the clones, expressed as a percent of the population mean, were total height 9.3 percent, stem diameter 11.5 percent, number of branches 19.0 percent, and incidence of *Melampsora* rust 72.7 percent. Phenotypic correlations were obtained among several characters: Stem diameter with total height (0.61), stem diameter with number of branches (0.39), foliation date with height increment (—.52), and incidence of *Melampsora* rust with defoliation (0.69) and with the following season's growth in both height (—.38) and diameter (—.45).

Height increase in the second year was not correlated with first-year height growth, although both characters were highly heritable. Perhaps separate developmental phenomena are involved, or the clones responded differently to the environments encountered during the two growing seasons.

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Reproductive Behavior in Sugar Maple: Self-Compatibility, Cross-Compatibility, Agamospermy, and Agamocarpy

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One of the important requirements of a plant-breeding program is an understanding of the reproductive behavior of the experimental material with which the breeder plans to work. In developing a breeding program in sugar maple (Acer saccharum Marsh.) at the Northeastern Forest Experiment Station, the flowering and fruiting habits of the species were explored. Self- and cross-compatibility, agamospermy, and agamocarpy in sugar maple were investigated from 1958 through 1961.

Materials

The trees used in these experiments were mature native specimens, selected for their ability to consistently produce large crops of easily reached flowers. The trees were located near Burlington, Vermont, and at Williamstown, Massachusetts.

In the cross-compatibility study, six tester pollens were applied to three trees used as female parents at Burlington; and four tester pollens were applied to two trees used as female parents at Williamstown. Trees used as females also served as male parents in reciprocal crosses.

Observations on self-compatibility were made on five trees at Burlington and on two trees at Williamstown. Agamocarpy was studied in conjunction with agamospermy on each of four trees at Burlington and two trees at Wiliamstown. The number of fruits and seeds set in the absence of any pollen source was used in determining the incidence of agamocarpy and agamospermy.

Flowering, Fruiting, and Phenology

Individual sugar maple trees in the Northeastern part of the species' range produce both male and female flowers that are pseudo-hermaphroditic. Although bisexual, only one sex is functional. Extended observations made in New England show that these trees may be divided into two groups based on differences in blooming sequence. One of the groups is characterized by a blooming sequence that is primarily male-female or protandrous; in the other the sequence is primarily female-male, or protogynous.

Between the two groups, the blooming times of flowers of the opposite sexes generally coincide, which results in reciprocating pollination. Thus a heterodichogamous[1]) condition, similar to that described in A. *platanoides* by Stout (1938), is present in A. *saccharum*.

Most trees in the Burlington area bloom within 1 or 2 days of each other. However, a few trees may be found that consistently bloom 3 or 4 days earlier.

Under normal weather conditions, budburst in the Burlington area usually occurs during the first week in May. At Williamstown, some 140 miles to the south, budburst occurs 7 to 10 days earlier.

Under average humidity and temperature conditions, dichogamy is incomplete in sugar maple. The periods of time when male and female flowers on the same tree are mature and functional overlap slightly, thus making self-pollination possible. A more detailed account of dichogamy in sugar maple will be published later.

The compound pistils of sugar maple flowers are composed of two fused carpels and two locules, each locule containing two ovules. The number of ovules that develop into mature seed varies among trees and among flowers on the same tree. Two classes of trees were observed in this respect. In one class, one of the paired carpels is consistently filled with a seed and the other is always empty. In the second class three conditions may be found in the paired carpels on the same tree: (1) one carpel empty and one filled, (2) both carpels empty, and (3) both carpels filled. The first condition is most prevalent.

Normally only one of the ovules in a locule develops into a seed. Instances where both ovules develop have been observed but are extremely rare.

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[1]) Terms used with reference to dichogamy are according to Stout (1928).

Methods

Twigs bearing flower buds were covered with cellulose sausage-casing bags about 2 weeks before budburst. At that time the flower buds were enlarged and were easily distinguished from the smaller vegetative buds.

Pollination began when the female flowers reached stage II (Gabriel 1966). This stage is characterized by stigmas slightly divergent, 3—5 mm. in length, somewhat flattened greenish yellow, wings on ovary not plainly visible. Pollinations were discontinued after stage IV, characterized by stigmas widely divergent, obtuse angle, 5—6 mm. in length, quite broad and silvery, twisted, wings on ovary visible above calyx. Isolation bags were removed when the stigmas had completely withered.

