Ash Seed Collection and Emerald Ash Borer (EAB)

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September 26, 2006
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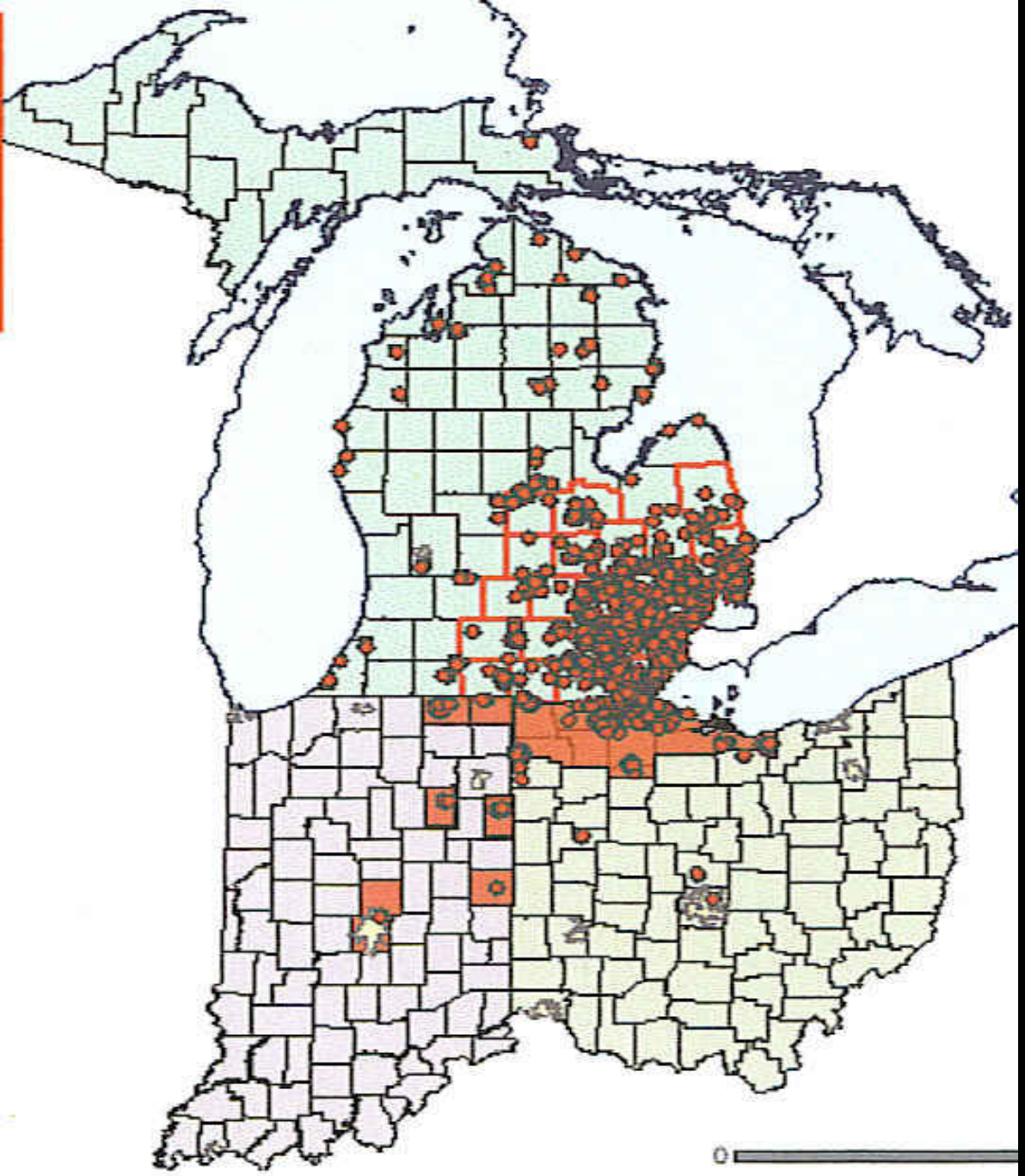


The EAB Viewer is a GIS map showing were the EAB has been confirmed to occur. It, therefore, also indicates the areas where there is the greatest chance of loosing ash trees and subsequently their genes which are adapted to the local growing conditions. This map is compared to Omernik ecoregion level III map to develop seed collection areas.

More information on EAB can be found on the web at http://eabviewer.rigis.msu.edu/viewer.htm and by searching for emerald ash borer using a search engine.

Omernik ecoregions are defined according to precipitation, soil types, temperatures, and similar factors that determine the growing conditions to which plants, e.g. ash trees, must adapt to survive and grow well. Combining the information on this map with the EAB viewer map helped define seed collection areas for ash. A seed collection area is a practical grouping of 2 to several counties. 25 to 50 trees per collection area per species of ash will be considered adequate to preserve most of the ash genes in the collection area.

Seed Collection Areas

The next slide is a list of the priority seed collection areas for 2006 for Indiana. For each seed collection area the following information is provided: the counties included in the area, the Omernik ecoregion in which the area is located, and the species expected to be found in the area. The species listed are taken from published range maps of the species of ash. List have also been created for Michigan and Ohio, and follow the same format as used for the Indiana list.

Range maps for some species of ash can be viewed at http://www/na.fs.fed.us/spfo/pubs/silvics_manual/table_of_contents.htm

Indiana Seed Collection Areas

Collection Area Identifier	Counties included in the collection area	Omernik Ecoregion Level III	Species to collect in this area
1	Elkhart, LaGrange, Steuben, Noble, De Kalb (that part in ER 56)	56	White, green, black
2	De Kalb (that part in ER 55), Allen (that part in ER 55), Wabash, Huntington, Wells, Adams, Grant, Blackford, Jay	55	White, green, black, blue
3	Tipton, Madison, Hamilton, Boone, Hendricks, Marion, Hancock	55	White, green, black, blue
4	Delaware, Randolph, Henry, Wayne	55	White, green, black, blue
5	White,		
P 1	Starke	54	Pumpkin
P 2	Wells	55	Pumpkin
P3	Marion	55	Pumpkin
P4	Shelby, Bartholemew, Jackson	55	Pumpkin

Species Identification

The first step in the collection process is to locate ash trees and identify their species. The next several slides will help do this. The species identification is important so that the seed is completely identified. Leaves, twigs, seeds and the location of the tree are all important to identify it. A voucher specimen, a leaf, a twig, and a picture, will be taken from each mother tree to verify the species identification. This voucher specimen will be stored at the National Arboretum in Washington DC.

The characteristics can vary within a species and look like another species sometimes. Therefore, it is necessary to look at all characteristics to make an accurate identification.

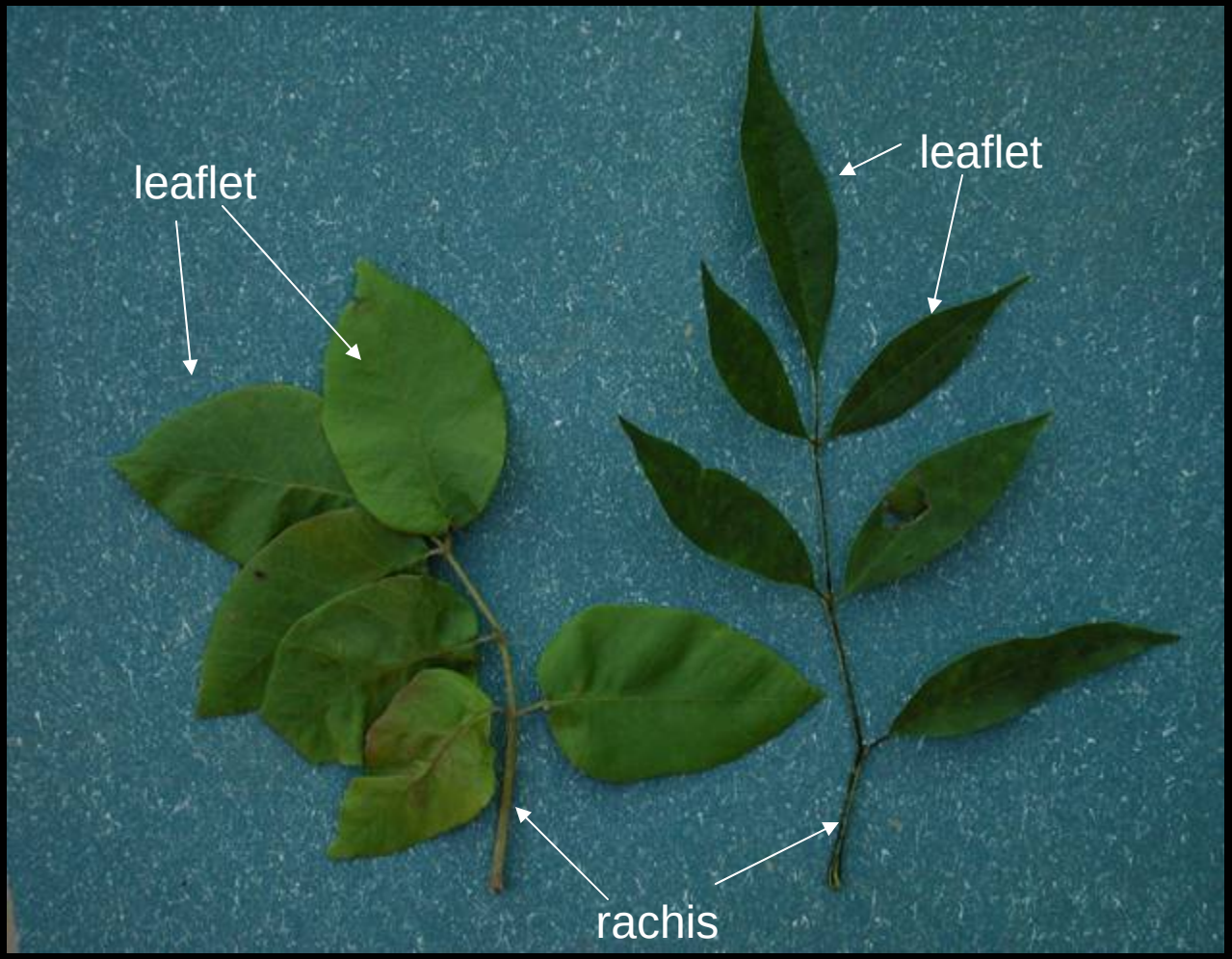
Ash trees have alternate branches.

Maples, ashes, dogwoods, and horse chestnuts (mad horse) have opposite branches but only ash has a pinnately compound leaf. Horse chestnut has a palmately (like a hand) compound leaf.

White ash showing opposite arrangement of branches and leaves.

Where species grow can help identify them.

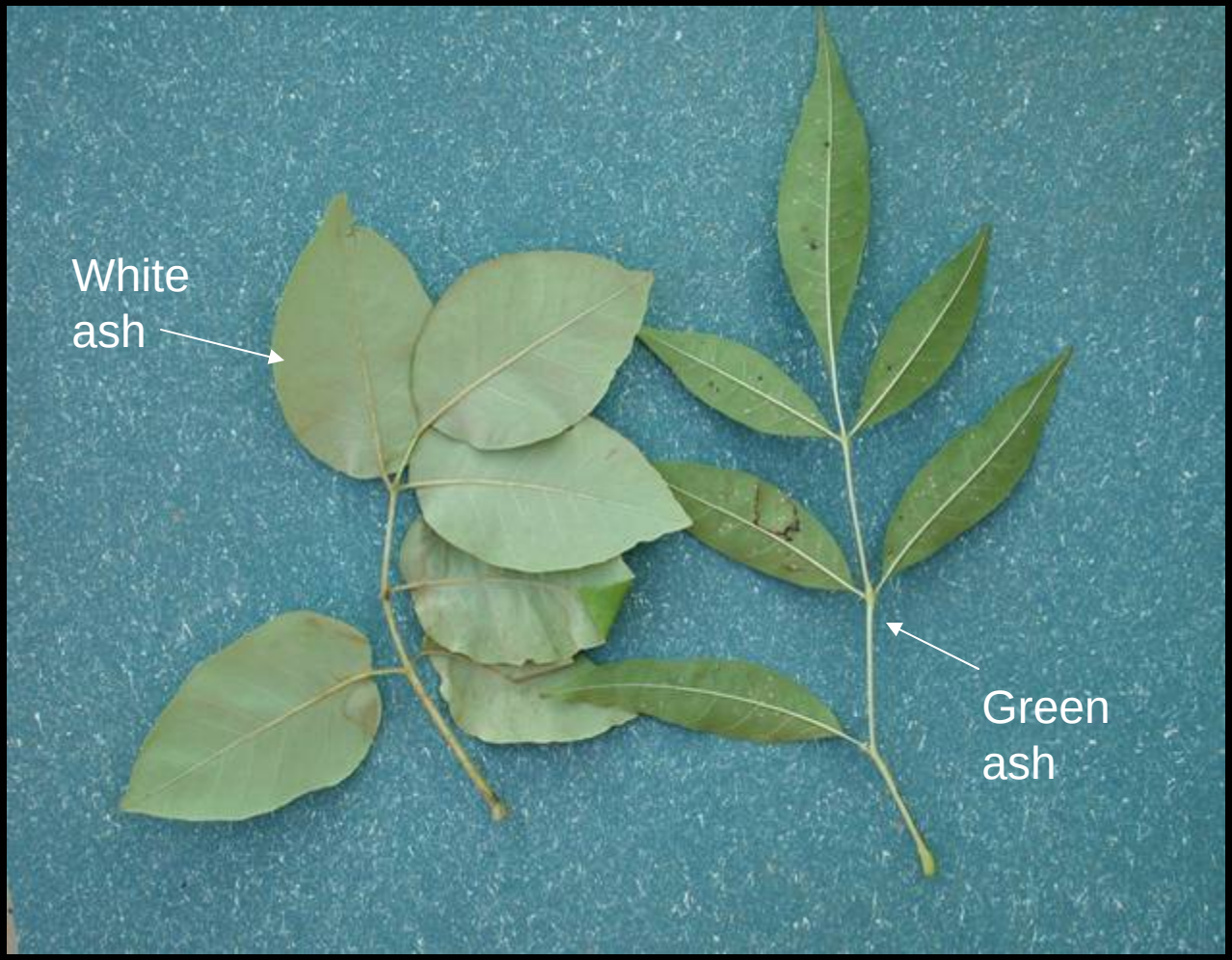
- Green ash and black ash are found on wetter sites (places) with black ash growing in standing water for part of the year.
  - Green ash might be found near black ash.
- White ash is an upland species growing on moist but more well drained sites (places).
  - Green ash might grow close to white ash.
  - White ash and black ash will not normally grow near each other in nature.



White ash left, green ash right.

Ash leaves are pinnately compound.

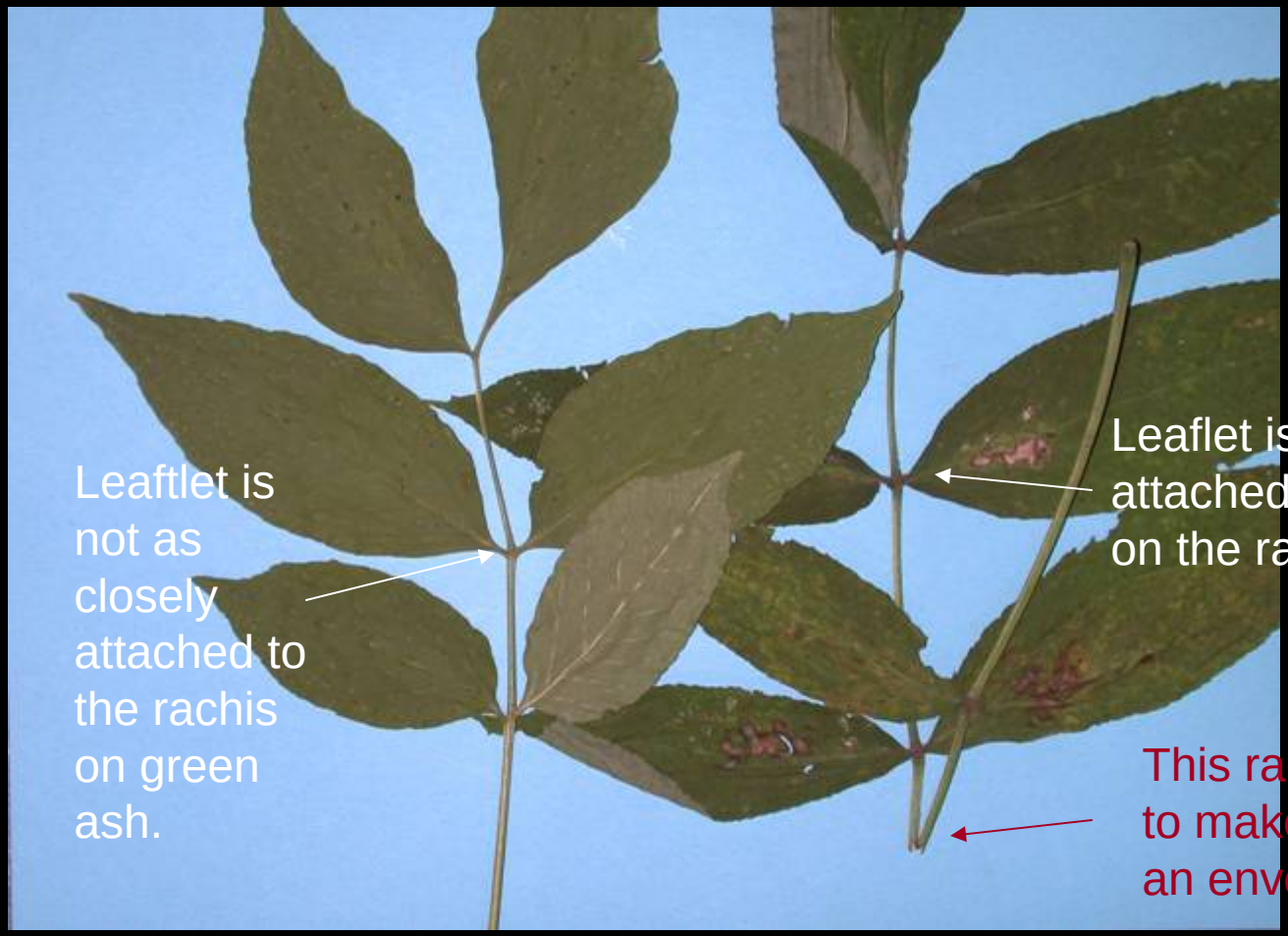
Ash leaves have a central stem called the rachis, with leaflets branching off of it somewhat like a feather.



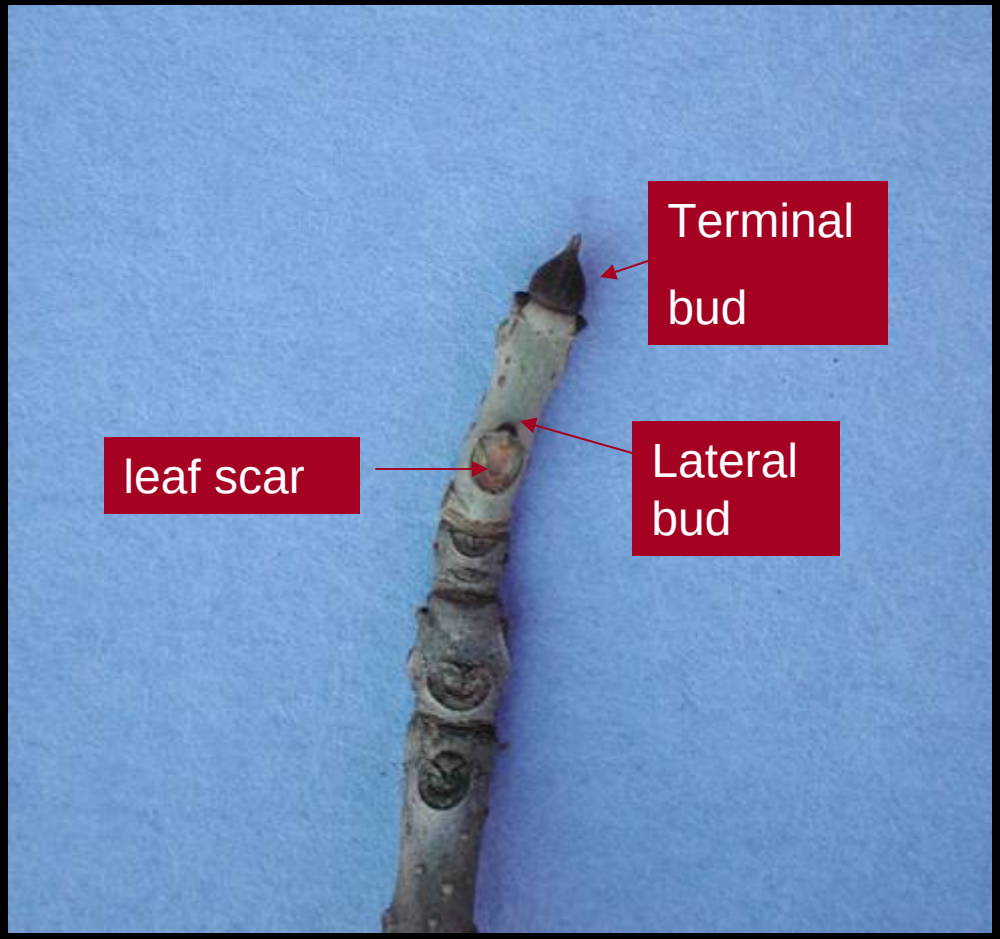
This is the underside of the leaves. White ash is lighter colored beneath than is green ash.

