Controlled Pollination of Chestnut Trees

Steps of Controlled Pollination

1. Find your trees and have them properly identified

2. Bag the female flowers
mid to late June, depending on location.

3. Collect Pollen

4. Pollinate 10-14 days after bagging
early to mid-July.

5. Wait

6. Harvest
late September to early October, depending on location.

Finding Chestnuts

- The best time is during blooming season, particularly when male flowers are in full bloom
  - Smell
    - Wet rags; bleach
  - Sight
    - Drive around forest roads
    - Stand on top of a ridge to look for bloom in valleys and on other ridges
- Tree Locator Form
  - Leaf and twig sample
  - Contact Chapter Mother Tree Coordinator/Breeding Program Coordinator

Tree Locator Form (PA&NJ)

Purpose. This form is to help TACF record, map, and analyze chestnut-trees across its range. This form should be printed and filled out with as much information as available and submitted with a leaf and twig sample to the office listed below. An analysis of the characteristics and microscopics will be completed by a TACF identification expert and the results will be sent to the submitter.

Leaf and Twig Sample. Please cut a mature leaf that has been growing in the full sun and a twig with some leaf buds and place them in an envelope. Do not use plastic due to the molding effect. Wrap the samples in a single paper towel to cushion in the mail.

We appreciate your participation!

SUBMIT SAMPLE TO:
PA Chapter The American Chestnut Foundation (PA-TACF)
691 Pumping Station Road
Hanover, PA
e-mail: operations@patacf.org

Copies of this form are available:
http://chestnut.cas.psu.edu/forms.html
Or at: http://www.patacf.org

Location:
County: _____ Town: _____ State: ____
Latitude (N): _____ Longitude W):_____

Location information is crucial. The closer you can get us to a tree with your directions, the better. Lat/Long measures are the best way to give us good location info. A good website to use to obtain Lat/Long is http://www.topozone.com. If you can't obtain Lat/Long measurements, then please attach a map and directions to the tree from the nearest road.

Tree Information:
Diameter (inches @ 4.5ft): _____ Height (feet): _____
[ ] Isolated Tree [ ] Group of Trees (number):_____
[ ] Clear cut— estimated number of acres: _____

Burs: [ ] None [ ] Few [ ] Many [ ] Unknown
Catkins: [ ] Present [ ] Absent [ ] Unknown
Surroundings: [ ] Full Sunlight [ ] Partial Shade [ ] Full cover
Blight: [ ] Not Visible [ ] Visible [ ] Sunken Canker(s) [ ] Swollen Canker(s)
Could we reach the tree with a large truck? [ ] Yes [ ] No
Comments: _____
_____
_____

Owner of Property:
Name: _____ Phone: _____
Address: _____
City: _____ State: _____ Zip: _____
E-mail: _____

Are there any restrictions to viewing, pollinating or harvesting the tree? [ ] Yes [ ] No
Is permission needed from the owner? [ ] Yes [ ] No

Submitted by:
Name: _____ Phone: _____
Address: _____
City: _____ State: _____ Zip: _____
E-mail: _____

TACF Analysis Information Checker: _____ Date: _____

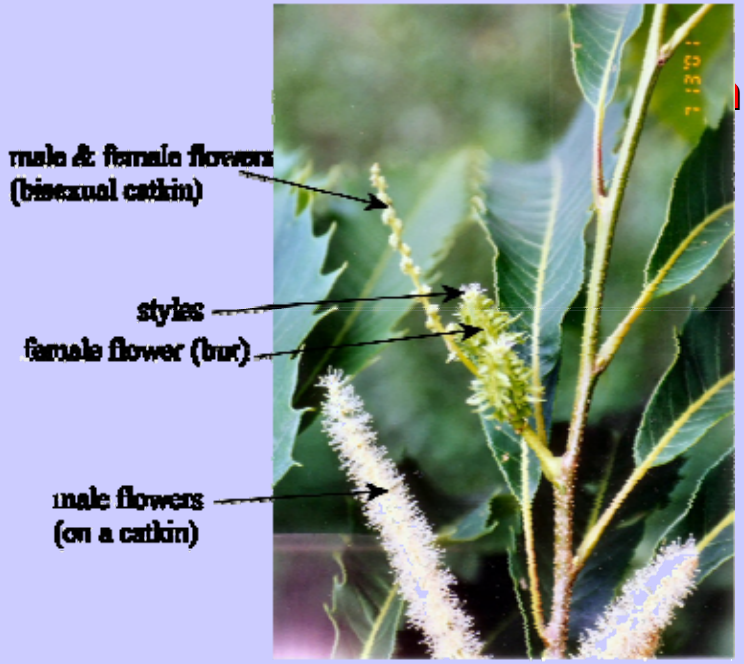
American [ ] Class A -Classic
American/Hybrid [ ] Class B -Slightly Hybridized
Hybrid/American [ ] Class C -Heavily Hybridized
Non– American [ ] Type: _____