Pollen was obtained by forcing flower-bearing branches in a warm, humid greenhouse. Branches from experimental trees were collected several weeks prior to natural budburst and placed in buckets of water. Following dehiscence of the pollen sacs, small flower-bearing twigs were clipped from the larger branches and the pollen was extracted by holding the twigs over a sheet of paper and striking them sharply at their bases with a pencil. After several hours of drying at room temperature to minimize clumping, the pollen was stored in cotton-stoppered vials in a dessicator at 28 percent relative humidity at 2° C. Humidity was maintained by use of sulfuric acid diluted to a specific gravity of 1.45. Earlier experience has shown that pollen stored under these conditions will maintain satisfactory viability for at least 2 or 3 weeks.

Because of the difficulty experienced in obtaining even small quantities of sugar maple pollen, pollinations were made with a small camels'-hair brush to conserve the supply. The brush and the pollinator's hands were washed in alcohol between pollinations to prevent contamination.

The stored pollen from forced flowers was used for those pollinations where the desired pollen did not mature naturally at the proper time. Fresh pollen was used where blooming times of flowers of female and male parents coincided. The data indicated no significant variation in seed-set attributable to the method of handling pollen.

Emasculation of bagged inflorescences scheduled for use in the studies of cross-compatibility, agamospermy, and agamocarpy was carried out before anthesis; it was done by severing the pedicels of the functionally male flowers with splinter tweezers. Stamens were not removed from the functionally female flowers because earlier exploratory studies have shown them to be contabescent. No emasculations were necessary among inflorescences to be used in the study of self-compatibility.

Seed set to cross-pollination was determined by cutting open the fruits. Where it was desirable to save the seed for other phases of the study, the fruit coats were first softened by soaking them in water. An opening wide enough to determine the presence or absence of a filled seed was then forced along the suture line at the placental end of the fruit. On release of pressure, the suture would spring back to a closed position without damage to the fruit or seed.

No protandrous2) trees were pollinated at Williamstown. Flower buds were bagged only on the lower branches, and these branches subsequently were found to bear flowers that were entirely male in function. Female flowers appeared only in the upper part of the crown. This distribution of flowers did not occur in protandrous trees at Burlington. There flowers of both sexes were uniformly distributed over the entire crown. The reason for this difference in sex distribution among the flowers of protandrous trees in the two localities is not known.

Table 1. — Comparison of fruit- and seed-sets from self-pollination and without pollination.

Tree number	Self-pollination			Without pollination		
	Flowers	Fruit-set	Seed-set	Flowers	Fruit-set	Seed-set
	Number	*Percent*		*Number*	*Percent*	
M—1	108	50.9	11.1	50	36.0	0
M—4	148	58.8	8.1	36	94.4	2.8
M—6	68	73.5	20.6	40	92.5	0
M—7	78	74.4	10.3	50	68.0	2.0
M—13	104	59.0	18.3	—	—	—
H—509	100	54.0	13.0	50	68.0	0
H—573	118	71.2	27.1	46	76.1	2.2

Fruits were enclosed in early August in nylon mesh bags to minimize losses due to windstorms and to squirrels. They were harvested in late September and early October, allowed to dry for several days at room temperature, and then stored in airtight containers in a refrigerator at 2° C.

Results and Discussion

Self-compatibility. — All trees used in the experiment produced seed in varying proportions when self-pollinated. Seed-sets, expressed as a percent of flowers that were artificially self-pollinated on each tree, ranged from 8.1 to 27.1 percent (*Table 1*). Differences among trees were statistically significant.

Neither the gametophytic incompatibility system described by Prell (1921) and by East and Mangelsdorf (1925), nor the sporophytic incompatibility system described by Gerstell (1950) are operating in *A. saccharum*. Both of those systems are based on the interaction of S alleles in the pistil and pollen grain. Pollen tubes would be unable to grow in the diploid stylar tissue because the latter would carry all alleles for incompatibility. Thus, a partial seed-set to self-pollination precludes the operation of either of these systems.

Examination of the interior of the carpels for early seed development suggested that the low set of seed to selfing may be related primarily to post-zygotic abortion. During the first 2 weeks after self-pollination, growth and coloration of the four ovules in each pair of carpels generally was uniform. Between the second and third weeks, either three or all four of the ovules would change from flesh color to brown and begin to atrophy. In carpels destined to produce filled seed, one ovule would remain plump and smooth and would continue to grow. However, in a large proportion of the paired carpels that were examined, all four of the ovules died.

Cross-compatibility. — In the study of cross-compatibility the hypothesis was tested that seed-set would not differ in controlled cross-pollinations where (1) only the pollen parent was varied and (2) only the female parent was varied.

Certain parental combinations produced significantly higher seed-sets than others. Variations in seed-set from cross-pollinations ranged from 16.7 to 46.8 percent at Burlington and from 18.5 to 47.0 percent at Williamstown (*Table 2*). Chi-square tests performed on the data showed significant differences in seed-set (1) due to pollen parent when trees M-4 and M-6 were used as female parents and (2) due to female parent when trees M-5, M-7, M-13, and H-507 were used as male parents.

