

**Field Guide for locating, pollinating, and harvesting
nuts from flowering American Chestnut Trees
(*Castanea dentata*)**

The American Chestnut Foundation
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Introduction

American chestnut trees, *Castanea dentata*, still number in the millions in the forests of Appalachia, but most of them exist as small stump sprouts on the forest floor -- mere remnants of the giant trees they were before the coming of the chestnut blight, *Cryphonectria parasitica*. When the sprouts are exposed to full sunlight, however, they can grow up to 20 - 30 feet tall and flower profusely before again succumbing to the blight.

A major goal of The American Chestnut Foundation is to make pollinations on these native chestnut trees as they flower, so that we can bring as much genetic diversity as possible into our breeding program. To do this throughout the original range of the chestnut we need volunteers to help us locate, pollinate, and then harvest nuts from these native American chestnut trees.

This Field Guide is intended to provide the information that our volunteers will need to help us. Please give us feedback as to whether you found the guide to be clear and complete, so that we can improve it in future editions. Above all, thanks for helping us to restore the American chestnut to the eastern forests!

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Where to look

American chestnut trees are mostly found in wooded areas of the Appalachian mountains and foothills.

The surviving sprouts are more likely to be found in acidic soils on dry, well-drained hillsides (facing south and east, where the sun is hotter and the soil drier). Also look on sandy soils that have good drainage.

Plants associated with American chestnut include mountain laurel (*Kalmia latifolia*) and chestnut oak (*Quercus prinus*; formerly *Quercus montana*).

Flowering American chestnuts are most likely to be found in areas exposed to a lot of sun -- along roadsides, at the edge of the forest, and in areas where a lot of the overstory trees have been removed (e.g. Clearcuts).

How to identify American chestnut trees

Chestnut trees are most easily located while they are in full bloom, from early June, in the southern part of the range, to the weeks around the Fourth of July in the North. The great mass of conspicuous white catkins on larger trees is visible at great distances. The odor of the blooms is also quite distinctive, especially on still mornings and evenings. Later in summer, bur-laden trees are fairly obvious. In early fall, chestnut leaves turn yellow sooner than the leaves of many other deciduous trees with yellow leaves. In late fall, the brown leaves tend to stay on the trees. In fall and winter, to locate trees that had female flowers the previous summer look on the ground for fallen burs.

American chestnut leaves are long in comparison to their width, the teeth on the edges of the leaf curve inward, and the stems usually have a reddish color

Chinese chestnut leaves are more oval in shape, thicker and more leathery than American chestnut leaves. Chinese chestnut leaves grown in the sun have a whitish cast on the back of the leaf, and the stems are gray with large white "bumps" or lenticels on them.

European chestnut leaves look much like American chestnut leaves, but their teeth tend to be triangular in shape rather than curved inward.

Japanese chestnut leaves are often dark, shiny green on top and the sides of leaves are somewhat parallel.

You should collect leaf and twig samples from every tree you pollinate, label them carefully, and send them to TACF for positive identification.

Other trees that might confuse you

The Horse Chestnut



The Horse Chestnut

fat twigs and buds
very few spines on the husk of the burr
glossy dark brown nuts without a pointed tip like a chestnut
7 leaflets to a leaf rather than one

(cont. next page)

Chestnut Oak & Chinkapin Oak



Chestnut Oak & Chinkapin Oak

leaf teeth may be pointed, but never have a bristle at the tip

Chestnut oak leaves are thinly haired underneath

acorns rather than chestnuts

leaf stalk longer than a chestnut's

long droopy flower catkins that are less fragrant and not as creamy white compared to a chestnut catkin

3 buds with many scales at twig tip rather than one bud with few scales

the chinkapin oak has very hairy undersides of leaves whereas the chestnut has smooth leaf undersides

(cont. next page)

Beech



Beech

very long thin buds

the husk of the burr is tiny, and the beechnut even smaller

leaves are broad and fat, only 3-5 inches long

The male flowers are not in a catkin form

The Beech is known as the "initial tree" and is often carved.

The chestnut does not have the smooth silvery bark that the beech has.

