Bulletin 1

Badgersett Research Corporation

Chestnut Pollinator's Guide

CONTENTS

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Introduction

Pollinating chestnut trees is not always simple or straightforward. I know quite a number of folks, even those with experience pollinating other species, who have blithely assumed that chestnuts should be easy to work with and ended the growing season with no nuts at all to show for their pains. We do not yet entirely understand all aspects of chestnut pollination, either natural or artificial, but don't let that discourage you. If you follow the methods described here, you will certainly have some success.

Harvest methods and techniques for storing nuts are also covered here- all your pollination work will be wasted if the nuts are lost or improperly handled!

Please do not take everything you read here as gospel. Improvements on these methods are certainly possible. Use your common sense!

Avoid Spreading the Blight

Today the blight fungus is no longer as common as it was during the epidemic. There is a small but real possibility you could carry the infection from one place to another. Wherever you are working; in the East where blight is widespread, or in a region where there is no blight yet, it is well to be aware of the dangers, and to take as few chances of spreading the blight to currently undiseased trees as possible.

Identifying the blight: The best and easiest way to learn to identify the blight is to have someone experienced show you the fungus in the field, and show you how to find it. Lacking that, the following points may help you know that blight is present.

• Dead chestnut trees. Little else really kills them. However, particularly outside the major chestnut range, trees planted on unsuitable sites may be healthy for years, but eventually succumb to poor drainage, other environmental factors, or other diseases.

• Red-orange color showing in bark cracks or at the base of dead branches. This is the color of the sporulating fungus.

• Dead branches, or a partially dead crown. Lightning strikes a fair number of chestnut trees in the midwest, and shade can kill branches, so this is not a sure indication.

• Many new shoots growing from a point just below a dead part of the crown (also can be confused with lightning or wind damage).

• A branch with dead, dry leaves clinging tightly to it, while other branches around it have healthy green leaves ("flagging").

If you see one of these signs, suspect the blight; if you see two or more signs, expect it.

While there are few certainties about how to avoid spreading the blight, pathologists generally agree on the following points:

• There is almost no chance of spreading the blight through the pollen we use.

• There is almost no chance that a human will directly infect a tree by carrying spores from one tree to another. You would probably have to put the spores directly into a fresh wound.

• Avoid wounding the tree- whether you are likely to have spores on you or not, any wounds you make are potential infection sites. This is important for those working with relatively healthy trees within the blight regions; there is always a chance of starting a new, lethal infection. Chestnut trees are thin skinned, and easily wounded.

• An unquantifiable risk is associated with spreading spores to the vicinity of chestnut trees. The blight fungus can grow on dead wood lying on the ground. Scientists have not decided how well or how often it does this, but all agree it can. The risk here is that in just walking in an area with blight, your clothing will pick up a few spores; if you then visit an unblighted site, you might drop a few spores on the dead twigs and leaves. If they start growing there, then the area will have an active source of spores, which will sooner or later infect the trees. Until this aspect of the fungus' life is better understood, do not visit "clean" sites right after visiting blighted ones. If you must, then you should change clothes, including shoes and socks. It is probable that dry-cleaning possibly contaminated clothes will kill any spores they might contain. Cleaning shoes may be more difficult; removal of dirt and wiping with a strong chlorine bleach solution may help.

Be careful! You can't see the spores, of course, so there is sometimes a tendency to forget they are there. Don't forget!

Species Identification

One of your first tasks will be to confirm the species of the tree you are working on. It is best always to recheck previous identifications, as chestnuts are quite variable, and identification of some species and their hybrids can be difficult. All of the species listed here have been planted in the East for decades, some for centuries; and many of their hybrids can also be found there, in very unlikely places, even in what looks like wild woods. While leaves are notoriously variable in form, and ultimately unreliable as proof of species, they are useful in simple cases. Hybrids will always need additional characters for identification.

The basic rules for simple identifications are as follows:

Never look at just one leaf! Always examine several branches, and include leaves from the base of a branch and new leaves from the tip of the current growth. If a tree has leaves or twigs with noticeable hair, it is not pure American. If the leaves do not usually have large, regular teeth, it is not pure American.

If the guidelines below still leave you uncertain about a particular tree, more study may be necessary to make the identification. Gather old and new leaves, and about a foot of fresh twig, and either send them to someone for another opinion, or consult the references listed in the bibliography. Please forgive the crude graphics- I make no claim to be an artist!

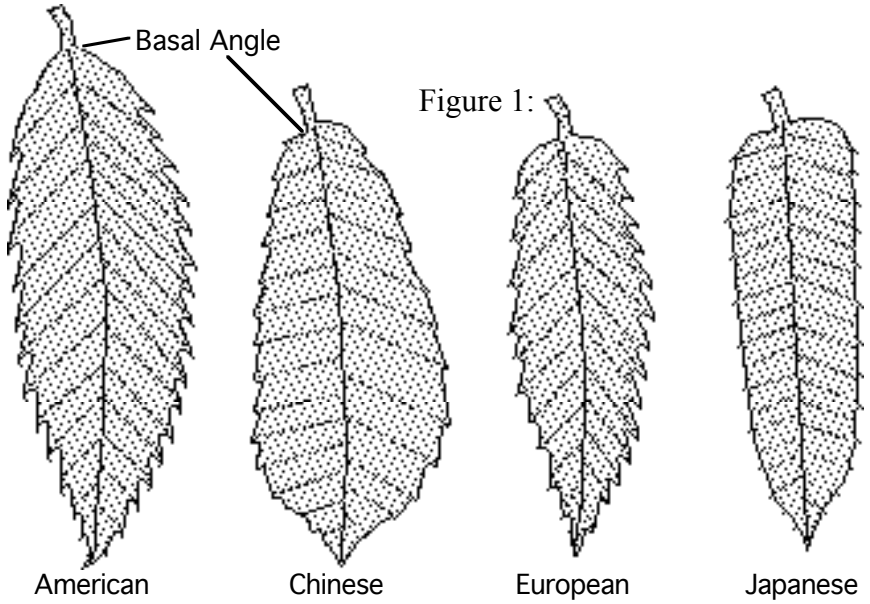


Figure 1:

American chestnut- leaves may be large, usually thin and limber, with distinct teeth. Most importantly, they are essentially hairless; a few simple hairs can usually be found on the underside of the mid-rib, but they have to be searched for, with a hand lens. Basal angle usually sharp, but variable, even on the same branch. Although the illustration shows American leaves larger than other species, they are quite variable, and often a particular tree or part of a tree will have only small leaves. Teeth sometimes decoratively re-curved, something seldom seen on other species. Twigs are hairless, thin, and buds pointed.

Chinese chestnut- Leaves are thicker than American, often stiff and shiny, and the underside is often white with a soft mat of hair (I call it the Oriental rug); but, older leaves commonly appear hairless- the hairs wear off. Teeth may be large or small. Basal angle is often nearly 90° to the mid-rib, and the tip-ward third of the leaf is frequently (not always!) markedly broader than the basal third, abruptly tapering to the tip. This leaf broadening is often found in hybrids with Chinese, also. (Annoyingly, it is not hard to find pure American leaves with this basic shape, but the taper is usually less abrupt.) Twig characters are unique- the winter twig is buff-yellow in color, with easily visible simple hairs near the tip of the twig- no other species has those traits.

