Receptivity of the Pistillate Flowers and Pollen Germination Tests in Genus Castanea

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Introduction

The process of hand-pollination is at best cumbersome and time consuming. It therefore is important that the pollination be as efficient as possible, yielding the maximum number of seed with the minimum amount of work. This is particularly true in the large-seeded species such as the chestnut, Castanea spp., where only a few inflorescences can be enclosed in a single bag, necessitating the use of a large number of bags to obtain enough nuts for a progeny test.

In the Connecticut chestnut-breeding program, which has been in progress since 1930, it has been the practice to pollinate each bag three times at two-day intervals in order to obtain maximum nut yield. This procedure has resulted in an average nutset of from 30 to 40 per cent, with many pollinations yielding 70 to 80 per cent. If a similar nutset could be obtained from a single pollination, more and larger pollinations could be made.

Chesnut pollen has been considered difficult to germinate (CLAPPER, 1954), although SCHAD et al. (1952) describe a technique which they have used successfully. A simple test for pollen viability would be most helpful in a chestnut breeding program where pollen has to be used after long-distance shipping.

During the summers of 1953 and 1954, a number of experiments were conducted to determine the length of the receptive period in Castanea, and to determine the effectiveness of single pollinations. The effects of emasculation on nutset were studied and various germination tests for chestnut pollen were tried out. The results of these experiments are presented herewith.

The Flowers and Flowering Sequence in Castanea

Chesnuts are monoecious; the flowers are borne on the current year's growth. Two types of inflorescence are found: the unisexual male catkins, which are located on the lower parts of the shoot, and the bisexual catkins towards the terminal end of the shoots (see Fig. 1). Staminate flowers are spirally arranged along the axis of the catkin in clusters of from three to seven; each consisting of a 6-lobed calyx and ten to twenty stamens with long filaments. Pistillate inflorescences appear solitary or in clusters of two or three at the base of the bisexual aments. Three pistillate flowers surrounded by the many bracted involucre are normally found in the true chestnuts, although an occasional involucre with only one or two flowers may be found. The chinkapins, i.e. C. pumila, have only a single pistillate flower in each involucre. The calyx is 6-lobed adnate to a 6-celled ovary. The styles are 7 to 9 in number. The stigmatic surfaces are limited to the extreme tips of the styles (HARLOW and HARRAR 1941; REHDER 1949; VILKOMERSON 1837).

The lower unisexual catkins are the first to start opening. At New Haven, Connecticut, the true chestnuts begin opening in the latter half of June, varying as much as a week from year to year. The Japanese chestnut, C. crenata, is the most precocious; C. mollissima and C. dentata commence flowering from four to as much as fourteen days later. The pistillate flowers are next to open, and not until eight to ten days after anthesis of the unisexual catkins do the male flowers of the bisexual catkins begin to open. This sequence of flowering has been described as duodichogamy (STOUT 1928).

VILKOMERSON (1937) studied the flowering behavior of twenty-five chestnuts, including seven C. mollissima, eleven C. crenata, three C. seguini, and four hybrid trees. Using visual observation of the pistils and taking the separation of the styles as an indication of receptivity, she concluded that a protandrous type of flowering (as described above) as well as a protogynous flowering habit are common; of the twenty-five trees examined, fourteen found.
were proliï¬cous, nine protogynous, and two synchronous. Clapper (1954) cites Vilkomerson's work, but does not discuss her results; his own data do not indicate the occurrence of any protogynous trees. Hand-pollinated involucres produced nuts only if pollinated three or more days after anthesis of the unisexual catkins.

Self fertilization is rare in chestnuts, the trees usually being completely self-sterile (Clapper 1954, Vilkomerson 1937). Vilkomerson selfed 464 involucres on the twenty-five trees mentioned and obtained four nuts, two of which were smaller than normal. The duodichogamous flowering-habit cannot be considered the reason for this high degree of self-sterility, as there is ample overlap in the flowering periods of the male and female flowers. The cause must be sought in some form of self-incompatibility.