A black ash leaf. The leaflets of black ash are attached very closely to the rachis. They are sesile on the rahcis.

Another black ash showing leaflets that were more pointed (acute) than the leaf in the previous slide. Species characteristics can be variable.

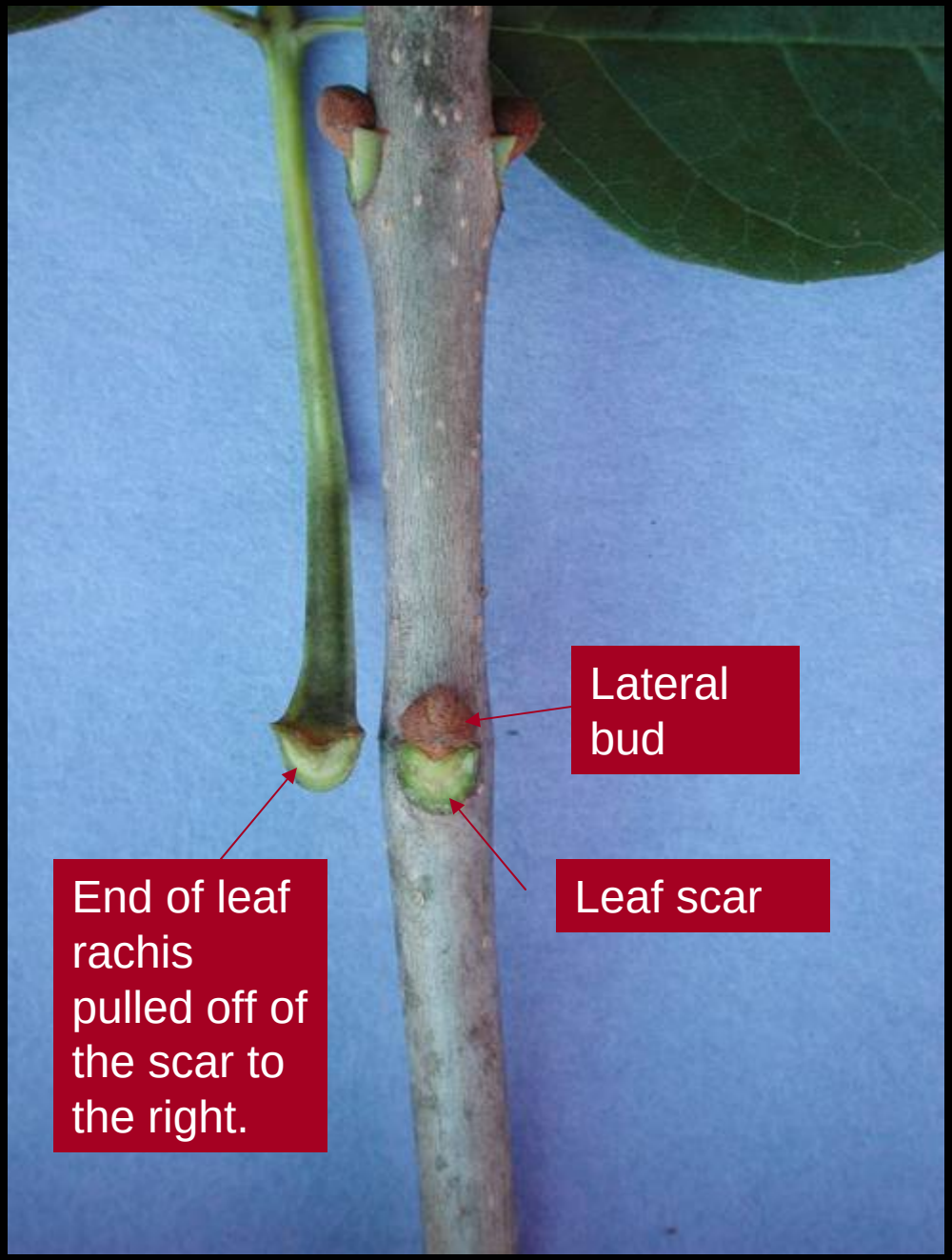


A green ash leaf on the left and a black ash leaf on the right. Note the difference in how their leaflets are attached to the rachis.



Black ash twig

A green ash twig. The bud scar on green ash is typically straight across the top and does not wrap around the bud.



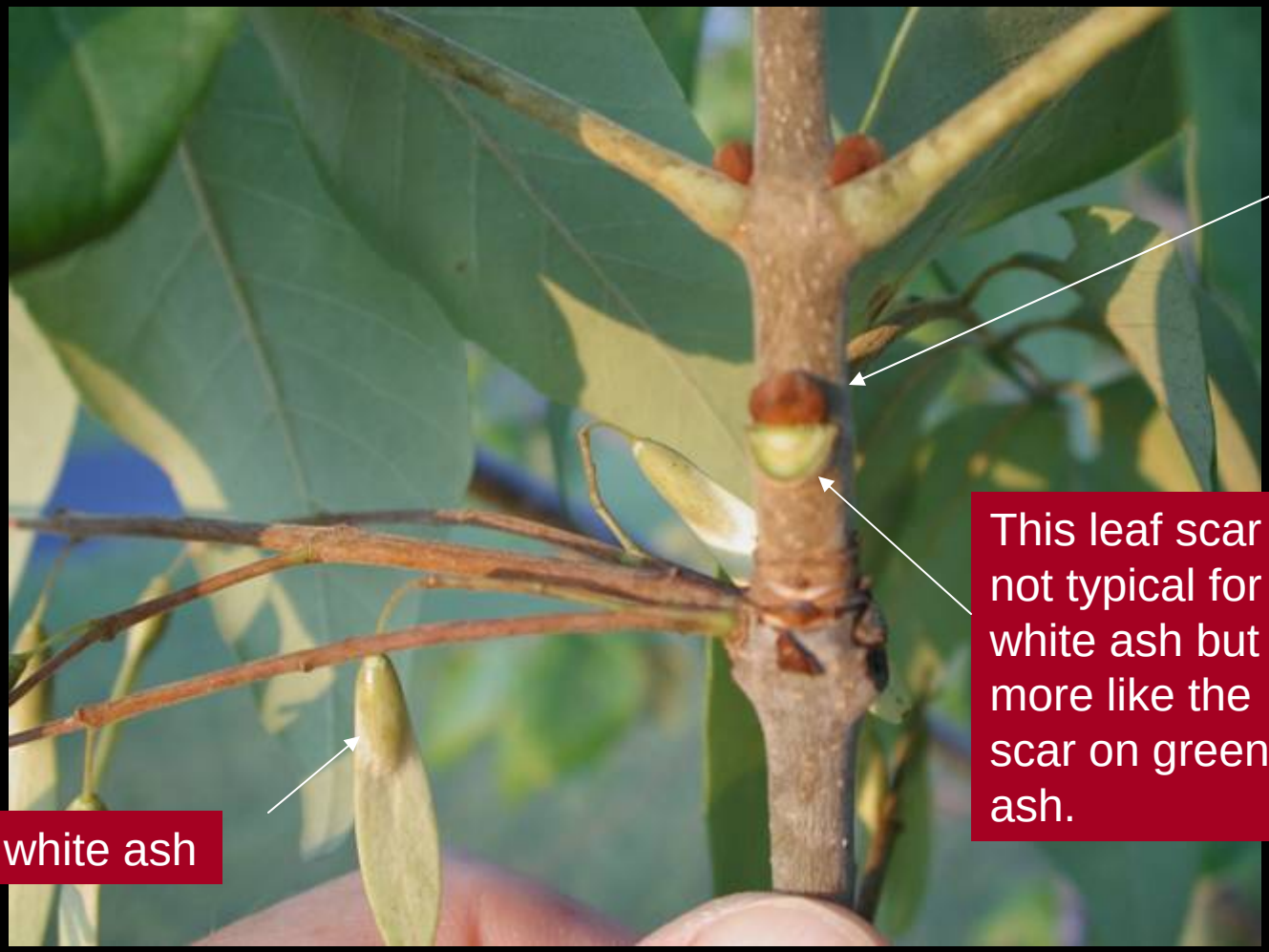
White ash twig showing how the leaf scar comes up on the sides of the bud.

Seeds of green ash are born in tighter pannicles than white ash. The seeds are also narrower and more pointed.

White ash seeds are born in looser pannicles than are green ash. The seeds generally are not as pointed and sharp as green ash are.

Black ash have wings that surround the seed and are easily distinguished from the green and white ash.

Comparison of black, green and white ash seeds



This tree was called white ash because it was more white than green. This is an example of how traits can vary within a species or on one tree.

Monitoring and Locating Seed Trees

Where will seeds be found?

Predicting if seeds will be found in the fall of the year.

Identifying trees from which to collect.

Ash flowers are formed at the base of the new growth each spring. Abundant amounts of seeds will most likely be found from trees that have full crowns with good light exposure. These will be trees growing in open areas.

Another view of ash flowers blooming at the base of new shoot growth in the spring of the year. The new growth is bright green in spring.

Immature white ash seeds growing from the base of the current year's growth. The flowers and immature seeds are indicators that seeds will be available in the fall.

This tree would be good to collect from. It is growing wild next to a residential yard. Unless the tree is positively known to have been a wild tree sprouting up naturally or has been transplanted from the local forest, trees in residential yards are not good trees because their genetic background is unknown. Trees from parks, streets or other public places are not acceptable for the same reason.

Dioecious: only male or female flowers are produced.

Monoecious: Both male and female flowers are produced.

White and green ash are dioecious.

Black ash is polygomous: maybe male, maybe female, maybe both on the same plant.

Outcross: two trees involved.

Selfed: The tree's own pollen pollinates the tree.

Trees growing along rural roads are good trees to collect from. White and green ash are either male or female and cannot self pollinate themselves. Although this tree is isolated it has been outcrossed and will have good seeds. Isolated trees of a monoecious tree species might have self pollinated seeds that are inbred and will give poor seedlings.

This is the tree from the previous slide. Many seeds can be hand stripped from the tree while simply standing on the ground.

Although this tree has lots of seeds this year it may not have many next year. Trees are cyclical in seed production and may not have seeds every year.

Trees growing along the edge or out in farm fields are good seed producers .

This tree is acceptable for collecting because it was a strong tree at one time. The top has died back most likely because the farmer has struck it several times with farming implements. The bark at the base of the tree has been broken off about half way around the tree.

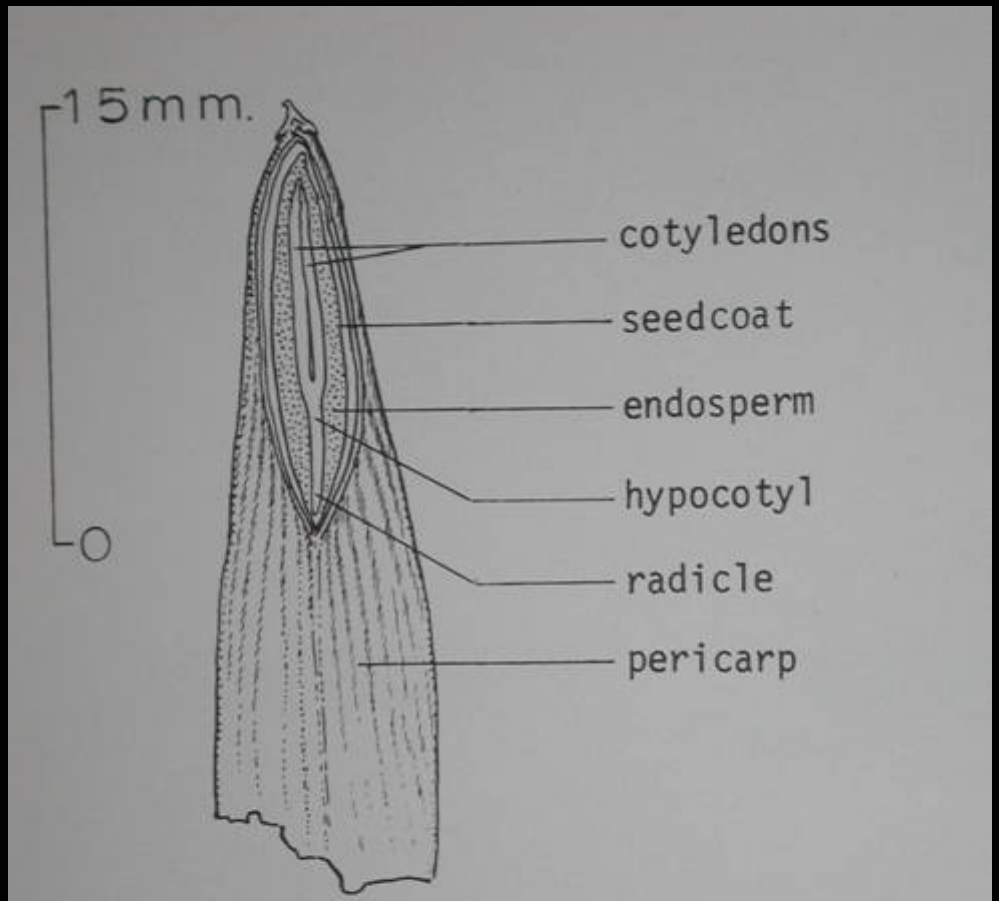
This tree has an abundant seed crop.

When are seeds ready to collect?

- Must first know the seed structures to observe if they are developing and maturing.
  - The following slide shows the structure of a mature ash seed
  - Subsequent slides show seeds in different stages of development and the changes they undergo as they mature.

It is important to known the structure of the seed for determining when seeds are mature and good for collecting.

What is called the seed in practice is actually the fruit, samara, of the ash tree. The true seed is inside the fruit.



RE 3.—*Fraxinus pennsylvanica*, green ash: longi-dinal section through the embryo of a samara, ×.

The pericarp in this drawing is the fruit wall. This fruit type is a samara. A samara is a one-seeded, dry, indehiscent, winged fruit.

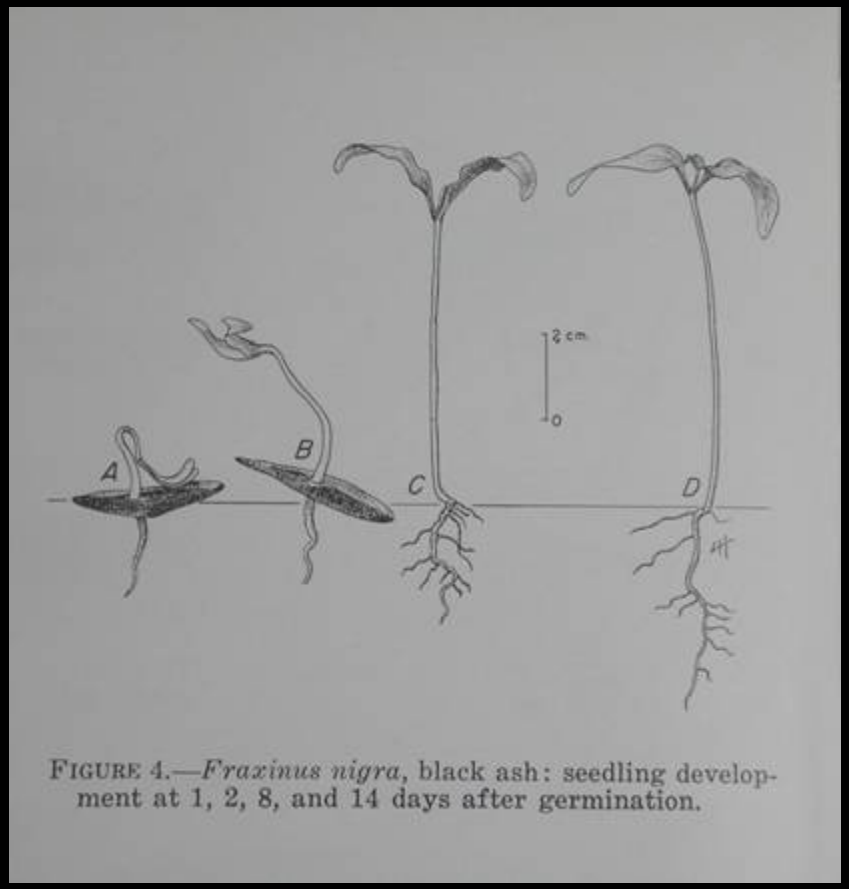
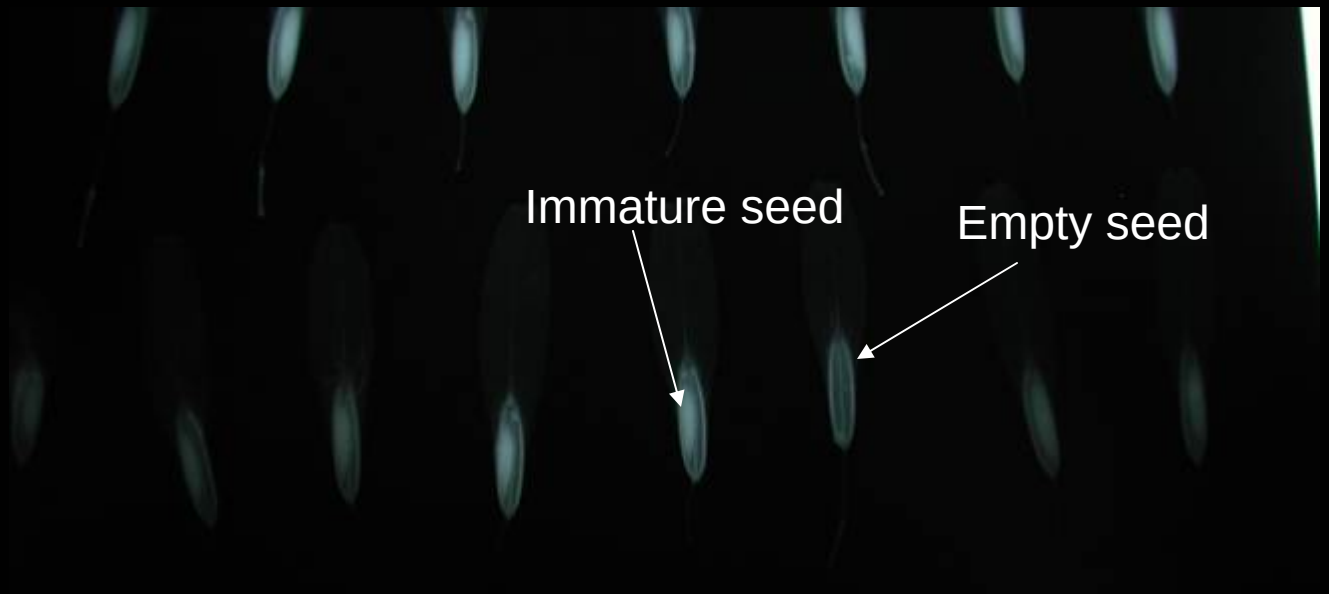


FIGURE 4.—*Fraxinus nigra*, black ash: seedling development at 1, 2, 8, and 14 days after germination.

Drawing of black ash seed germinating.

