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

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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 **<u>do</u> <u>not</u> <u>collect</u> <u>seed</u> <u>which</u>**:
   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 <u>permanent</u> 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 ***<u>indicating silica gel</u>***. 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^{o}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^{o}F nights and 86^{o}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/liesner/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
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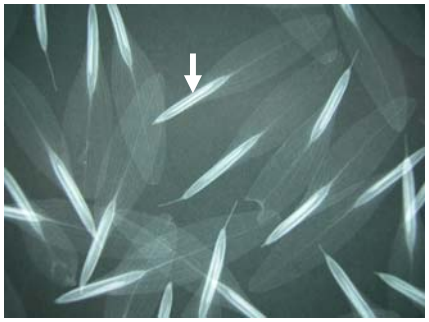
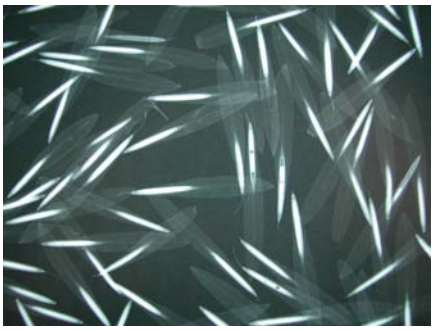
Ash seed clusters.

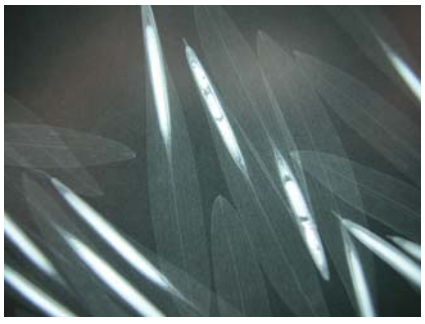
X-ray of filled ash seed
Note large white area which is the embryo.



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.



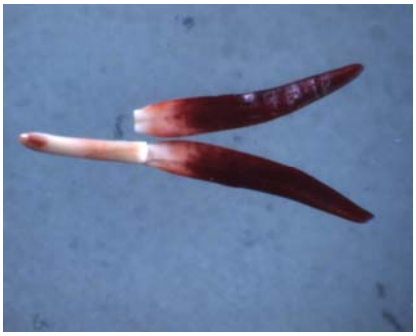
Close-up of X-ray of insect damaged ash seed.

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.