University of Maine and the

USDA ARS Colorado accept either the NSL or NGFP data sheet, and the USDA ARS Iowa requires the NGFP data sheet. These data sheets are overviewed in the "Documentation for trees and seed" section above, and copies of them can be found in the appendices. USDA ARS Colorado also requires the Black Box Template, which is a Microsoft Excel form to be discussed in the "Shipping for seed storage" section below. The University of Maine requests at least 1,000 seeds per mother tree (Figure 13). The USDA ARS "Black Box" does not have a required number of seed as it is specific to the sender and project submitting seed to the facility. The USDA ARS in Iowa requires at least 1,000 seeds but recommends 3,000 to 5,000 seeds per mother tree (Figure 13). Each seed storage facility has different focuses for storage and abilities for seed removal, or the ability for someone to request seeds from the facility, for research or propagation. Figure 14 highlights the differences between the locations for more information on deciding where to send seed.

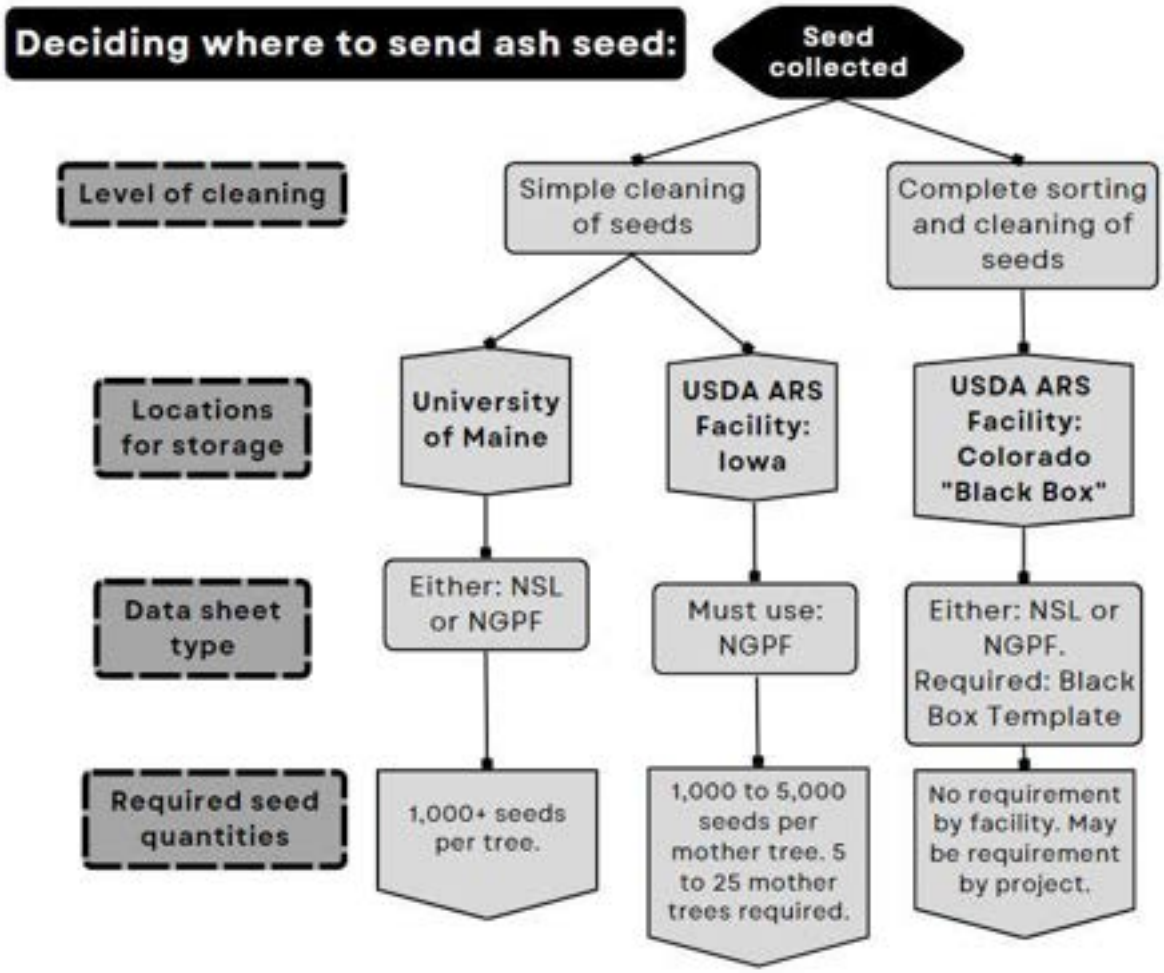


Figure 13. "Deciding where to send ash seed flowchart" identifies the aspects of seed preparation for each facility.

Storage facility: University of Maine, APCAW

The University of Maine is just starting a seed program and currently is not a long-term storage facility. There are plans to create a network with the Wabanaki Tribal Nations in present day Maine to hold seed for them, and when designated as such that seed may be used for University of Maine led research projects. Seed donation to the university will allow seed to be used for Tribal use or research and seed may be send to another seed facility after cleaning for long term storage. The capacity for storage is small currently, but scaling up to larger storage is the goal, as well as a potential brown ash tree nursery for all of our Tribal partners,

and to propagate trees for other planting purposes. Therefore, the quantity required, see Figure 14, is set at least 1,000 seeds per mother tree, which is around 1.5 liters of seed. This is not a strict requirement, but enough to provide sufficient seed for testing viability before storage.

Storage facility: USDA ARS - Iowa

The USDA ARS Iowa facility is where the National Plant Germplasm Ash Conservation Project is located, and any seed sent here will be housed under this research group. Seed sent here must be simple cleaned, as the researchers will sort and store the seeds thoroughly, checking for viability for future growth and the overall health and quality of the seed. This facility requires as much seed as you can, at least 1,000 seeds per mother tree, but preferably between 3,000 to 5,000 seeds, see Figure 4. This is almost 2 liters of seed for brown ash (USDA: Agricultural Research Service 2018a). It is possible that the Iowa facility may choose to store high quality seeds at the Black Box, as well as their own, and the final storage location of seed can be seen on the Germplasm Resources Information Network, GRINs (USDA: Agricultural Research Service 2018b). Seed stored in or by USDA ARS Iowa can be requested back to the collector, however, any researcher can request seed from here and your seed may be provided to another requestor without your knowledge. The seed submitted here will be added to the GRINs database, although this may be negotiable for tribal member's privacy of brown ash stand locations for basket harvesting.

Storage facility: USDA ARS - Colorado

The final location is the USDA ARS Colorado Black Box, which is the Agricultural Genetic Resources Preservation Research center. At this location seed is submitted to be held in deep

freeze for long-term storage. We recommend seed to be sent for Deposit for Security Duplicate Collections, which is the formal name for the Black Box. This depository receives a box of seed that must be completely sorted and cleaned for long-term storage. The box received by the facility is simply placed in cold storage without any further action. Therefore, seed must be completely ready for long-term storage when shipped and the box must be prepared for cold storage. Finally, the sender of the seeds may request the box back at any time, which will be removed from storage and mailed back. This is a great resource for researchers without the capacity to store seeds themselves and would like to take advantage of the security of a federal cold storage facility. There is no requirement for the number of seed to be stored, due to the seed being unopened and kept until it is requested back by the sender, therefore, it is up to the sender to decide what the purpose of the storage is, what type of research, and the needs for that in the long-term, see Figure 14.