Reproductive life-cycle of the American chestnut

Flowering American chestnut trees will either have only male flowers (catkins) or both male and female flowers (small burrs). Female flowers usually have male flowers on the same branch. Chestnut trees bloom from mid-June to early July, depending on latitude and elevation. A chestnut tree rarely self-pollinates. Therefore at least two chestnut trees need to be near each other for viable nut production.

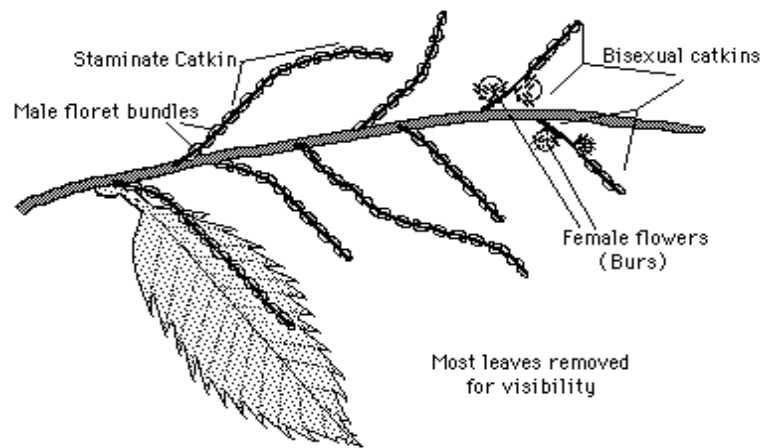


Fig. 3. Male and female chestnut flowers

Female chestnut flowers develop into burs that contain up to three nuts at maturity. The nuts are ready for harvest when the burs begin to open up.

Pollination Techniques

Female tree operations

Bagging Chestnut flowers to be pollinated will have to be "bagged" to exclude random airborne pollen from fertilizing or blocking them. Use bag #401, a brown corn tassel bag (the brown color doesn't seem to impair bur development), from Lawson Bags, P.O. Box 8577, Northfield, IL 60093, (800) 451-1495 or (847) 446-8812. Pricing is available at the Lawson website; if you're only doing a few, write the Meadowview Farms at the above address and we will send you a few). Order these before you start! You can carry supplies up the ladder in your pockets, in a folded pollination bag attached to your pants with paper clips, or in a carpenter's apron.

Bag female flowers when they have exerted their styles 2-4 mm. The styles are white or yellow, whereas the remainder of the female flower is green. In this manual, we also call female flowers burs, which is what they develop into as they mature. It is safe to bag for only 5 days after style emergence. Another good rule of thumb, which applies in most years, is to begin bagging when green catkins on 50% of the flowering branches begin to exert stamens and turn white or creamy yellow.

a) Remove all male catkins and leaves and the male part of the bisexual catkins, using scissors or hand pruning shears.

b) Puff up the bag, place over shoot, twist on base and secure with a jumbo paper clip or a twist'em. When using paper clips, try to have at least one free end of the clip "sprong" over the shoot to lock it in place; do not spread the paper clip before slipping it over the bag and stem. Try to place the bag

so it does not touch the female flowers leave an inch of free space at the tip. Otherwise make the bag extend down shoot as far as possible.

c) When a ladder is in place, branches may be pulled over to the ladder with a pole pruner and tied off to the ladder to increase the number of flowers bagged without moving the ladder. Make a loop in one end of a small rope and either tie off the pole pruner or else the branch itself. It can be helpful to have two ropes, one to secure a thicker part of the branch and the second to tie off branch tips above the thick part.

d) Leave a few branches unbagged so you can judge when the tree is ready to be pollinated. Don't use your worst branches for this!

Pollinating - In general, pollinate 12 days after bagging. The female flowers are receptive when the styles turn yellow and spread out across the top of the flower. At that time, the base of the styles can often be seen protruding from the nascent bur. Peak receptivity generally occurs when all the male catkins are in bloom but before anthers emerge from the bisexual catkins. The 12-day rule is a reliable guide to the best time for pollinating.