European chestnut- this species is the most difficult to separate from American on the basis of the leaves; they may be large, and have large teeth. The teeth may be rounded, but are not always. (The illustration shows rounded teeth on the left leaf margin, sharp teeth on the right.) Luckily, the leaves are usually hairy, and will feel fuzzy; but like the Chinese, older leaves may feel smooth. Also, the basal angle is usually near 90°, like the other Old World species. The twigs and buds are typically "fatter" than any other species.

Japanese chestnut- leaves tend to be small and narrow, with very small teeth, sometimes reduced to just bristles. The underside of the leaf will have "glands" visible with a hand-lens as light colored dots. Basal angle similar to Chinese.

Record Keeping

Maintaining concise and inclusive records is crucial: any nuts of undocumented or even questionable origin are worthless for scientific breeding. I keep a separate sheet for each tree, with clear identification and an entry for

each visit to the tree with a note of every action and observation made on each visit. Make each entry at the time of the visit; don't try to do it later from memory.

Example:

Hesper, Iowa

Species- American
Finder- Bernard Larson, Newburg, MN
Owner- Hesper Friends' church
Location- Behind Friends' church, on street
Contact- in Hesper-Robert & Joyce Street, Rt. 4, Decorah, IA 52101- 319-555-5829
dbh- 41"
crown spread-63'
height-about 50'
condition- excellent- one dead branch, no sign of blight
comments- flowers & burrs, no nuts- neighbors complain of smell when flowering- origin - possibly from Caledonia, MN trees? soil pH ~ 6.2
1987

June 23- visit at 6 PM- 15/50 (30%) bisexuals shedding; most staminates (90%) browned; some few dropping.

6/27- POLLINATED- using Douglass 1A from John Gordon, airdried one day, sieved and shipped overnight with desiccant. Crude germination test showed some viable pollen, with good tubes, as long as the pollen grain.

Arrive 12:00, 13/50 bisexuals not shedding (74% bisexual anthesis).
high mid west branch unpollinated control- ~ 160 burrs
entire W side pollinated dry, 390 burrs, including S branch
NE side wet, 72 burrs, pollen mixed into 1% sucrose in distilled water

68° F; 53% RH; barometer 29.67 and falling. clear, moderate wind
many staminates browned & dropped- ~50%; some bisexual males dusting pollen

high SE branch is mostly dead, for no apparent reason- scanned with binoculars for blight, no sign of orange-red or cankering.

Suggestions on specific observations to make: What % of ultimate staminate catkins (last catkin before the bi-sexuals) are shedding? (count 50, record #s) What % of bisexual catkins are shedding pollen? (count 50, record #s) Weather conditions. Time of day. Any observation of actual visible pollen shed. Actual counts are far more valuable than guesses! Others: blight activity, changes from last year, etc.

When kept for several years, such notes can teach us a lot about the activity of particular trees and also about trees in general: we still have a lot to learn.

Chestnut Reproductive Biology

Chestnut trees have both male and female flowers on the same tree. However, like many other plants chestnuts are self-incompatible: a tree usually cannot fertilize its own flowers. "Selfing" in chestnut does occur, as a rare, though regular phenomenon; genuinely isolated trees self-pollinating from less than 1% to perhaps 5% of their flowers. Trees grown from such selfed nuts tend to be severely dwarfed or non-viable.

The chief method of natural chestnut pollination is still a matter of debate: the morphology of the flowers appears superficially similar to wind pollinated plants (although the tiny size of the stigma is atypical). Experiments have indicated that wind can be an adequate pollinator within orchards. However, the wind does not carry pollen very far— trees only 100 feet apart will experience reduced pollination success, and trees 1,000 feet apart are essentially reproductively isolated from one another. Insects apparently play a role, but how important they are is uncertain. The male catkins are white, scented with a musky odor attractive to many insects, and provide nectar. On the other hand, the female flowers have no color, no odor, and no nectar. Many types of insects are seen on the male catkins, but they are seldom seen on the females…

Chestnut flowers are formed on the present year's new growth. Male flowers have no petals, and are reduced to clumps of anthers strung together on a long catkin. Several long, purely male (staminate) catkins are formed first, and at the tip of the branch, shorter structures bearing 1-5 female flowers at their base and male flowers at their end are called the "bisexual catkins". The little female flowers will become the burrs. Usually, each burr will contain three separate ovules, which if fertilized will become the nuts. Burrs with only one or two ovules are not uncommon. The number of ovules a burr contains can be easily determined by looking at the protruding styles.

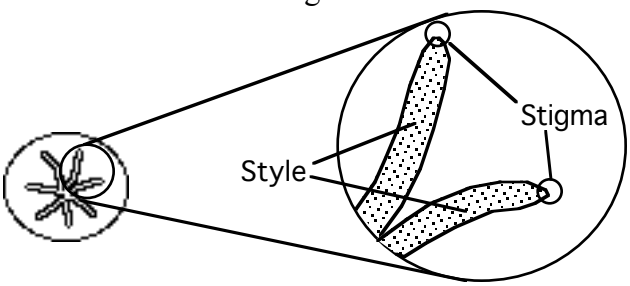
Figure 2: Top view, young burrs and spread styles (about twice life size)

1 ovule 2 ovules 3 ovules

The styles, when first visible, are short and stick straight up from the top of the burr. Before the flowers become receptive, most styles spread out, as in the illustration. Sometimes, however, American chestnut styles will not spread, but remain short and nearly straight up throughout receptivity.

It is critical to understand the difference between the style and the stigma. The stigma is the portion of a female flower which is actually receptive to pollen. The style is merely an inert stem which supports the stigma. In chestnut, the actual stigma is one of the smallest known, and is virtually invisible. It is located at the very tip of each style.

Fig. 3



Chestnuts flower late, compared to most trees. For much of the chestnut range flowering takes place from June to early July; local differences will depend on latitude and altitude. The following series of illustrations show typical flower development, over a period of 3-4 weeks.

Figure 4 (1/3 to 1/2 life size)