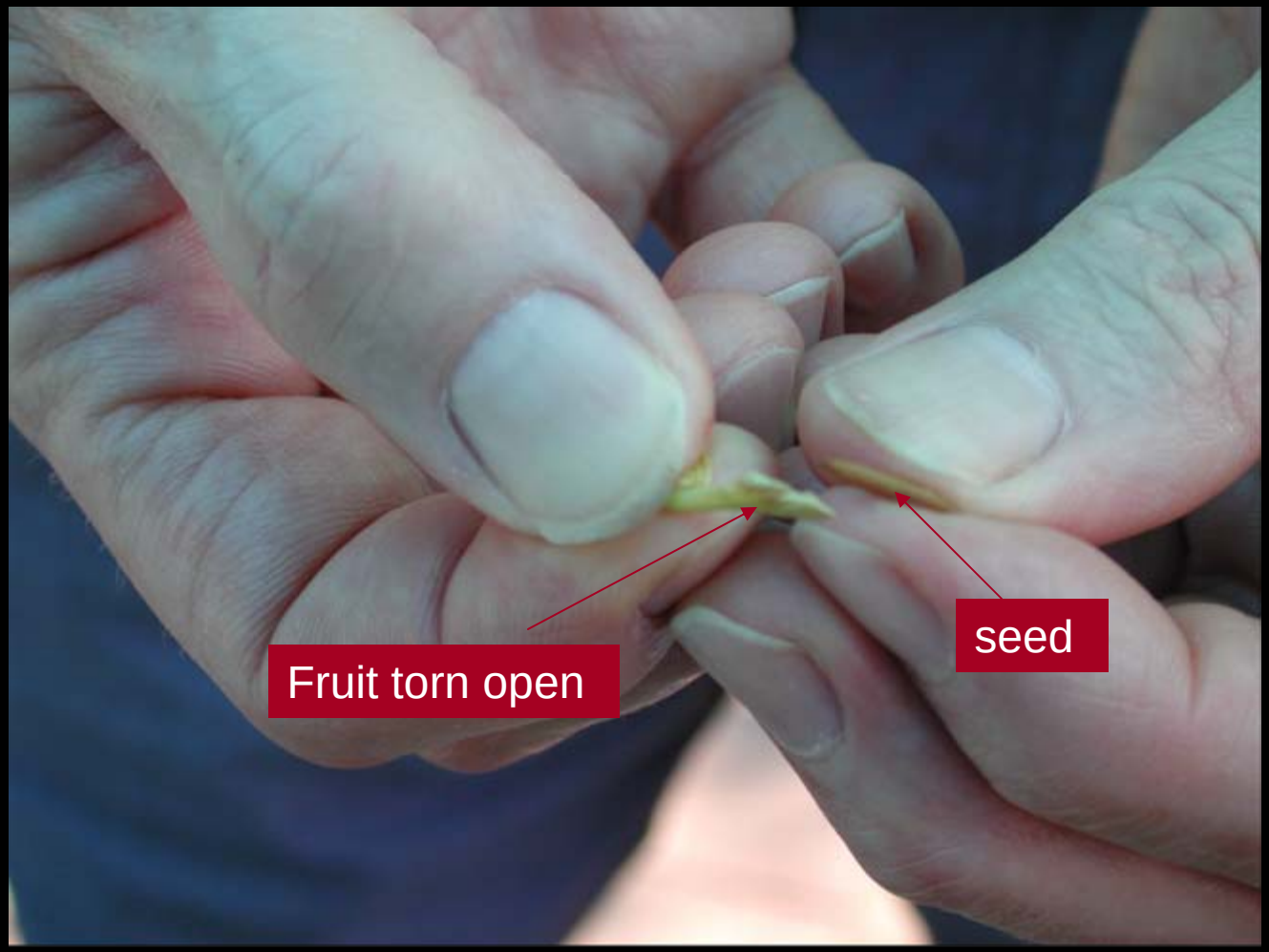
The first seeds to fall are empty or damaged by insect. This is an x-ray of seeds fallen from a tree in late August.



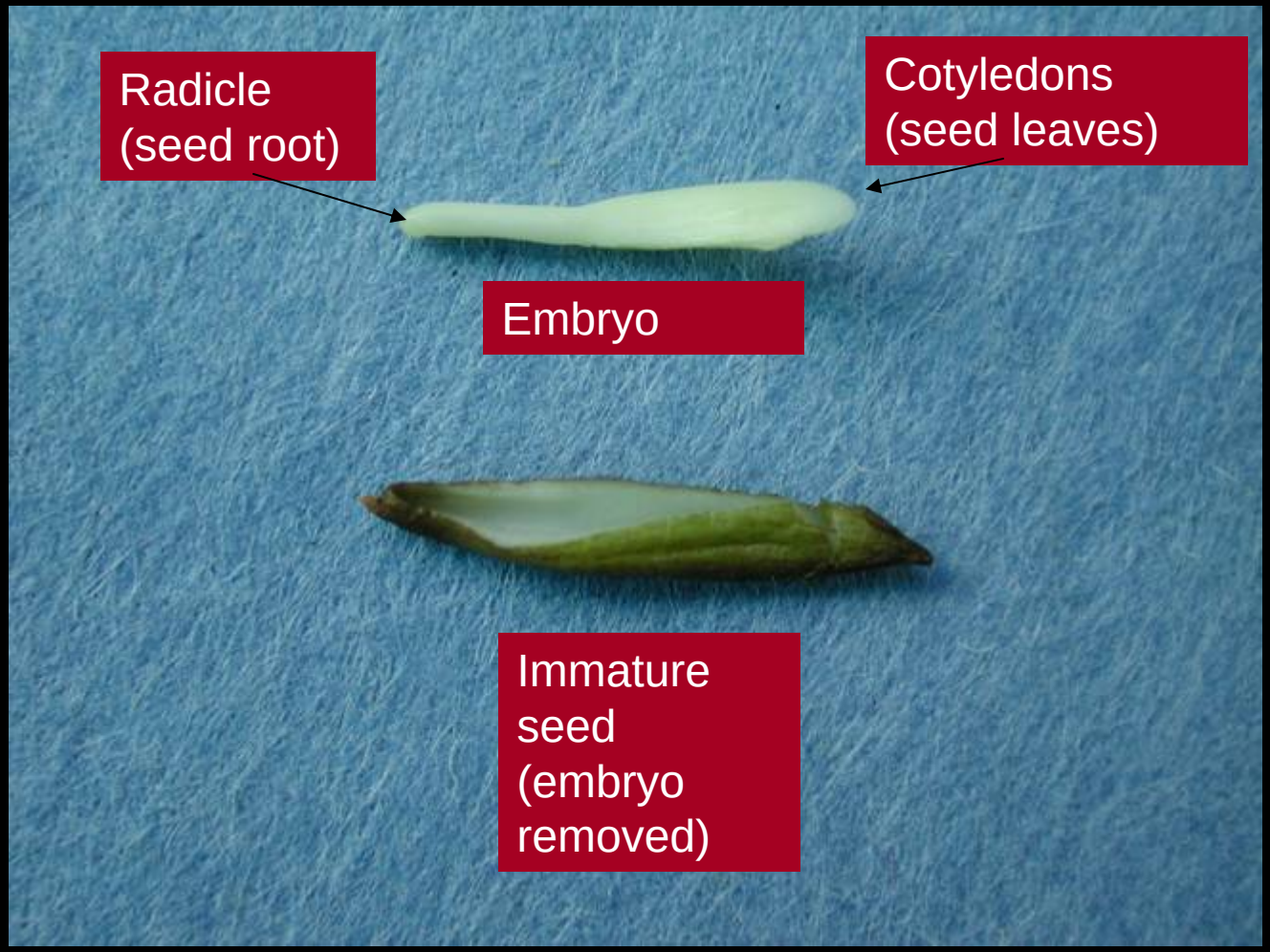
Fruit reaches full size before the seed and embryo. Cut seeds open to make sure embryo is full sized and firm, not soft or milky before collecting. The white image in this x-ray is the developing seed. It is about 1/3 of its mature size.

To examine the seed, first grasp it as shown here. The fruit can then be torn open with the thumb nail of the other hand to expose the developing seed.

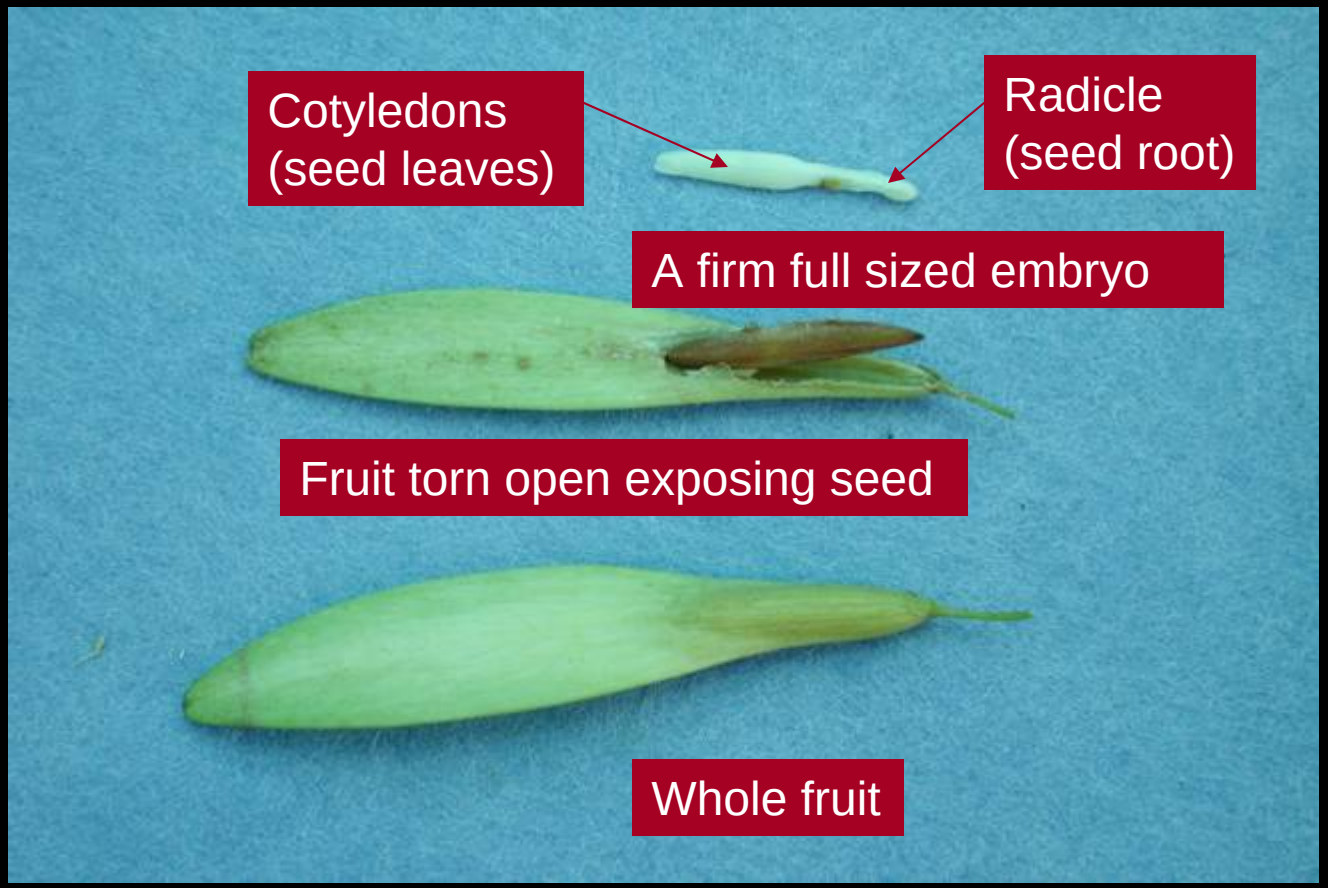
This fruit has been torn open to expose the seed for examination.



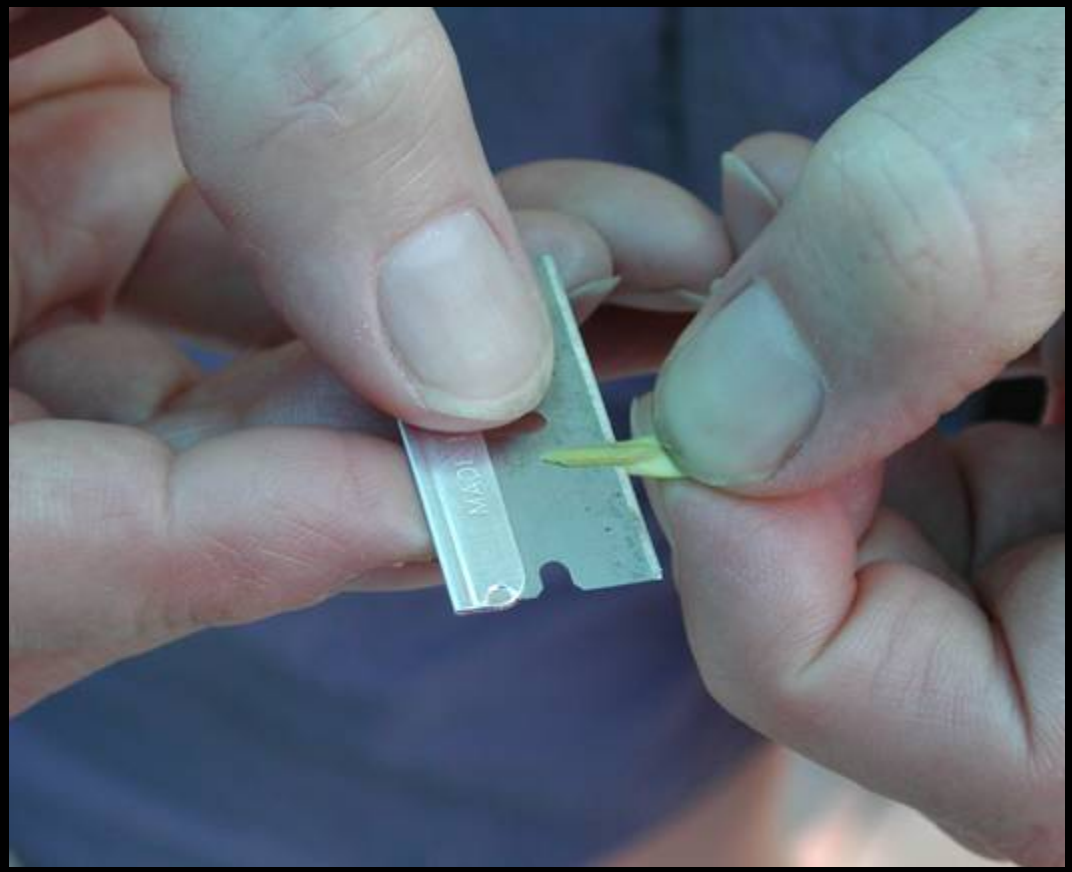
This seed has been pulled from the fruit after tearing the fruit open. The brown seed color indicates the fruit is ready for harvest.



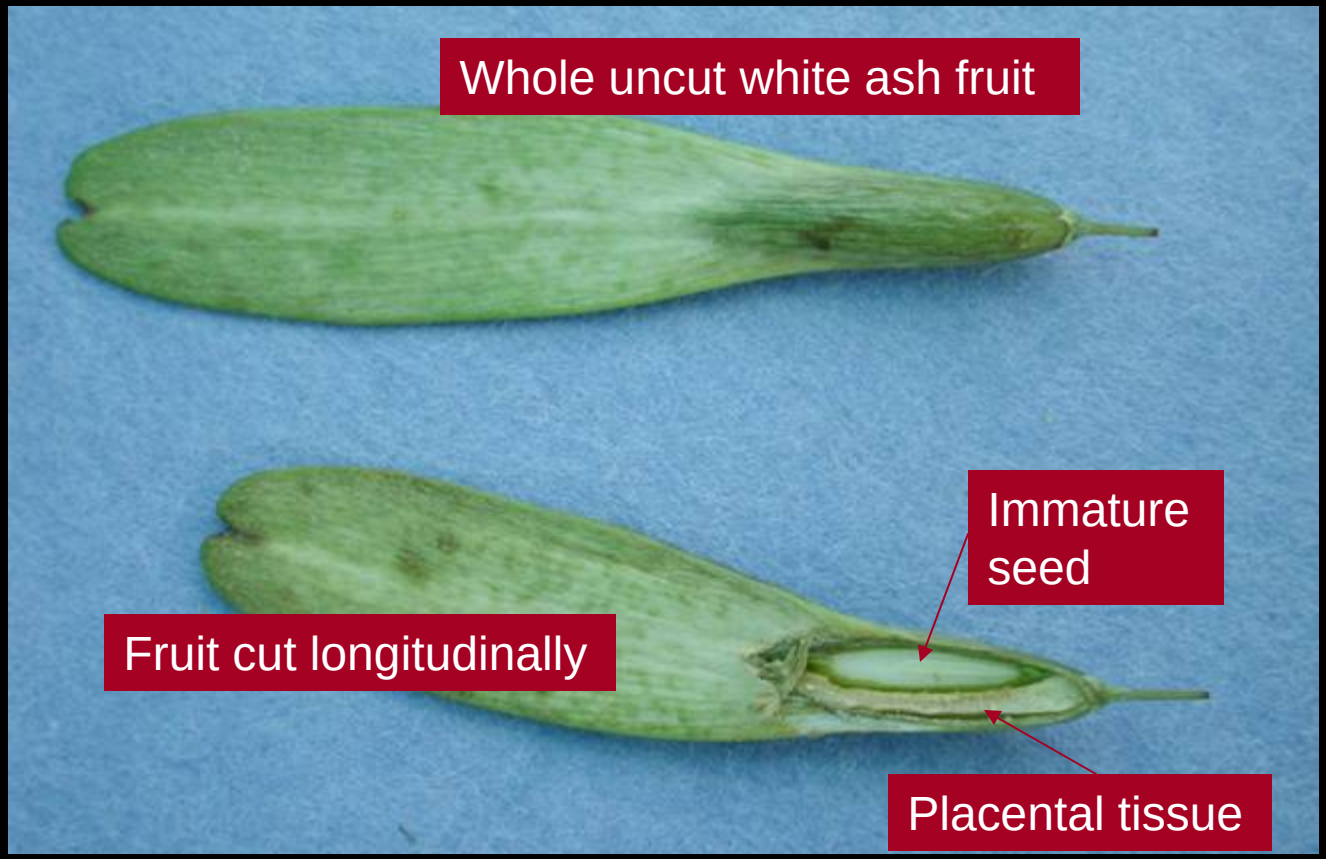
A green immature seed excised from the fruit with the embryo excised from the seed. The embryo is firming up but is still immature.



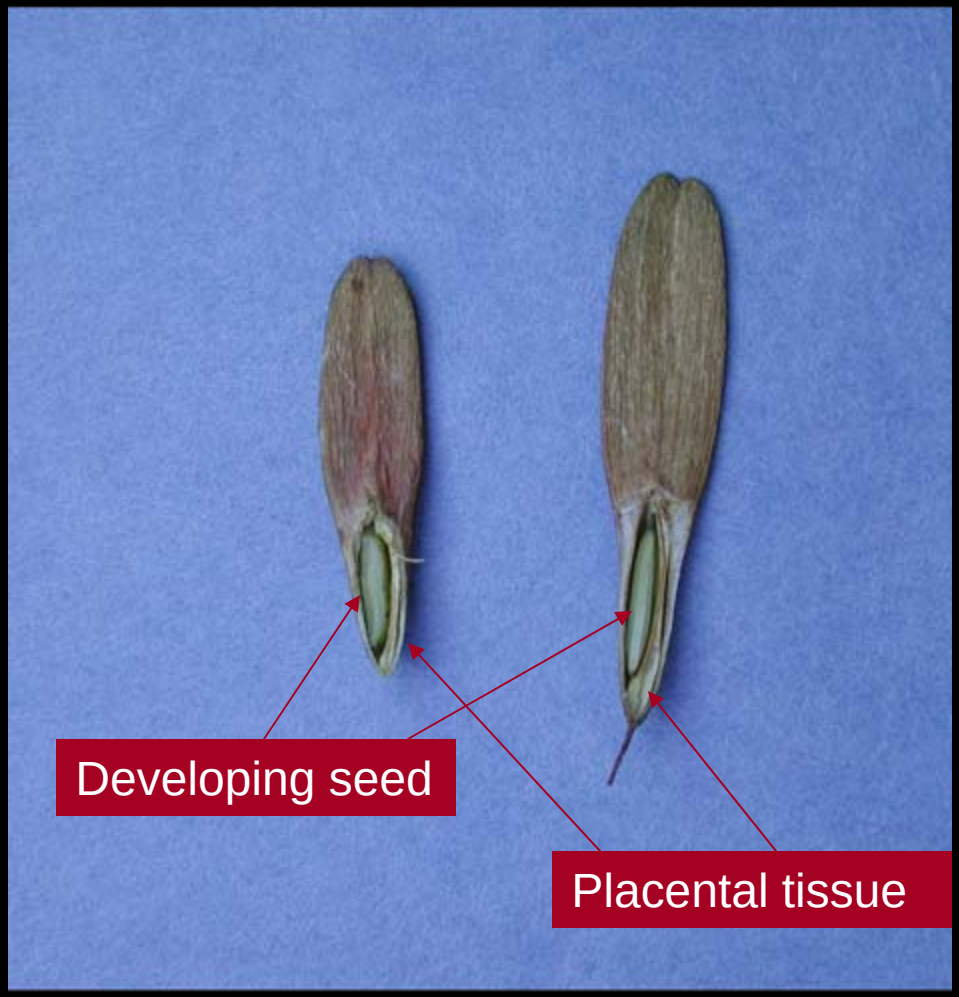
A white ash seeds at different stages of examination. These seeds are ready to collect because the seed coat is brown, the seed fills the fruit, and the embryo and endosperm are firm and not soft or milky.