**Female Receptivity**

**Material and Methods**

In June 1953, six trees were selected for the study of the receptivity of the female flowers; these included two Chinese chestnuts, two Japanese chestnuts, and two hybrid trees of the same combination ([C. crenata × C. pumila] × C. crenata)] × C. crenata. In the following, these hybrids will be mentioned as S-8 × J.

The trees were selected on the basis of size and time of flowering. Fairly large trees were necessary to get the number of bags required to conduct the experiment. They were bagged on the day of anthesis of the unisexual male

![Image](image-url)

**Fig. 1.** - Flowering chestnut. This is the type of flowers typical for C. mollisimum. The duodichogamous flowering-habit is clearly shown; the lower male flowers have been shedding pollen for some time. The female flower has just reached receptivity, while the male flowers of the bisexual catkins still are tightly closed.


catkins with exception of S-8 × J, 4—6, and C. crenata, 7—7, which were bagged the day after, and two days before anthesis, respectively. Before bagging, the branches were completely emasculated in order to make sure that nuts would not develop from self-fertilization.

The trees were pollinated on the 5th, 9th, 13th, 17th and 22nd day after the anthesis of the first male catkins, the total of three weeks being considered long enough to cover the entire possible length of female receptivity. Each female parent was pollinated with three trees of the same species or hybrid combination, the male parents being chosen for their progressively-later anthesis of the male catkins so that long storing of pollen would be avoided. The following diagram shows the pollination schedule used. This schedule was used in order to detect differences in the effectiveness of the pollen sources by direct comparison.

<table>
<thead>
<tr>
<th>Pollen source No.</th>
<th>Time of pollination in days after beginning of anthesis</th>
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<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>X</td>
</tr>
<tr>
<td>2</td>
<td>X</td>
</tr>
<tr>
<td>3</td>
<td>X</td>
</tr>
</tbody>
</table>

The catkins were bagged with Kraft paper bags at the time of anthesis and the bags were not removed until just before pollination. Figure 2 shows the actual pollination done by brushing the catkin over the styles. The bags were finally removed from the female flowers ten to fourteen days after the final pollination.

![Image](image-url)

**Fig. 2.** - Handpollinating chestnut flowers. The bags over the emasculated flowers are removed and the pistils brushed with a fresh catkin just removed from the male parent-tree. The bags are replaced immediately after pollination. One or more catkins are used for each bag depending on the number of females in the bag, and the condition of the anthers.

The experiment in 1954 were limited to three trees: 1—9, C. mollissima; 7—7, C. crenata; and 4—6, S-8 × J. Also, a few of the pollen parents were changed. The pollination schedule was the same, except that pollen from the first male parent was collected indoors, cleaned, and stored over calcium chloride under refrigeration at approximately 2°C. This stored pollen was used in bags, additional to those pollinated with pollen sources 2 and 3 (see diagram).

In both years’ experiments, ten bags were used in each of the individual pollinations except on S-8 × J, 4—6, where only 8 bags were available in 1954, and on S-8 × J, 16—1, where only 8 bags were available in 1953. A similar number of bags were left unpollinated as a control on the effectiveness of the bagging.

**Results and Discussion**

A summary of the results for the 1953 and 1954 experiments is shown in Figure 3. The nutset, expressed as percent of the number of female flowers developing at the time of debagging, ranged from 49.0 per cent for the flowers pollinated on the 5th day after the beginning of anthesis to 17.2 per cent for those pollinated on the 22nd day. These values are comparable to the average value of 30—40% for the nutset resulting from hybrid crosses which were mentioned in the introduction. The values compare favorably with this value and the immediate conclusion must be that one pollination is sufficient if the timing is right and good pollen is used. No nuts were produced in any of the control bags; this shows that the females were not receptive at the time of bagging and that the bags were effective as pollen barriers.

A somewhat injurious effect of the bags also is apparent from Figure 3. Between the time of bagging and the first pollination five days later, 7.0% of the females were lost; by the 17th day, 32.5% had dropped off. Lack of fertilization will, of course, eventually result in the wilting of the flowers, but there seems to be no doubt that part of the loss is due to the effects of bagging. This harmful effect of bagging varies considerably from one tree to another; past experience has shown that it is particularly evident in some of the Japanese-American hybrids and their backcrosses to the Asiatic parent.