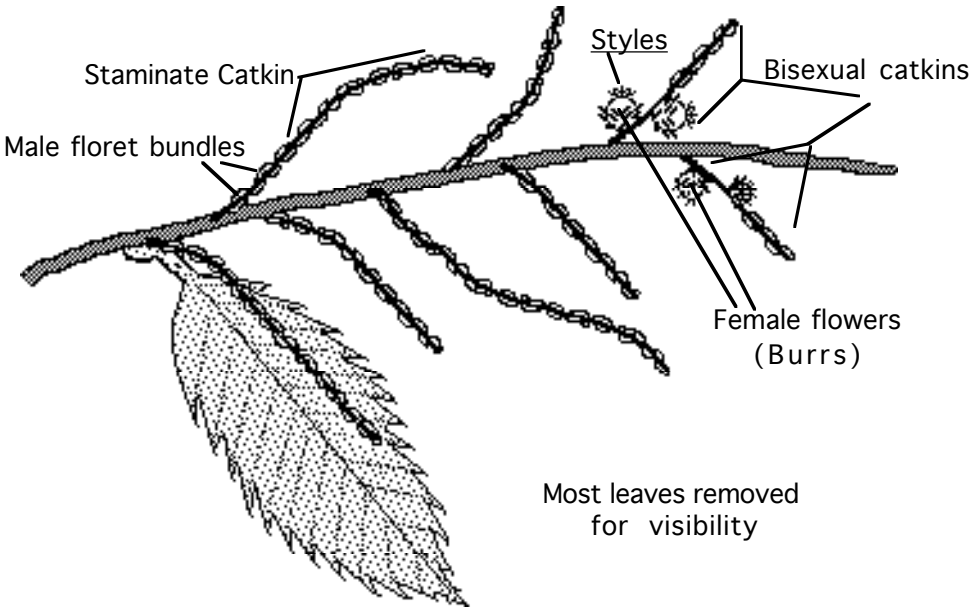


Figure 4: Chestnut branch with immature flowers. The staminate catkins are not yet shedding pollen, but are covered with the small bumps that are actually developing clusters of anthers. Female flowers on the younger bisexual catkin are visibly smaller and less mature than those on the older one. Catkins commonly originate at the base of leaves, but may originate at a bud node with no leaf. In vigorous trees, branch growth continues well beyond the final bisexual catkin, but in older trees or less vigorous branches, growth for the season may end at the bisexuals.

Pollen shed (anthesis) begins at the lowest catkin on the branch (the proximal) and proceeds up to the bisexual catkins. The male parts of the bisexuals shed pollen much later than the staminates.

Figure 5

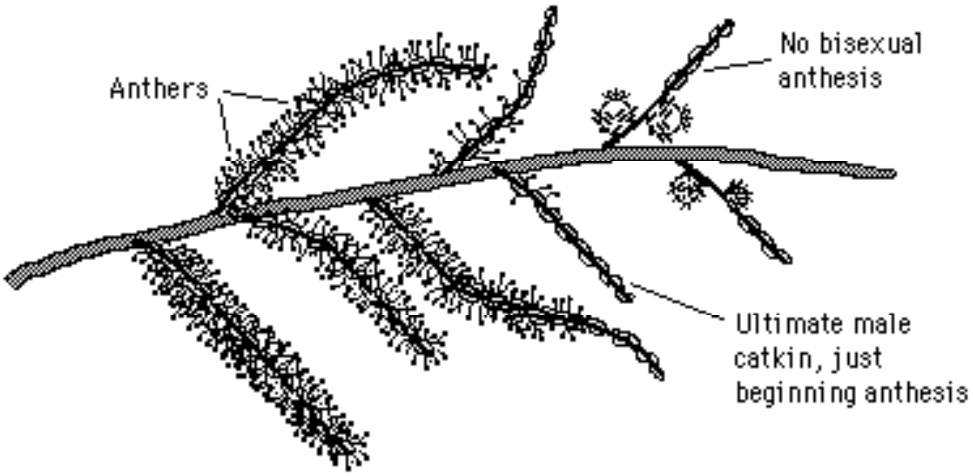


Figure 5 shows a branch in an early stage of flowering; not all the staminate catkins are blooming yet. The females are unlikely to be receptive at this point. Unlike these graphics, the blooming staminate catkins are a creamy white color. Before anthesis, the catkins are green and inconspicuous, but during the bloom chestnut trees are easily spotted in the woods.

Figure 6

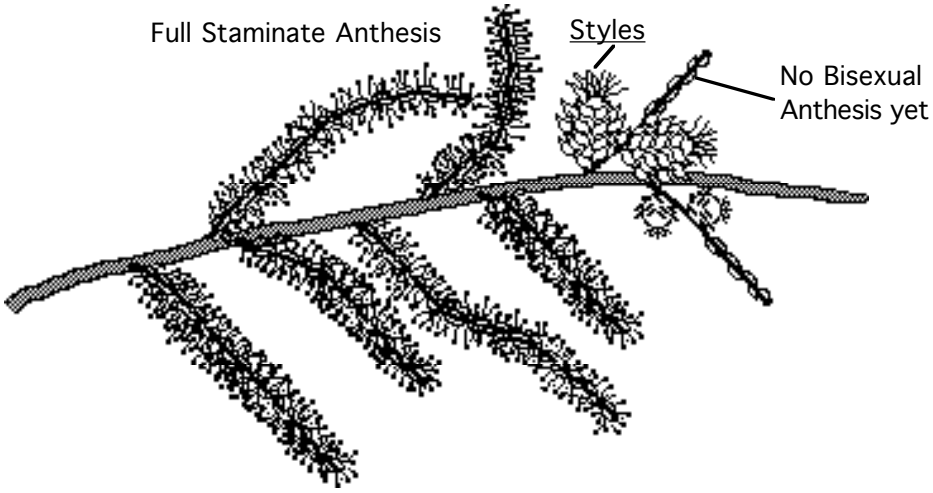
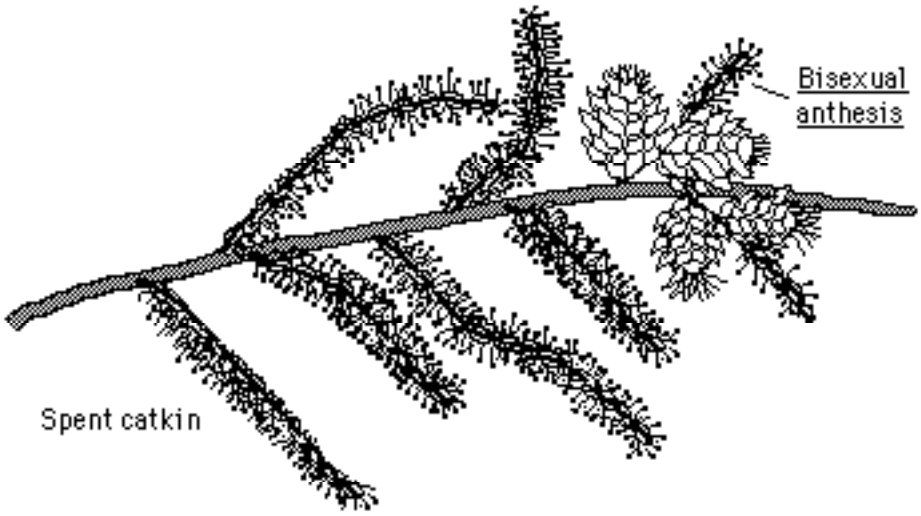


Figure 6 is a branch at full staminate anthesis; note that the burrs are larger, and the styles have grown longer and spread out somewhat. The older burrs are probably receptive at this point, the younger burrs might be, too. This stage may indicate the peak of female receptivity.

It is common for chestnut branches to progressively activate new catkins every day for 2 weeks; then there may be a week when the tree appears the same from day to day, after which the bisexuals begin to shed pollen and the older staminates begin to brown and drop off. My present belief is that peak female receptivity usually occurs during the time when the trees do not change their appearance; after the ultimate male catkins are shedding pollen, and before the bisexuals begin to shed pollen. Most trees seem to follow this rule, but occasionally an unusual tree will be found where the females are receptive before or during the main anthesis.