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2) Trees with protandrously or protogynously blooming flowers will hereafter be referred to as protandrous or protogynous trees.

Unexpectedly low seed-sets were observed in the following:

♀	♂	Percent seed-set
M-6	× M-7	16.7
M-4	× M-13	18.4
H-509	× H-573	18.5
M-4	× M-5	19.4

Partial incompatibility may occur between trees in cross-pollinations. Depending upon the genotypes of the trees in the cross-pollinations, it would be possible for all, part, or none of the pollen from a particular male tree to function effectively in the stylar tissues of the flowers of a given female tree. Thus, the unexpectedly low seed-sets observed in the cross-pollinations could be due in part or entirely to the operation of an incompatibility system.

Theoretically, the number of seed that would be set in each direction of a specific reciprocal cross would be identical if incompatible alleles were solely responsible for reduced seed-set. However, the differences in seed-set within reciprocal crosses made between trees M-4 and M-6, M-4 and M-13, and H-509 and H-573 indicate that other factors may be acting to reduce seed-set.

A macroscopic examination was conducted of developing embryos. The results seem to indicate that the cross- and self-compatibility embryo failures follow the same pattern. All embryos start developing externally in what appears to be a normal manner. However, 2 to 3 weeks after pollination embryo failures begin to appear.

Trees having lower general combining ability also had lower seed-set on selfing. Rankings of female parents according to seed-set to self- and cross-pollinations are as follows:

Tree	Cross-pollination		Self-pollination	
	Rank	Seed-set	Rank	Seed-set
		(percent)		*(percent)*
M-6	1	41.2	2	20.6
H-573	2	40.9	1	27.1
M-13	3	40.5	3	18.3
M-4	4	27.5	5	8.1
H-509	5	25.2	4	13.0

Correlation between rankings was significant at the 5 percent level. From this it may be inferred that self-sterility and cross-sterility are associated among the trees used in this experiment. As pointed out by Riley (1932), such relations may be found among other plant species.

This experiment was not designed to partition the effects of heredity and environment on seed set in *A. saccharum*. However, it is common knowledge that trees immediately adjacent to one another and of comparable age and form may differ consistently in seeding habits. Such differences imply genetic control. Nevertheless, environment probably exerts some effect. Stout (1923) and Markarian and Olmo (1959) reported that increases in self-fertility in *Brassica* and *Rubus*, respectively, were associated with changes in environment.

Viability was not determined for the seed from either the cross- or the self-pollinations. However, seedlings were grown from the selfed seed. These seedlings exhibited extremely wide variability in morphological characters and in initial vigor. Some were exceptionally strong and robust, and some never developed beyond the emergence of a radicle from the seed (Gabriel 1962).

Table 2. — Seed-set from cross- and self-pollinations made at two geographic locations, with chi-square analyses of data on flowers setting seed, according to parent.[1])

Female parent	Male parent						χ^2
	M—1	M—4	M—5	M—6	M—7	M—13	
			BURLINGTON				
M—4	52 36.5	148 *8.1*	98 19.4	62 46.8	76 26.0	76 18.4	20.32**
M—6	80 45.0	96 38.5	96 45.8	68 *20.6*	42 16.7	108 46.3	13.20*
M—13	96 44.8	100 39.0	100 38.0	100 42.0	100 39.0	104 *18.3*	1.28^0
χ^2	1.15^0	0.0^0	15.93**	2.85^0	8.34*	15.28**	

Female parent	Male parent				χ^2
	M—11	H—509	H—573	H—507	
		WILLIAMSTOWN			
H—509	92 29.4	100 *13.0*	92 18.5	86 28.0	3.37^0
H—573	100 40.0	42 28.6	118 *27.1*	100 47.0	5.70^0
χ^2	2.4^0	—	—	7.14**	

Legend: 52 flowers pollinated
36.5 percent flowers setting seed
^{0} Not significant
* Significant at the 5% level
** Significant at the 1% level

[1]) Seed-set from self-pollinations *(in italics)* were not included in chi-square analyses.

Agamospermy. — Agamospermy has been described as the reproductive process by which seed is produced without benefit of fertilization (Stebbins 1941). Its occurrence has been reported in nut trees by Morris (1913) and by Stuckey (1916), and in the birches by Duffield (1942).

In the present study of agamospermy, 272 flowers distributed among 6 trees were completely isolated from all sources of pollen *(Table 1)*. Three filled fruits developed from these flowers — one on each of three trees. Thus the seed-set for the entire sample of flowers was roughly 1 percent. The three fruits were not examined internally when collected; hence, it is not known whether or not the embryos were fully developed and normal.