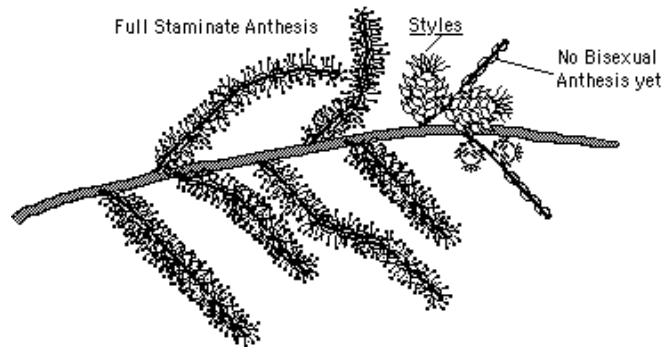


Fig. 3. A single flowering chestnut branch with male flowers in full bloom. Time to pollinate.

a) Remove bag, pollinate (see below), and resecure bag with a new paper clip or twist'em.

b) Save every tenth bag as an unpollinated control; do not remove the bag. Mark unpollinated bags with a metal or plastic tag twisted on above the paper clip. You can write on the tag to indicate the male and female parents of the real crosses. Do not save your worst bagged flowers for unpollinated controls. It is important to do these checks. Despite careful attention and experience from more than ten-thousand crosses, I almost always have a few trees with nuts in the check bags. It is important to know who the father of a cross is; that's why you do all these manipulations! The check bags tell you whether or not your cross is what you hope it is.

c) Branches or trees should be labeled with information about what pollen or

treatment was applied. Label the branches or trees as you go, not later! Plastic or aluminum tags can be used. Use a black Sharpie™ to write on plastic tags. Both tend to disappear over the summer- wind, birds, and curious people take them off. Write a description of what branch was pollinated with what or make a map of separate trees in your notes.

d) Bags may be left on till harvest. In weevil-infested orchards, this reduces weevil damage so that nuts needn't be hot-water treated nor trees sprayed. It also can save some nuts in burs which open before you harvest.

Pollinating with fresh catkins - You can carry catkins up the tree in a cup or tin can in a carpenter's apron, or in a folded corn-shoot bag tied to your belt or pants with paper clips. Use a clean bag or can for each type of pollen. Rub one catkin over all the styles of each female flower 4-5 times. Use a new catkin when all the anthers have been removed; every 5-10 female flowers at most. Use the whitest catkins available. This is the easiest and possibly the best method of pollinating.

If fresh catkins are in short supply, tap a previously bagged catkin on a microscope slide and proceed as indicated in the next section, "Pollinating with Dried Pollen." Catkins can be tapped repeatedly on slides over a series of days. See more below under "Male Tree Operations, From trees with few branches and catkins"

Pollinating with dried pollen - Place a small sample of pollen in a 1-inch-diameter vial. Don't carry your entire supply of pollen up the tree! Clean a microscope slide with alcohol (Vodka or 70% Pure Grain) and dry thoroughly with kleenex. Cover the mouth of the vial with the slide. Holding vial and slide tightly together, turn the vial upside down, and shake pollen onto the slide. Turn the vial right side up, shake all the pollen you can off the slide and back into the vial. A film of pollen will remain on the slide, which is now "loaded". Re-cap the vial. Gently rub slide on tip of style (Figure 4). You might want to mark the loaded side of the slide with a grease pencil or crayon. Make sure style tip leaves streaks in the pollen. Reload slide every 5-10 flowers. Use a clean slide every 50-60 flowers. Keep your pollen cool in the shade.

Male tree operations

Male flower collection - Collect flowers optimally when they are creamy white-yellow, before they have started to turn tan or brown. If you are going to be collecting in the morning and expect dew, cover the flowering shoots with brown paper grocery bags at dusk the previous night so the flowers will be dry when you pick them.

From large trees - Cut flowering branches about 2 feet long and place in a 1-gallon, very clean plastic milk jug with a 3-inch-diameter hole cut in the top. Prune leaves and twigs off

the base of branches which go inside the jug. Fill the jug three-fourths full with cool (not ice cold) tap water, so all branch bottoms are well immersed. Label the jug with a black Sharpie™. Cover flowers and branches with a large paper grocery bag and place milk jug in a 5-gallon plastic bucket, to prevent tipping over and spilling. Label the bag and bucket. Keep the bucket of flowers cool, out of direct sunlight. Try to keep them away from wind and excessive vibration. Flowers will keep 3 to 7 days, longer if refrigerated. Catkins may be obtained directly from the cuttings. Remove the grocery bag gently.