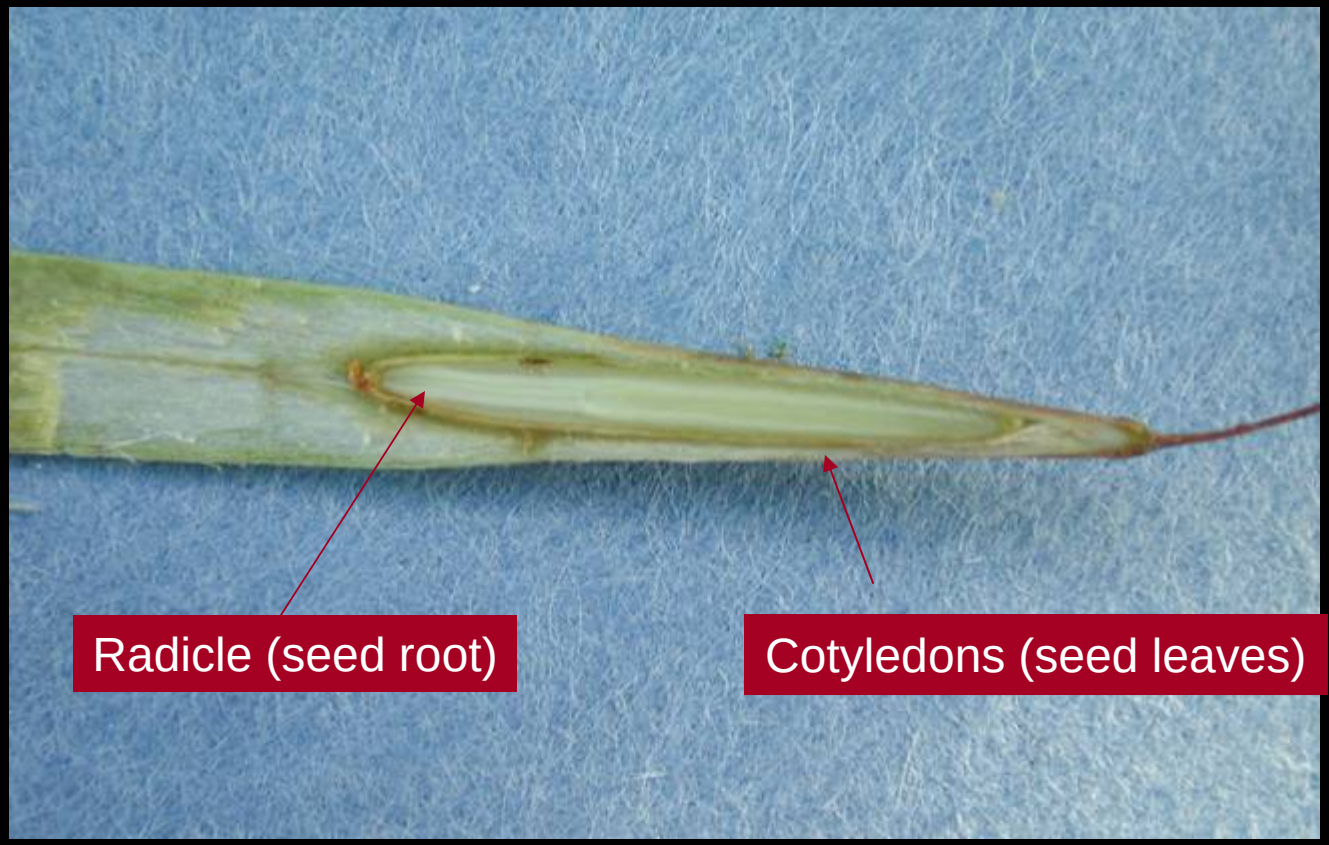
Fruits can also be cut longitudinally with a razor blade to make a clean cut that sometimes makes it easier to see more detail.



Longitudinal cut showing the developing seed. This seed is about half of its mature size. It is important to distinguish between the seed and the placental tissue next to it. Otherwise it could be concluded that the seed fills the fruit when in fact it only about half fills the fruit with the other half filled with placental tissue. This seed is not mature enough to harvest. The seed must develop further.



Longitudinal cut showing the developing seed. This seed is almost mature size, but is not mature enough to harvest. The seed coat is still green in color. Seeds from their mother tree must develop further before picking.

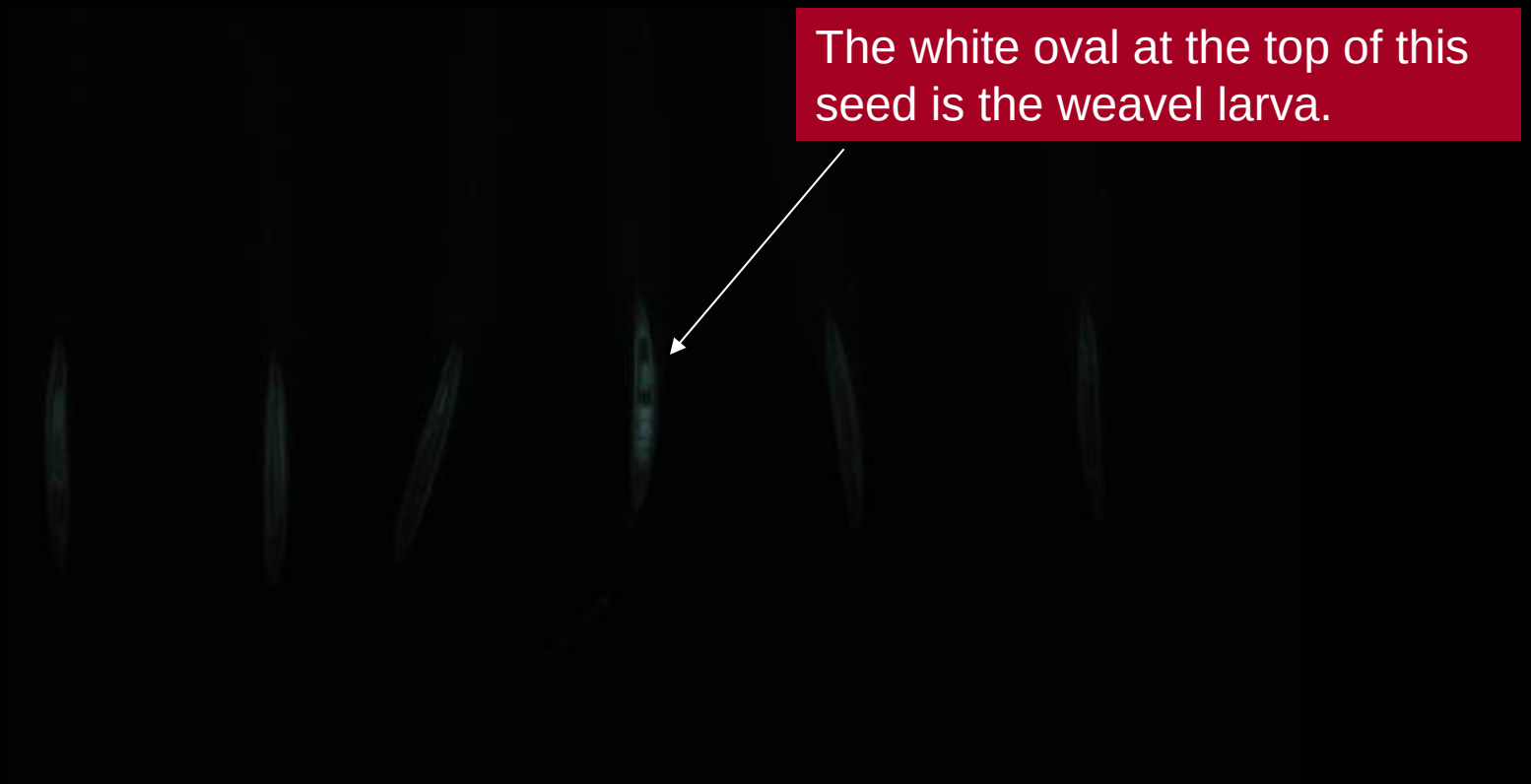


This longitudinal cut of a green ash seed shows that the seed fills the fruit cavity and the embryo has reached full length. It is ready for harvest.

Longitudinal cuts on green ash seeds showing that the seed coats have matured and turned tan colored. These seeds are ready for harvest.

Insects damage on seeds

- When insects feed on seeds the seeds are often killed.
- A longitudinal cut with a razor blade or knife will show if the seed is damaged.
- Insect damage can also be seen in an x-ray
- Weavels and seed bugs cause damage to ash seeds.



An x-ray of green ash seeds. The seed 3rd from the left contains a weavel larva.

A weavel larva has destroyed this seed.

These fruits apparently have been attacked by a seed bug.

Longitudinal cuts showing the seeds in the previous slide have been damaged at the same point as the fruit was damaged.

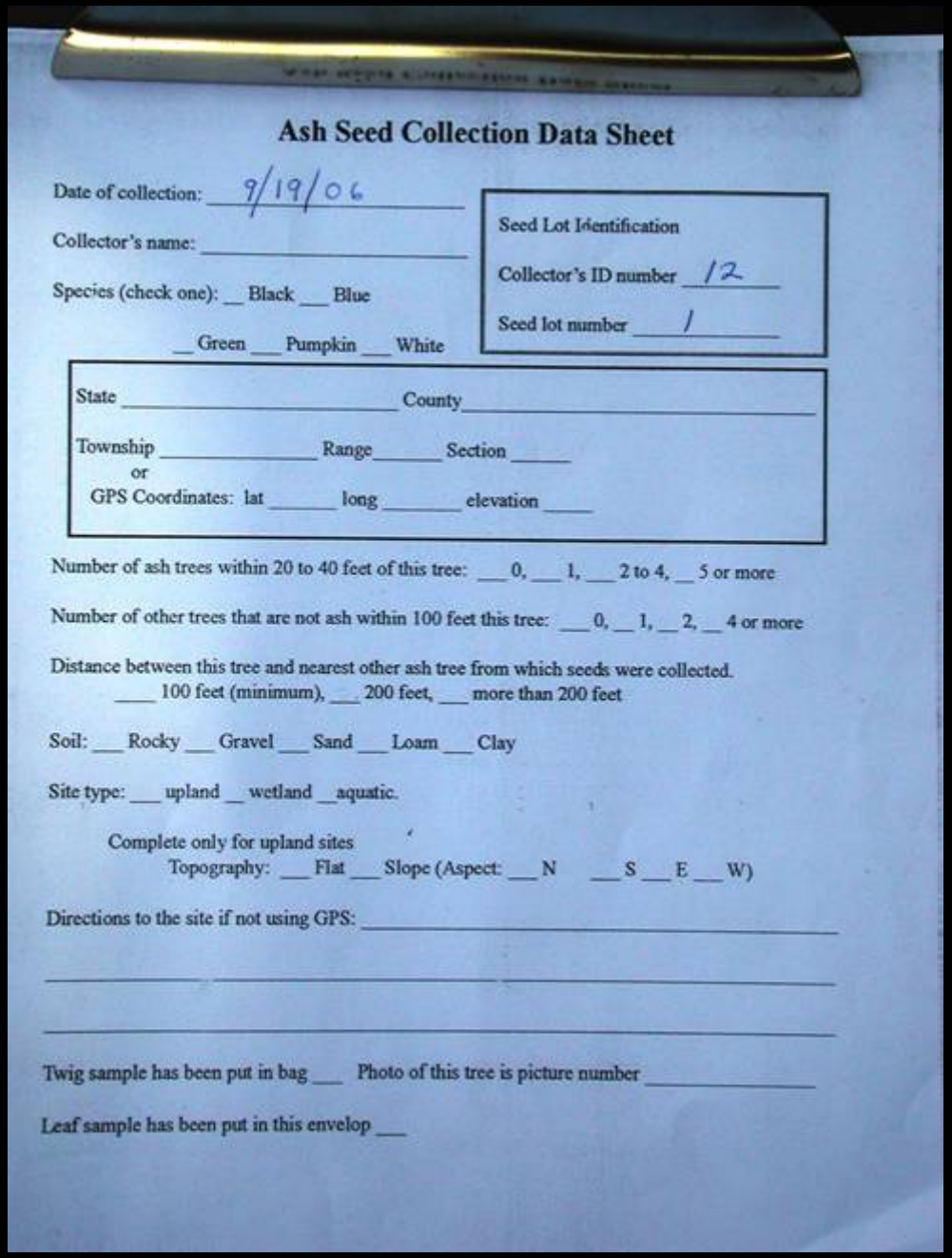
Do not collect from trees with heavy amounts of insect damaged seeds. These seeds are if poor quality and not likely to germinate.

The Actual Seed Collection

- Prerequisites
  - The species of ash has been identified
  - A tree with an abundant seed crop is located.
  - Examination of the seeds shows they are full, mature, not damaged by insects
  - Assembled the collection materials

Step by Step Procedures

- This section of the presentation covers a line by line completion of the data collection sheet and the picking of the seeds.



Ash Seed Collection Data Sheet

Date of collection: 9/19/06

Collector's name: _____

Species (check one): __ Black ___ Blue

__ Green ___ Pumpkin ___ White

Seed Lot Identification

Collector's ID number 12

Seed lot number 1

State _____ County _____

Township _____ Range _____ Section _____

or

GPS Coordinates: lat _____ long _____ elevation _____

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

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

Distance between this tree and nearest other ash tree from which seeds were collected.

____ 100 feet (minimum), ___ 200 feet, ___ more than 200 feet

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

Site type: ___ upland __ wetland __ aquatic.

Complete only for upland sites

Topography: ___ Flat ___ Slope (Aspect: ___ N ___ S ___ E ___ W)

Directions to the site if not using GPS: _____

_____

_____

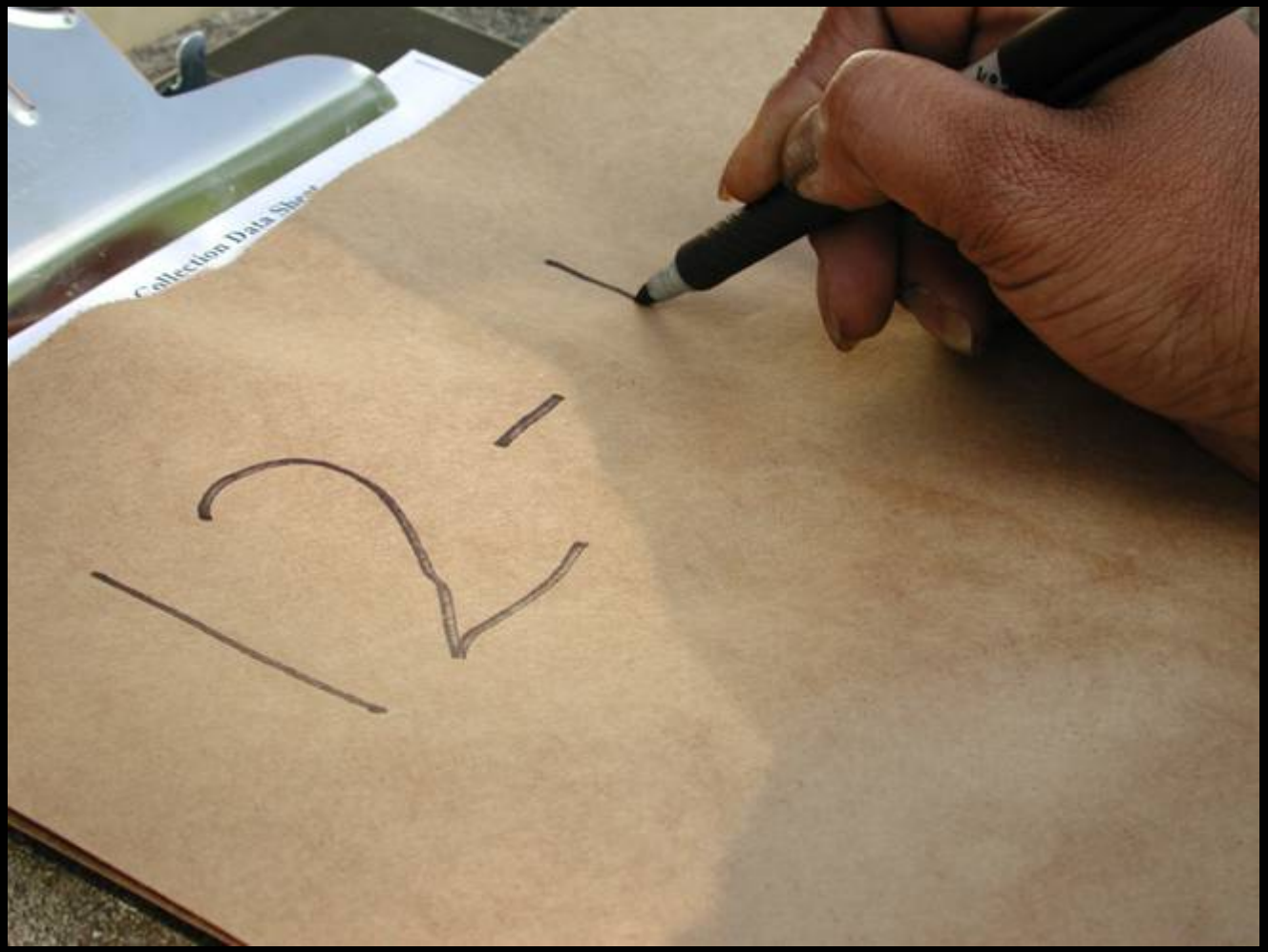
Twig sample has been put in bag ___ Photo of this tree is picture number _____

Leaf sample has been put in this envelop ___

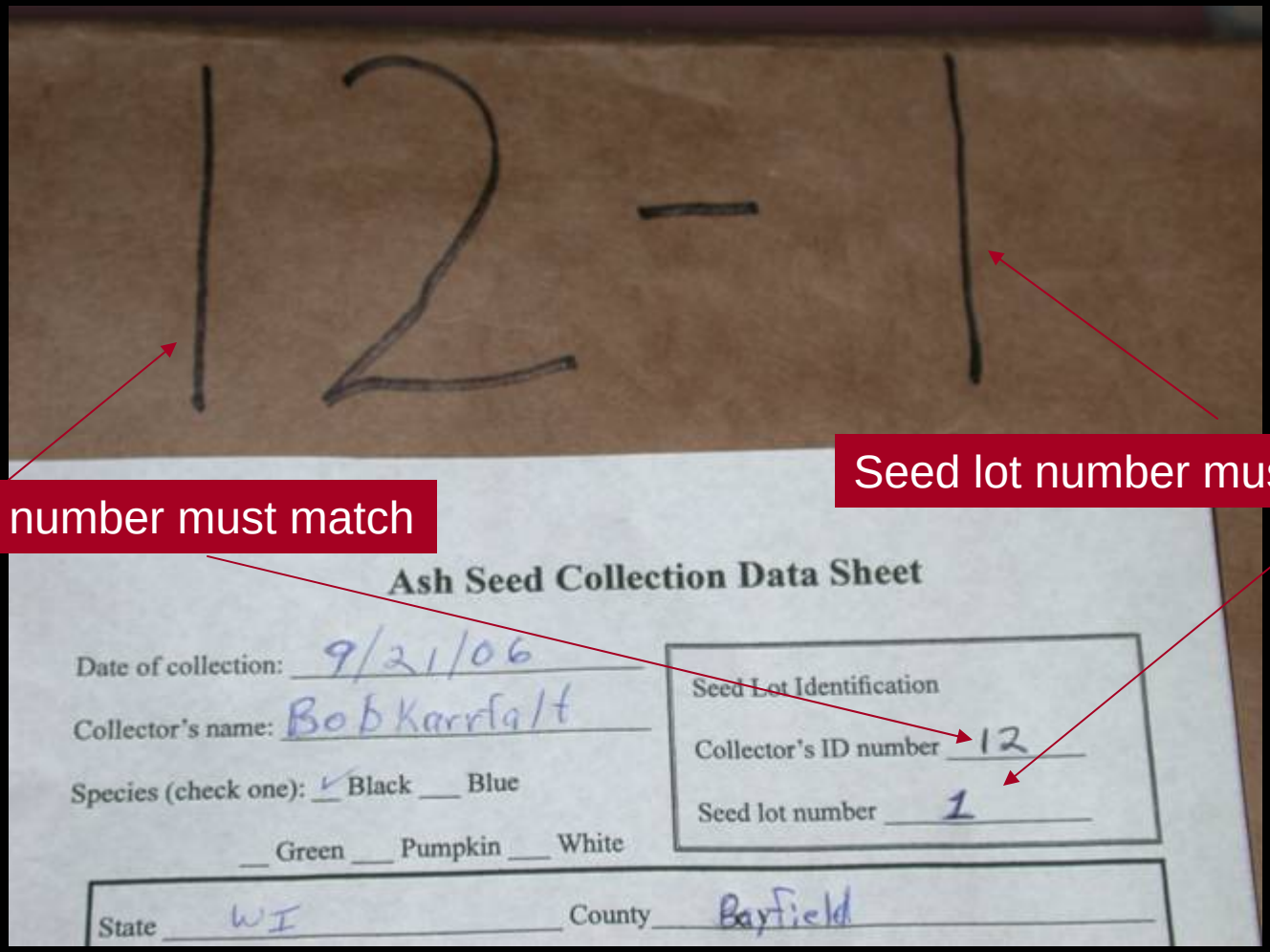
A data collection sheet is needed for each seed lot collected in order to maintain its identity. These sheets are found in the accordion folder.