On each tree, individual flowers will differ in their status- young flowers whose development was retarded by heavy shade may be found when most staminates have dropped. Sometimes whole regions of a tree will differ in their status from the majority, apparently mostly depending on how much sun each part receives.

Figure 7



The above branch is quite far along in the season; the oldest catkins are browning, no longer producing pollen, and will soon drop. The male parts of the bisexual catkins are now shedding pollen. The older burrs have grown considerably, and the styles are being overgrown by the expanding burr. Most of the female flowers are probably no longer receptive.

Bagging Flowers

Whether you are trying to make a controlled hybrid or just pollinating to get any kind of nut you can, you will probably have to "bag" your female flowers. If the flowers are left unbagged, they may get pollinated by any air or insect borne pollen available. You will have "open pollinated" nuts, which means you cannot be certain what the male parent was. It also now seems certain that while a tree cannot pollinate its own female flowers, long exposure to its own pollen may "block" them, so that when you pollinate, the pollen you apply cannot reach the ovum. Bagging eliminates both these problems, but causes several of its own. (If you have a small, truly isolated tree, you might avoid bagging by cutting off all the male flowers before they shed pollen.)

There are 3 special problems in bagging chestnut flowers; Heat, Wind, and Soft Twigs. Most other trees flower in early spring, and bags put on them can be fastened onto well-hardened wood. Chestnuts flower in June and into July, on fresh shoots that may be rather soft, particularly if you are working on a vigorous young tree. Winds accompanying thunderstorms will tear some bagged shoots off, or in some cases may injure the stigmas by constant rubbing. And very hot days can "cook" some shoots when they are enclosed in bags.

Leave some flowers unbagged- so you can judge the correct time to pollinate.

Try to bag female flowers as late as possible, to decrease heat and moisture damage and mortality of flowers. If possible, bag flowers just as soon as the first chestnut trees in the area start to shed pollen, but not earlier. For isolated trees, you should bag no later than the stage shown in Figure 5.

The bag to be used should have no pores that can pass pollen grains, but should "breath" to decrease moisture build-up. Brown paper grocery bags can be used to enclose entire branch tips, and can be fastened on hardened wood. Smaller white "corn shoot" bags specifically designed for pollination (Lawson Bags #421) keep the flowers cooler and may be less susceptible to wind damage, but must be fastened on soft wood. These bags are available from: Lawson Bags, PO Box 8577, 318 Happ Road, Northfield, IL 60093; phones: 800-451-1495, 312-446-8812. A minimum order is 3,000 bags-

Remove all the male catkins that would otherwise be included inside the bag, including the male portion of the bisexual catkin. They can be pinched off, but it may be easier, and do less damage, to use scissors. If using small bags, it may help to cut off the tips of some of the leaves, or remove them, so the flowers are not too crowded in the bag.

Wrap the point on the twig where the bag will be fastened with an unwound cotton or polyester ball (available at any drugstore). This will prevent abrasion and allow some moisture to escape without allowing pollen in.

Secure the bag around the branch, making certain the bag is closed and no pollen can enter. I use common staples, at least one going through some of the cotton. If you use wire, be careful not to get it too tight. If you are working an isolated tree and only trying to exclude self pollen, you need not worry about making the closure absolute. It may help, in fact, to leave it a little more open, so heat-buildup will not be such a problem.

When to Pollinate: Determining Receptivity

Clapper-" Female flowers are… receptive when the styles have turned from a green color to straw-yellow, and have spread more or less at right angles to the axis of the flower. Another method of determining the receptive period is to note when staminate catkins on the mother tree begin to blossom; about 12 days after that date the stigmas will be receptive."
Jaynes-"Pistillate flowers are not receptive until five days after anthesis of the first staminate flowers and remain receptive up to the 17th day. Best results with controlled crosses can be expected if the pollinations are made between the tenth and 13th day after anthesis."
Schad- "It is not possible at the present time to pinpoint from what moment on the female flower is ready for fertilization and during how long a time the stigmas are receptive. One can only conclude that under natural conditions, the majority of fertilizations take place a little after the full expansion of the stigmas of the lateral flowers and lasts about three weeks."
Chinese- "Pollination is carried out when the female styles branch".

A difficulty with all these methods is that they require the observer to see the tree every day, or rely on phenomena which have not proven reliable for some American trees. When working with scattered trees it is not possible to see them every day, nor is it possible for a new worker to estimate how long a tree has been shedding pollen. And I have waited more than once until staminate catkin drop for the styles to spread- sometimes they never do.

What is needed is a method which will allow anyone to walk up to any tree and assess its reproductive status. For such a method a consistent phenomenon is required, one easily quantified. At the moment, the pollen shed

sequence appears to have the desired qualities. I now have several years of data which indicate (not conclusively yet) that the period between the anthesis of the ultimate male catkin and the anthesis of the bisexual catkin is the time when most females will be receptive. Figure 6 illustrates this stage clearly- it may last a week or more.

Trees that do not follow this sequence have been reported in several papers; although I have never seen one, chestnut trees in which the females are receptive before any males shed pollen do exist; keep an open mind.

Pollen Application

The easiest and best method is to use a freshly picked mature catkin, and brush this lightly over the female flowers being pollinated. This can be very effective if the male parent tree is close to the female, and not more than a few hours pass between the time the catkin is picked and the time it is used.

In many cases, of course, fresh pollen may not be available. Then you must use preserved pollen, and the necessary techniques will become more complex.

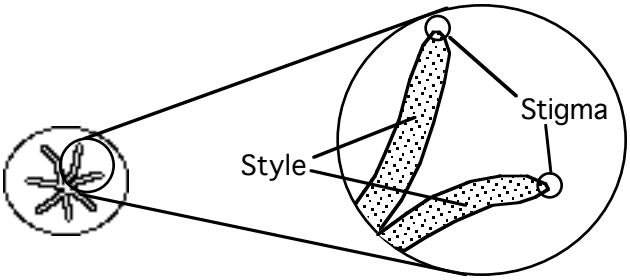
A common technique used for many species of plants is to apply pollen with a camel hair brush. Camel hair brushes are readily available at artist's supply or book stores; a #2 brush is about right. Divide your pollen supply, putting some into a "working" container (vial), as it is a frequent occurrence for vials to get dropped in the process of working among the branches. Leave your "stock" supply in a safe place, possibly on ice in an ice chest. When ready to apply the pollen, work the brush around in the vial and get it covered with pollen; then wipe the stigmas with the loaded brush. Be sure to wipe the stigmas of all the ovules present. This technique is effective, and fairly conservative of your pollen supply. It is frustrating to watch so much pollen fall off the brush and the flowers, however.

A more effective method is to use a glass slide. I have used this technique for several seasons, and it has produced better results than with anything else I have tried.

Get several clean glass microscope slides. Wipe them with lens paper before using.
Separate your pollen supplies into "stock" and "working" vials. You will probably drop your working vials, and slides, in the process of working through the branches, and it would be disastrous to lose your entire supply. Keep your stock secure.
Take a clean slide, open your working pollen vial, and 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.

Carefully remove the bag from the branch to be pollinated. The bag will have to be replaced immediately when you are through pollinating. If it is in good shape, the same bag can be reused, otherwise a new bag will have to be put on.

