

	<h2>POLLINATION</h2> <h3>Making Controlled Pollinations of Chestnut</h3> <p>Literature available on care of chestnuts: Harvesting, Mudpacking, Planting, Pollination, and Quick Guidelines. To request these, write The American Chestnut Foundation, P.O. Box 4044, Bennington, VT</p>
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In the mountains from Georgia to Maine, you should start looking at your trees twice weekly in the beginning of June, so you can judge which trees will be flowering in the current year and when you will need to begin pollinating. At lower elevations in the south, it can be as early as the beginning of May. In many areas of the country, American chestnut trees begin blooming between June 15 and June 28. Chinese chestnut trees tend to begin blooming about a week earlier.

1. **Records-** Write down everything you do; how, when and where. Any nuts you make might be unidentifiable and worthless, otherwise.

2. **Species Identification-** Check the species identification of your trees; there have been plenty of long-standing misidentifications. We will be glad to determine what species of chestnut you have; send leaves and twigs of your trees to the ACF Wagner Research Farm, 14005 Glenbrook Ave., Meadowview, VA 24361. In winter, you can send old fallen leaves from around the base of your tree.

Female Tree Operations

1. **Bagging-** Chestnut flowers to be pollinated will have to be "bagged" to exclude random airborne pollen from fertilizing or blocking them.

Use bag #421, a corn-shoot bag, from Lawson Bags, P.O. Box 8577, Northfield, IL 60093, (800) 451-1495 or (847) 446-8812. (The minimum order is 3000 bags, costing about \$120; 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.

In general, you can expect to harvest one nut for each bag placed over several female flowers.

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. See Figures 1 and 2.

a) Remove all male catkins and leaves and the male part of the bisexual catkins, using scissors or hand pruning shears. I used to leave a terminal vegetative bud, the first beyond the last female flower, but have found that shoots from these buds sometimes set late burs, which can be pollinated once the bag is worn out, so it is better not to leave any terminal buds.

b) Puff up the bag, place over shoot, twist on base and secure with a jumbo paper clip or a twist'em. 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.

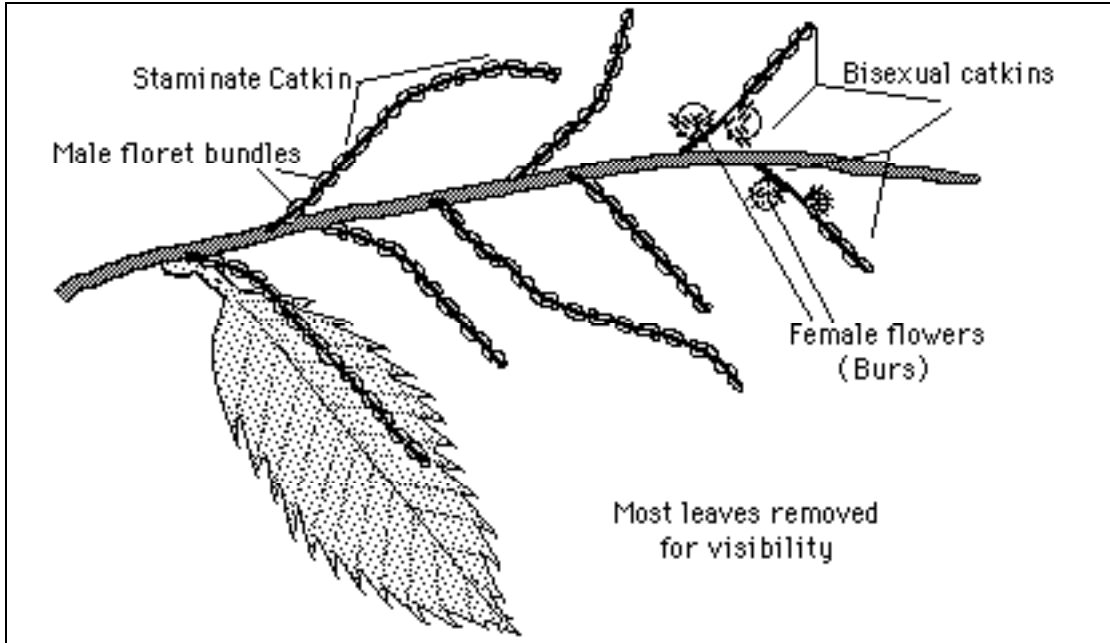


Figure 1

A single flowering branch of chestnut, prior to emergence of styles and anthers. Flowers not ready to bag.

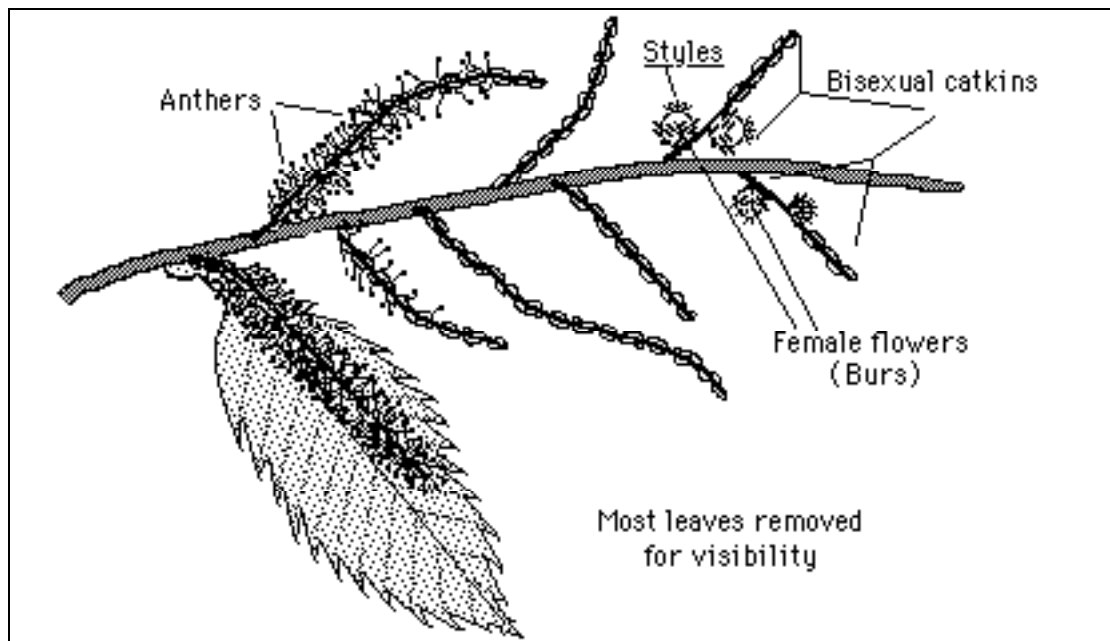


Figure 2.

Chestnut flowers 3-5 days after first emergence of anthers. Ready to bag.

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!

2. **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. See Figure 3.

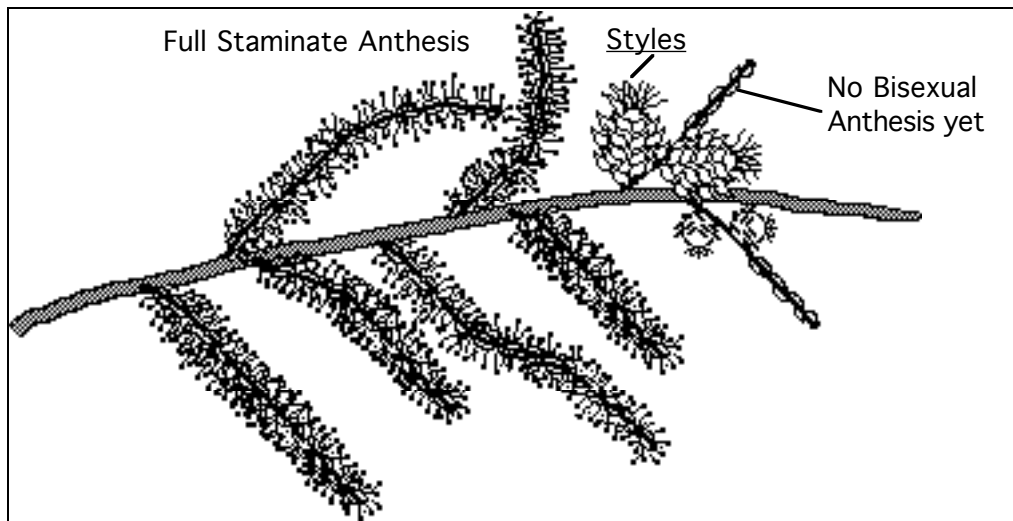


Figure 3.

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 two-inch square of glass with alcohol (Vodka or 70% Pure Grain)

and dry thoroughly with Kleenex or paper towel. Cover the mouth of the vial with the glass. Holding vial and glass tightly together, turn the vial upside down, and shake pollen onto the glass, very vigorously. Turn the vial right side up, shake all the pollen you can off the glass and back into the vial. A film of pollen will remain on the glass, which is now "loaded". Re-cap the vial. Gently drag the tips of the styles through the film (Figure 4). Make sure style tip leaves streaks in the pollen. You might want to mark the back side of the glass with a bit of electrical tape; if the edges are sharp, wrap those with electrical tape. Try not to get fingerprints on the business side of the glass. Reload glass every 5-10 flowers. Clean the glass every 50-60 flowers. Keep your pollen cool in the shade.

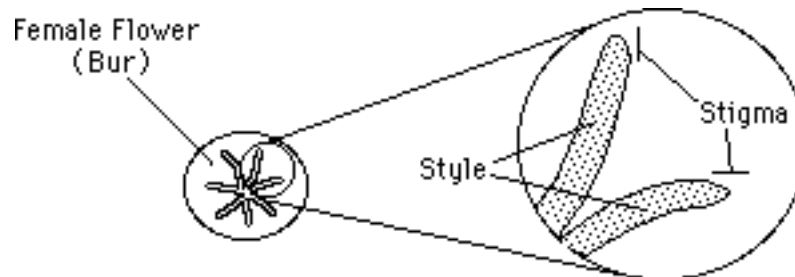


Figure 4.

Female chestnut flowers showing the style and stigma. Apply pollen to the tip of the style, where the stigma is located.

Male Tree Operations

1. **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.

a) 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.

b) 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.

2. Pollen Collection & 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.

d) 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.

e) 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.

f) 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.

3. 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.

a) 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.

b) 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 tie 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.

c) 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.

d) 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.

e) 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.

g) 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-

ladder

pole 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