Filling out the data sheet is the first step in taking the seed from the tree.

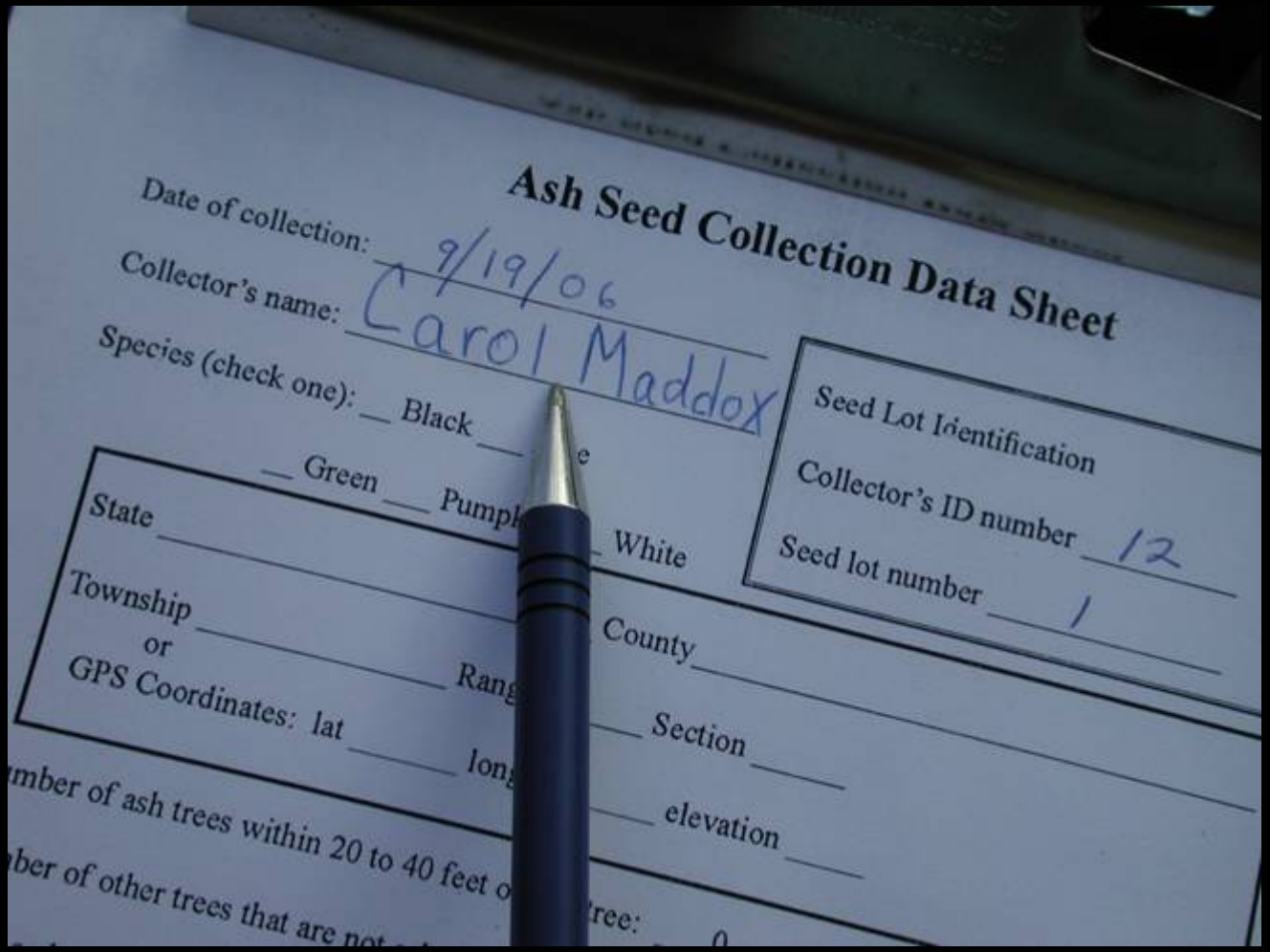
"Collector's ID number" and "Seed lot number" will be filled in by the seed lab before the data sheets are sent to you.



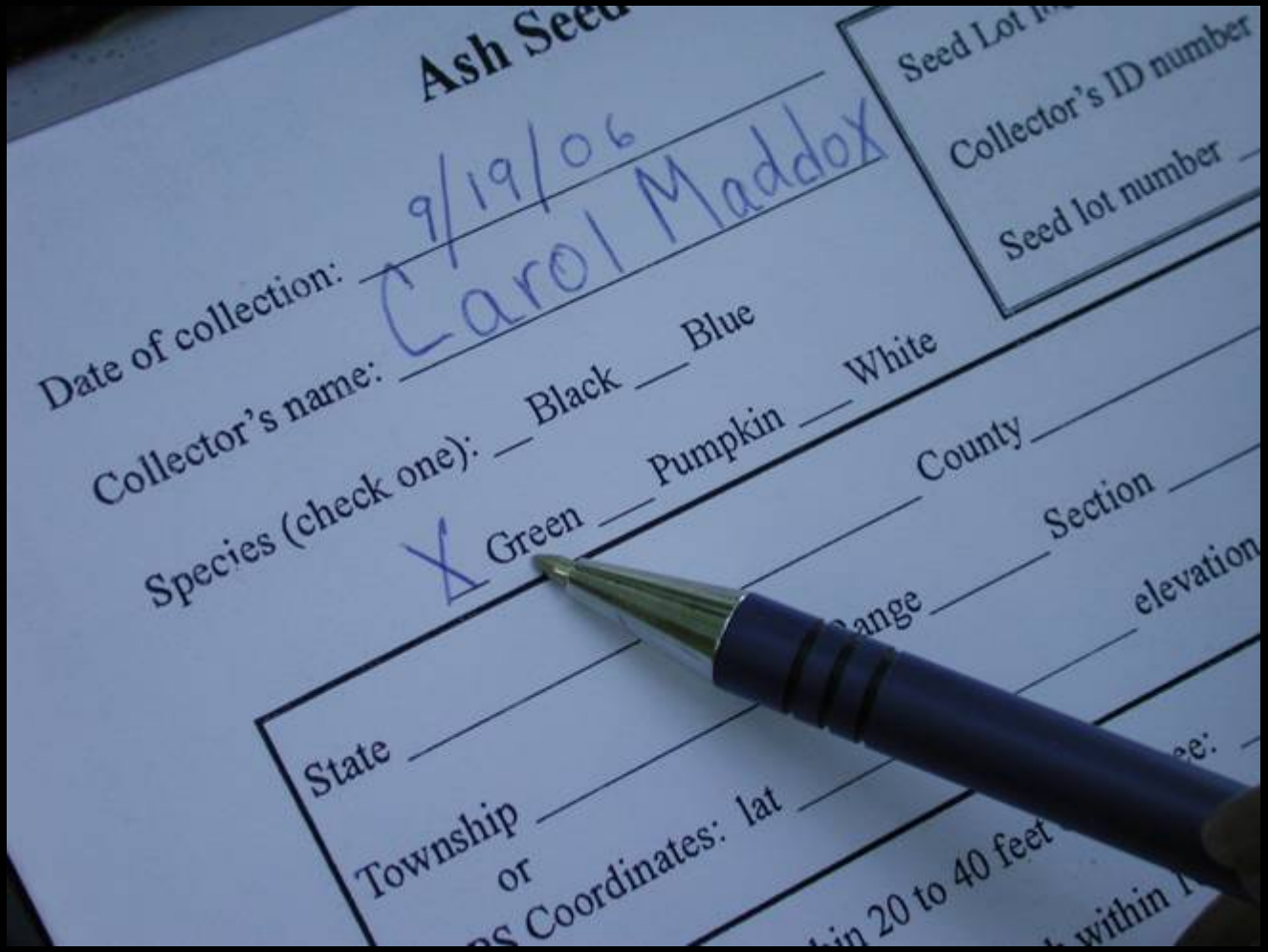
Clearly write the collector identification number and seed lot number on the collection bag for the seeds. Use a dash as shown to separate them. Write the collector's ID number first followed by the dash and the seed lot number.



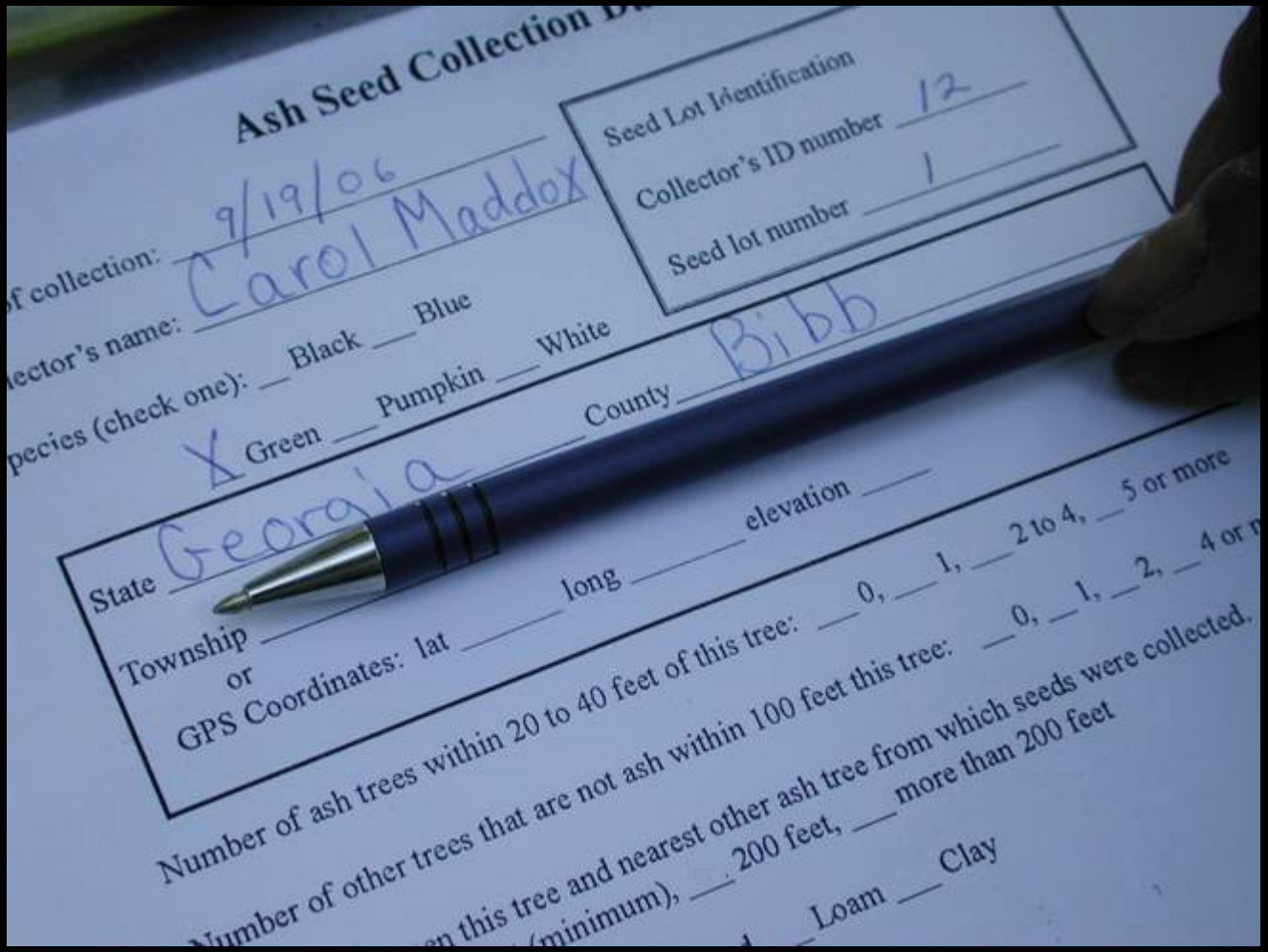
Bag number and data collection sheet numbers must match exactly for the seed to be positively identified and useable in the gene preservation program.



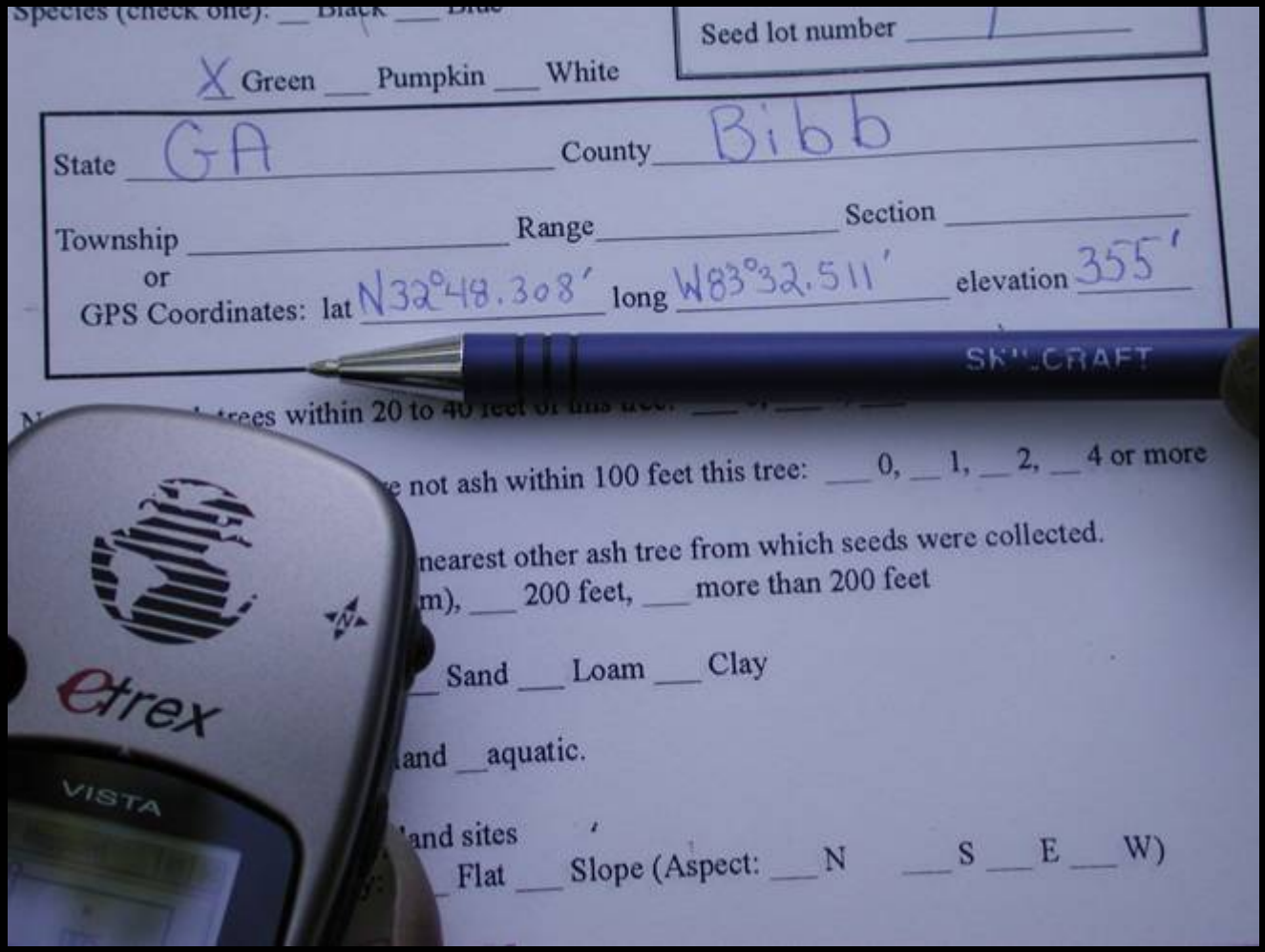
The name of the actual person picking the seeds from the tree is written on the “Collector’s name” line. Just one name is needed if more than one person is picking from the same tree.



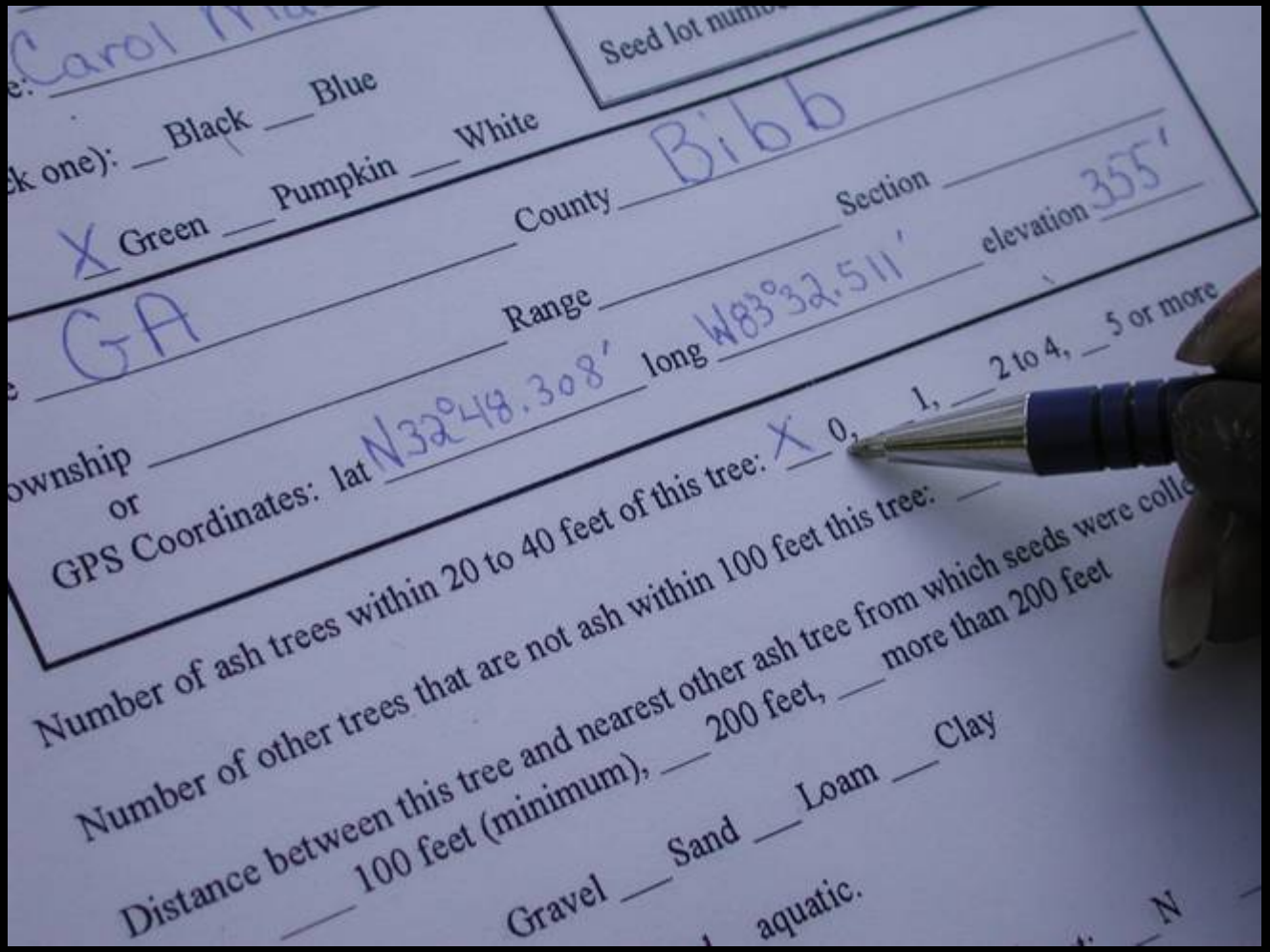
Place an “X” or a check mark indicating what the species of the tree is.