Since the only receptive part of the female flower is at the very tip of the style the pollen must be put there. Chestnuts have one of the smallest stigmas of any tree.



Take the loaded slide, and drag it lightly over the style tips (stigmas) of the flowers. Try to hit all the stigmas. 2 or 3 drags are plenty; more might conceivably damage the stigma. You may be able to see the stigmas leaving "tracks" in the pollen film. BE SURE you touch the LOADED side of the slide to the flower! This seems obvious, but the pollen film is nearly invisible, and it is easy to lose track of which side is the right one. A mark with a crayon or grease pen will help.

After 5-10 flowers, re-load the slide with pollen. After 50-60 flowers, get a new slide or completely clean the old one. It is likely that the old one will be accumulating "self" pollen from your tree, which might interfere with fertilization. If possible, re- pollinating the tree 4-6 days later can increase the number of "takes".

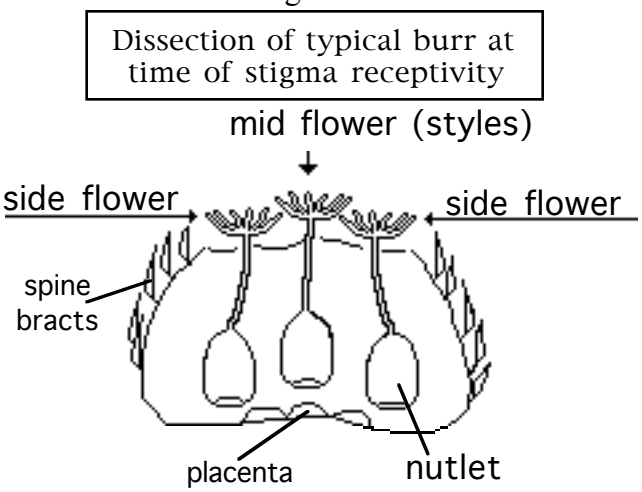
This technique works, and works well. It also makes the absolute most of your pollen supply- no pollen goes anywhere but on the stigmas, whereas with a brush most of the pollen gets stuck in the brush, or goes into the air, or gets put on an unreceptive part of the flower.

I use a small styrofoam block, with slots cut to hold slides, on which I can place several "loaded" slides. I place this inside a closed, clean, pollination bag, to prevent contamination. This way, I can load several slides at once, have immediate replacements for spent or dropped slides, and juggle no pollen bottles while up on a ladder.

If you have only a small amount of pollen, the glass slide technique is the most conservative. The brush technique may give a lower percentage of takes, wastes a lot of pollen, and in my experience, is not be any faster to perform.

Exactly where should the pollen go? The stigmas of the chestnut flower are specifically connected to the individual ovules. If pollen is applied to styles on only one side of the flower, the ovule(s) on the other side will receive no pollen. Make an effort to hit all the stigmas.

Figure 10



(Approximately 5X natural size)

Controls- Whatever method you use, it is imperative to always leave a small group of flowers unpollinated, as a "control". If your unpollinated flowers develop nuts, you will know there is a natural pollen source nearby. Designate a specific branch as a control; count the flowers on it, and leave them unpollinated; 50-100 burrs should be adequate. If bagging, leave several bagged flowers unpollinated, but otherwise treat them just as you do the pollinated flowers. We have already had several instances where previously unknown trees suddenly matured and began supplying pollen to trees which had been assumed to be isolated; without the control, those open pollinated nuts would have been considered hybrids, and would have seriously confused information on the inheritance of blight resistance.

Quantity- it might be possible to put too much pollen on- there are indications in other species that too much pollen applied can depress fertilization success.

Using more than one type of pollen per mother tree- if there is a reason to use more than one pollen on a mother tree, it is probably safe to do so- the pollen does not travel far; certainly not from glass slides. Several years of observations have shown consistently that unpollinated control regions immediately adjacent to pollinated parts of a tree show no increase in nuts over unpollinated areas far from pollinated branches. If you do put more than one pollen on a tree, always separate them as much as possible, and put unpollinated control regions between types.

Pollen Gathering and Preservation

If you are lucky, you may be able to get someone to send you the pollen you will need. It is quite possible that you will have to gather your own, however, since good pollen is in short supply. Once you learn how to handle pollen, you will be able to supply it to others.

Chestnut pollen is microscopically small, only about 12 microns long (about the size of a red blood cell), and has proven more difficult to gather and preserve than pollen of many other tree species. Attempts to ship large numbers of fresh catkins have usually led to pollen that is mostly dead. Warm, humid conditions can kill fresh pollen fairly quickly, sometimes in less than 4 hours (Schad et. al.)

The male catkins produce less pollen than one would guess from looking at them. Most beginners think they are seeing plenty of pollen, when in fact they are only seeing the pin-point sized anthers. These are abundant, but do not always mean pollen is present. If you stroke a catkin, pollen will occasionally be visible on your hands as faint streaks of very fine, pale yellow dust. You may have to examine and turn your hand carefully in full sunlight to see it. Some pollen will be present on mature catkins on still days even if you cannot see it; if you can see pollen, then it is actively being shed and may be easily gathered by the glass plate method. Trees may not shed in this fashion every day. On windy days, the freshly produced pollen is quickly blown off the catkins.

Some pollen can be gathered from any fresh, light-colored catkin, as soon as anthers protrude. It is easiest to gather at early full anthesis. When the catkins change color from cream-white to yellow or brown, they are past their prime, and may have no pollen at all.

Ideally, catkins to be gathered for pollen should be bagged to exclude pollen from other nearby trees; if this is feasible, it should be done. Jaynes states that pollen yields from bagged catkins are better than from unbagged, due to exclusion of wind and insects. He recommends pollen collection bags be dusted with Malathion to kill insects which otherwise consume a great deal of the pollen (personal communication). If the quantity of pollen being gathered precludes the extra time and work bagging requires, it can be bypassed, but the possibility of contamination from other trees should be noted.

Preservation of chestnut pollen is accomplished by drying (desiccating) it, and then refrigerating or freezing the dried material.

The easiest collection and preservation technique is to simply gather good catkins and dry them whole. The dried catkins can then be used as pollen application "brushes". This is convenient for small quantities (a handful or two), but drying large quantities of catkins rapidly becomes difficult. They contain a good deal of water, and can overwhelm small desiccators.

Collection in the field of pure pollen on a sheet of glass has proven very effective, provided large numbers of catkins can be reached easily from the ground. Use a moderate sized pane of absolutely clean window glass (wipe with lens paper just before collection), a razor blade, and a clean vial for the pollen. Gently strike the catkins on the glass until the glass looks dusty. Tilt the glass and dump bugs and "trash" off before scraping the pollen together and into the vial with the razor blade. A piece of slick, glassine weighing paper can be rolled into an excellent funnel. Pollen gathered this way is very pure, highly viable, requires no further sieving, and is easily preserved. In addition, the same catkins can be harvested for pollen every day for weeks, instead of just once. Repeatedly scraping the pollen together with the razor blade will seem tedious, but it is lightning fast compared to putting catkins through a sieve.