The results of the pollinations on the different trees are presented graphically in Figure 4. A pronounced variation between the trees is immediately apparent. For example, C. crenata, 7—7, reached maximum receptivity only 5 days after anthesis and remained fully receptive at least until the 13th day; C. crenata, 13—2, on the other hand, did not reach full receptivity until the 13th day, and its period of receptivity appeared to be much shorter. The data for the S-8 × J hybrid, 4—6, are not reliable for 1954, as a large number of labels were lost during the September hurricanes; considering the data for 1953, there is little doubt, however, that maximum receptivity is not reached until the 9th or 13th day, that it remains high until the 17th day, and thereafter drops off sharply. In the case of the Chinese trees, the results are less clear as there is a considerable difference between the different pollen sources; however, it seems that maximum receptivity is reached about 9 days after anthesis and remains high for a longer period than in the other trees, with no clear drop-off between the 17th and 22nd day.

There is no doubt that weather conditions have a marked effect on the relative success of the pollinations, and Clapper (1954) has shown...
that late morning and early afternoon offer much better conditions for pollination than does late afternoon. He considers the higher humidity in the early part of the day as partial explanation of this. Better contact and germination of the pollen grains on the stigma may result from this higher humidity. Some of the irregularities in the curves may be the result of pollinations at different times during the day, or they may be ascribed to the weather conditions. Some of the irregularities finally may be ascribed to partial pollen-incompatibility; for example, pollen source No. 3 was used with good results on S-8 × J, 4—6, but failed almost completely on S-8 × J, 16—1.

Clapper (1954) concludes that the later the pollinations are made, up to the 17th day after the anthesis, the more successful they will be. This seems to be an over-simplification of conditions, and applies only to Chinese chestnut, the species primarily studied by Clapper. From the above results, it would seem that pollinations between the 10th and 13th days after anthesis would give reasonably good results on all trees studied. To what extent this conclusion can be extrapolated to include other species and hybrids is not certain; but, from the past 20 years breeding experience, it seems safe to include all the four true chestnuts: *C. mollissima*, *C. crenata*, *C. dentata*, *C. sativa*, and their hybrids.

The Effect of Emasculation on Nut Yield

In the course of the controlled pollination work in chestnut, it has often been found that the female flowers drop off in the bag before the buds start to develop. This has been encountered especially in Japanese × American hybrids and their backcrosses. It was thought that this, perhaps, was due to injuries resulting from emasculation and to the bagging itself. The following small experiment was carried out in order to test this hypothesis.

Two trees were used in the experiment. A Japanese × American, 4—4, hybrid was bagged as follows: 12 bags were emasculated completely; 12 bags had only unisexual male catkins removed, while the bisexual catkins were left intact; 24 bags were bagged without any emasculation; and 12 flowering branches were just tagged. The number of female flowers were counted in all cases. The other tree, a Japanese × (Japanese × American), 7—4, hybrid was bagged similarly, but only 7 and 14 bags of each respective type were put on. Pollinations were repeated three times at two-day intervals. Chinese pollen was used on both trees. The nutset, expressed as a per cent of the number of pollinated flowers, was taken as a measure of how successful the pollinations had been. The results are shown in Table 1.

<table>
<thead>
<tr>
<th>Type of emasculation</th>
<th>No. % at time of first pollination</th>
<th>No. of nuts in % of no. % at time of first poll.</th>
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<tbody>
<tr>
<td></td>
<td>4 parent</td>
<td>7 parent</td>
</tr>
<tr>
<td></td>
<td>4 parent</td>
<td>7 parent</td>
</tr>
<tr>
<td>Complete</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only unisexual</td>
<td>117</td>
<td>51</td>
</tr>
<tr>
<td>catkins removed</td>
<td>69</td>
<td>57</td>
</tr>
<tr>
<td>Not emasculated</td>
<td>84</td>
<td>54</td>
</tr>
<tr>
<td>but pollinated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not emasculated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not pollinated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not bagged,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>open-pollinated</td>
<td>84</td>
<td>75</td>
</tr>
<tr>
<td></td>
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</table>