Analysis Notes:
_____
_____
_____
_____

Tree Name:_____ Tree Code: _____

Chestnut Flowering

- Chestnuts are monoecious
  - Both male (catkins) and female flowers (burs) on same tree
  - Catkins and Bisexual Catkins
- Self-incompatible
  - 99% of the time



- Chinese trees generally flower before American trees
- Catkins bloom before burs; catkins bloom before bisexual catkins.
- Chestnuts need a lot of light to bloom – you'll most often find flowering chestnut trees in clearcuts of 8-15 years of age and along roads or fields – areas where they will receive ample amounts of sunlight.

Getting to the Flowers

This is the most difficult part!

- **Caution: *Ladders can be very dangerous.***
- Ladders
  - Aluminum tripod orchard ladders (Stokes)
    - Lightweight and fairly cheap
    - They come from the West Coast, so order more than 2 at a time.
    - 16’ works best
  - Werner Trestle ladders
    - More expensive, but higher and safer.
    - Can get up to about 30’ in the air.

Werner Ladders

http://wernerladders.com

1-866-3Werner

Stokes Ladders
4545 Renfro Drive.
P.O. Box 445
Kelseyville, CA 95451

800-842-7775

http://orchardladders.com

Getting to the Flowers

- Scaffolding
  - Only for the adventurous!
  - Build your own from scrap (at right)
  - The Kentucky folks have used a large triangular metal scaffold that is used for TV antennae. Although large, heavy, and dangerous, the structure worked quite well.

Getting to the Flowers

Bucket Trucks

- Utility Companies (Allegheny Power, United Electric)
- Tree Utility Companies (Asplundh)
- Telephone and Cable companies (short booms)
- Towable Booms (JLG Rentals)
- Contact local arborists

Supply List

1. Way to get up in the
2. Apron
3. Pole with hook (teles
4. Bags
5. Clips / Cable Ties / T
6. Grease Pencil / Sharp
7. Scissors
8. Pollination Form

http://chestnut.cas.psu

DOCUMENT WHAT YOU D

Bagging Female Flowers

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

2) Puff up the bag, place over shoot, twist on base and secure with a jumbo paper clip or a twist'em (the latter tends to be preferable). 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.

3) Number the bags with a permanent marker or grease pencil (it is helpful to do this before you start bagging). Numbering makes it easier to keep records on how many bags are placed on the tree, which bags have been pollinated, and which bags have been collected during the harvest. Placing a mark on every tenth bag helps keep track of which bags are **controls.**

4) 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.

5) 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!

CONTROLS - We don’t take of the control bags when pollinating. Therefore, if there are nuts in the control bags, we know we bagged too late. The cross, then, would be contaminated.

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
  - The minimum order is 3000 bags, costing about $120
  - If you're only doing a few, write the Meadowview Farms, 14005 Glenbrook Ave., Meadowview, VA 24361
  - Order these before you start!

- You can carry supplies up the ladder in your pockets, or in a folded pollination bag attached to your pants with paper clips. But a carpenter's apron works best (see pictures). You can get these from Lowe's or Home Depot for about $1-$3

Lawson Bags
P.O. Box 8577
Northfield, IL 60093
(800) 451-1495 or
(847) 446-8812
http://lawsonbags.com/

Nail apron

Cotton Bib

Bagging Female Flowers

American Chestnut Branch Ready to Bag
Remove the Male Flowers (catkins)