From trees with few branches and catkins - If there are only one or two catkins on your tree, you may want to visit it early every morning while it is flowering and pick off exerted stamens, placing them in a vial. Then proceed as indicated under step e) in the next section. This is a tedious method.

If there are 10 to 20 catkins on the tree, and you have a receptive tree nearby, simply put the catkins in a tin can or corn-shoot bag and pollinate the receptive tree with them. It is best to collect the catkins early on a still morning, but after dew has dried off (or to have covered them the previous evening as discussed above). Collected catkins should be stored in a sealed bag or can to prevent dessication: fold over the top of a corn-shoot bag and secure with a paper clip or put plastic film wrap over a tin can. Catkins will keep 1-2 days, longer if refrigerated. If you want to collect and store pollen from 10 to 20 catkins on a tree, repeatedly tap each catkin onto a small sheet of glass very early on still mornings. Then proceed as indicated under step c) in the next section.

Pollen collection and preservation

- a) Very gently remove paper bag from flowers and flowers from jug.
- b) Wrap wet base of branches with several dry paper towels to avoid water drops on glass. Vigorously shake the bundle of branches over a 2 to 3-foot square or rectangular piece of glass. It is best also to unfold a few brown paper grocery bags and place them under and around the glass to catch pollen that falls around the glass. This process can be repeated over a number of days, 2-3 times a day. If the flowers are in a cool, still room, it is not necessary to replace the paper bag. They can be stored on top of their piece of glass and will drop anthers on it.
- c) Pick out the obvious trash and bugs with clean tweezers. Scrape the pollen into a pile with a single-edge razor blade (Gem). Use alcohol and kleenex to remove oil from the razor blade before use. You can separate most of the remaining fluff, trash, and bugs from the pollen and anthers by scraping off the top of the pile of pollen and "marching" it away from the rest of the pile. Then scrape what is left on the marching trail back into the main pile of pollen. Repeat as necessary. Bugs frequently will crawl out of the pile if you disturb it with the razor blade.

Scrape the pollen pile into a labeled vial. Cap the vial with a labeled lid which has a 0.25-inch diameter hole in top and place in dessicator, over fresh

silica gel or calcium chloride. Use a paper punch or similar tool to make the hole in the lid. The dessicator can be a plastic peanut butter jar. Desiccate the pollen for at least 4 hours, more if there is a lot of pollen; not more than 24 hours. Do not store fresh (undesiccated) pollen in high humidity or at room temperature any longer than absolutely necessary.

Wrap a small amount of dessicant securely in dessicated paper and place in vial; make sure there's no dessicant on the outside of the paper. Recap vial with a lid with no holes. Tape the lid to vial to make sure the lid won't come off in shipping! After this the pollen can be safely mailed to other pollinators. Pollen should be refrigerated if it is to be used in the next week or so; frozen at 0°F if it is to be saved for next year. Do not freeze fresh pollen.

Pollen testing - It does you no good to pollinate your tree with dead pollen. It is best to test pollen if possible. It may be advisable to test it every day or so during the pollination season. Chestnut pollen is easily germinated if floated on drops of 1% table sugar (or glucose) in non-chlorinated water, and held at 85°-90° F for one hour. Examine under a microscope at 30-100x magnification. Good pollen should show 15-60% of the grains with tubes (as long as the grains) growing out of them.

Harvesting

Harvest the nuts when the burs begin to open. This is around the last week in September, first week in October in the mountains from Georgia north to Maine. In the Piedmont of the Carolinas, Georgia, Alabama, Mississippi and Tennessee, it can be as early as mid August. If possible, check your trees at least weekly two weeks before the local harvest date.

The main reason for this is to check for squirrel predation. If squirrels are clipping off the burs and eating through them, place a "peace offering" of several pounds of chestnuts under the tree. This will have to be repeated up to thrice weekly. Frequently, Chinese and Japanese chestnut come in early enough to yield a supply of nuts for the "peace offering." Squirrels do not attack chestnuts in the bur every year, only when there is a high squirrel population and a poor acorn crop. Shooting, trapping and poisoning have proven ineffective in controlling squirrel predation.

Use heavy leather or rubber gloves. If the burs still penetrate the gloves, put two pairs on. Some of us put rubber gloves on underneath leather gloves.