Bagging apparently has a injurious effect; open-pollinated flowers yielded 44.9 and 17.4 per cent, whereas the highest yields from hand-pollinated flowers were 29.1 and 13.7 per cent respectively. There is no evidence, however, that emasculation in itself causes a decrease in nut yield; rather it seems to be somewhat beneficial. Complete emasculation yielded 29.1 and 13.7 per cent as compared to 14.5 and 0.0 per cent where only unisexual male catkins were removed, and 25.0 and 3.7 per cent where no emasculation was done.

Emasculation entails the removal of a considerable amount of tissue from the flowering portion of the branches, and undoubtedly has some effects on the metabolism in the remaining plant parts. Perhaps a temporary accumulation of metabolites benefits the female flowers left on the plant, or perhaps the hormonal balance is changed with the result that abscission is less frequent.

The difference in the nut yield on the two trees is very striking. This lowered fertility in the Japanese × (Japanese × American) hybrid is, perhaps, the result of the chromosomes failing to pair regularly at meiosis, thus interfering with the normal development of the eggcells.

Apparently, Japanese × American hybrids and their backcrosses are inherently low yielders. Thus, the yield from the open-pollinated flowers is only 44.9 and 17.4 per cent of the possible number, whereas the yield normally ranges from 65 to as much as 80 per cent in other hybrids.

Germination of Chestnut Pollen

A germination technique for chestnut pollen has been described by Schad et al. (1952). They germinated the pollen in petri dishes on a 2% agar gel. Immediately before seeding, the plates were moistened with a sugar solution. Concentrations used were 5%, 10%, 15% and 20%; of these, 10% and 15% gave the best result. Counts were made after 48 hours, and germination percentages varying between 65% and 54%, depending on the tree and species assayed, were observed. Staining with potassium iodide and using the presence of starch as an indication of viability, they found cases where apparently-viable grains failed to germinate. The technique seems rather laborious, and a simpler method was therefore sought. The experiments can only be considered preliminary.

Chesnut pollen is difficult to extract because it cakes easily and therefore does not fall freely from the anthers; furthermore, the male catkins produce large amounts of nectar. This tendency to, cake, together with the presence of nectar in the extracted pollen, was undoubtedly one reason for the frequent occurrence of contamination in the pollen-germination test.

The pollen was extracted by dropping the male catkins on a glass plate and scraping it into a vial with a razor blade. Better results can, perhaps, be obtained if the catkins are allowed to dry partially before the collecting the pollen.

In a series of tests, the effects of temperature and of the sugar content and boric-acid concentration of the medium were studied. Pollen of *C. henryi*, *C. crenata*, *C. mollisima*, and an S-8 × J hybrid was germinated.

In the first test it was found that germination took place if the pollen was suspended in the medium and placed in droplets on a microscope slide, using Petri dishes as moist chambers. This technique is simple, and it was used in all the tests.

It was found that chestnut pollen germinates at relatively high temperatures. The best results were obtained
at 26° and 37° C. Germination at 22° C. was much lower, and there were indications that the optimum temperature was between 26° and 37° C. although it may differ somewhat between different species and hybrids. This may indicate that warm, rather than cool, weather will give the most successful pollinations in the field.

Whether or not the addition of sucrose to the medium has beneficial, or retarding effects on germination is not clear from these preliminary experiments. Pollen of C. mollissima, C. crenata, and the S-8 X J hybrid all germinated in distilled water; and while the addition of 10 and 20 per cent sucrose seemed to be somewhat beneficial in some tests, it was not so in others. Pollen of C. henryi definitely germinated better in 10 and 20 per cent sucrose solutions.

A stimulation of pollen germination by the addition of low concentrations of boric acid to the medium has been observed in the case of rose pollen (CALVINO 1951).