Bagging Female Flowers

Remove the leaves to accommodate the bag and keep as many leaves as possible

Bagging Female Flowers

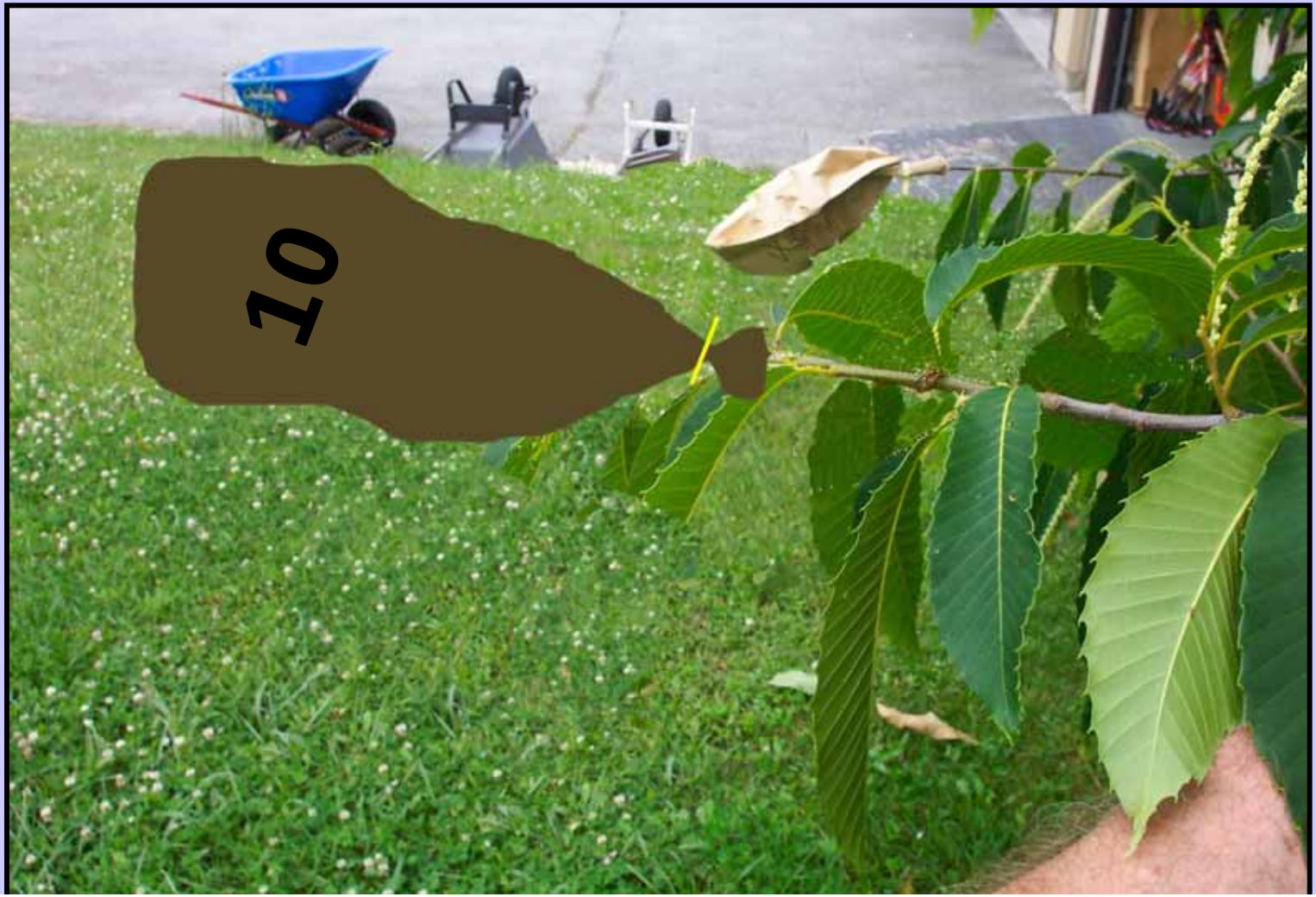
Remove the male end of the bisexual catkin