Pollen can also be collected by picking catkins and bringing them inside to extract the pollen. This is time consuming, and may result in pollen of decreased viability if the drying process is slow, but may be your only choice if the catkins you want are high up in a tree. The picking process often knocks much of the pollen present off the catkin; you may get a better yield if you can air-dry the catkins for 24 hours before proceeding.

Spread the catkins 1 deep on tinfoil or wax paper. An air-conditioned room works well; avoid a hot, humid, or windy room.

When catkins have been dried somewhat (1-2 days at 68-75° F), the pollen is then removed by rubbing them through a sieve. Several sieves have been tried, the best method so far being to use a medium hard toothbrush to put the material through a #35 USA Standard testing sieve (500 micrometer mesh).

If more than one kind of pollen is being handled, care must be taken to prevent cross contamination. Containers and brushes should be rinsed with 70% ethanol, (and dried carefully) between types of pollen. Since the grains are tiny and easily airborne, different pollen types should not be stored in the same room during catkin drying.

To dry whole catkins, glass collected, or sieved pollen, place the pollen over fresh desiccant (CaCl2 or silica gel) in a tightly sealed container, at room temperature. Partly depending on the quantity of pollen, times ranging from 2 hours to 2 days have been recommended. I have measured significantly lowered viability of American chestnut pollen with longer desiccation times.

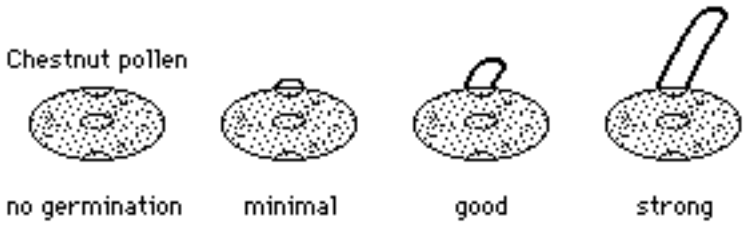
Following desiccation, pollen is placed in tightly sealed vials, labeled in a moisture-proof fashion, and placed in a refrigerator until shipping or use. Dried catkins can be sealed in tinfoil. Enclosing a small amount of fresh desiccant, carefully wrapped in paper, with the pollen will guard against moisture damage.

Shipping- Desiccated pollen can be shipped First Class mail. If fresh or air-dried catkins must be shipped, or undesiccated glass-collected pollen, all shipping should be done via some over-night method, with arrangements for the pollen to be refrigerated immediately on receipt. Beware of shipping on weekends, when no one may be there to receive it.

Storage- Pollen needing long term storage (a week to a year or more) is generally simply stored in a standard freezer, following desiccation. Undesiccated pollen can safely be refrigerated to hold it for a day or two. Desiccated pollen in daily use should only be refrigerated each night, not stored in the freezer. It may be that the temperature shock of freezing and thawing would do more harm than good.

Testing pollen viability- 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. Pollen can easily be germinated by floating a small sample on a drop of 0.5-1% sugar (glucose is slightly more effective than sucrose) in non-chlorinated water (deionized or distilled both work), and incubating it for 1 hour at about 30° C (90° F). I have germinated pollen in my kitchen, and counted grains under a child's microscope. Cover your sample so it won't dry out. Place a droplet with pollen on it under the microscope, and examine for pollen tubes at 100-400X magnification. Good pollen should show 15-60% of the grains with tubes (as long as the grains) growing out of them. Whenever possible, a sample of each batch should be tested for viability, preferably just before use in pollination.

Figure 9: (about 400 X)



Vital staining is also useful for indicating viability, if you have access to the necessary chemicals. The technique is as follows:

Bring pollen sample to room temperature, about 22° C.
Put pollen on a glass microscope slide.
Add a few drops of a 0.5% aqueous solution of:
3-(4,5 dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide (Sigma M2128, 1g $23.55).
(store this solution in darkness, and refrigerate)
Cover with coverslip, tapping out air bubbles
Leave slides at room temperature for 30 minutes.
Examine under a microscope, scanning at 100X, counting at 200X.
A bluish light source of fairly high intensity usually works best.

Viable pollen will stain magenta
semi-viable pollen will stain lilac
dead pollen will be either yellow or empty.

In general, germination tests tend to underestimate the actual viability of the pollen, and vital staining tends to overestimate it. Thanks to Dr. Mark Widrlechner for the vital staining methodology.

Timing Pollination Visits

A difficulty with using trees scattered around the countryside is that any attempt to assess the reproductive status of the tree really requires that the tree be visited. In order to reduce the number of trips, it will be helpful to try to judge when the next visit will be required. Phenology is variable from tree to tree and with the weather- if it is hot, speed up the visits, if cool, spread them out. Experience will quickly help you.

For non-isolated trees, bags should be applied at the beginning of anthesis, and removed 1-2 weeks following staminate catkin drop. For isolated trees, bags should be applied not later than 50% staminate catkin anthesis, and should be left off following the final pollination. There is no reason to leave the bags on until catkin drop, since any "self" pollen reaching the stigma after your pollination will not be able to do any harm- this will save you one trip to the trees.

Labels

Obviously, you must be able to remember which flowers were pollinated with what. Try to describe a major set of branches in your notes, and use those branches for only one purpose. If smaller groups of flowers are used, each set of bagged flowers must be labeled. Use something loose, to prevent girdling; and inconspicuous, to prevent vandalism by people or crows and jays. Aluminum tags tend to wear out with wind flexing, and drop

off before harvest. One that does not is an aluminum tag with cardboard backing and a rivet for the wire to go through: #79201 from Forestry Suppliers, $29.25/500- phone 800-647-5368.

Weather

Effects of weather on pollination success are unclear. Successful pollinations have been made on sunny and cloudy days, in cool and hot weather. Clapper reported slightly higher success rates for pollinations performed in the morning than for those done in the afternoon. There is some possibility that high humidity may contribute to better fertilization success. On one occasion, when circumstances allowed no other chance to pollinate, successful pollinations were even made during a light rain (the success rate was not good, however). In general, it is much easier and safer to work on calm days rather than windy days. Always note the weather in your records.

Harvest

Harvest is as critical as any other part of the process! Chestnuts are eaten by everything that crawls, walks, and flies- as soon as the burr opens. Even if your tree has never had viable nuts before, bluejays and squirrels will find the opening burrs immediately, and steal your valuable nuts before they ever hit the ground. I know!

The remedy is simple- as soon as nut-bearing burrs just begin to open, pick all the burrs which may possibly have a nut inside. The seeds will be viable.

Chestnut trees develop burrs whether they contain good nuts or not, which makes it difficult to find the good ones. Burrs which have not been pollinated will start to open and drop as much as a week before the fruitful burrs. When many of the empty burrs are opening it is then possible to spot the fruitful burrs- they will not only be closed, but will commonly still be bright green, in contrast to the brownish color of the empty burrs.

The state of the fruitful burrs can be assessed by opening one or two (if there are many available to work with). When the developing fertilized nuts begin to show brown color, the burrs will start to open very soon. I have, however, had trees start to drop nuts while they were still white. (Nuts from burrs opened for inspection, even when completely white, will germinate if not damaged- save them, preferably by leaving them attached to the burr and waiting for them to color up before storage.)