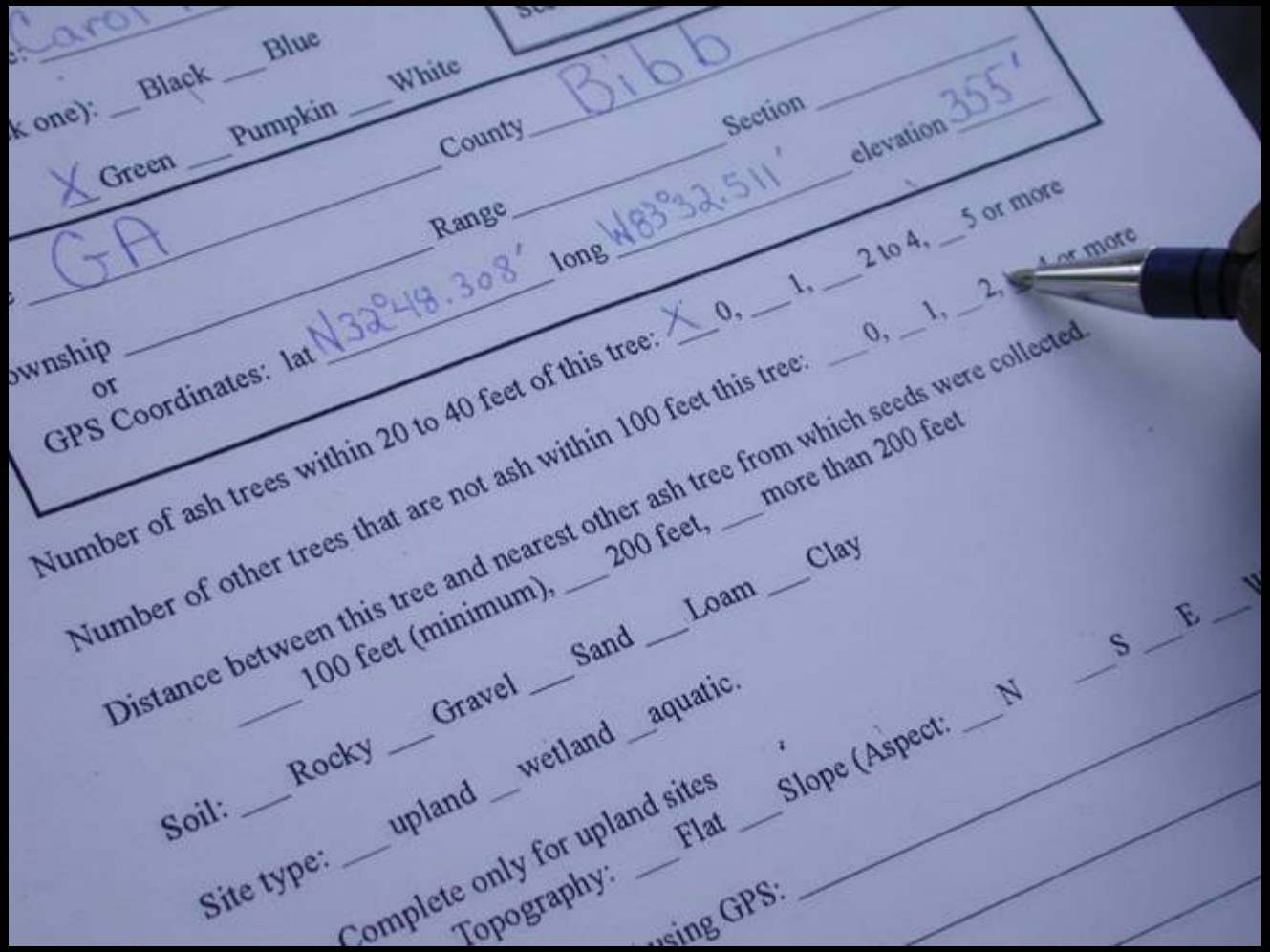
Fill in the state and county where the tree is located. Two letter abbreviations for state can be used (i.e. MI, IN, OH).



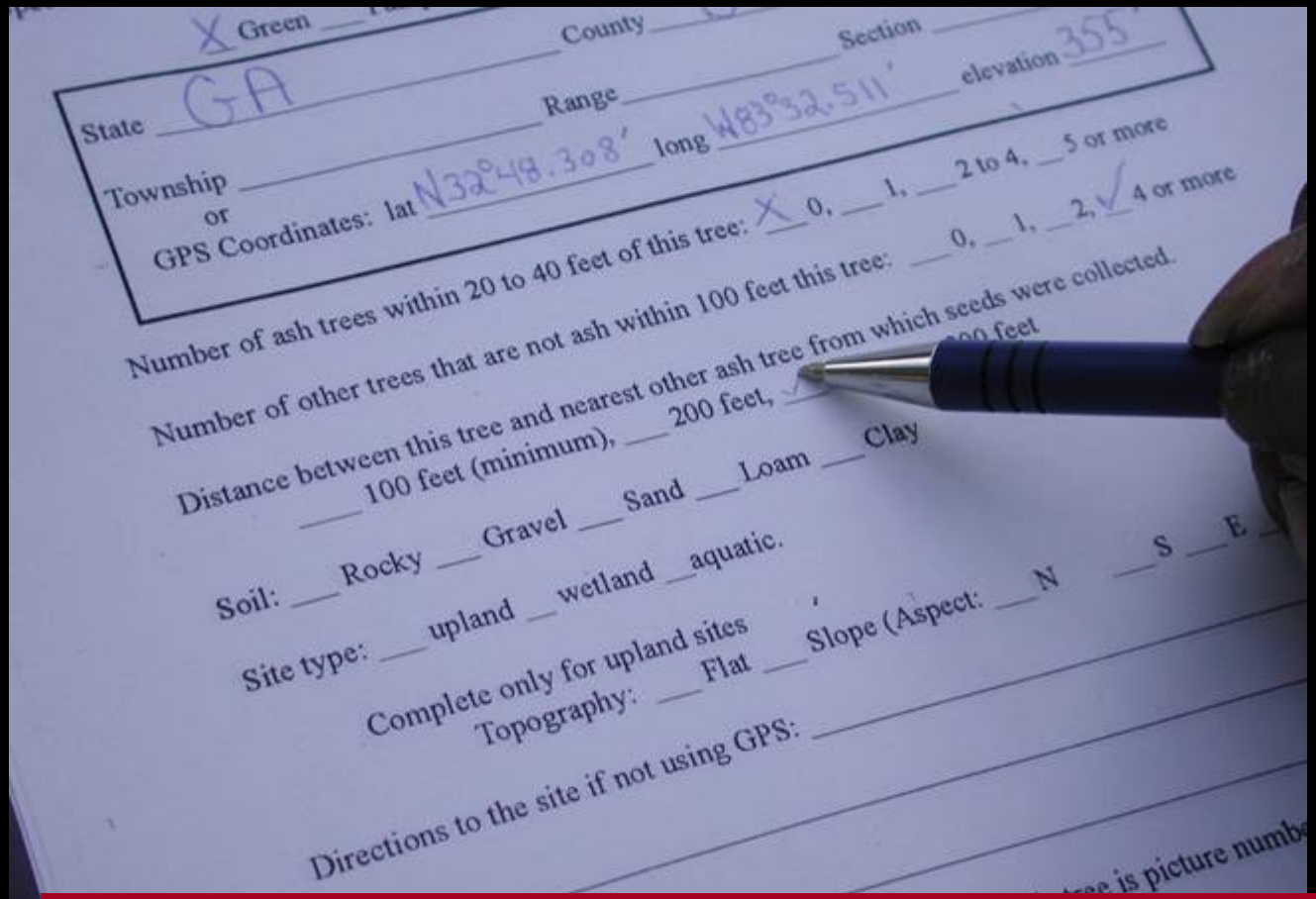
Fill in either the “Township, Range, Section” line or the GPS line. Both lines can be left blank if the directions to the site are recorded below on the sheet.



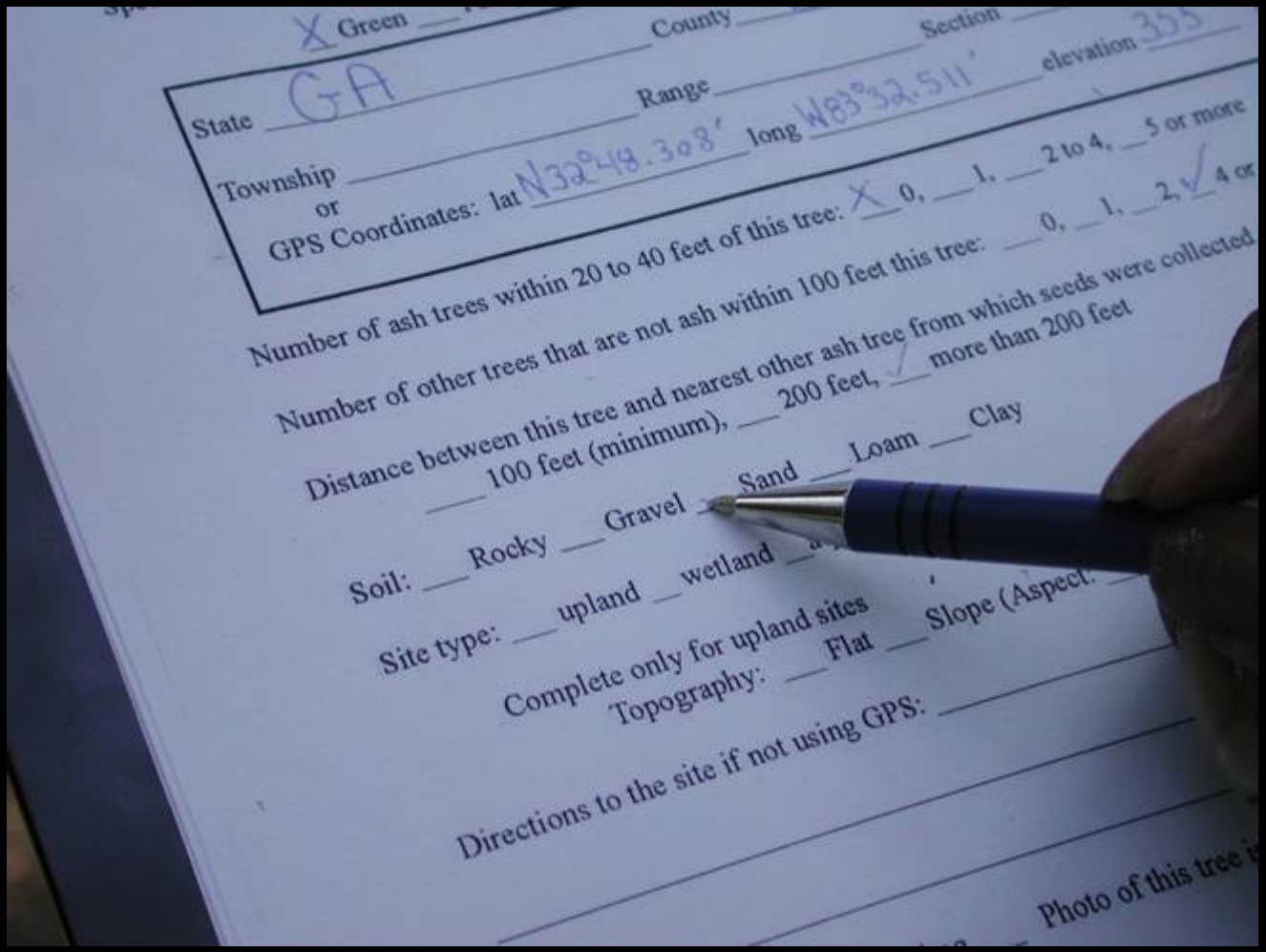
Mark the correct number of ash trees near the collection tree. This information helps us understand the background on the mother tree.



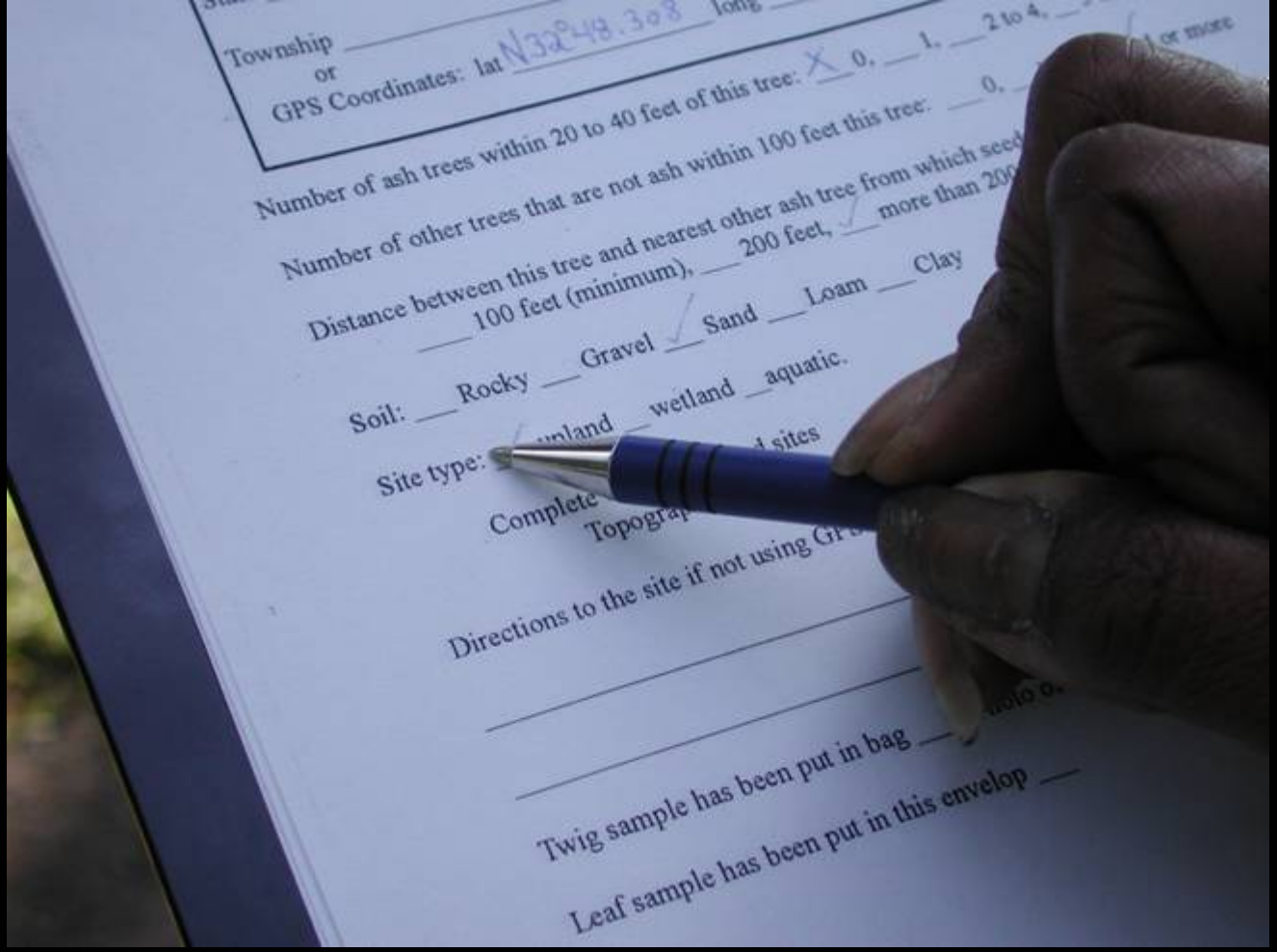
Mark the correct number of other trees near the collection tree. This information helps us understand the background on the mother tree. For example, is it an isolated tree or growing in a stand of trees.



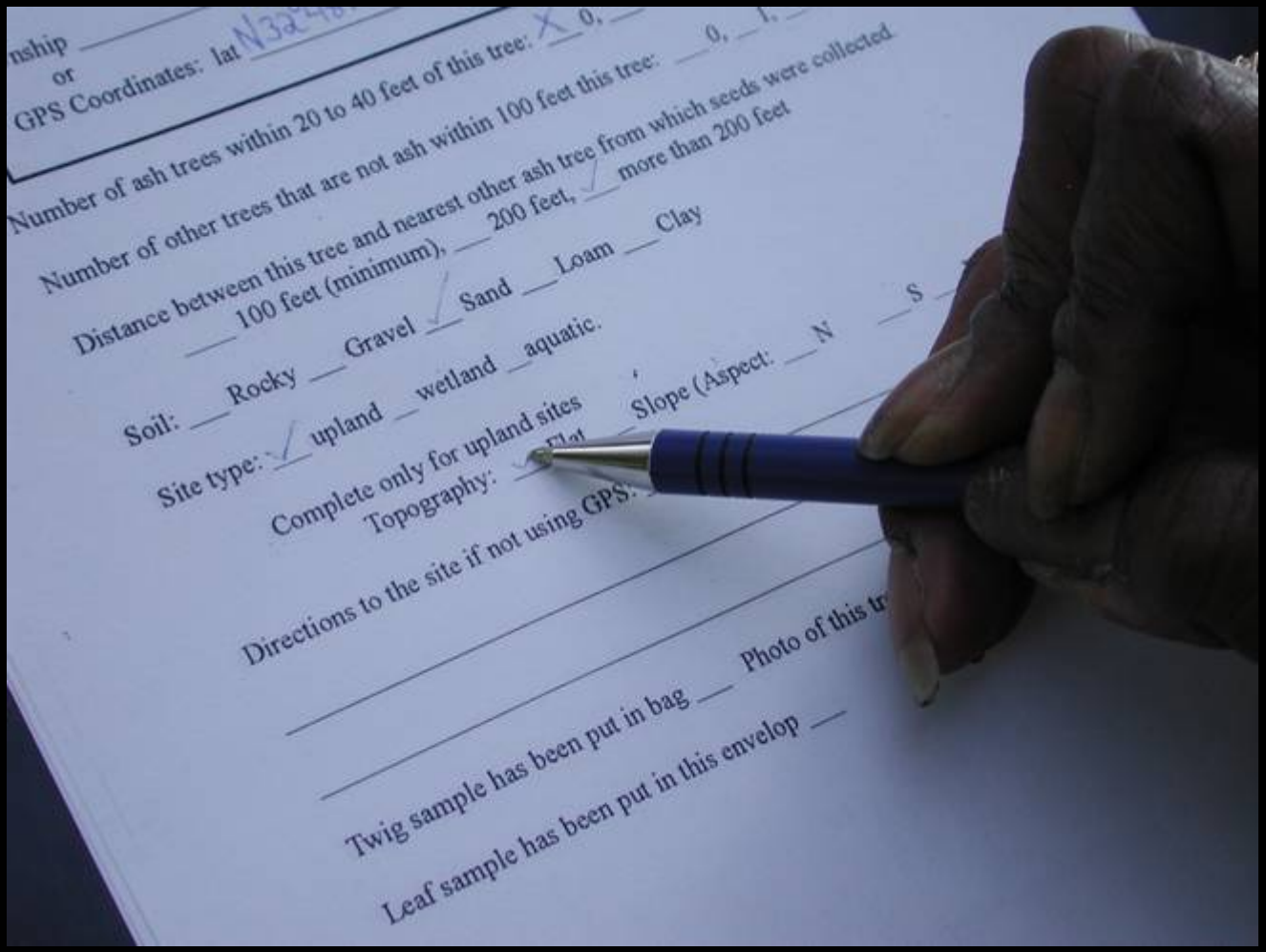
Mark the distance to the nearest other ash from which seeds are collected. A minimum of 100 feet between trees is requested so that related mother trees are not collected. This especially important for black ash which can root sucker, put up sprouts from the roots. Several trees growing close to each other might in fact even be the same tree or clone. Aspen is another tree that propagates itself with root suckers.



Mark the type of soil the tree is growing in. Soil type can be estimated by simply turning over a small amount of soil and estimating what type it is. Soil type shows where this trees progeny might grow well.



Mark the type of site. An aquatic site is a pond or stream. Pumpkin ash actually grows in standing water and therefore lives on an aquatic site. A wetland site is one where the soils are saturated with water for a good portion of the year. These are along streams, near lakes and ponds, and low spots. An upland site is one where water does not stand normally but drains away.