Bagging Female Flowers

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

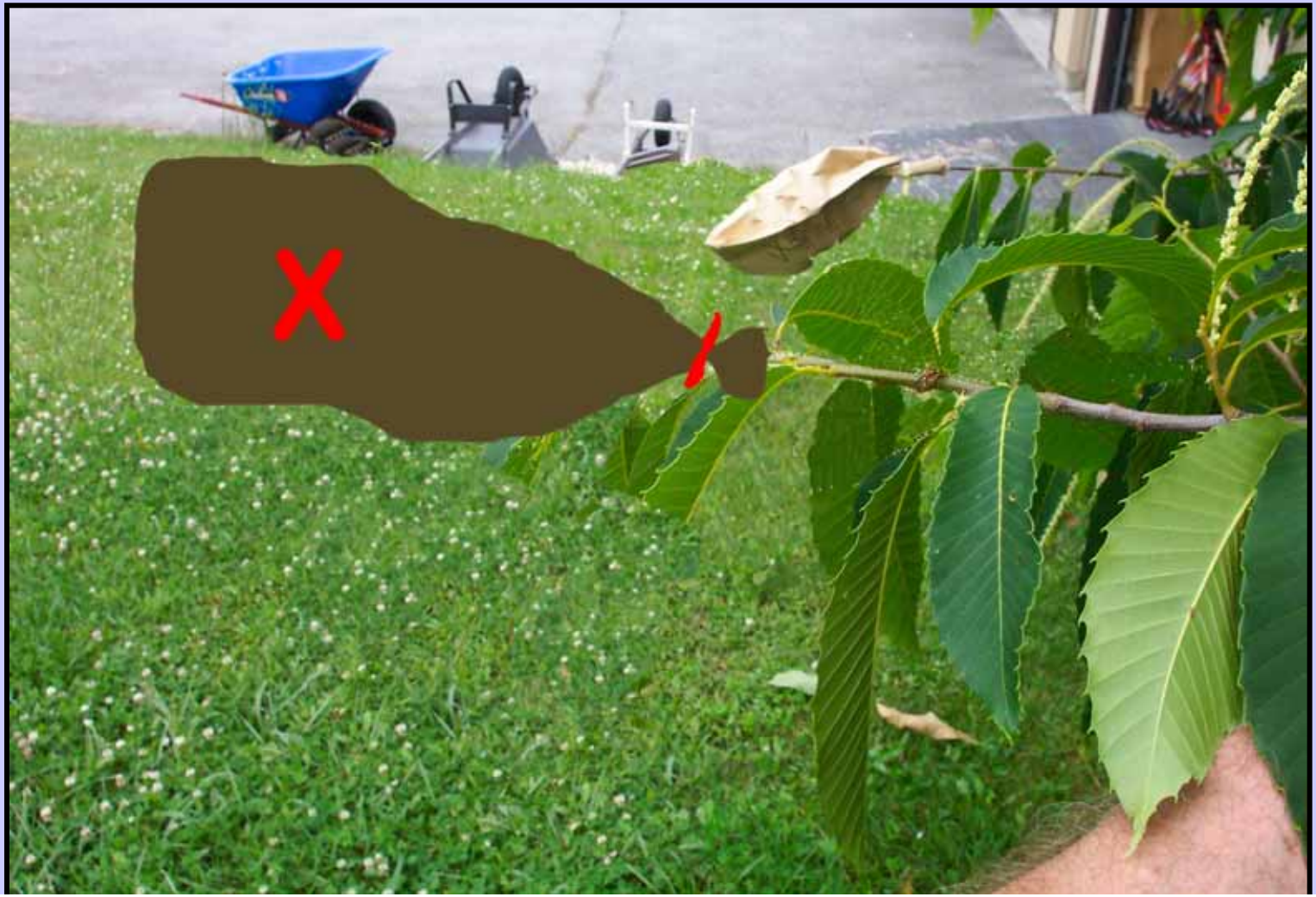
Bagging Female Flowers

Mark the bags and document the details



Bagging Female Flowers

Placing a mark on every tenth bag helps keep track of which bags are controls.



Bag Female Flowers

- Don’t forget your controls!
  - 1 Control Bag for every 10 Pollinated Bags.
- Timing is very important!
- Too early – flowers may abort
  - Too late – flowers may have already been pollinated.

Timing!

- After catkins fully extend, observe female flowers every few days; every day if possible
  - Good binoculars
- Southern part of Pennsylvania
  - Generally start bagging in mid-June
  - Later as increase in elevation and latitude

When to Bag??

- Bag female flowers when the styles are exerted 2-4 mm and are still grouped close to one another.
- 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.