Start looking at the trees in early or mid September. When nuts inside closed burrs are averaging 50-80% brown, or fruitful burrs are just starting to split open, start picking. A "fruit picker" basket on a pole will reach many burrs without requiring a ladder. I line the basket with mesh, so it will not allow loose nuts from partly opened burrs to fall through and be lost. A net bag like those grocery stores use for oranges works very well.

Be sure to keep burrs from different crosses and different control and experimental areas of a tree in separate, labeled bags. Use baggies or trash bags, depending on how many burrs there are; leave the tops open to allow the burrs to breathe. Be careful bags don't tip over and spill together, confusing the counts.

Store the bags so they will not dry out. The burrs generate a lot of moisture; be sure to allow it to escape. Overhaul the bags on a regular basis and remove nuts as they ripen. If the bags are stored in a cool, dark place, overhaul at least every 4 days; if stored in light at room temperature, overhaul at least every 2 days, as they will ripen (and dry) faster.

Storing Nuts

Following harvest, the seeds must be carefully stored. Chestnut seed has unusually strict requirements for storage; people used to working with other kinds of seeds usually kill their first batch of chestnut seed. It must not dry out too much, or get soggy. It must not freeze repeatedly, or go below 26° F, or be stored very long above 40° F. A little drying (curing) is beneficial, but too much will kill the germ. Nuts are cured enough when the shells start to dent slightly when squeezed between thumb and forefinger. One day's inattention can result in the death of many nuts, as most chestnut workers at some time or other have proven to themselves. If you store them like you would carrots, you will be on the right track, and have excellent chances for success.

Chestnuts have been stored successfully packed in sand, pasteurized sawdust, peat moss, unmilled sphagnum, or plain plastic bags with a few holes. The storage medium must be barely damp, and have some ability to retain moisture, but if unattended must also be able to drain, as the nuts "sweat" water during storage, and can become too wet in a totally closed container. The acid nature of sphagnum or fresh sawdust is useful in slowing the growth of spoilage fungi. The storage site, or container, must be mouse-proof.
Label each container carefully, inside and out, with the identification of the mother tree and the pollen used, in waterproof ink and/or pencil, on a durable surface. Put only one kind of cross in a container.

Recommendations- For fresh, uncured nuts, use barely damp or dry sphagnum, pack the nuts so that they don't touch each other, to limit spread of decay from bad nuts, and place in a tightly lidded plastic or metal container (not mouse proof) with holes punched in the bottom. For storage over winter, a refrigerator can be risky, as it may dry the seed too much. A mouse-proof metal container, buried and covered with straw bales or an 8" deep layer of leaves works fine. Under these conditions, the nuts will start to sprout in February.

or: Air dry freshly harvested nuts for 5-10 days indoors at room temperature to cure them. Then store in the vegetable crisper section of a refrigerator, in sealed plastic bags, mixed with an equal volume of nearly dry peat moss (caution- some inexpensive sandwich bags are so thin-walled that moisture will escape over the winter, killing the nuts). Bags should be turned over every few weeks to prevent excessive moisture condensation forming on the bottom, and to visually check the moisture content. Nuts in containers with free moisture may mold, and if allowed to dry too much, will not germinate (from Jaynes, personal communication).

Weevils

In many eastern parts of the US chestnut weevils are serious pests. Jaynes states that infested nuts can be saved by refrigerating them for 2 or more months- constant temperatures that cold interfere with the ability of the weevil to feed, and nuts can be planted without loss (personal communication). In some instances, however, nuts are apparently damaged not only by egg laying but by adult weevils feeding on them, in which case the holes they leave may allow fungi and bacteria to invade the nut rapidly. Tom Dierauf has suggested replacing the pollination bag with a fine mesh bag that would exclude the weevils- this sounds like a very promising approach.

Shipping Nuts

Watch the weather before shipping; if there is danger of the seeds being frozen in transit, keep them until it is safe. If necessary, wait until Spring.

Glossary

anthesis- the shedding of pollen
bisexual catkin- the catkin bearing both female flowers at its base and males at its tip
isolation- when a tree is so far from others that they cannot pollinate it- 100 yards.
phenology- the progressive change of an organism with the seasons; short for "phenomenology"
pollen- the male cells responsible for fertilizing the female- microscopic in chestnut
staminate catkin- the long string of reduced male flowers (See Figs 3-6)
stigma- portion of the female flower that actually accepts pollen; tiny in chestnut
style- the visible white "fingers" on the female flower, not receptive to pollen, but carrying the stigma at the tip
ultimate staminate or male catkin- the last all-male catkin, just before the first bisexual

ACKNOWLEDGEMENTS

I greatly appreciate the support, additions, criticisms, and time of Drs. Richard Jaynes and Mark Widrlechner, who thus contributed to the creation of this handbook.

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 day. Use the checklist every day, and change it as needed.

Pollination-
Ladder
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
camera, film
field notebooks
spare pens, pencils
knife
tape measure
insect repellent

sun screen
toilet paper
water
lunch
thermometer
pollen
application equipment-brushes, slides, pipettes
bags
bag & branch labels
handbook

Harvest-
ladder
thick leather gloves
tweezers
field notebooks
plastic bags- large and small
label material- moisture proof
keys
maps, compass
money
phone numbers
Foundation brochures
hat
raincoat
binoculars- for seeing the burrs in the crown of the tree, examining for blight
camera
spare pens, pencils
knife
insect repellent
sun screen
toilet paper
water
lunch
thermometer

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Quick Guide to Making Controlled Pollinations of Chestnut

(4 Pages)

These tasks are presented in the order in which you will probably have to perform them. In most parts of the country, you should start looking at your trees in mid June or earlier, so you can judge when you will need to go to work.

1. Records- Write down everything you do; how, when and where. Any nuts you make will be unidentifiable and worthless, otherwise. Check the species identification of your trees; there have been plenty of long-standing misidentifications.

2. Bagging- Chestnut flowers to be pollinated will have to be "bagged" to exclude random airborne pollen from fertilizing or blocking them. There are 3 special problems in bagging chestnut flowers; heat, wind, and soft twigs. Most other trees flower in early spring, and bags put on them can be fastened onto well-hardened wood. Chestnuts flower in late June and into July, on fresh shoots that may be soft. Leave some flowers un-bagged- so you can judge the correct time to pollinate.

a) Bag female flowers as late as possible, to decrease damage and mortality of flowers. Try to bag just as soon as the first chestnut trees in the area start to shed pollen, but not earlier. The female flowers should not be receptive yet.

b) The bag to be used should have no pores that can pass pollen, but should "breath" to decrease moisture build-up. Brown paper grocery bags can be used to enclose entire branch tips, and can be fastened on hardened wood. Smaller white "corn shoot" bags specifically designed for pollination (Lawson Bags #421) keep the flowers cooler and may be less susceptible to wind damage, but may have to be fastened around soft wood.

c) Remove the male catkins that would be included inside the bag, including the male portion of the bisexual catkin; use sharp scissors, or pinch. With small bags, it may help to cut off the tips of some of the leaves, or remove them, so the flowers are not too crowded in the bag.

d) Wrap the point on the twig where the bag will be fastened with an unwound cotton or polyester ball (available at any drugstore). This will prevent abrasion and allow some air in and moisture out without allowing pollen in.

e) Secure the bag around the branch, making certain the bag is closed and no pollen can enter. I use a common stapler and several staples; at least one staple going through some of the cotton. If you must use wire, be careful not to get it too tight.