If the site is upland, mark whether it is sloped or flat. White, blue, and sometimes green ash are found on upland sites.

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

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

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

__ Rocky ___ Gravel ___ Sand X Loam ___ Clay

pe: X upland __ wetland __aquatic.

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

ions to the site if not using GPS: 400 feet south of a spot on

rner Road, ½ mile east of Frank Road.

ample has been put in bag ✓ Photo of this tree is picture number

If the site is upland and flat, the aspect is not marked. Aspect in this case does not exist. If the site is upland and sloped it is necessary to mark the aspect. The aspect is the direction the slope faces. With your back to the slope what direction are you facing. (You may have to use a compass.) In our example we face north.

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

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

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

Site type: X upland __ wetland __aquatic.

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

Directions to the site if not using GPS: 400 feet south of a spot on Turner Road, ½ mile east of Franklin Road.

Twig sample [illegible] Photo of this tree is [illegible]

Leaf sample has been put in this envelope ✓

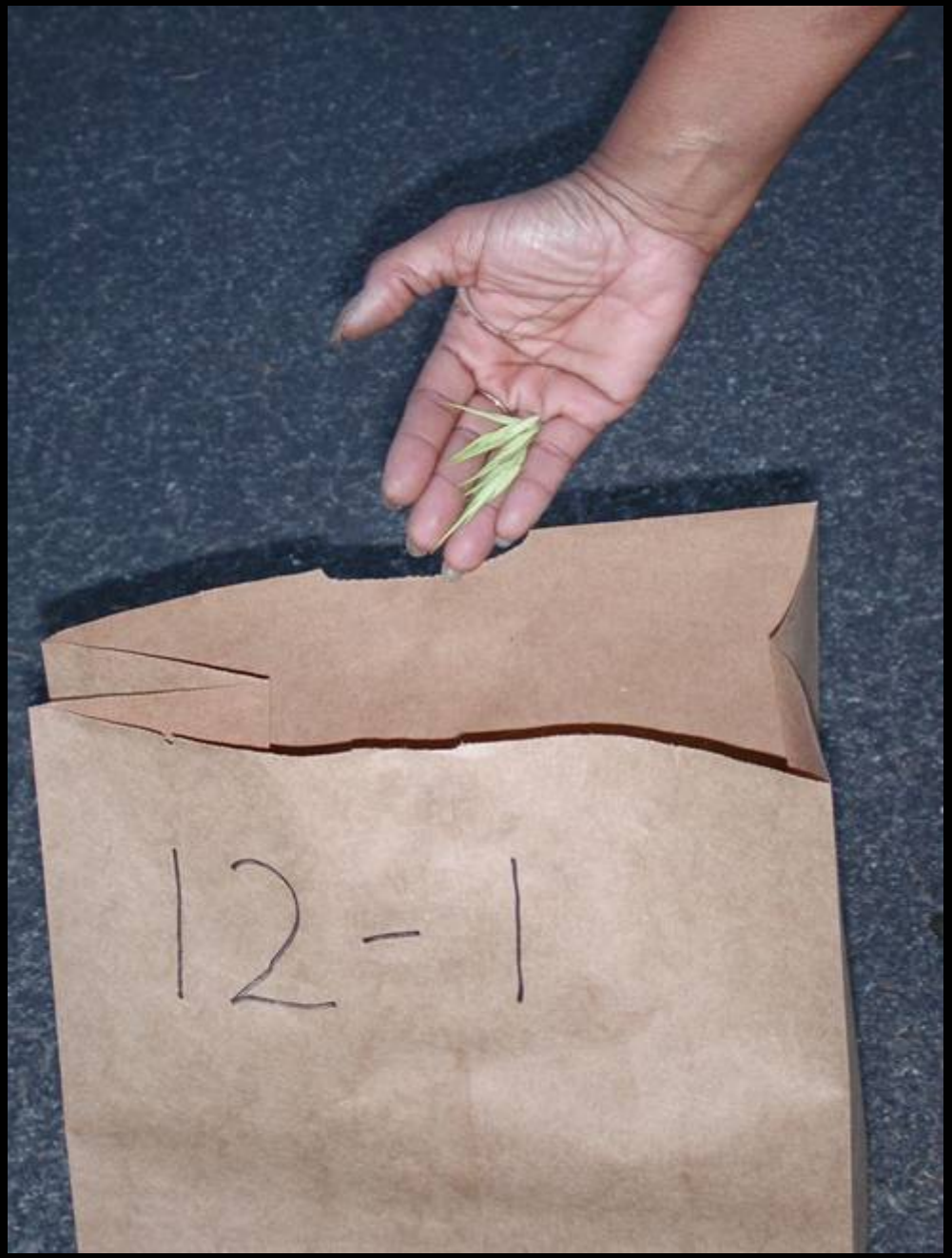
If you recorded a GPS reading, this section can be skipped or filled in at your preference. Distances need not be precise. If for some reason you wish to return to this tree, make the directions as precise as possible to assist your return.

Picking the Seeds

- Seed picking can begin once the data sheet is filled out through to the “Directions to the site …”

For seeds that can be reached from the ground, hold the branch in one hand and pull or strip the seeds from the tree with the other hand.

Seeds pulled from the tree.

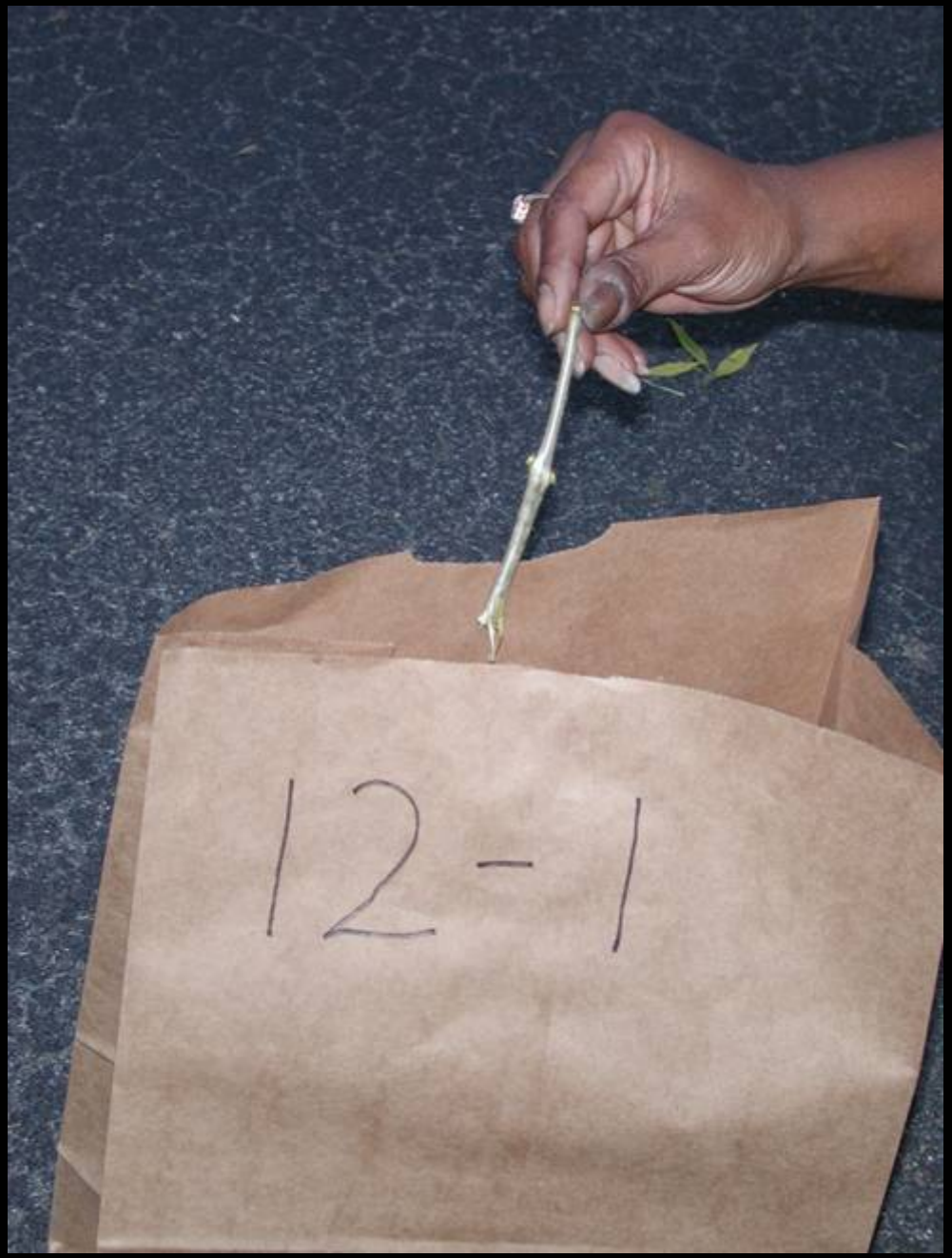


The seeds are next put into the collection bag that was marked earlier with the Collector’s ID number and Seed lot number

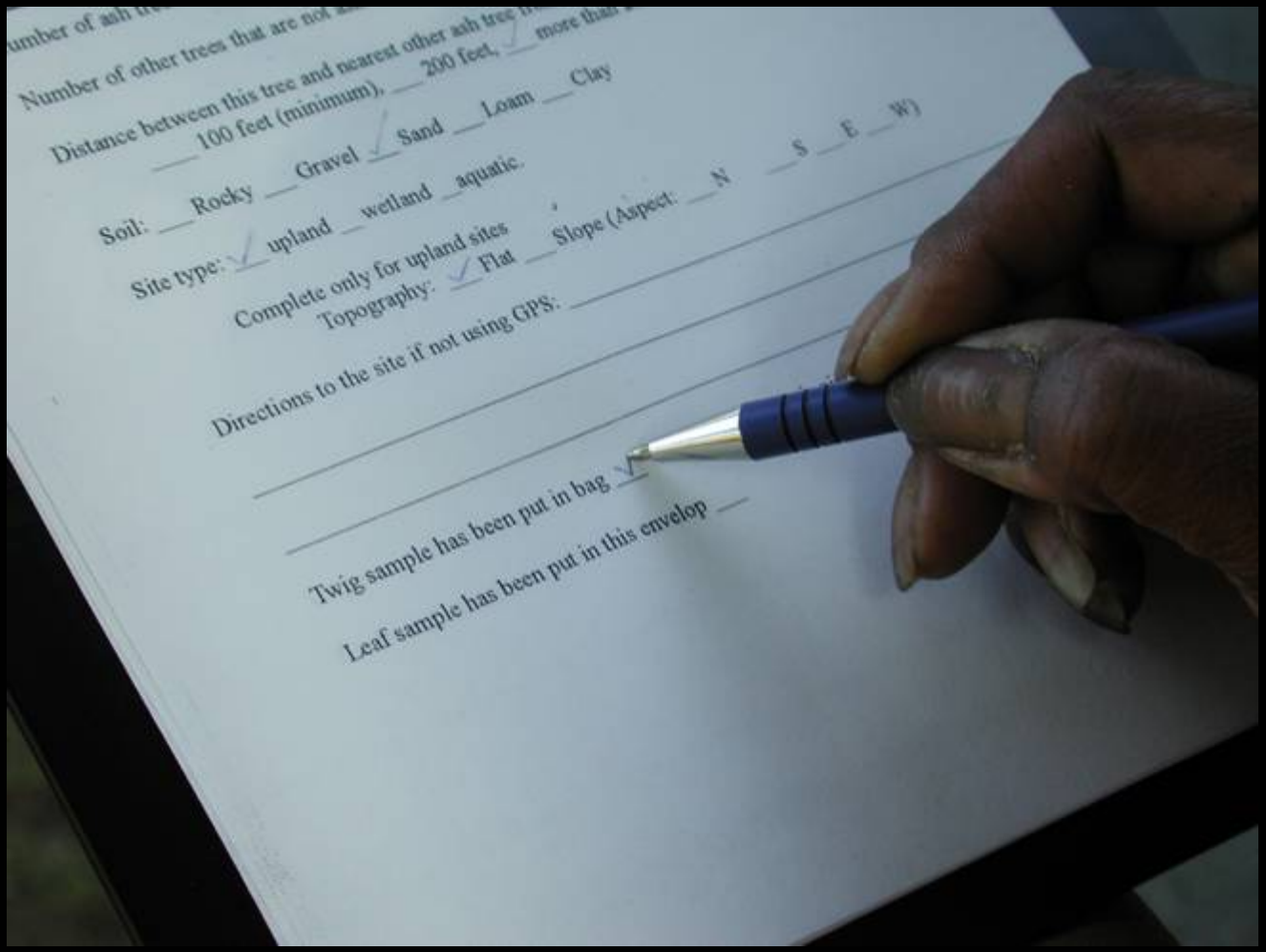
It is desired to have a minimum of 1000 seeds per tree. That is about one large 24 oz. drink cup filled to the very top as shown here, or seeds a minimum of ½ inch deep completely covering the bottom of the collection bag. As many seeds as reasonably possible should be taken from each tree. This will maximize the benefit from the effort of finding the tree by making the most research possible.

After picking all the seeds that can be reached, a twig sample is taken for identification purposes. To do this grasp the twig with both hands and break off a the end of the twig (about a 6 to 9 inch long piece).

The twig should snap off relatively easily.



Pull all the leaves off of the twig and drop it in the bag with the seeds you have just collected.



Mark the data collection sheet that you have put a twig sample in the bag. This is done just to make sure the twig sample was taken and not forgotten.

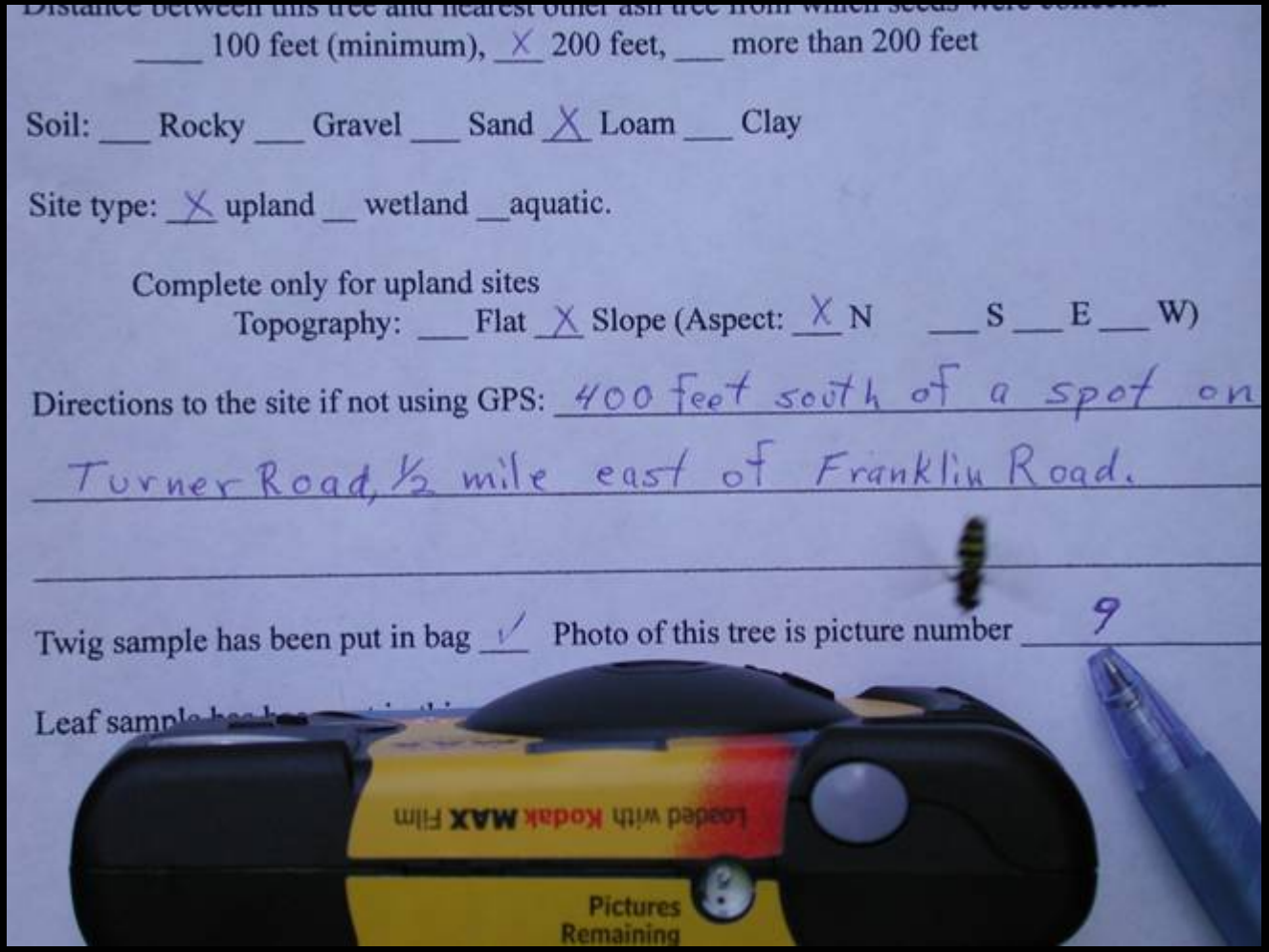
With the twig sample in the bag, fold the top of the bag over once and staple it shut in three or four places to be sure the seeds will not spill out.

When using the small stapler, be sure the staples pass beneath the little “ears” shown with the arrows. Otherwise the staples will not feed correctly and the tool will not work.

Bags can be folded down to make them easier to ship and carry.

After taking the photo, do not advance the film. Wait until just before you take a picture of the next tree before advancing the film. You will need to record the current number on the data sheet.

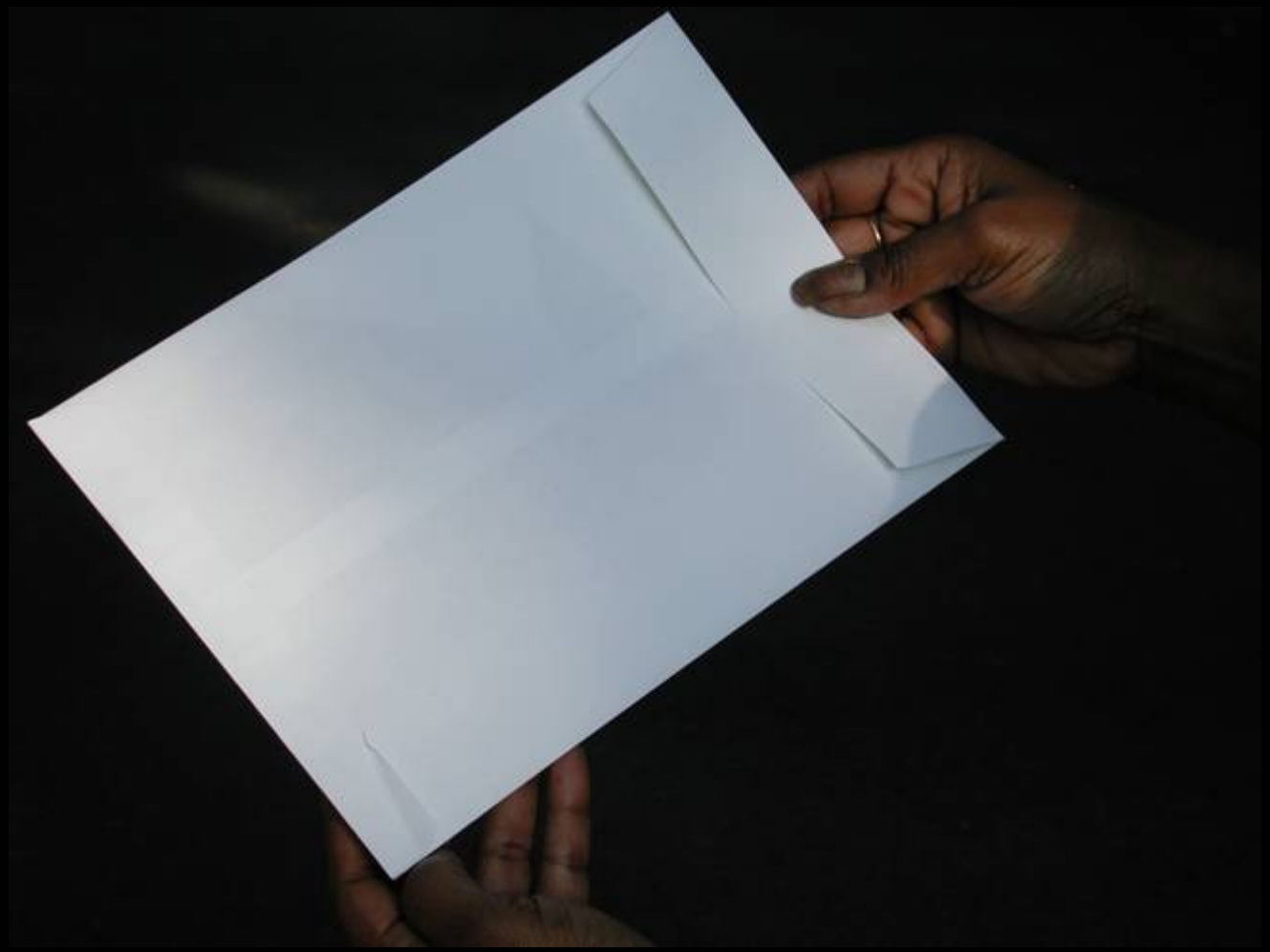
Take a picture of the tree. The photo provides back-up information about where the tree was growing, its size, condition, and what other trees were growing nearby.