In a test on chestnut pollen, boric acid at the rate of 1, ½, ¼ and 0 per cent was added to 10 and 20 per cent sugar solutions. The two higher concentrations of boric acid definitely have a retarding effect on germination and actually cause a distortion of tubes of the few grains that do germinate. At ¼ per cent it had some beneficial effects on the pollen from some trees, but none on others. More tests will be necessary to determine the effects of lower concentrations of boric acid.

**Summary**

The period of pollen receptivity of two Japanese, two Chinese, and two hybrid chestnuts was studied. All the trees were protandrous, and the female flowers did not become fully receptive until from 5 and 13 days after the beginning of the anthesis of the male catkins. Most trees remain fully receptive for a week or more. The Chinese trees especially have a long receptive-period; however, even within species there is a considerable variation between trees.

The results indicate that one pollination, rightly timed, will give as good results as two or three pollinations on alternate days. If the results of these experiments are taken as the norm for other species and hybrids, pollination should give the best result if it is performed between the 10th and the 13th day after the beginning of the anthesis of the male catkins.

Emasculation does not injure the female flowers, and cause a reduction in the nut yield; on the contrary may even have a somewhat beneficial effect. Bagging with brown Kraft paper bags does, however, cause some reduction in yield, and it may be advisable to try bagging the more tender trees with a type of bag which permits more light penetration and air circulation. Perhaps, bags of a cotton material with a high thread count would be suitable.

A simple technique for the germination of chestnut pollen is described. The pollen can be germinated in drops of distilled water or in a sucrose solution on microscope slides, using Petri dishes as moist chambers. Good germination was obtained at 26° and 37° C. That the pollen germinates best at these relatively high temperatures may be an indication that pollenation on warm, rather than cool, summer days will give the best results. This, however, will have to be demonstrated in field experiments. Boric acid at ½ and 1 per cent concentrations retarded germination and caused a distortion of the pollen tubes.

**Zusammenfassung**

Titel der Arbeit: Über die Empfängnisbereitschaft der weiblichen Blüten und die Pollenkeimfähigkeitsteste der Gattung Castanea.


Die Ergebnisse zeigen, daß eine einzige Bestäubung, zum richtigen Zeitpunkt durchgeführt, gleichgute Resultate liefert, wie zwei oder drei Bestäubungen an einem Tag um den anderen. Wenn die Ergebnisse dieser Versuche auch als Norm für andere Arten und Bastarde angenommen werden können, würden diejenigen Bestäubungen die besten Resultate liefern, die man zwischen dem 10. und dem 13. Tag nach Anthesebeginn der männlichen Kätzchen durchführt.

Kastration schlägt die weiblichen Blüten nicht und verursacht auch keine Minderung des Nuß-Ertrages; im Gegenteil, manchmal erhält man sogar dadurch einen günstigen Effekt. Die Einbeutelung mit Packpapiertüten (Kraft) verursacht jedoch eine Reduktion im Ertrag, und es kann ratsam sein, bei empfindlichen Bäumen die Einbeutelung mit einer Tütensorte zu versuchen, die einen größeren Lichtzutritt und Luftzirkulation gestattet. Vielleicht würden sich dafür Beutel aus Baumwollmaterial mit hoher Fadenanzahl eignen.


**Résumé**

Titre de l'article: Réceptivité des fleurs femelles et tests de germination de pollen dans le genre Castanea.

On a étudié la période de réceptivité de deux châtaigniers japonais, deux chinoises et deux hybrides. Tous les arbres étaient protandres: les fleurs femelles ne devaient complètement réceptives que 5 à 13 jours après le début de l'anthesis des chatons mâles. La plupart des arbres restaient complètement réceptifs pendant une semaine ou plus; en particulier les châtaigniers chinois avaient une longue période de réceptivité. On note à l'intérieur d'une espèce, une variation considérable entre les arbres.