Timing Is the Key to Success

NOT READY

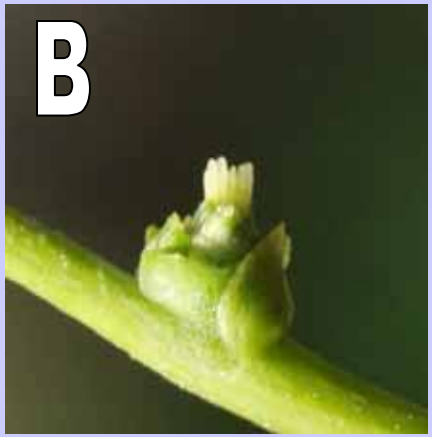
Timing Is the Key to Success

READY TO BAG within 3-5 Days

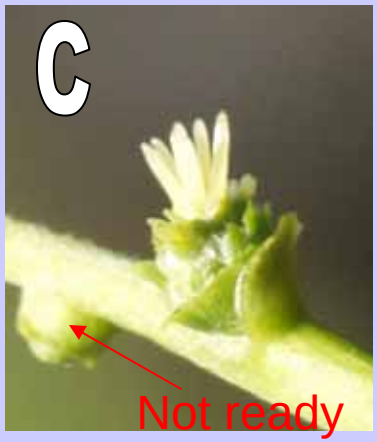
Receptive - Pollinate

Maturation of Female Flowers

- Too early



- Still too early



Almost there – start panicking now; wait about 1 day.

Bag Now.

- A tad late.

- Too late.

- Too late.

- Way too late.

Look at the Male Flowers

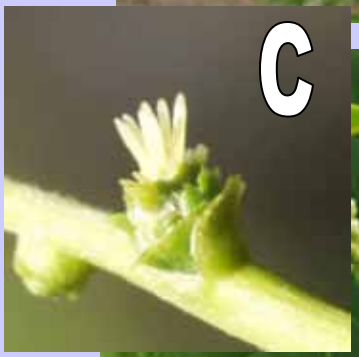
Another good rule of thumb, which applies in most years, is to begin bagging when green catkins on 50% of the flowering branches begin to exert stamens and turn white or creamy yellow.

Timing Is the Key to Success

READY TO BAG within 3-5 Days

Look at the Catkins

- Too early



Almost there – start panicking now; wait about 1-2 days.

Collecting Pollen

- Use Fresh Catkins

or

- Use Dried Pollen
  - Collect branches with catkins
  - Keep fresh until ready to strip from branch
  - Strip catkins and leave on table to dry overnight at air temperature
  - Strip catkins of pollen
  - Collect pollen in prescription bottles
    - Label bottles!!
      - Tree from which it came and date of collection
      - Use a permanent marker. Place masking tape on vial and write it on the masking tape.

-- If dried properly, dried pollen tends to yield greater nut yields than fresh catkins --

Sifting and Collecting Dried Pollen

Drying/Preserving Pollen

*Make sure your hands and all of the materials you use are sterilized with rubbing alcohol or hard liquor while drying pollen

- Collect catkins from desired tree – take a whole branch and place the branch in a jug/5-gallon bucket filled with water until you are ready to collect the pollen. Cover the branch and catkins with a paper bag. Don't leave them too long in the jug – just overnight if possible.
- Very gently remove paper bag from flowers and flowers from jug.
- Separate the anthers from the catkins. You can do this by shaking the catkins, stroking the catkins with your fingers, or, and this is a great method, using a frying pan splatter screen. This method is faster, yields more anthers, and cuts down on the amount of debris in the sample
  - Place several individual catkins on top of the splatter screen and hold the screen over a piece of glass. Shake the screen horizontally causing the catkins to roll and drop their anthers. You can roll the catkins gently with your hands across the screen as well (don't roll hard enough to rub off the hair like filaments onto the plate as well).

- 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.

Frying pan splatter screen

Drying Pollen without Dessicant

Dr. Hill Craddock at UT-Chattanooga has a simple method for drying pollen.

1. Pluck catkins from the branches and spread them on a glass sheet – don't let the catkins touch.
2. The catkins will dehisce over night.
3. The next morning, strip the catkins by running you fingers over them and/or shaking them, or by using the frying pan splatter screen.
4. Throw away the used catkin.
5. Sift the pollen and collect them in pill bottles.
6. Store them in the refrigerator for eventual use. Try and use it as soon as possible.

Pollination

- Pollinate
  - Don’t Pollinate Control Bags!
  - Pollinate 10-14 days after bagging
    - (14 days are preferable to 10)
    - Take off bag
    - Apply pollen
      - Fresh Catkins
      - Dried pollen
        - » Tip bottle
        - » See pollen on cap
        - » Rub cap on styles of flower
    - Replace bag

POLLINATING

1. Pollinating with fresh catkins

While pollinating with fresh catkins or dried pollen, make sure your hands are sterilized and that you sterilize again when you switch trees. Do this with rubbing alcohol.