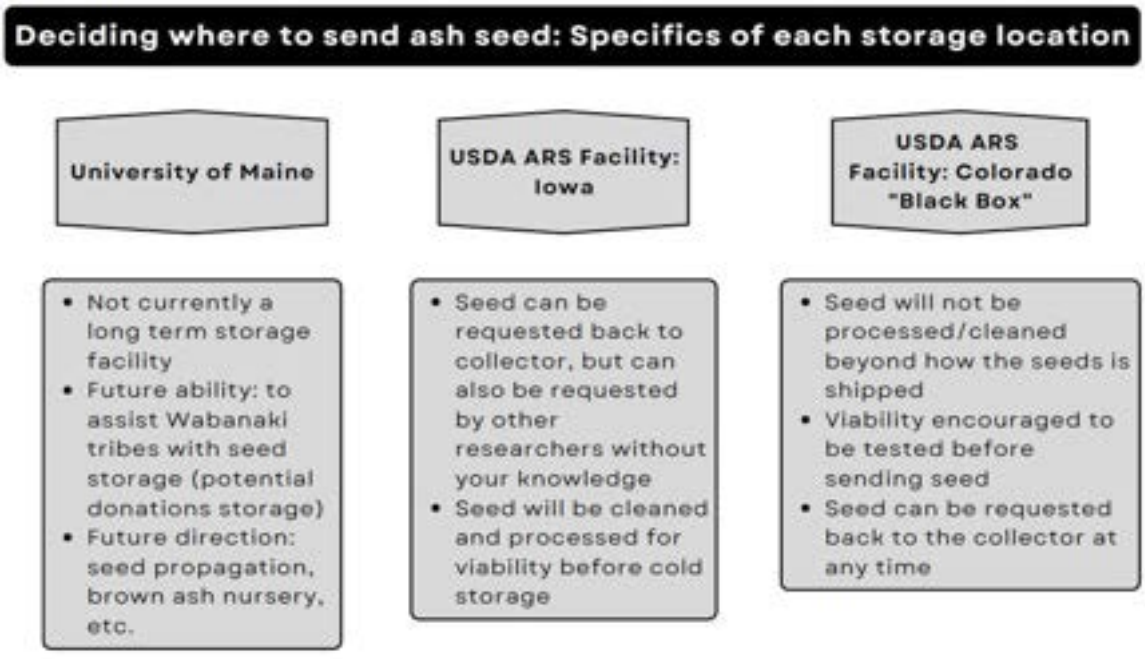


Figure 14. Highlights for each of the recommended ash seed storage locations.

Seed cleaning and sorting

This classification of seed cleaning was created for this document to provide a simplified description of the process. Three types of seed cleaning that we have distinguished in this document for ease of explanation and for final storage options are as follows: simple cleaning of seeds and complete sorting and cleaning of seeds. Figure 13 dictates which facilities will accept which level of sorting and cleaning: the University of Maine will accept limited and simple cleaning of seeds; USDA ARS Iowa accepts simple cleaning and USDA ARS Colorado accepts complete sorting and cleaning.

Simple cleaning: this has been partially modified but is what is recommended by the USDA ARS Iowa facility for submission. It is very important to limit the amount of moisture that the seeds encounter during collection, post-harvest handling, and final deposition of seeds in storage or for research, as seeds can rot and be ruined. During the entire time that seed is collected, all of the bags that contain seed need to be kept between 65- and 70-degrees F, this includes the vehicle that the seed is being transported in (USDA: Agricultural Research Service, 2018b). It is extremely important not to overheat the collection before it has been fully processed for storage. It is best to find an area to work in with good ventilation and where insects, which may be in or among the seeds, can get out, like a garage. Hotel rooms and the backs of cars are not good places for this work. It is best to secure a workspace where the seed can be spread out on a table or in a tub. Each bag needs to be opened, but not ripped, it will need to be stapled shut again, and the seed spread to where it can be looked over. Now when we say seed, what we really mean is the samara, which is the case that the seed is inside of, see Figure 1. The samara needs to be removed from the twig that it is attached to and placed back

into the paper bag. When looking over the samaras on the table, please remove any woody debris, like larger sticks, twigs, leaves, grass, or anything other than samaras that may have gotten into the bag in the field. Leaving leaves in the bag can increase the moisture content of the samaras while the seeds are being transported and must be removed. If the samaras are wet, like if they were collected in the early morning, they must be left out to be completely dry before putting them back into the paper bags and sending them to the facility. This may take 24 hours or more depending on how green the seeds are when collected (USDA: Agricultural Research Service, 2018b). If samaras look like they might have insect damage or mold, they must not be submitted to a facility. Remove the samaras and dispose with the rest of the non-samara material. Once all the woody debris has been removed, the samaras must be placed back in their original bags with corresponding datasheets, with the bags stapled closed. Then the bags need to be prepared for packaging and shipping to the storage facility. Bags going to the University of Maine, may be dropped off at the facility and instructions for this can be found in the next section.

Complete sorting and cleaning of seeds: this is what is required to send seed to the USDA ARS Colorado facility. This location requires the seed to be ready for cold storage upon arrival, and therefore must be completely prepared by the sender beforehand. The instructions provided for the submission of seed to the USDA ARS Colorado facility can be found in the following document, which is included in the Appendix C, “Recommendations for the collections, storage, and germination of ash (*Fraxinus spp.*) seed” (Ellis 2006). This document outlines how to collect, sort and test seed for viability before sending to the facility and should be followed as closely as possible for the best results. Please focus on section “Collecting seeds

(samaras)" starting at number 12, through to "Testing ash seed (samara) viability" section 1. Instructions for submitting seed to this facility can be found in the next section.

Packaging and shipping seeds for storage at chosen facility

As for packaging collected seeds, each facility has specific criteria for shipping seeds. Seeds can be brought to the University of Maine in-person to Nutting Hall and the School of Forest Resources administrative office, or to the drop-off bin outside of Room 118 on the first floor. Materials must be in paper bags with a completed datasheet stapled to each bag with information regarding who collected the seed, who dropped off the seed, a phone number and email address to get in contact with the collector for questions, and to what level the seed was sorted. Please contact John Daigle, PhD via email, jdaigle@maine.edu, to let the team know you have left seed. If you are dropping off many bags of seed, please contact John Daigle before dropping off, so someone can meet you and receive the bags. To ship seeds to the University of Maine, please use the address found in Table 3. Seed must be placed in a cardboard box that will fit all of the paper bags and shipped with the proper datasheets and paperwork as recommended for dropping off in person. Ground transportation shipping is recommended, as shipping via airline can affect the moisture content of the seeds.

To send seed to the USDA ARS facility in Iowa, you will need to get in touch with Jeff Carstens, PhD, the contact at the facility. It is recommended to ship via FedEx, which the USDA ARS will be able to cover the cost of, otherwise, you can ship via the US Postal Service. Please choose ground transportation shipping to prevent changes in moisture content of your package, which can happen with airline shipping. Seeds must be placed in properly marked paper bags with corresponding datasheets and securely stapled shut to prevent seeds from

coming out of the bags and mixing in the box. Please see chart below for addresses for shipping, which depend on which carrier you are choosing to use.

The USDA ARS Colorado “Black Box” facility does not open the box that seed is shipped in and has a different shipping procedure. The contact for this facility is NLGRP-Blackbox@usda.gov, and you will need to notify this email address before shipping that you intend to send seed. You will need to fill out, sign and submit a Material Transfer Agreement (MTA), which can be found in the Appendix of this document. By filling out the MTA, you will be provided with a Black Box MTA#, which will be required on your documents going forward. The MTA requires a Microsoft Excel spreadsheet to be included, which is called the NLGRP Black Box Deposit Template, also in the Appendix. This spreadsheet will identify the taxon, Inventory Identifier, cultivar of the species, along with other information that gives the facility the information of what is being shipped to them. The Instructions tab of the worksheet explains what each of the fields mean and how to fill out the worksheet on the Blackbox Shipment tab. The Blackbox Shipment tab must be filled out and included within each box for each shipment, as well as the MTA and submitted to the facility before shipment. After sending your first shipment, all other shipments must include the Letter of Transmittal, see Appendix. Your MTA# covers subsequent future shipments, so this entire document only needs to be filled out the first time, the Letter of Transmittal and the Black Box Deposit Template must be included in every shipment. Packages must be sent under expedited shipping services.

As it has been mentioned, seeds submitted here must be completely sorted, dried and ready for cold storage. The boxes shipped cannot be larger than 86 cm wide by 75 cm deep by

55 cm high, as this is the size of the space they will be held in. Each bag of seed must be in "moisture proof packaging such a heat-sealed foil laminate pouches'' and labeled with the sender's information, MTA# and name of the species within. Boxes must be labeled according to the number total boxes shipped, example: Box 1 of 3, etc. Finally, the shipment information must be emailed to the contact email: NLGRP-Blackbox@usda.gov. Further questions regarding this process may be answered on the website of the USDA ARS Colorado, along with access to all documents mentioned: https://www.ars.usda.gov/plains-area/fort-collins-co/center-for-agricultural-resources-research/paagrpru/docs/plants/pages/deposit-germplasm/, under "Deposit of Security Duplicate Collections (Black Box Storage)".