Wrap a good-quality (Hefty, etc.) black plastic garbage bag around your belt and secure it with a paper clip or twist'em shoved through the bag and around your belt. Keep a white plastic kitchen trashcan bag in your black plastic garbage bag and put all the unpollinated controls in that. Carry several spare bags in a pocket. If the burs can be grabbed so that the nuts will not fall out, rip them off and put them in the garbage bag. Take the pollination bags and ties off the tree so it will not be unsightly and so you can count the number of bags. Place them in the garbage bag too. If the burs have opened too far or some nuts have fallen into the pollination bag, cut or break off the whole

branch while holding the nuts, or else bend it into the garbage bag to save the nuts. Put all the burs in the bag too so you can count them. Try to avoid cutting off too many branches to get the burs, for this removes many of next year's flower buds.

If possible use a separate sheet of glass for each type of pollen collected, or else, between pollen types, clean and dry

it thoroughly as you would a drinking glass, using plenty of dish detergent. Rinse very well. Keep branches with different pollen types as widely separated as possible, especially when the paper bags are not in place. Do not mix the jug, bucket, paper bag, razor blade, and paper towel between types of pollen. Clean the tweezers thoroughly with alcohol (Vodka or 70% Pure Grain) and dry thoroughly between types of pollen.

Put the metal tags with which you labeled the branch or tree into the white trash bag so you can identify the contents of both bags. The tag will be less likely to fall out of a hole in the bag if it's inside the white bag which is inside the black bag. Tie both bags securely shut.

When you get home, remove the burs from the plastic bag, count them and record the count for that cross. Also count the number of pollination bags and record that count. Put the unopened burs and the free nuts in a large or small paper grocery bag, depending on the number of burs. Also put the label in the paper bag, and write the cross identification on the paper bag. Keep the controls separate in the white garbage bag with tag inside. Record their bag and bur counts also.

If you have a walk-in cooler, put the bags of unopened burs in there to wait for them to open. Otherwise put the bags in a room out of sunlight and reach from mice! Every two to three days, go through the bags removing nuts from opened burs, but do not remove nuts which are still sticking to an opened bur. After a week to ten days, remove all the nuts from all the burs, whether opened or not, sticking or not.

Immediately count and store the nuts in moist, but not wet, peat moss (2-3 cups water per gallon of dried milled peat moss) in a plastic bag into which you have placed numerous holes with a tooth pick or paper clip. Make sure each nut is surrounded by peat moss and not touching other nuts or the side of the bag. Put the label in the plastic bag and also write the cross id and the number of nuts in the bag on the outside with a black Sharpie™. Refrigerate the nuts at 34 F until planting or shipping time.

Field equipment checklist

Some useful items in the field- If you are 30 miles from home base and have to go back for something you forgot, you have lost a good part of the day. Use the checklist every day, and change it as needed.

Pollination

- ladderpole pruner
- small rope
- keys
- maps, compass
- money
- phone numbers
- Foundation brochures- to introduce yourself
- hat
- raincoat
- binoculars- for seeing flowers in the crown of the tree, examining crown for blight
- field notebooks
- spare pens, pencils, black Sharpie™ marking pens
- knife
- insect repellent
- sun screen
- toilet paper
- drinking water
- lunch
- carpenter's apron
- pollen
- fresh pollen application equipment - pollination bags
- dried pollen application equipment -
 - microscope slides
 - vials
 - alcohol
 - kleenex
- grease pencil
- pollen collection equipment -
 - milk jugs
 - 5-gal buckets
 - water
 - large paper bags
 - glass plates
 - tweezers
 - razor blades
 - alcohol
 - kleenex
 - vials & lids
 - hole punch
 - scotch tape
 - labels
 - dessicator
 - pollination bags (corn-shoot bags)

- thousands of jumbo paper clips or twist'ems
- metal branch tags
- pruning shears
- scissors

Harvest

- ladder
- pole pruner
- hand pruning shears
- rope
- thick leather and/or rubber gloves
- field notebooks
- good-quality garbage bags (black and white)
- label material- moisture proof
- keys
- maps, compass
- money
- phone numbers
- Foundation brochures
- Raincoat/hat
- binoculars- for seeing the burs in the crown of the tree, examining for blight