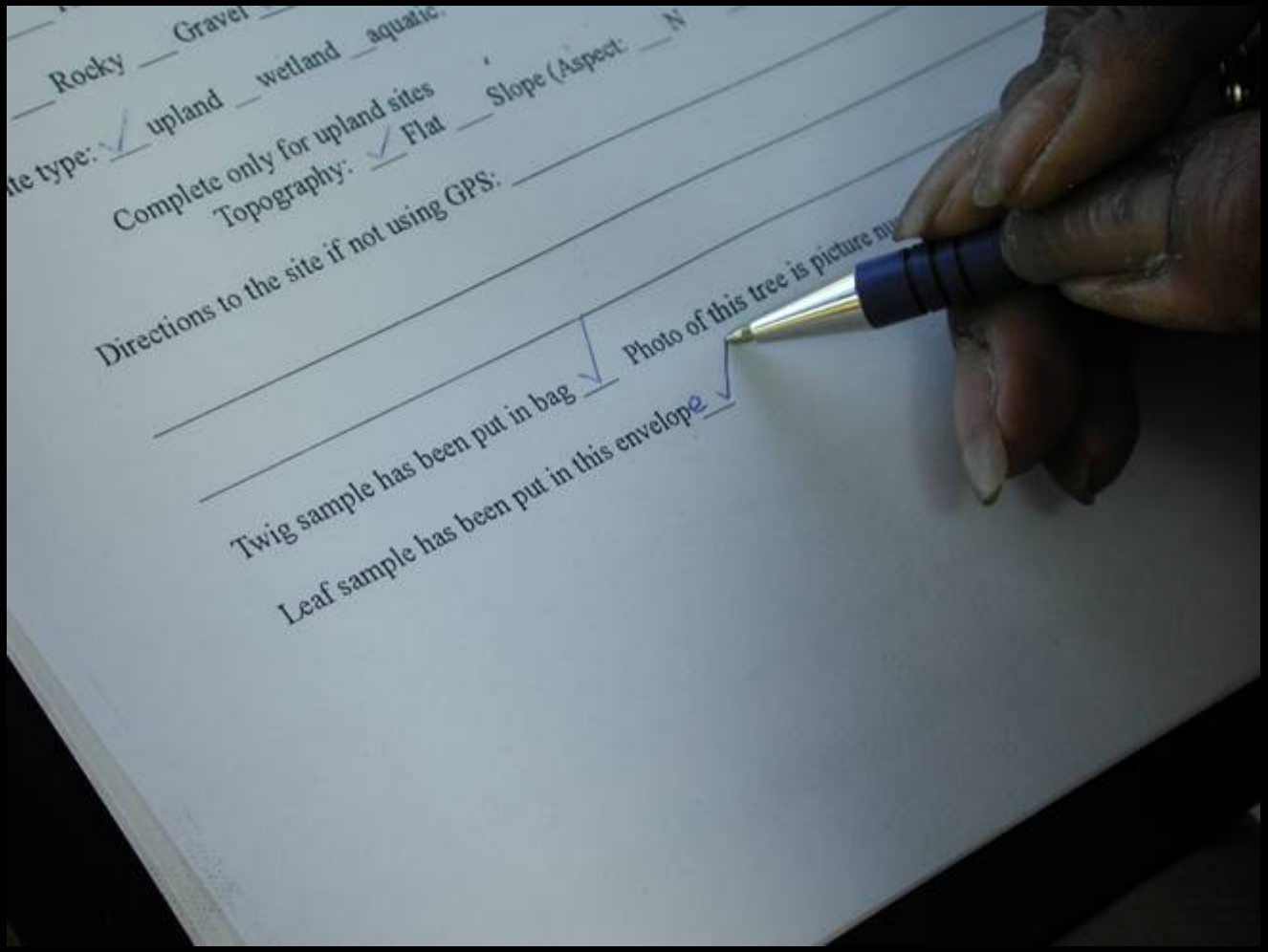
Record the number of the photo for this tree on the data collection sheet. This will be the number in the window on the camera that tells the number of "Pictures Remaining" or the number of "Exposures Remaining." Use the number that shows in the window after you take the picture and before you advance the film for the picture of the next tree you collect from.

Place one of the leaves pulled from the twig sample into the data collection sheet/envelope. Pick a leaf that fits the envelope diagonally. If necessary the rachis of the leaf can be folded to make the leaf fit the envelope.

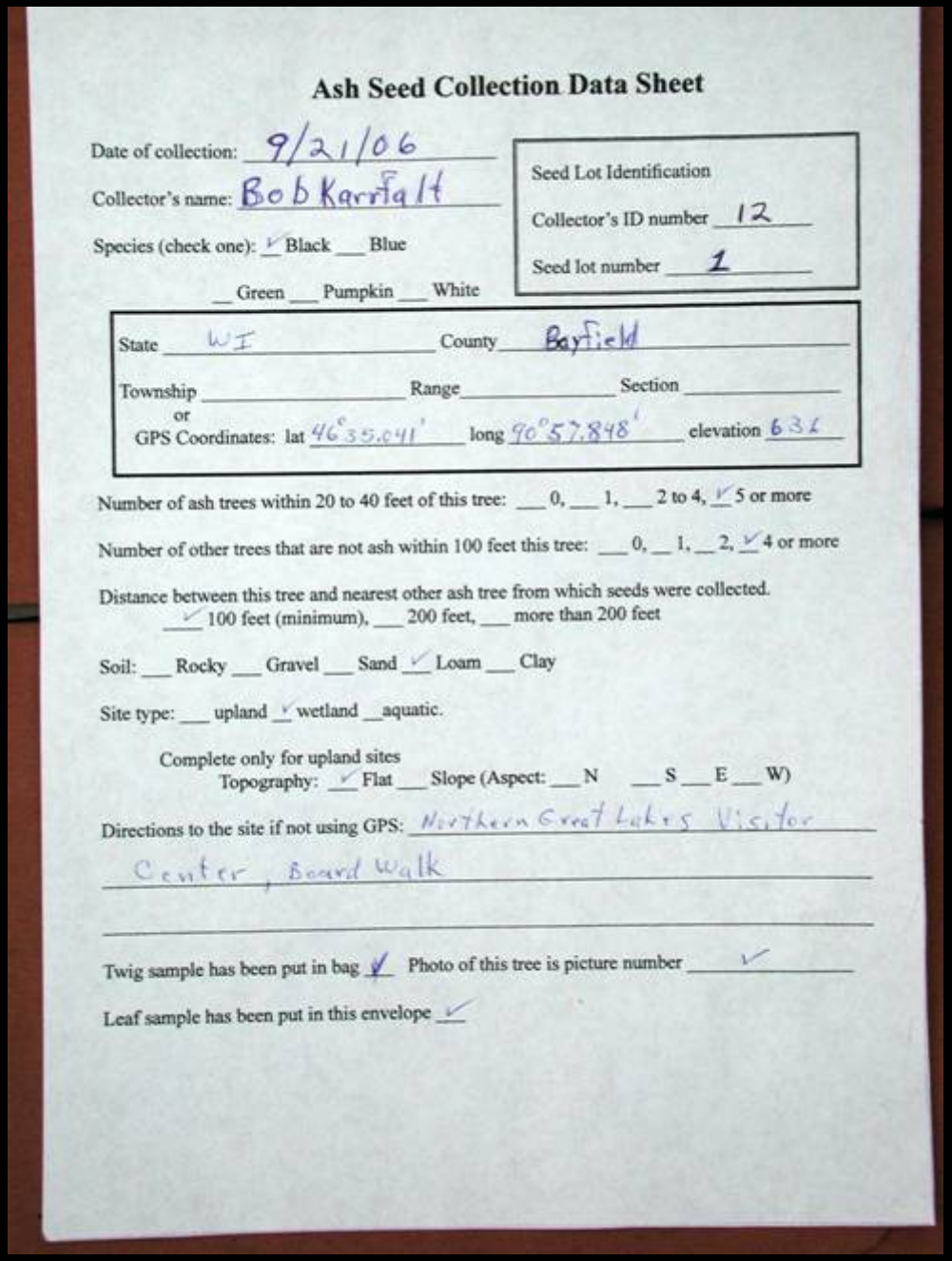
Slide the leaf into the envelope diagonally making sure that all the leaflets are flat and not folded over on themselves. This will allow the leaf to dry flat and more easily identifiable.



Close the envelope after inserting the leaf. It is not necessary to moisten the flap. Moisture from the leaf will do this for you.



Mark the data sheet showing that the leaf sample was put into the envelope.



Ash Seed Collection Data Sheet

Date of collection: 9/21/06

Collector's name: Bob Karrfalt

Species (check one): ✓ Black ___ Blue

___ Green ___ Pumpkin ___ White

Seed Lot Identification

Collector's ID number 12

Seed lot number 1

State WI County Bayfield

Township _____ Range _____ Section _____

or

GPS Coordinates: lat 46°35.041' long 90°57.848' elevation 632

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

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

Distance between this tree and nearest other ash tree from which seeds were collected.

___ ✓ 100 feet (minimum), ___ 200 feet, ___ more than 200 feet

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

Site type: ___ upland ✓ wetland ___ aquatic.

Complete only for upland sites

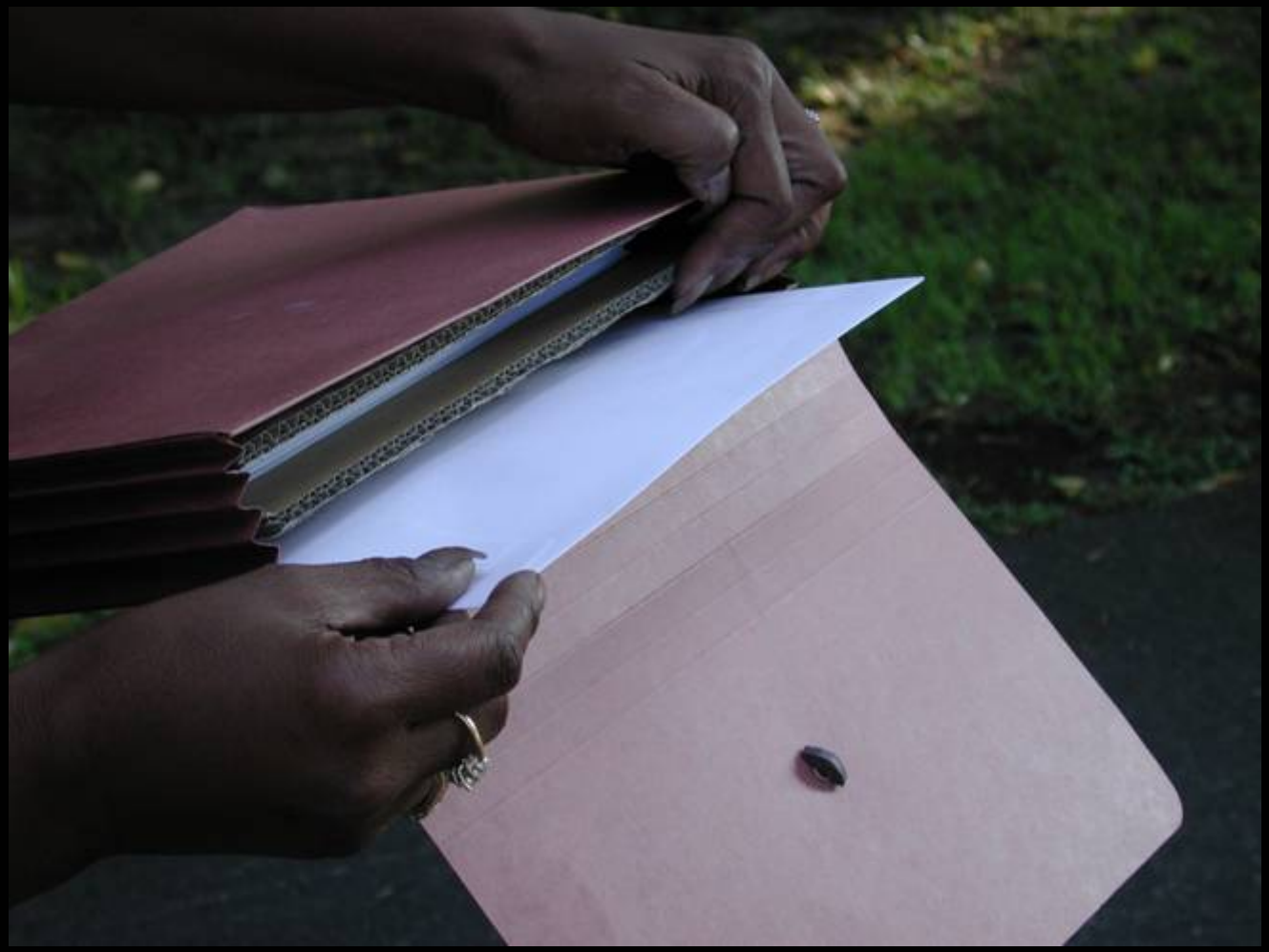
Topography: ___ ✓ Flat ___ Slope (Aspect: ___ N ___ S ___ E ___ W)

Directions to the site if not using GPS: Northern Great Lakes Visitor Center, Board Walk

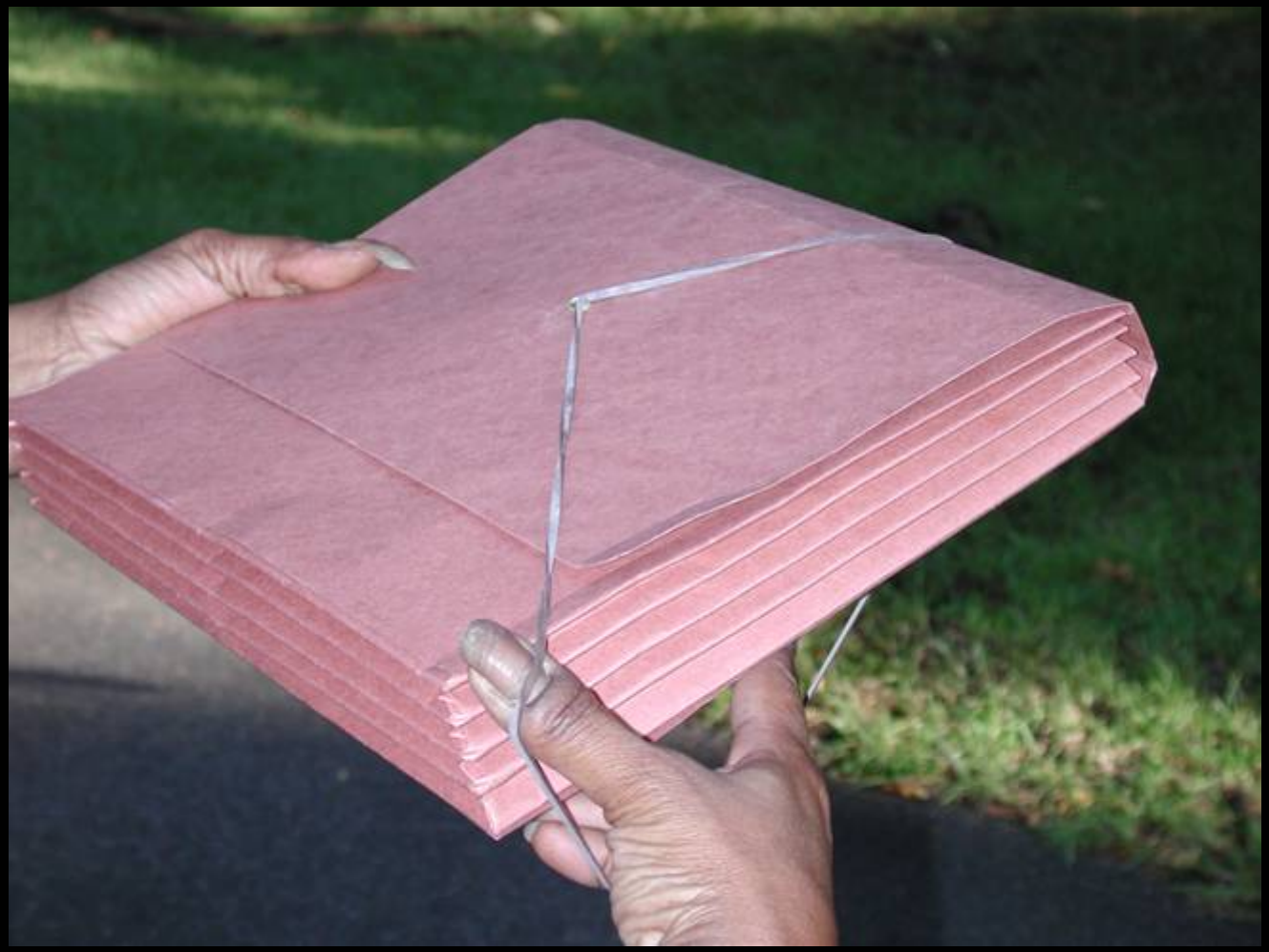
Twig sample has been put in bag ✓ Photo of this tree is picture number ✓

Leaf sample has been put in this envelope ✓

The entire data sheet is now reviewed to be sure all data has been recorded and all samples taken.



Put the completed collection data sheet/envelope back into the accordion folder. Use the cardboard to divide the completed sheets from the unused ones.



Close the folder and secure it with the rubber band before moving on.

The closed folder is now safe to transport without worry of spilling the contents.

If you need to mark the tree for a second visit it can be marked by tying a piece of flagging to a branch and writing the Collector’s ID number and the seed lot number for the tree on the flagging (e.g. 12-1).

Often branches are beyond reach when simply standing on the ground.

When seeds are beyond reach from the ground

- A tarp can be spread beneath the mother tree will catch seeds.
  - The tree is then shaken by or several persons to cause the seeds to fall.
  - A rope can be thrown over a branch and the branch can be shaken.
  - A pole pruner can be used to cut the pannicles from the tree. The seeds land on the tarp.
- Ladders, or the back of a truck can be used to elevate the pickers up to the seeds. Extra care is always needed when using these methods.
- The next few slides also give another idea on reaching seeds from the ground.

A hook, such as this pole pruner, can be used to pull the branch down to within reach of a person on the ground.

The seed bearing branch is hooked with one hand and pulled to within reach of the other free hand.

The branch is held down with one hand while the seeds are stripped from the branch with the other hand.

Ash seeds can be hand stripped from the tree if they are with in reach of the ground.

Post harvest handling

- Keep the seed out of the heat (over 90^{o}F)
  - Do not leave in the car in the sun
  - Store them so they can continue to dry and not heat up.
- Drying the seeds soon after harvest will preserve their viability.
  - Spread bags of seeds apart so air can reach the bag. Paper bags are use so moisture can escape from the sees. (Never use plastic bags for ash seeds.)
  - Ship the seeds frequently to the address provided so they may be fully dried for storage.
- Dry the leaf samples as shown to prevent them from deteriorating.

Bags of seeds should be spread apart to allow moisture to escape. This will keep the seeds from heating which is necessary to maintain high seed viability. Caution! Opening the bags may release many benign insects into the room. Ideally the seeds will be held in a cool dry location until shipped to the NSL.

The data collection sheet/envelopes will need to be put between newspapers so the leaf samples can dry. The papers need to be changed daily. The moist papers can be spread out to dry for the next day's change. Do this for several days until the leaves feel dry.

The newspapers containing the data envelopes with the leaf samples should be stacked and weighed with a heavy item like a phone book to keep the leaves flat while they dry.

How the Seeds Will Be Stored Long Term

- Dried with air 30% relative humidity or less until dry (10% seed moisture content or less)
- Seal in a moisture proof container
  - 4 to 6 mill poly-foil bag
  - Plastic bottle with a tight lid
- Freeze the seed at either – 8^{o}C or below