2. Pollen Collection & Preservation- In many cases you will not have to collect pollen. If you do, however, there are two primary methods for collecting pollen; on glass or by picking catkins.

a) Catkins will produce more pollen if they are bagged some days ahead of time. This excludes wind and insects, also pollen from other trees. Jaynes recommends this, and recommends using Malathion dust (insecticide) in the bags to kill the small insects that are constantly grazing on the catkins. It is possible to collect good

amounts of pollen from unbagged catkins, if the collecting is done on a calm day; early morning may be your best bet. Pollen is available on cool or hot days, morning or afternoon. Don't try to collect it if there is dew on the trees. If you have collected pollen from any other species, you will be surprised at how little pollen chestnuts produce.

b) Get a clean, smooth piece of window glass, preferably as large as you can comfortably handle (not more than 2' square). Take this (and a spare) into the field with you, and just before collecting pollen, wipe it down once more with new lens cleaning paper, but no liquids. This eliminates dust and unwelcome pollen from other trees. Don't cut yourself on any sharp edges.

c) With your hand balancing the glass like a waiter does a tray, hold the glass under a bunch of catkins, and using your other hand, lightly strike the catkins against the glass, several times. Chestnut pollen sticks to glass well, but it can be knocked off the glass into the air or onto the leaves if you continue striking too long.

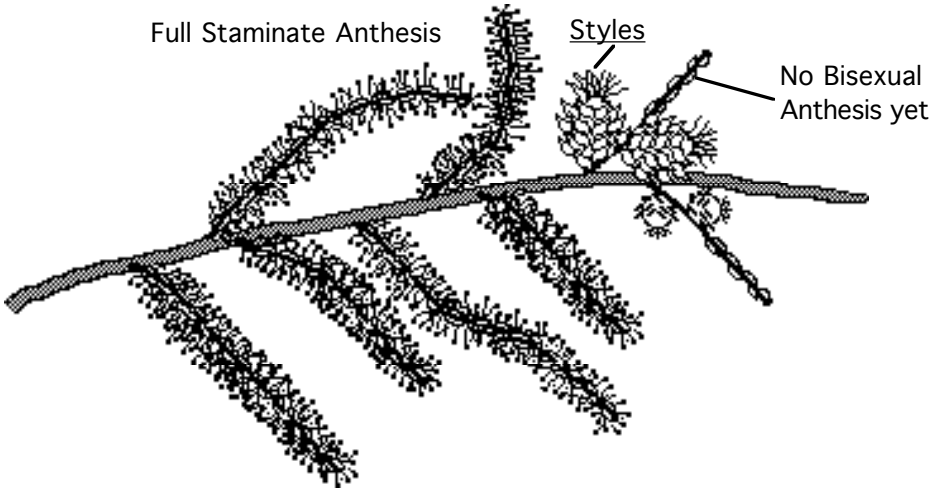
d) hold the glass up to the sunlight after several hits- if you can see a faint haze on the glass, collect the pollen before continuing. Chestnut pollen is very tiny; you cannot see the individual grains. There will be plenty of "trash" on the glass, including anthers, anther filaments, and bugs. If you have plenty of catkins to collect from, you may want to tilt the glass and tap it to dump the trash off; most of the pollen will stay on the glass. Leaving the trash on will probably not hurt anything if you pick out the bugs.

e) using a one-edged razor blade, scrape the pollen together. If the process is working, you will collect small piles of brilliantly yellow dust. Scrape these into new or carefully cleaned vials. The vials should be tightly sealable, and suitable for long term storage.

f) repeat the collection procedure until you have the pollen you need. One group of catkins may be good for 2 or 3 collections before you harvest another group.

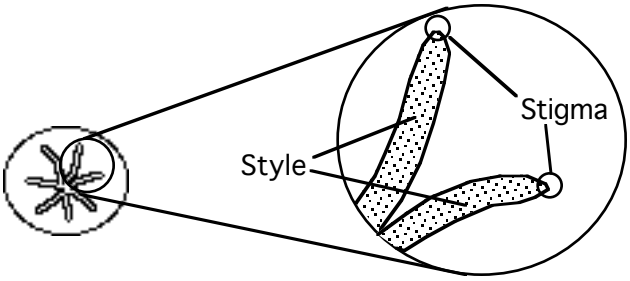
g) to preserve the pollen, get it as quickly as possible (not more than a few hours if unrefrigerated) into a room-temperature desiccator, over fresh silica gel or calcium chloride. Desiccate the pollen for at least 4 hours, more if there is a lot of pollen; not more than 24 hours. After this the pollen 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. Do not store fresh (undesiccated) pollen in high humidity or at room temperature any longer than absolutely necessary.

3. When To Pollinate- pollinate your trees when most of the flowers look like this:



4. 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 100-400X magnification. Good pollen should show 15-60% of the grains with tubes (as long as the grains) growing out of them.

5. Pollen Application- The only receptive part of the female flower is at the very tip of the style; the pollen must be put there. Chestnuts have one of the smallest stigmas of any tree.



a) pollen can be applied with a camel hair brush, or better, with a glass slide.

b) get several clean glass microscope slides. Wipe them with lens paper before using.

c) separate your pollen supplies into "stock" and "working" vials. You will probably drop your working vials, and slides, in the process of working through the branches, and it would be disastrous to lose your entire supply. Keep your stock secure.

d) take a clean slide, open your working pollen vial, and 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.

e) carefully remove the bag from the branch to be pollinated. The bag will have to be replaced immediately when you are through pollinating. If it is in good shape, the same bag can be reused, otherwise a new bag will have to be put on.

f) take the loaded slide, and drag it lightly over the style tips (stigmas) of the flowers. Try to hit all the stigmas. 2 or 3 drags are plenty; more might conceivably damage the stigma. You may be able to see the stigmas leaving "tracks" in the pollen film. BE SURE you touch the LOADED side of the slide to the flower! This seems obvious, but the pollen film is nearly invisible, and it is easy to lose track of which side is the right one, even in full sunlight. A mark with a crayon or grease pen will help.

g) after 5-10 flowers, re-load the slide with pollen. After 50-60 flowers, get a new slide or completely clean the old one. It is likely that the old one will be accumulating "self" pollen from your tree, which might interfere with fertilization. If possible, re-pollinating the tree 4-6 days later can increase the number of "takes".

6. Labels-Branches should be labeled with information about what pollen or treatment was applied. Aluminum or waterproof cardboard tags can be used. 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 in your notes.

7. Controls- Remember to leave a few of your branches as controls: bag them, take off and replace the bag when you pollinate, but don't pollinate them. If they develop nuts, there has been a slip somewhere. This is important, don't neglect it.

8. Removing Bags- Remove the bags as soon as pollen is no longer shedding from any of the nearby chestnut trees; or if that is too long and you are worried about heat damage to your flowers, when all the male catkins of your tree have dropped.

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