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.

1. Take off the bag.
2. 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.
3. Replace the bag.
4. Mark the bag as pollinated by placing a check mark or X with a Sharpie or grease pencil.
5. Repeat for all bags **except** the control bags. You should have marked them as control bags as you bagged a couple of weeks ago.
6. 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 Dried Pollen

2. **Pollinating with dried pollen**

- Place a sample of pollen in a small vial, film canister, or prescription pill bottle (flip tops are the most useful). Don't carry your entire supply of pollen up the tree. Make sure that the container is dry and sterilized before placing the pollen inside (sterilize with rubbing alcohol or hard liquor).
  - Take off the bag.
  - To pollinate, invert the container and then return it to an upright position; static electricity will hold a film of pollen on the cap. Pop off the cap and Gently rub it on the tips of the styles. Place more pollen on the cap at least every 5 or so flowers. Make sure you keep pollen cool and dry in the shade or in a cooler.
  - Replace the bag – secure with new twist tie / cable tie / paper clip.
    - 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.

- Place a small sample of pollen in a clean and sterilized bottle. Don't carry your entire supply of pollen up the tree!

Document all of this!

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.

If only one pollen is used per tree (and this is recommended), you'll just need one or two labels for the tree. Be sure to write all of your information on the Pollination form.

Supply List for Pollination

- Way to get up in the tree
- Pole with Hook
- Pollen
- Carpenters apron
- Sharpie / grease pencil
- Paper clips / twist ties / cable ties
- Pollination form
  - Remember the one you filled out when you bagged the tree?

- Steady Hand
  - When using vials of dried pollen, do not take entire bottle with you at once . . .

Maturation of Burs

Step 5 - Wait

Step 6
Harvest

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, rubber gloves or gloves covered with latex (find them at any home improvement store). 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.

- Label or bags well or, 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.

Harvest Time!

- Timing is not so vital
  - But get to them before the squirrels!
- Report Harvest Details on your Pollination Form (*you didn't lose it over the summer did you?)*
  - Collect Information on:
    - Bags collected
    - Number of burs collected
    - Number of seed collected

Documentation: Pollination Form

	Tree Name		Received from Mother Tree Coordinator
	Tree No/Code		Received from Mother Tree Coordinator
	Bag Date		Date of Bagging
	Poll. Date		Date of Pollination
	Male (Pollen) Parent		Code of Pollen Parent - should be on vial
	Dried or fresh?		Is the pollen dried (in vial) or a fresh catkin?
Pollinated	**Bags ()**		Number Bags placed (How many recovered during harvest)
	Burs		After harvest, how many burs were recovered
	Nuts		After harvest, how many nuts were recovered
Unpollinated Checks	**Bags ()**		Bags placed (How many recovered during harvest)
	Burs		After harvest, how many burs were recovered
	Nuts		After harvest, how many nuts were recovered
	Harvest Date		Date of Harvest
	Comments		Anything extra??

http://chestnut.cas.psu.edu/forms.html

Supplies for Harvest

- Pole with Hook
- Scissors / Pruning Shears
- Pollination Form
  - Yep. The one you took with you during bagging and pollinating. Hope you kept it in a safe place over the summer.
- Many bags for collection of burs
- Keep separate genotypes in separate bags!
  - Mark bags!

STORAGE

- 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.

Storage

- For eating
  - Chestnut weevils
    - Soak in water of 117-120 degrees Fahrenheit
    - Do this for 20 minutes
  - Yes, the weevils will still be in there. But . . .
    - Their generally still in egg/small stage – you'll never know or taste them
    - Think of them as extra protein.
- For planting: Several options for storage
  - Breathable ziploc bags with damp (not wet!) peat moss.
  - Deli Containers
  - Produce bags

Take Home Message

- Timing is Everything!
- Nothing can compare with experience.
- Take careful notes.
- Don’t eat your entire inventory.

- The chapter also needs open-pollinated nuts from confirmed: Chinese, Japanese, European, and American trees
  - Demonstration plots
  - Control trees (checks) in advanced hybrid orchards

Questions?

More Information on Pollination can be found at:::
http://chestnut.cas.psu.edu/procedures.html