The three fruits were stratified according to recommended procedures to determine viability. However, no germination occurred, possibly because of incomplete or abnormal embryo development. When examined after stratification, the fruit contents had decomposed to the extent that embryo structures could not be distinguished.

Agamocarpy. — Gardner and Marth (1937), Hagemann (1937), Gustafson (1940), and others have found that agamocarpy may be induced in various species by treating female flowers with auxin-containing compounds. Gustafson (1939) hypothesized that the production of agamocarpic fruit is induced under natural conditions by the presence of sufficient auxin in the ovaries to cause them to grow without fertilization. According to the results of this experiment, some such phenomenon must occur in *A. saccharum*.

All trees used in the study were agamocarpic to a rather high degree *(Table 1)*. Fruit-sets ranged from 36.0 to 94.4 percent of the completely isolated female flowers. Obviously, pollination is not required for fruit-set in sugar maple.

Trees differed significantly in the set of mature agamocarpic fruit. Two to 3 weeks after budburst, nearly 100 percent of the isolated female flowers on all trees were developing into normal-appearing fruits. Then, in the following

2 weeks, a substantial but variable fruit-drop was noted. Although no data were collected on premature fruit-drop, this phenomenon would seem to offer the most logical explanation for the large discrepancies noted between trees in agamocarpic fruit set. Determination of the causes of premature fruit-drop was beyond the scope of the study.

Summary

Cross- and self-compatibility, agamospermy, and agamocarpy were studied in native mature trees of sugar maple (*Acer saccharum* Marsh.) at Burlington, Vermont, and Williamstown, Massachusetts from 1958 through 1961.

Incompatibility systems did not appear to be operating in the trees used in the selfing experiment. Seed-sets, expressed as a percent of the total number of artificially self-pollinated flowers, ranged from 8.1 to 27.1 percent. Reduced seed-sets on self-pollination appeared to be related primarily to post-fertilization abortion of ovules.

Statistically significant differences in seed-set to controlled cross-pollinations were found *(1)* when the female parent was held constant and the male parent was varied and *(2)* when the male parent was held constant and the female parent was varied. Thus, variation in seed-set was not consistently related to the performance of either the male or female parent. Results of reciprocal crosses indicated that partial cross-incompatibility exists between some parents. Factors, such as post-zygotic abortion, may be contributing to the unexpectedly low set of seeds observed in certain of the crosses that were made.

The correlation of rankings of female parents for seed-set to self-pollination and to cross-pollination was statistically significant, indicating that self-sterility and cross-sterility are related phenomena.

In the study of agamospermy conducted among 6 sugar maple trees, 3 filled fruits were formed from 272 unpollinated flowers. None of the seed germinated. The species was found to be highly agamocarpic; fruit-sets from unpollinated flowers ranged from 36.0 to 94.4 percent among the trees used in the experiment.

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Successful Controlled Pollination on Detached Cuttings of Coast Redwood[1]

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Introduction

In the course of our work on the vegetative propagation of the Coast redwood *(Sequoia sempervirens* D. Don [Endl.]) we observed the development of male and female strobili on cuttings in the rooting bench. In 1964, several cuttings bearing female strobili were collected several weeks after pollination occurred in nature. Some cones matured and a few seeds germinated, producing apparently normal seedlings. These observations prompted us to test the possibility of control-pollinating female strobili on detached cuttings, and maintaining such cuttings until viable seeds could be harvested.

Variations of this method have been used with several hardwood genera such as *Populus, Salix Ulmus* and *Acer* (von Wettstein-Westersheim, 1933; Johnson, 1945; Wright, 1962, page 338). To our knowledge, Chiba's (1952) report on his experiment with *Cryptomeria japonica* is the only published reference to the application of this method to a conifer species. He referred to his report as "preliminary", but our search for subsequent articles and information has been to date unsuccessful.

Chiba collected 15 cuttings bearing one-to-five strobili from each of two trees, one 12 years old (tree A) and the other 40 years old (tree B). Pollination with pollen from a single tree was done in a greenhouse. Eight months after pollination, 21 cuttings had rooted but only five had mature cones. Five "A" cones yielded 320 seeds, of which 23 germinated (0.7%), and four "B" cones yielded 240 seeds, of which 19 germinated (0.8%). Chiba also grafted and pollinated three scions bearing female strobili, but obtained viable seeds from only two cones on one graft.

Materials and Methods

Coast redwood requires nine months from pollination until the ripe seeds are shed. In the Berkeley area, male and female buds become visible in October, pollination occurs during January-February, and the ripe cones shed seed in October-December. Cuttings with unopened buds containing female strobili were collected from three trees (20 to 30 years old) near Berkeley on 13 January 1965. Two of these trees (8 and 10) had been the most prolific producers

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