Addresses for shipping seed to each location:		
University of Maine	**USDA ARS: Iowa**	**USDA ARS: Colorado**
School of Forest Resources Attn: Ash Protection Collaboration Across Wabanakik (APCAW) 5755 Nutting Hall, Room 118 Orono, ME 04469-5755	*FedEX (contact Jeff first):* USDA-ARS North Central Regional Plant Introduction Station Attn: Jeff Carstens 1305 State Avenue Ames, IA 50014 *USPS:* USDA-ARS North Central Regional Plant Introduction Station Attn: Jeff Carstens Iowa State University G212 Agronomy Hall Ames, IA 50011-1170	USDA, ARS, NLGRP Attn: Black Box Storage 1111 South Mason Street Fort Collins, CO 80521

Table 3. Addresses of seed storage locations for shipping of seed.

Future Uses of Seeds

There are many uses for ash seed and there are several organizations getting involved with this effort. To briefly discuss the three main options of seed usage, we will overview the

following: conservation of seed for storage (both long and short term), collecting seed for propagation, and collecting seed for scientific research. In one event of seed collecting, there could be multiple uses, and often this is the case with researchers. All the reasons discussed here for collecting ash seed are important, and there are more beyond these. For the brevity of this document, this is just a few reasons or projects.

Seed storage, propagation, and scientific research

Conservation of seed can happen for several reasons. Short term storage of seed may be for holding seed until a research project is ready to use the seed, or for the season to be right to plant the seed, or for a project that might not yet be decided upon. We would categorize short term storage as: months to a few years. Most seed is stored in cold storage, and smaller facilities even use home refrigerators. The health and viability, or ability for the seed to be successfully stored for future planting, depends on how the seed was prepared for storage and the capabilities of the refrigerator or freezer used to store the seed. We hope to provide more information on this type of storage process in the future. Long-term storage is another option to hold seed in freezers under specific conditions for an extended period. This storage could be for years with the correct conditions. This kind of storage can be used to hold genetic material for long periods of time to protect against threats, like EAB, with the hope that there may be a more favorable time to repopulate the species. There is also holding the genetic material for a record of what is available now.

Collecting seed for propagation means the seed is intended to produce seedling stock that can then be planted in the forest at suitable sites. There are many preservation goals that drive these efforts; brown ash is culturally significant, has ecological values, and economic

values to Tribal artisans. Because of these values there has been a continued interest in ensuring that there will be brown ash on the landscape for generations to come. The planted cohorts of brown ash in these forests are ensuring the critical continued recruitment of brown ash in the overstory of these forests. The propagation and development of seedling stock can be achieved in the following ways: individuals can grow their own seedling stock at home, if your community has one established, a Tribal nursery can grow this seedling stock for their community and forests, nonprofits and environmental groups can grow seedling stock for reforestation efforts, and the research community can produce seedling stock for use in management trials to improve our knowledge base on this strategy in the face of EAB. There are even situations where that suitable site is not in the forest but in a controlled environment for ease of access for seed collection, these orchard-like settings serve the purpose of a living seed bank, and this is something already being practiced by the Saint Regis Mohawk Tribe at Akwesasne. There are many reasons for propagating and growing ash trees from seed and it is all made possible with collection of seed right now.

Finally, collecting brown ash seed for scientific research. All the above strategies could combine with research regarding brown ash seeds, however, there are those who collect solely for research purposes. Two scientific projects will be overviewed to provide insight into the type of research that utilizes ash seeds. The first examined the effect of insecticides used on ash trees to prevent EAB and what happens to the native weevils who use ash seeds to lay their eggs (Mwangola et al. 2022). Ash seeds were collected from trees that were treated with insecticides for EAB and compared with trees that were not treated to see which trees had less weevils in their seeds. This is one kind of research that requires ash seeds to be collected. It

should be noted that according to this study, trees that were treated had fewer weevils, meaning that more seed was available to potentially produce new ash trees (Mwangola et al. 2022). This is a rare positive outcome of non-target impacts from insecticide treatments.

A second research project that focuses on ash seed collection, looked specifically at collections from lingering ash trees, or trees that have survived the initial wave of EAB. The researchers state on their website that they are "targeting seed collections from areas in which the emerald ash borer was detected in 2010 or earlier, since those areas have likely already seen widespread tree death," (Holden Forest & Garden 2022). Ash trees that are still producing seed in the Great Lakes region survived the initial wave of EAB and have been living with the insect present since. It is essential to collect seed anytime trees produce them in this area, as any genetic information can greatly inform science on what might be the key to EAB resistance for this species. This is an excellent example of collecting seed for genetic research. More information about this research can be found at: https://holdenfg.org/news/ash-trees-are-exploding-with-seeds-researchers-are-capitalizing-on-it/.

For more information regarding the collection and storing of ash seed, we encourage you to visit the USDA Ash Conservation Research webpage (https://www.ars.usda.gov/midwest-area/ames/plant-introduction-research/home/npgs-ash-conservation-project), hosted by the Plant Introduction Research program in Ames, Iowa. This is a great location for more information on the National Plant Germplasm System and introduces the Germplasm Resources Information Network, or GRINS, which is the federal database that holds information on seed stored at federal facilities.

Ash Foliage Collection: Pennsylvania State University partnered research

To assist other research projects involving the protection of ash trees in the Northeast, our team at the University of Maine has agreed to help with Dr. Jill Hamilton's collection of ash leaves at Penn State. The goal of this research is to collect leaves from many ash trees across Maine, to capture a wide range of genetic variation, of which the research team can explore key genomic differences. The protocol that Dr. Hamilton has provided for this collection can be found in the appendix. If you are interested in collecting ash foliage, please use the contact information in that section to receive the materials needed to harvest and ship samples.

Conclusion

This document was created to assist in the collection of brown ash seed against the threat of EAB, which is spreading throughout the state of Maine and beyond. Efforts to save this great tree species need to be made by all of those interested in its survival. A call for direction on how to collect brown ash seed was made by that community, specifically the Tribal artisan community, enabling this document to be written. This document is meant to be a living one where it can be updated as new information regarding seed collection, storage, and uses for seed development. At the time of its first publicly released version, it has been written and reviewed by the University of Maine APCAW team in the School of Forest Resources, as well as key partners in the US Forest Service, tribal basket makers in Wabanaki Tribal Nations, researchers at the University of Vermont, non-profit partners at Wild Seed Project, and others. Great effort has been made to include those interested and invested in protecting ash, on the landscape and its deep cultural importance, in the creation of this document. All reviews and input by our network have been taken into consideration to ensure the information provided

here is accurate, useful, and done with care and dedication to the task of protecting ash for the future. Updates to this document and inquiries regarding this project can be found at **www.umaine/apcaw/**. Thank you for your time and interest in this very important project.

If you interested in allowing access for seed collection on your property, please contact APCAW. We are interested in connecting those interested in seed collecting with places to access ash to increase the availability of genetic material being harvested. We are greatly appreciative of those willing to allow for seed collection for the many reasons that have been mentioned in above.

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Appendix A: Ash Collection Kit and Identification Information

Ash Seed Collection Kit: Recommended Checklist

This checklist was developed for a small group of two to four people to work together on the two stages of seed collection: scouting a forest stand for ash and collecting the seed. The quantities suggested for this checklist reflect what can fit in about one large plastic tub, other than the pruning poles, to be taken to the field for scouting trees and collecting seed. Cost and quantity of items will vary depending on the number of people collecting, the quantity of seed collected, and quality of certain items. For items that are more expensive, like the GPS units, we have provided a suggestion of the model, but that is not required. Particular information regarding certain items are explained here below. Please note that items or companies referred to on this list are not endorsed by the University of Maine School of Forest Resources or APCAW.

GPS unit: For a Global Positioning Systems unit we recommend a Garmin eTrex 10. This is an easy-to-use GPS unit that is great for all range of users, particularly beginners. While a GPS unit is not required, it provides the best data for seed collectors to return to ash tree locations for collection and for researchers to spatially analyze the data of ash tree location. In 2021, these cost about $110.

Binoculars: The Nikon Trailblazer 8×25 ATB binoculars are lightweight and small for easy packing and use. When these were selected, they came with a case and cleaning kit for about $90. Binoculars are important to be able to identify the sex of an ash tree from the ground in springtime, as well as to see seed throughout the summer and fall. Decent binoculars are essential for seed scouting and collecting.

Flagging tape, aluminum tags, roofing nails, and hammer/mallet/hatchet: All of these items can be purchased online from a forestry supplier or hardware store. It is important to know what colors of flagging tape are already being used in the forest, as they can mean different things to different people. If you have access to scouting and collecting on land that you do not own, it is best to find out what color flagging may already be used in the forest and discuss what color you plan to use before going into the woods. It is important to choose a color that you and your collecting team can see well. We suggest putting a piece of this flagging tape on equipment you bring in the woods, as the bright color can help you find dropped or misplaced items. Aluminum tags should be large enough to mark them if needed and the roofing nails that can be pounded through to secure to a tree. A hammer, mallet or hatchet can be used to place these.

State gazetteer or paper map: The Maine Atlas and Gazetteer, originally produced by DeLorme, now Garmin and published by Rand McNally, are about $25. These are used widely by locals and travelers alike and provide excellent maps for both driving and topographic backcountry uses for those scouting ash on unknown forests. It is very important to have paper maps when in the forest, as Maine is very rural and service for cell phones is not guaranteed.

Safety vests: These do not need to be expensive and can be purchased relatively cheap. Forestry safety vests are specialized to have pockets on the front and back and can be used to bring items into the forest without needing a backpack but can be upwards of $80 each. Regardless of price, it is important to have bright safety gear all times of the year, especially in the fall when it could be close to hunting season. Always wear bright colors for safety, as you

can be seen by the rest of your team or safety personnel if there was an emergency or you got lost.

Backpack: If you do not have a safety vest with pockets, a backpack may be important to carry smaller items, like flagging tape, clipboards, pens, and pencils. This does not need to be expensive or kept specifically with your seed collecting kit. It is your preference to how you want to carry your materials into the forest and keep yourself organized.

Pruning pole (head, adapter, extensions): These items were recommended to us by experienced seed collectors and are at the high end of quality and were purchased by our team from a forestry supplier store. The pruning head was a Jameson PH-11 Tree Trimmer Head, which was about $51. The adapter, which fits the pruning head to the extension pole, is specific to the Jameson PH-11 and costs about $17. The extension poles were 6-foot Jameson JE Series Foam Core Dielectric Fiberglass, and it is recommended to purchase at least three poles. Each pole cost about $80, making the cost for poles $240, and the total cost for pruning pole equipment around $308. It is important to mention that these poles are recommended for their quality and for the safety of the user. If you are a private landowner, you may already own your own pruning equipment, and therefore, not need to purchase this for your kit. Due to the height of mature ash tree branches which produce seed, it is recommended to have a pruning pole that can safely and securely reach and remove these branches. Pruning poles with all their extensions are heavy and require strength of the user to safely operate. The specific items are recommended for their ruggedness and use by experienced seed collectors; however, their brand is not required for seed collecting. A note on the Jameson JE Series Foam Core Dielectric

Fiberglass: dielectric fiberglass was prioritized because it is non-conductive to electrical force. This type of pole will act as an insulator protecting the user of the pole if for some reason it came in contact with power lines. This could occur because some brown ash trees are roadside and located adjacent to electrical power lines.

Tarp: There is not a specific brand of tarp recommended. The tarp should be large enough to place under a tree while collecting seed to provide a falling branch a place to land and prevent seed from spreading on the ground. This is important for late season collection when seed clusters dry out and fall from the branches far easier than earlier in the season. A tarp may be able to collect more seed as branches are being taken down, and less seed could get lost on the ground. This makes it easier to pick up seed on a tarp, rather than the forest floor.

Safety glasses: There is not a specific brand or type of safety glasses recommended, but it is stressed that everyone in the seed collection group has eye protection. Falling tree branches and seed can be dangerous and eye protection is a must.

Rope: We recommend at least 200 feet of 650 lb. paracord/parachute cord, which can be purchased by the spool. At the time this list was put together, a spool cost about $20 online. A bright color is recommended to be able to see easily in the forest. This will be used with the pruning pole.

Hardhats: Hardhats are personal protective equipment that is specific to each person, and were not purchased for the seed collection kits given to the Wabanaki tribes by our

research team at the creation of this list. Therefore, we do not have a recommendation for hardhats, but we do strongly recommend the use of hardhats and head protection when harvesting ash seed to protect the collectors.

Loppers/branch cutters: This is an item that homeowners may already have. The Corona lopper that we have chosen is 29 inches long to be able to reach smaller branches closer to the ground, but also to clear brush around the base of a tree. Clearing the base of the tree from brush will make it easier to place the tarp.

Collection Stage	Item	Quantity and notes	Cost (approximate)
SCOUTING TREES	GPS unit	1 GPS and batteries / Garmin eTrex 10	*$110 each*
	Pens/pencils	5	*Less than $0.50 a pen or pencil, depending on how large of a box purchased*
	Clipboard	2	*Less than $2 a clipboard if purchased in bulk*
	Binoculars	1 / Nikon Trailblazer 8x25 ATB	*$90 each*
	Flagging tape	3 rolls	*Less than $3 per roll when purchased in bulk*
	Aluminum tags (for trees)	30+	*100 tags can be found for around $25*
	Roofing nails (and container for nails)	1 box	*100 pack of 1 inch rooking nails can be found for around $4. Box to store can be*

			found for under $5 at a dollar store.
	Hammer/mallet/ hatchet	1	*Forestry Suppliers has hatchets for $52*
	State gazetteer or map of area	1 / Maine Atlas and Gazetteer	*$25 each*
	Safety vests	As many as in the group	*Up to $80 each for forestry safety vests*
	Backpack	1	*Less than $20*
	Maine Forest Service: “Forest Trees of Maine”	1	*$15 from Maine Dept. of Ag., Conservation and Forestry*
COLLECTING SEED	Tarp	1 (10ft x 12ft)	*Less than $15*
	Pruning pole head trimmer	1 / Jameson PH-11 Tree Trimmer Head	*$51 each*
	Pruning pole adapter	1 / specific to the Jameson PH-11 head trimmer	*$17 each*
	Pruning pole extensions	3 / 6’ Jameson JE Series Foam Core Dielectric Fiberglass	*$80 each x 3 = $240 total*
	Safety glasses	1 for each member of the group	*Less than $2 each when purchased in bulk*
	Paper lunch or grocery bags	50 small bags, 20 large bags	*Less than $10 for 100 small (4lb) bags. Less than $13 for 25 large (57lb) large bags.*
	Stapler and staples	1 stapler, 1 box of staples	*Less than $10 for a stapler with staples. Box of 5,000 staples is less than $5.*

	Rope	200’ of 650 lb. para-cord/parachute cord	*$20 a spool*
	Hardhat	It is recommended that those using pruning poles protect their heads while cutting branches.	*Less than $35 on ForestrySuppliers.com*
	Loppers/handheld branch cutters	1 / Corona loppers 29”	*$35 each*

Table 4. Ash Seed Collection Checklist of recommended items with quantity and recommended cost of items, with costs valued in 2021 and 2022.

Ash Identification Table

ASHES *The Important Distinctions*

ASH

	White Ash *Fraxinus americana*	Green Ash* *Fraxinus pennsylvanica*	Black Ash *Fraxinus nigra*
LEAVES			
LEAFLETS	5–9, usually 7	7–9	7–11
DESCRIPTION	Leaflets are mostly entire, borne on stalks, without hairs below. Turn purple in autumn	Leaflets borne on stalks. Hairy below and on rachis. Turn yellow or bronze in autumn.	Toothed leaflets which are without stalks except the one at the end. Hairs lacking below except for buff-colored hairs at the junction of the leaflets and the rachis. Turn yellow in autumn.
BUDS			
SIZE	¼ inch	⅛ inch	Less than ¼ inch
SHAPE	Blunt-pointed	Cone-shaped	Sharply-pointed
COLOR	Brown	Brown with rusty or dull red hairs	Black or very dark
FRUIT			
WINGS	Wing terminal	Seed body grading gradually into wing	Flat, completely surrounds seed body
SEED BODY	Cigar-shaped	Funnel-shaped	Slightly twisted, less than half the length of the fruit
TWIGS			
TEXTURE	Smooth and shiny, often with slight bloom, very brittle	Somewhat covered with downy hairs	Smooth, not shiny
COLOR	Gray or greenish-brown, inner bark bright brick red	Greenish-gray, inner bark cinnamon-colored	Pale gray, inner bark dirty white

*Specimens of green ash which lack hairs on the twigs or leaflets, but otherwise fit the above description, were formally designated as var. lanceolata. They are now designated under the species due to the many gradations of the hairiness character.

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Appendix B: Ash Seed Data Sheets

National Seed Laboratory Data Sheet

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 (Decimal degrees) : lat ____._____ long ____._____ elevation _____meters

Directions to the site if not using GPS: _____

_____

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

Emerald ash borer (EAB) present in surrounding area: Yes_____ No _____

Signs of EAB present on collection tree (check all that apply):

_____ epicormic sprouting _____ canopy thinning _____ blonding _____ 'D' shaped exit holes

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

Site type: ___ upland __ wetland __aquatic.

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

_____

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

National Plant Germplasm Facility Data Sheet

DATA COLLECTION FORM

Site # _____ Date Harvested (DD/MM/YY)_____

Genus_____ Species _____

Location Name _____

Directions_____

Lat/Long source: ☐GPS ☐Map Map Datum: _____

Plant Description _____

_____

_____

_____

Collector(s) Contact Information:

Name(s)_____Institution_____

Distance to planted/cultivated ash trees (miles): _____

No. plants found _____ No. plants sampled _____ Site size (m^2) _____

Herbarium specimen: ☐yes ☐no Herbarium Specimen # :_____

Pop. abundance: ☐abundant ☐frequent ☐occasional ☐rare

SITE DESCRIPTION:
Exposure _____ Slope _____ Aspect _____

Site Physical _____

_____

Site Vegetative _____

_____

_____

Soil Type _____

GPS COORDINATES FOR SAMPLES:
Tree: _____Lat: _____ N S Long: _____ E W
Tree: _____Lat: _____ N S Long: _____ E W
Tree: _____Lat: _____ N S Long: _____ E W
Tree: _____Lat: _____ N S Long: _____ E W
Tree: _____Lat: _____ N S Long: _____ E W
Tree: _____Lat: _____ N S Long: _____ E W
Tree: _____Lat: _____ N S Long: _____ E W
Tree: _____Lat: _____ N S Long: _____ E W
Tree: _____Lat: _____ N S Long: _____ E W
Tree: _____Lat: _____ N S Long: _____ E W

National Plant Germplasm Facility Data Sheet – Definitions

Site #: Typically, a three digit number assigned by the collector to represent a single locality (e.g. state park, watershed, natural area, or any other type of defined area, etc.) or population.

Date Harvested: Date when the sample was harvested

Location Name: Name for the location or population being sampled (e.g., state park, watershed, natural area, or any other type of defined area, etc.)

Directions: General description on how to find the location/population. Typically provided as a legal description or approximate distance from a specific landmark (e.g., city, lake, etc.)

Lat/Long source: Indicates how GPS coordinates were obtained (map or GPS unit)

Map Datum: Indicated type of datum used with GPS unit, typically recorded as NAD83

Plant Description: Brief description of the plan that was harvested, typically includes approximate size of specimen, may also include additional interesting comments referring to growth habit, fall color, phenology, etc.

Exposure: full sub/shade

Slope: Slope or gradient of the sampled habitat

Aspect: Direction in which the slope faces of the sampled habitat (e.g., north, east, south, west)

Site Physical: Description of the immediate surrounding (e.g., habitat type(s))

Site Vegetation: Listing of associated plant species growing in the immediate surroundings

Soil Type: Description of the soil type for the immediate surrounding

Tree#: Typically, a three-digit number assigned by the collector to represent a single mother tree sample

Lat. And Long: GPS coordinates marking the location of the sample harvested

Appendix C: Ash Seed Shipment Supplemental Materials

Instructions for sending seeds to the Black Box

U.S. DEPARTMENT OF AGRICULTURE

NATIONAL LABORATORY FOR GENETIC RESOURCES PRESERVATION

Instructions for Sending Seeds to NLGRP for Black Box Storage

Before sending a shipment, please ensure:

1. A Material Transfer Agreement (MTA) is in place. The final fully executed copy will come from the Technology Transfer Assistant at USDA and will have a Black Box MTA # (located at the bottom of the document) and all signatures.
2. The Black Box Deposit template (MTA Appendix 1) has been filled out according to the instructions, and the file has been sent to NLGRP-Blackbox@usda.gov prior to shipment.
3. If you have already sent us your first shipment under the current MTA, you have included a Letter of Transmittal.

International Depositors: Before shipping, please email NLGRP-Blackbox@usda.gov for import instructions. NLGRP will provide depositor with an appropriate permit/shipping label (if applicable). The NLGRP Import Instruction Letter provides the current instructions needed to import material in accordance with USDA, Animal and Plant Health Inspection Service (APHIS) regulations. Because regulations change, please request an Import Instruction Letter each time you send us a deposit. Please follow all instructions carefully. **Material may be destroyed or returned if conditions and regulations are not met.**

Domestic Depositors: Any required state and/or federal permits must be in place prior to shipment.

Packaging & Shipping Instructions

- Please ensure seed is dried and package in moisture proof packaging such as heat-sealed foil laminate pouches. If seed drying is not possible, please contact NLGRP-Blackbox@usda.gov to discuss having NLGRP equilibrate and package seed for storage.
- Label each packet with depositor organization, depositor inventory identifier and genus species.
- Our seed storage racks have an opening of 86 cm wide x 75 cm deep x 55 cm high. Please send your seed in boxes sized to be stacked in this space. Our recommended box size is 42.5 cm wide x 37.5 cm deep x 15.25 cm high.
- If multiple boxes are shipped, each must have a packing list of the box contents (this can be a print out of the Black Box deposit spreadsheet (MTA Appendix 1 inventory), by box). Numerically label each box (1 of 5, 2 of 5, etc). Please be sure the corresponding box number appears in the spreadsheet.
- Ship seed via expedited service. Email NLGRP-Blackbox@usda.gov with shipping date and tracking information.

NLGRP Shipping Address (for domestic shipments only. International shipments refer to Import Instruction Letter):
USDA, ARS, NLGRP
Attn: Black Box Storage
1111 South Mason Street
Fort Collins, CO 80521

Current as of 11/10/2022

Material Transfer Agreement for Black Box submission

U.S. Department of Agriculture
Agricultural Research Service

BLACK BOX GERMPLASM STORAGE DEPOSIT AGREEMENT

PARTIES:

ARS: USDA, ARS, Plains Area
National Laboratory for Genetic Resource Preservation (NLGRP)
1111 S Mason St.
Fort Collins, CO 80521

ARS Scientist:
Tel:
E-mail:

Depositor: Organization:

Address:

City: State: Zip:

Depositor Scientist:

Tel:

FAX:

E-mail:

PREAMBLE:

The U.S. Department of Agriculture, Agricultural Research Service, established the National Plant Germplasm System (NPGS). The mission of the NPGS is to support agricultural production by: (1) acquiring crop germplasm; (2) conserving crop germplasm; (3) evaluating and characterizing crop germplasm; (4) documenting crop germplasm; and (5) distributing crop germplasm. The National Laboratory for Genetic Resource Preservation (NLGRP), with extensive capacity and infrastructure, provides security back-up storage of seeds for other governmental agencies, botanical gardens, national genebanks in other countries, international genebanks, NGOs and Native American tribes. Free, back-up storage at NLGRP protects genetic resource collections from irreplaceable loss and ensures long-term benefits of genetic resources.

PURPOSE:

To provide "Black Box storage" (defined jointly by CONDITIONS 1 through 19) at ARS NLGRP for packets of seed sent by Depositor (defined as 'Material')

Brief Description of Material(s):

PROCESS:

For newly deposited Material, Depositor shall:

1. Provide a seed packet inventory spreadsheet identifying the material being deposited (Appendix 1) and include a paper copy of the seed packet inventory in each shipment. This paper copy included in each shipment shall also cross-reference the contents of each individual box/seed packet in the shipment with each individual item listed in the electronic version of the seed packet inventory;

2. E-mail a complete Seed Packet Inventory covering the contents of each shipment to ARS in xls format to NLGRP-Blackbox@usda.gov before shipment;

3. Ship Material to the ARS NLGRP in mutually agreed on packaging to the ARS address listed in the Deposit Instructions (Appendix 1); and

4. Pay all costs associated with shipping the Material.

Depositor may:

1. Deposit additional Material with ARS over the term of the Agreement. Additional Material shall be shipped as detailed above with a Letter of Transmittal that shall refer to this Agreement and bind Depositor and ARS to the terms of this Agreement for the additional Material shipped to ARS. (See Appendix 1 – Black Box Deposit Instructions, Template for Letter of Transmittal)

CONDITIONS:

The Material is deposited with ARS under the following conditions:

1. The Material shall only be used for "Black Box" storage and shall not be part of the NPGS collection.

2. ARS shall not use the Material for any other purpose including but not limited to research, breeding, training, propagation, characterization, viability testing and regeneration.

3. ARS shall not transfer the Material, in whole or in part, to a third party. Any third party requesting a sample shall be referred to Depositor.

4. The Material is available from the Depositor in a manner that facilitates access for research, conservation and sustainable use in compliance with national laws and applicable international treaties.

5. Rights of the Depositor over the Material are not changed by this Agreement.

6. ARS will provide optimal storage conditions. ARS will be responsible for all storage costs pertaining to the Material.

7. ARS shall not be liable for any damage caused to the Material by any reason. In the event of loss or destruction of Material at NLGRP, NLGRP will inform Depositor in writing of the loss and the reasons therefore.

8. ARS will return Material to Depositor at Depositor's written request. Any written notice given under this Article shall identify the specific seed lots of Material that are to be withdrawn. ARS will return the requested

seed lots of Material within a period of one (1) year from the date of receipt of such written notice. The costs of packaging and shipping in respect of the return of the seed lots of Material shall be borne by the Depositor. ARS will continue to exercise all care and diligence over the seed lots from the time notice is received until actual shipment of the seed lots of Material.

9. Depositor shall not in any way state or imply that this Agreement or the results of this Agreement is an endorsement by ARS of its organizational units, employees, products, or services; except to the extent permission is specifically granted by an authorized representative of ARS.

10. ARS shall not in any way state or imply that this Agreement or the results of this Agreement is an endorsement by Depositor of its organizational units, employees, products, or services; except to the extent permission is specifically granted by an authorized representative of Depositor.

11. The Parties acknowledge and agree to comply with all applicable laws and regulations of the Animal Plant Health and Inspection Service, the Center for Disease Control, and/or Export Control Administration pertaining to possession or transference of technical information, biological materials, pathogens, toxins, genetic elements, genetically engineered microorganisms, vaccines, and the like.

12. The provisions of this Agreement are to be deemed severable and the invalidity, illegality or unenforceability of one or more of such provisions shall not affect the validity, legality or enforceability of the remaining provisions.

13. ARS will treat all information generated or gathered under this agreement in accordance with the Freedom of Information Act.

14. This Agreement may be executed in any number of counterparts, each of which when so executed shall be deemed to be an original and all of which taken together shall constitute one and the same agreement. Signature by facsimile shall also bind each of the parties to this Agreement.

15. ARS is an agency of the U.S. Government and any rights or obligations created under this Agreement are freely transferable within the U.S. Government and shall not be deemed a "transfer."

16. This Deposit Agreement shall be construed in accordance with United States of America Federal Law as interpreted by the Federal Courts in the District of Columbia.

17. Before the expiration of this Agreement, Depositor and ARS will determine if the agreement will be renewed. If not renewed, ARS will return the Material to the Depositor.

18. Either party may unilaterally terminate this entire Agreement at any time by giving the other party written notice not less than sixty (60) calendar days prior to the desired termination date.

19. This Agreement constitutes the entire agreement between Depositor and ARS and supersedes all prior agreements and understandings between them with respect to its subject matter.

This Agreement shall become effective upon date of final signature and shall continue in effect for a period of ten (10) years.

Appendix 1

Instructions for depositing black box collections, a spreadsheet template for collection inventory and a template for the letter of transmittal (needed for shipments after the initial deposit), can be found at this website:

https://www.ars.usda.gov/plains-area/fort-collins-co/center-for-agricultural-resources-research/paagrpru/docs/plants/pages/deposit-germplasm/

For further information, please contact: NLGRP-Blackbox@usda.gov

ACCEPTED FOR THE AGRICULTURAL RESEARCH SERVICE

_____ _____
Signature (Scientific Technology Transfer Coordinator) Date

ACCEPTED FOR THE COOPERATOR:

_____ _____
Signature Date

_____
Typed Name

. . . . . .

By signing below, the ARS Scientist and ARS Research Leader acknowledge that they have read, understood, and agreed to the terms and conditions of this Agreement.

_____ _____
Signature (ARS Scientist) Date

_____
Typed Name

_____
Signature (ARS Research Leader) Date

_____
Typed Name

ARIS # _____
Storage Agreement: version 11/10/2022

Letter of Transmittal for Black Box Storage

Letter of Transmittal
NLGRP Blackbox Storage

Instructions:

- Depositors can send material to NLGRP for the duration of the MTA. However, after the first shipment under a given MTA, a letter of transmittal must accompany subsequent shipments.

- For subsequent shipments, cut and paste the text below onto letterhead and sign letter. Email letter along with Appendix 1 spreadsheet to NLGRP-Blackbox@usda.gov before shipping seed.

[Institute Name]
[Shipping date]

Under the USDA-ARS MTA Blackbox Storage # _____, Depositor is sending ARS additional germplasm for black box storage. Attached is an additional Appendix 1 – Seed Pack Inventory that shall be added to the Germplasm Storage Deposit Agreement as acknowledged by ARS.

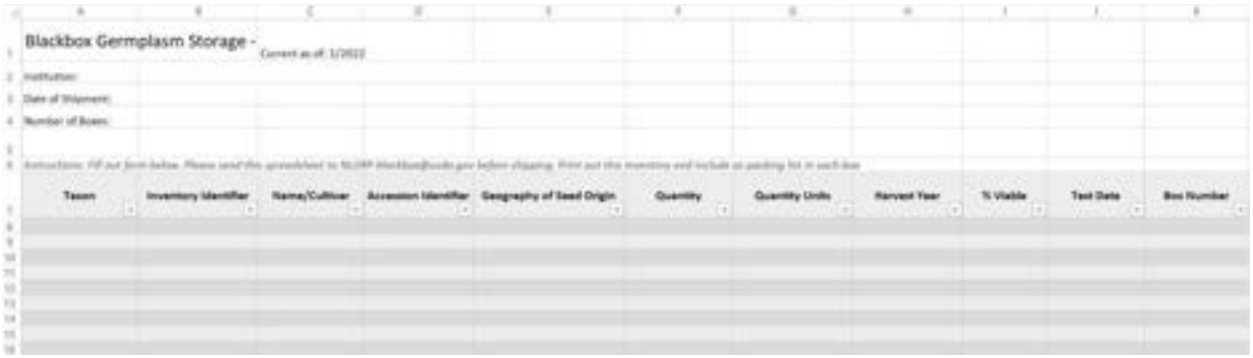
- MTA # can be found on the last page of the executed agreement.

- Contact NLGRP-Blackbox@usda.gov if you have any questions.

NLGRP Blackbox Deposit Template – Instructions

	A
1	**NLGRP Blackbox Germplasm Storage - Appendix 1: Instructions**
2	
3	**Institution***: Complete institution name
4	**Date of Shipment***: Please fill in date of shipment.
5	**Number of Boxes***: Total number of boxes in shipment.
6	
7	
8	
9	**Taxon***: Genus species (Do not include species authority)
10	**Inventory Identifier***: Institute inventory identifier for the seed sample being sent to NLGRP
11	**Name/Cultivar***: Name/cultivar associated with the inventory sample
12	**Accession Identifier**: Institute accession identifer
13	**Geography of Seed Origin**: Fill out country of seed origin if known.
14	**Quantity***: Number of seed in each packet or wt in grams
15	**Quantity Units***: Count, Gram
16	**Harvest Year**: Year of seed harvest if known.
17	**Percent % Viable**: Percent of seed viability.
18	**Test Date**: Date viability tested.
19	**Box Number***: Indicate the box number the sample has been packed in. All boxes should be numerically labeled on the outside (x of x total boxes).
20	
21	
22	*** Indicates field is required**

NLGRP Blackbox Deposit Template



Recommendations for the collections, storage, and germination of ash (*Fraxinus spp.*) seed

RECOMMENDATIONS FOR THE COLLECTION, STORAGE, AND GERMINATION OF ASH (*FRAXINUS SPP.*) SEED

Dave Ellis
Plant Genetic Resources Preservation Program
National Center for Genetic Resources Preservation
1111 South Mason Street
Fort Collins, CO 80526
telephone - 970 495 3227
fax - 970 221 1427
elvis@ars.usda.gov

Seed that is harvested when mature and processed immediately has the greatest life span during storage. Seeds infested with fungus or insects do not survive very long and may potentially infect other seeds. The recommendations below will help to acquire the highest quality seed for long term storage.

Identifying trees for collection

1) There are several species of interest: *F. americana* (white ash), *F. nigra* (black ash) and *F. pennsylvanica* (green ash) are among the ash native to the Lake States.
2) It is always a good to collect leaf samples along with seed samples so that identity can be confirmed.
3) Collect seed when it is mature. Seed maturation dates differ among ash species: Sept-Oct is generally a good time to collect black and white ash, while green ash can be collected into December. Also note that ash trees may only produce large seed crops once every 3 to 5 years.
4) Seeds are contained within fruiting bodies called *samaras*, with the seeds are at the thicker base end of the samara.
5) Collect seeds when the samaras are faded from green to yellow or brown. Seeds within samaras should be firm, crisp, white and fully elongated. Avoid collecting samaras that have signs of mold or insect infestation.
6) Record date, location (lat/long data from topo map and relevant landmarks and/or GPS coordinates) for sampled tree in field notes. Use one bag for each tree
7) Collect seeds on a non-rainy day

Collecting seeds (samaras)

1) Ash trees can be very tall. Make sure proper safety protocols are used.
2) You may want to spread sheets under the tree to collect seed that falls.
3) Clusters of samaras from low lying branches can be clipped with pruning sheers. Rope, pole pruners, shotgun or bow and arrow can be used to dislodge samaras from higher branches.
4) Pick seeds (samaras) off tree as late in the year as possible to ensure collection of mature seed. Avoid picking seed up off the ground. The trick may be to pick as late as possible but once mature, the longer you wait, the more prone the seed is to weathering, insects or fungal contamination. It is more efficient to harvest the seeds as clusters rather than picking individual seeds..
5) If leaves are available on the tree, prepare a pressed dry herbarium sample for positive identification of the species at a later date. See websites for proper preparation of herbarium samples.
6) Samaras should be a natural brown and no longer green (if seed is pale green it is ok where you cannot get back to the site in a week or two).
7) Visually inspect seed prior to collecting. When possible **do not collect seed which**:
   A) is black or dark green.
   B) has evidence of insect damage. This would appear as tiny entrance or exit holes in the seed.
   C) is non-uniform – avoid distorted, twisted seed.
   D) does not have a solid base which is thicker than the wing.
   E) is mildewed or has evidence of fungal infection (spotted/mottled-looking seed).
   F) is on the ground.
8) Once seed is picked off the tree, place the seed in a paper lunch bag and label the collection information (see number 10 below). A paper bag open at the top will provide necessary air flow to naturally dry seeds. Do not place in plastic bag or other container which does not allow air flow.
9) One paper lunch bag full of seed (4-6 cups of seed) is plenty from any one tree.
10) Use a separate bag for each batch of seed (usually seed from a single tree/bag).
11) Collection notes are usually kept in a field book with collection numbers written on the bag containing the seed. However, since multiple people will be collecting seed over many years and storage will be long-term, we recommend labeling the bag (and any pressed leaves) so that the location and identification of the source is clearly marked. The portion of the paper bag containing the label can remain with the sample in storage for positive identification of the collection decades from now. The labeling should include:
   A) The species – place question marks around the species when identification is not certain (*Fraxinus ?pennsylvanica?*).
   B) A description of where the seed was collected. Examples include:
      i) Just inside gate to Bear Park - left side of the road in Lightfoot County, MI.

ii) Right side of Elk Creek Rd, ~2.7 miles N. from the traffic light in Red Deer, WI.

iii) Moline National Park, Brookside campgroup, behind campsite #7, OH.

C) GPS/GIS coordinates and elevation when possible.

D) Description of tree:

i) Shrub or tree.

ii) Healthy or sick looking.

iii) Any Emerald Ash Borer (EAB) evidence ("D"-shaped exit holes, dead branches, lesions in bark).

iv) Evidence of other borers or insect damage.

v) Approximate height of tree.

E) Date of collection.

F) Name and contact information of person(s) collecting the seed.

12) Once collected, keep seed out of direct sunlight but in an area that allows airflow (i.e. not in a sealed cooler). Do not leave in hot car or box in the back of a pick-up truck or car in the sun.

13) Leave seeds (samaras) in paper bags open at the top for about 3 days in a dark, cool, dry location. After 3 days, clean the seed. Break apart the seed clusters so that seed is individualized and remove any branches and debris.

14) Dry samaras by spreading them thinly in a single layer on newspaper in a shallow tray. Use one tray/seed collection to avoid any chance of mixing two different seed collections.

15) Place the trays with the seed in a dark, cool, dry location. If the weather is humid, place trays in a dehumidified room with lots of airflow. They will dry out within about a week or two.

16) When samaras are dry, seeds can be cleaned. Seeds can be isolated from dried samaras by rubbing them through your palms. Remove samara fragments by shaking sample through screens. Spread cleaned seed out on tray and inspect for insect or microbial damage

17) Place the seed in an air-tight moisture-proof container containing with the collection information written on the outside of the container and the original labeling information from the collection bag inserted into the container with the seed. The portion of the collection bag with the information can be cut and inserted in with the seed.

A) Air-tight moisture-proof containers include:

i) A kitchen "seal-a-meal" self-sealing bags.

ii) A screw-top bottle or jar.

iii) A plastic zip-lock bag (least preferred as they often do not seal tight).

B) To label the outside of the container use a permanent marker – Sharpie's work great.

18) To test moisture content of the seed, place the seeds in a screw cap container containing a small package of *indicating silica gel*. If silica gel turns pink within a few hours, seeds should be removed and dried in a drier environment. If silica gel turns pink in 1-3 days, replace it with freshly activated silica gel. If silica gel remains blue for a week in the screw cap jar, the seeds are sufficiently dry for storage. Many silica gels can be reactivated by

putting it in the oven at about 250F overnight – the granules should turn from pink (moist) back to blue (dry).

19) Place the air-tight container containing the seed in a cardboard box in a freezer. Locate the freezer where someone looks at it at least weekly (preferably daily). This way any problem with the freezer will be noticed immediately. May freezers can also be equipped with an audible alarm to notify you if it is not keeping things cold.

Testing ash seed (samara) viability

When possible it is always preferable to know how good the seed is that you are storing. There are two primary reasons for this. 1) You want to ensure you are storing good, live seed and 2) you will to know if the seed deteriorates during storage to enable you to pull the seed out of storage and germinate it prior to complete loss of viability. Below we mention two methods. The first requires specialized laboratory equipment that can be found in most high school biology classes. The second method relies on germination of the seed. We recommend testing a minimum of 100 seed. In collections where few seed are available, testing 5-10 seed will suffice.

There are other methods for testing seed viability that are available and used in seed testing laboratories, yet these generally require advanced laboratory facilities. These methods include terazolium staining of the embryos and x-raying the seed. For *Fraxinus*, both methods are used, with x-raying of the seed the quicker and easier method for facilities with the equipment.

1) Physical examination of the seed. Fresh or dried seed can be examined with a microscope or magnifying glass.
   A) The narrow, pointed end of the seed is where the embryo is.
   B) Carefully cut this end open by slicing length-wise and observe the embryo
      i) The embryo should be white, solid and fill the entire seed cavity. Is the seed "fresh and filled"? If the embryo looks wilted or off color, this is not a favorable sign.
      ii) You can also observe the presence of seed insects – these are usually gray with segmented bodies and a brown head.
      iii) Basically anything other than an embryo in the seed cavity is an indication of poor seed.
2) Germination tests. This is done in 2 phases: Phase1 - stratify to break dormancy; and Phase 2 actually germinate the seed. Stratification can be done in a common refrigerator (about 45°F) for 2 to 3 months, while germination can be done in a greenhouse or cold frame.
   A) Place 100 seed in a thin layer of moist sand or moist paper towels and let this sit for 60-90 days in a refrigerator.
   B) After the cold stratification treatment, place the seed in an area with 68°F nights and 86°F days. If moist paper towels were used for the cold stratification, spread these towels out in a thin layer of sand prior to placing in a greenhouse or cold frame. Keep the seed moist during this time
   C) The number of seed germinated should be counted after 40 and 60 days. The percent of seed germinated should be recorded and kept with the seed sample.
   D) Germinated seed can be planted and grown in pots.

WEBSITES FOR INSTRUCTIONS FOR MAKING HERBARIUM SPECIMENS

http://www.mobot.org/MOBOT/Research/Library/laesner/pressing.html
http://www.herbarium.unc.edu/chpt18.html
http://www.siu.edu/~ebl/prepare.htm
http://www.une.edu.au/botany/plant_collecting.htm
http://www.rmh.uwyo.edu/prelude/intro/rmcoll.htm
http://herbarium.usu.edu/K-12/Collecting/specimens.htm
http://www.uaf.edu/museum/herb/howtocoll.html
http://herbarium.ucdavis.edu/herbarium.html
http://www.montana.edu/wwwpb/pubs/mt8359.pdf
http://www.flmnh.ufl.edu/herbarium/voucher.htm
http://www.virtualherbarium.org/collecting.htm
http://www.herbarium.lsu.edu/makingherbspecimen.html
http://www.auburn.edu/academic/science_math/botany/herbarium/collecting.html
http://www.life.uiuc.edu/ib/335/CollectingPlants/CollectingPlants.html
http://www.montana.edu/wwwpb/pubs/mt8359.html
http://artemis.austincollege.edu/acad/bio/gdiggs/collecting.htm

The National Arboretum has offered to store herbarium voucher specimens for you. For more information or questions on herbarium specimens you should contact:

Kevin Conrad
Curator
Woody Landscape Plant Germplasm Repository
U.S. National Arboretum
ARS-USDA
10300 Baltimore Ave
Building 010A Room 233
Beltsville, MD 20705
Cell Phone 240 832 9415
ConradK@usna.ars.usda.gov

Ash seed clusters.

X-ray of filled ash seed
Note large white area which is the embryo.

X-ray of empty ash seed
Note light white area where embryo should be. Small under developed embryos are also noted (arrow).

X-ray of filled and insect damaged ash seed
Note segmented embryos (arrow) where insects have eaten.

Close-up of X-ray of insect damaged ash seed.

Evidence of insect damage to seed (arrow). Note also deformed seed (bottom).

Close up of insect entrance hole in seed.

Empty seed due to insects. Rip in seed is due to insect exiting.

Close up of grub found in ash seed.

Tetrazolium stained ash embryo. Red color indicates good (live) embryo.

Ash seed sliced longitudinally to examine the embryo. Middle embryo is good while top and bottom have insect damage.

Appendix D – Online Access to Ash Collecting and Shipping Documents

Where to find datasheets and shipping information online

Document	Document Location	Publisher/Creator of Document	Online Location
Ash Identification Table	Appendix A	Maine Department of Agriculture, Conservation and Forestry	https://www.maine.gov/dacf/mfs/publications/handbooks_guides/forest_trees/pdf/Ashes.pdf
National Seed Laboratory Data Sheet	Appendix B	USDA Forest Service, National Seed Laboratory	https://www.fs.usda.gov/nsl/seed_collection_data_page.pdf
National Plant Germplasm Facility Data Sheet	Appendix B	USDA Agricultural Research Service, Plant Introduction Research	https://www.ars.usda.gov/midwest-area/ames/plant-introduction-research/home/npgs-ash-conservation-project/data-collection-form/
National Plant Germplasm Facility Data Sheet – Definitions	Appendix B	USDA Agricultural Research Service, Plant Introduction Research	https://www.ars.usda.gov/midwest-area/ames/plant-introduction-research/home/npgs-ash-conservation-project/data-collection-form/
Instructions for sending seeds to the Black Box	Appendix C	USDA Agricultural Research Service, Agricultural Genetic Resources Preservation Research	https://www.ars.usda.gov/plains-area/fort-collins-co/center-for-agricultural-resources-research/paagrpru/docs/plants/pages/deposit-germplasm/
Material Transfer Agreement for Black Box submission	Appendix C	USDA Agricultural Research Service, Agricultural Genetic Resources Preservation Research	https://www.ars.usda.gov/plains-area/fort-collins-co/center-for-agricultural-resources-research/paagrpru/docs/plants/pages/deposit-germplasm/
Letter of Transmittal for Black Box Storage	Appendix C	USDA Agricultural Research Service, Agricultural Genetic Resources Preservation Research	https://www.ars.usda.gov/plains-area/fort-collins-co/center-for-agricultural-resources-research/paagrpru/docs/plants/pages/deposit-germplasm/
NLGRP Blackbox Deposit Template – Instructions	Appendix C	USDA Agricultural Research Service, Agricultural Genetic Resources Preservation Research	https://www.ars.usda.gov/plains-area/fort-collins-co/center-for-agricultural-resources-research/paagrpru/docs/plants/pages/deposit-germplasm/
Recommendations for the collections, storage, and germination of ash (*Fraxinus spp.*) seed	Appendix C	USDA Plant Genetic Resources Preservation Program	http://www.emeraldashborer.info/documents/Fraxinuscollection.pdf

Appendix E – Ash Foliage Collection Instructions

Ash Foliage Collection: Pennsylvania State University partnered research

Objective: Collect leaf tissue for population genomics analysis for ex situ collections

Equipment needs:

- Handheld GPS
- AA batteries
- Pencils and permanent black marker
- Coin envelopes (1 envelope used for each individual collected)*
- Plastic freezer bags (1 freezer bag for each population)*
- Silica gel*
- Clippers or pruning shears
- Leather gloves

  *Materials that can be provided by Dr. Hamilton for collection

Field sampling for population genetics requires between 20 and 40 different individual trees per population. Ideally, if there are male and female trees they should be identified and collected separately. For each species at each site, collection should happen from individuals relatively spread apart from each other in the population (at least 5 feet apart, but ideally more). Where possible, leaves collected should be fully mature: young bright green leaves with limited insect or fungal damage.

Overview of Methods

Creating a Site Label and Filling out Site Level Data Sheet

On a sheet of paper, you will record the state and location you are collecting in, for example you can indicate the town or some descriptive language of the area you are making these collections in (e.g., White Mountains National Forest). Using the state and the name of the location, you will create a three-letter code to identify the population. For example: Orono, ME will be written as: ME-ORO. Record the GPS location (latitude and longitude in decimal degrees) and elevation in meters for the **first** individual tree sampled at each site. Except for

the GPS coordinates, elevation and the final number of individuals collected from, most of the data can be filled out prior to going to the site.

On this datasheet of paper, you will need to record:

- Species name (Brown, green or white ash)
- The site location along with state-population codes (Orono, ME = ME-ORO)
- Data of collection (MM/DD/YY)
- Latitude (decimal degrees)
- Longitude (decimal degrees)
- Elevation (meters)
- Number of individuals collected

Labeling the Sample Envelopes and Plastic Freezer Bags

Labeling if the individual envelopes can be done prior to the site visit as well.

- For each species at the site, label 20-40 individual coin envelopes with **Species** (brown ash: BA, white ash: WA, or green ash: GA), **Population Code** and **Number** (1-40). For each envelope include an “F” for female and an “M” for male if you are able to identify the individual's sex. The final code might look something like this:

  [Species, Population Code, Number, and sex] = [BA ME-ORO-1-F]

  Label coin envelopes in pencil.

- Label a large plastic freezer bag with the following information in **permanent black marker.**
  - Species
  - Population Code
  - Date of Collection
  - Latitude
  - Longitude
  - Elevation
  - Total number of individuals collected (1-40)
  - Collector name
- Include site-label datasheet (same information from above) inside the plastic freezer bag.

Post-Sampling Storage & Data Management

These activities need to be completed the same day as collection.

- **For leaves:** Fill the plastic freezer bag associated with 'leaves' with about 3+ fingers width of silica gel in the bottom of the bag. Place coin envelopes of leaves in a bag of silica gel – you should be able to get all 40 individual coin envelopes in one (1) plastic freezer bag – although, for quick drying, fewer leaves per freezer bag is helpful. Try to ensure as much air as possible is removed from plastic bags to limit potential tissue degradation before it comes fully dried.
    - A note on silica gel: silica gel can be reused once leaves have completely dried. Email Dr. Hamilton first prior to silica gel reuse.
    - Silica gel has blue beads that will change color as they begin to soak up moisture (often from blue to pink). If silica gel beads appear pink prior to use *do not use that silica gel* – check with supervisor about oven-drying for re-use.
    - If the plastic freezer bag seems very full with coin envelopes, split the coin envelopes into two (2) plastic freezer bags and indicate: No. Plastic Bags (1/2 or 2/2, or 1/3, etc.) for each plastic freezer bag.

Shipping costs will be covered by the Schatz Center for Tree Molecular Genetics.

- Email Dr. Jill Hamilton (jvh639@psu.edu) for shipping information.

Shipping Information:
Address: Schatz Center for Tree Molecular Genetics
323 Forest Resources Building
University Park, PA 16802
Cell number: +1 (530) 312-3118