Les résultats montrent qu'une seule pollinisation faite en temps voulu, donnera d'assez bons résultats que deux ou trois échelonnées sur plusieurs jours. Si l'on peut étendre ces résultats à d'autres espèces et hybrides, la pollinisation
devrait donner les meilleurs résultats si elle est faite entre le 10ème et le 13ème jour après le début de l’antéthèse des chatons mâles.

L’émasculation ne blesse pas les fleurs femelles, et ne cause aucune réduction dans la fructification; elle a peut être au contraire un effet légèrement favorable. L’emploi de sacs en papier Kraft cause cependant quelque réduction dans la fructification et il pourrait être préférable d’esayer sur les arbres les plus délicats un type de sac qui faciliterait la pénétration de la lumière et la circulation de l’air. Des sacs de coton à tissage serré pourraient peut être convenir.

On décrit une technique simple pour la germination du pollen de châtaignier. On peut faire germer le pollen dans des gouttes d’eau distillée ou dans une solution de saccharose sur des lames de microscope, en employant des boîtes de Petri comme chambre humide. On a obtenu une bonne germination à 28° et 27° C. Le fait que le pollen germe mieux à ces températures relativement élevées peut indiquer que la pollinisation faite pendant les jours chaudes donnera de meilleurs résultats que pendant des journées froides. Cela doit cependant être vérifié par des expériences sur le terrain. L’acide borique à ½ et 1% retarde la germination et provoque une distorsion de tubes polliniques.

**Literature Cited**


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**Cone Characteristics and Natural Crossing in a Population of F1 Pine Hybrids**

By Jonathan W. Wright

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During the past 15 years workers at the Northeastern Forest Experiment Station have produced a number of artificial species hybrids in series Larchiones of the hard pines. The earliest hybrids produced involved Scotch pine (P. sylvestris L.), Japanese red pine (P. densiflora Suka. and Zucc.) and Japanese black pine (P. thunbergii Parl.). They are now old enough to fruit.

Natural crossing was studied in a plantation of these hybrids and their parent species; and cone characteristics were used as a means of identifying hybrid progeny. The vigor of these first hybrids and the ease of producing them by controlled pollination have already been reported (4, 5). (The trees originally identified as P. nigra Arnold in [4] are now considered to be P. thunbergii.)

An earlier study of this sort was reported by Sarto (3), who analyzed several natural hybrids between Japanese red pine and Japanese black pine grown in his nursery for 7 years. He does not state to which generation the hybrids belong, but concludes that trees tending toward one or the other parent in position of the resin ducts also tend toward the same parent in other characteristics.

All names and species limits used in this report are as used in Reemer (2).

**Material and Methods**

_Arboretum Trees_

The parents of the open-pollinated and F1 hybrid progenies were located on the campus of Yale University.

_F1 Hybrid Progeny_ New Haven, Connecticut. Cones were also received from authentic specimens located in the Arnold Arboretum of Harvard University, Boston, Massachusetts; Eddy Arboretum of the Institute of Forest Genetics, Placerville, California; Morris Arboretum of the University of Pennsylvania, Philadelphia, Pennsylvania; and the Wind River Arboretum of the Pacific Northwest Forest Experiment Station, Wind River, Washington. These cone collections were used to establish the correct identity of the parents and progenies.

**Standing Stone Plantation**

F1 hybrid and open-pollinated progeny (all adjudged non-hybrid) of the New Haven trees were started from seed in 1940 and were outplanted in 1945 in a non-replicated permanent field planting on Standing Stone Experimental Forest in Huntingdon County, Pennsylvania. At the time of the first measurement (4) all cones from all hybrid trees and one cone from each non-hybrid tree were harvested. The extracted seeds were sown in the spring of 1949 in the Philadelphia nursery of the Northeastern Forest Experiment Station.

The cones were scored for 11 different characteristics, for each of which up to 5 or more recognizable grades were established. Insofar as possible the differences between grades of the same characteristic were kept uniform and were defined mathematically. It so happens that in most characteristics there was a gradation from _P. sylvestris_ through _P. densiflora_ to _P. thunbergii_; the grades were numbered so that grades 1, 3, and 5 referred to cones of these three species respectively. The characteristics scored were: