SELF-AND CROSS-INCOMPATIBILITY IN BLACK CHERRY (*Prunus serotina*)

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INTRODUCTION

Black cherry (Prunus serotina, Ehrh.) is the largest native American cherry species in height and diameter and the only one that provides large amounts of material for woodworking. On better sites it may reach over 100 feet in height and upwards of five feet in diameter.

From earliest colonial times cherry has been a favorite furniture wood. Its dark reddish-brown heartwood finishes to a beautiful and distinctive luster giving it a quality look. Famous English and American furniture craftsmen such as Duncan Phyfe, Hepplewhite, and Chippendale used cherry for many of their creations. Those which remain today still retain their beauty and utility.

Cherry has been used in many ways from agricultural implements to wartime tank construction, but in recent years its use has become more and more restricted to high-value products because of its popularity and diminishing supply. As late as 1940 the two outstanding uses were furniture and backing blocks for use in printing. Today lumber and veneer for furniture manufacture lead the list.

It is not a slow-growing species but makes good growth only on the best of sites. On the Allegheny Plateau where sites are best, dominant and codominant trees average about 2.5 inches in diameter growth per decade between the ages of 13 and 33 (Defler, 1937, cited by Hough, 1960). Since these better sites are limited in number and the demand for cherry has been high, the best stands have long since been depleted in all but this small section of the natural range. To make

matters worse, regeneration has come from low-quality trees, leaving less desirable crop trees for future use.

The present outlook is not bright. Demand for high-quality material is rising despite competition from synthetics, while the supply is diminishing. Furniture manufacturers realize this and are promoting regeneration with better planting stock. It is a late start, but progress is being made.

If a sufficient supply of good-quality material is to be assured, future crop trees must be descendants of superior-quality trees now bearing seed. High grading[1] has removed almost all of these except those in a small area in the Allegheny Mountains of New York, Pennsylvania, and West Virginia known as the commercial range. But a few isolated individuals of high quality can still be located in relatively inaccessible and protected areas in most of the range. Along with better trees in the commercial range, these are the trees that tree improvement workers are using as parents in the first phases of black cherry improvement.

Any breeding program must be adjusted to fit the needs of the species studies. It appears that special consideration must be given self- and cross-incompatibility in the case of black cherry. Preliminary evidence indicates that in the Gainesville, Florida, area the species is probably completely self-incompatible under natural conditions, but also that under certain artificial conditions, it might be selfed. Near relatives of black cherry, the sweet and sour cherries and the plums (Prunus avium, Prunus cerasus, and Prunus domestica, respectively) all have a similar incompatibility system--one that

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[1]Removal of only higher quality trees during harvest operations.

prevents nearly all selfing and also some crossing in certain species but that allows for selfing in others.

Since forest tree improvement programs usually include establishment of clonal seed orchards in which natural cross-pollination is expected along with hand pollinations for production of progeny test material, any incompatibility system in the species used must be uncovered and cross-incompatible combinations pointed out. Also, such a system must be known and overcome if any close inbreeding program is to be successful.

The main purpose of this work is to determine if there is an incompatibility system in black cherry and if there is, how it compares to the one in the other Prunus species. But since black cherry breeding is in its early stages, new techniques will have to be adapted or new ones developed to accomplish the primary goal. So as a secondary goal several different studies aimed at adapting and developing techniques have been incorporated. These include observations on flower and flowering characteristics, natural and artificial pollination, pollen collection, isolation techniques, and determination of presence or absence of ploidy.

LITERATURE REVIEW

Early Work

Most people give Kolreuter (1964, cited by East and Park, 1917) credit for the discovery of what he called self-sterility in plants.[1] He found three Verbascum phoeniceum plants that failed to set seed with their own viable pollen but seeded readily when pollinated by V. blattaria, V. nigrum, V. phlomoides, and V. lychnitis. Darwin (1876) later verified that V. phoeniceum was self-infertile along with V. nigrum but found two related species, V. thapsus and V. lychnitis, that were self-fertile. Probably the next work on the subject was Herbert's (1837, cited by East, 1929). He found self-infertility very common among hybrids of the Amaryllidaceae and also that the non-hybrids Zephyranthes carinata and Hippeastrum aulicum were self-infertile. A little later Gartner (1844, cited by East and Park, 1917) found several additional examples in other species including Dianthus japonicus, D. barbatus, and Lobelia fulgens.

Several observers, including Mowbray (1830, cited by East, 1929) and Munro (1868, cited by East, 1929) reported self-infertility in Passiflora. Munro's work was especially important. He found self-infertile plants that were sometimes cross-fertile and sometimes

[1]Hereafter I shall use the term incompatibility when pollen and ovules were known to be functional and infertility when the reason for non-fertilization was at the time unknown.

cross-infertile with others of the same species and even the same generation. Some plants of P. alata crossed easily with their own progeny.

In Oncidium, Scott (1865) showed that stylar tissue was penetrated freely by tubes from self pollen of self-infertile plants, though fertilization did not occur. Muller (1868, cited by East, 1929) corroborated Scott's findings and also showed each self-infertile Oncidium flexuosum plant crossed with any other plant.

Attempts at Interpretation

Most work prior to the 20th century dealt with discovery of new cases. Many were reported in addition to the ones already mentioned including those by Bidwell, Bernet, Rawson (Darwin, 1876, cited by East, 1929); Lecoq (1862); Focke (1890, 1893, cited by East and Park, 1917); and others. Work in the early 1900's uncovered many new examples of incompatibility, including those reported by Backhouse (1911); Gardner (1913); Raves (1921); Sutton (1918); Detjen (1916); Heribert-Nilsson (1916, cited by East, 1929); Wada (1923, cited by East, 1929); Dahlgren (1922, cited by East, 1929); but was more concerned with interpretation of the phenomenon (East, 1929). Several noteworthy examples appeared.

Jost (1907) duplicated Hildebrand's (1866) earlier experiments on Corydalis cava. Where Hildebrand had found absolute self-infertility, Jost observed some self-fertility. Jost found that the different behavior of these plants and of Secale cereale and Lilium bulbiferum after self- and cross-pollination, was a difference in pollen tube growth. In Secale, tubes penetrated the micropyle in eight hours after cross-pollination but in 24 hours only reached the base of the pistil after self-pollination.

Correns (1912) showed that pollen of self-incompatible Cardamine pratensis germinated on stigmas of self-pollinated flowers but produced only short tubes that did not penetrate the stigma. However, cross-pollination produced pollen tubes that penetrated the upper ovary after 48 hours.

Correns (1912) and Compton (1912) suggested, as Jost (1907) had previously, that diffusible substances which stimulated or retarded pollen tube growth after cross- or self-pollination, respectively, were present in the pistil. Compton felt that self-incompatibility might be controlled by agents similar to those which govern immunity or susceptibility in plants and animals.

Results of these and many earlier experiments were explained when East and Mangelsdorf (1925) presented a new interpretation of self-incompatibility in Nicotiana. They called it the oppositional factor system. Filzer (1926) independently discovered the same system working with Veronica syriaca. This work cleared up some puzzling aspects of incompatibility and paved the way toward discovery of several additional systems.

Systems of Incompatibility

There are six known systems of incompatibility. Two are found in heteromorphic plants and four in homomorphic types.

Heteromorphic Plants

The first system (I) was first described by Darwin (1877, cited by Dnyansagar, 1963) in the genus Primula and is known as distyly. Two different flower types are borne on different plants. Thrum plants have short styles and high anthers; pin plants have long styles and short

anthers. Compatible pollinations occur only between the two different types. Genetic control is by one gene with two alleles, with sporophytic determination of the pollen reaction. This type control has been found in all distylic species so far analyzed (Dnyansagar, 1963) including Primula sinensis (Bateson and Gregory, 1905), P. acaulis (Gregory, 1915), P. officinalis (Dahlgren, 1916, cited by Lewis, 1949), P. viscosa and P. hortensis (Ernst, 1936, cited by Lewis, 1949), P. obconica (Lewis, 1949), Pulmonaria agustifolia (Darwin, 1876, cited by Lewis, 1949), and Fagopyrum esculentum (Dahlgren, 1922; Garber and Quisenberry, 1927).

The second system (II) is a more complex form of heterstyly characterized by short-, medium-, and long-styled flowers. Each flower has anthers at two different levels, each different from that of the stigma. Darwin (1877) showed that compatible pollinations are between stigmas and pollen from anthers at the same level. Fisher and Mather (1943) and Fisher and Martin (1947) found that genetic control was by two loci, each with two alleles which they called Ss and Mm. Long-styled plants are ssmm; medium-styled, ssM_; and short-styled, S_mm or S_M_ (Lewis, 1949).

Homomorphic Plants

Under the homomorphic class are two subclasses, gametophytic and sporophytic. Each includes two of the six incompatibility systems.

In the gametophytic subclass, mating type of pollen grains is determined gametophytically and alleles of incompatibility genes act individually in the style. Stylar pollen tube inhibition is the result of incompatible matings (Crowe, 1964).

In one system (III) genetic control is by multiple alleles at one locus as in Oenothera (Crowe, 1964). Prell (1921) first proposed this type and East and Mangelsdorf (1925) used it to describe incompatibility in Nicotiana sandarae. It was also used by Crane and Lawrence (1931) to describe incompatibility in sweet cherry (Prunus avium).

The other gametophytic system (IV) is controlled by multiple alleles at two loci as in Secale (Crowe, 1964). Present in several grasses (Lundquist, 1956, 1961a, 1961b, 1964a, 1964b; Hayman, 1956), it is also found in at least two members of the Solonaceae, Solanum pinnatisectum and Physalis ixocarpa (Pandy, 1957, 1960). Pandy (1962) concluded that the two-locus system is a derivative of the one-locus system and suggested that the second locus is a duplication arising from a structural chromosome change.

Under the sporophytic subclass, mating type of pollen grains is determined sporophytically and alleles of incompatibility genes express either dominance or individual action in both male and female parts. Site of the incompatibility reaction differs in the two systems included (Crowe, 1964). In the first system (V) genetic control is by a single locus with multiple alleles. Pollen tubes are inhibited in the style as in Parthenium. In the second (VI) genetic control is by one locus with multiple alleles and incompatibility is expressed between gametes after fertilization as in Theobroma. Type V is common in dicotyledons, but Type VI has been found only in Theobroma and Callistemon among the dicotyledons (Crowe, 1964).

Effect of Polyploidy on Incompatibility

An increase in chromosome number of an organism produces a drastic upset in both the genic balance and the physiological processes

they control. In self-incompatible species studied, the effect of doubling the chromosome number on incompatibility ranged from none to a change to self-compatibility (Lewis, 1949). Effects on these species are summarized in Table 1.

Lewis (1949) stated that the key to variable behavior of tetraploids is found in tetraploid Oenothera organensis. In its style the four S alleles, even when different, all operate without interaction to inhibit haploid pollen carrying the same alleles. In diploid pollen genic balance is unchanged if both alleles are alike; the pollen behaves like haploid pollen. However, complex interactions occur between unlike alleles, and diploid grains may show two different kinds of genic interaction depending upon the pair of alleles involved (Lewis, 1949).

With certain pairs of alleles, neither functions efficiently because of competitive interaction. In these cases there is a much reduced incompatibility reaction, and pollen tubes are only partially inhibited even in styles carrying both alleles. The result is either weak self-incompatibility or complete self-compatibility depending upon the strength of the original reaction (Lewis, 1949).

With other pairs of alleles there is no competitive interaction, but one allele is dominant to the other. Such pollen functions like it is homogenic for the dominant allele. Plants producing only this type pollen are completely self-incompatible (Lewis, 1949).

Competition and dominance in the diploid pollen grain explain most effects of polyploidy found in other species. Effects in species that show no change can be explained by dominance or lack of interaction, either dominant or competitive. Species that show a change to self-compatibility must exhibit competitive interaction (Lewis, 1949).

Table 1

Effects of Polyploidy on Incompatibility in Several Autotetraploids Arising under Controlled Conditions, by Species and Author (from Lewis, 1949)

Species	Origin of tetraploid	Effect	Author
Antirrhinum molle	Colchicine	Self-compatible	Straub, 1941
Campanula persicifolia	Spontaneous	Self-compatible	Gairdner, 1926
Petunia (axillaris?)*	Colchicine	Self-compatible	Stout & Chandler 1941
Pyrus communis	Spontaneous	Self-compatible	Crane & Lewis 1942
P. malus	Progeny of triploids	Self-compatible	Johansson, 1945
Solanum (3 species)	Colchicine	Self-compatible	Livermore & Johnstone, 1939
Trifolium repens	Colchicine	Self-compatible	Atwood, 1944
Ananas sativus	Colchicine	No effect	Kerns & Collins 1947
Brassica campestris	Colchicine	No effect	Howard, 1942
B. rapa	Colchicine	No effect	Howard, 1942
Oenothera organesis	Colchicine	Slight increase in self-compatibility	Lewis, 1943
O. rhombipetala	Colchicine	No effect	Hecht, 1944
Raphanus sativus	Colchicine	No effect	Howard, 1942
Taraxacum kok-saghyz	Colchicine	No effect	Bannan, 1946

* The species used was probably the garden form, P. violacea.

Variations of Incompatibility

Unilateral Incompatibility

This occurs when pollen tubes of a self-compatible species fail to penetrate the style of a self-incompatible species, but the reverse cross is compatible. Such results have been recorded by Mather (1943) with Petunia, McGuire and Rick (1954) with Lycopersicon, Harrison and Darby (1955) with Antirrhinum and Pandy (1962) with Solanum. Lewis and Crowe (1958) believe this interspecific incompatibility may be controlled by S alleles and that incompatibility substances in the pollen are superimposed upon an essential pollen growth substance. Further, these growth substances in pollen from self-compatible species are not protected by superimposed groups and are inactivated by "antibodies" in the style. Martin (1964, 1967) and Grun and Aubertin (1966) have pointed out that other genes account for unilateral incompatibility in some crosses. And Grun and Aubertin (1966) feel that mechanisms other than pollen tube inhibition may cause the phenomenon.

Somatoplastic Sterility

This is found mainly in interspecific crosses and results in decreased endosperm growth and eventual seed death in most cases. Cooper and Brink (1940) found that in the cross Nicotiana rustica x N. glutinosa death was caused by endosperm starvation due to an overgrowth of the nucellus stopping the food supply. The nucellus in this case blocked the gap adjacent to the chalazal pocket through which nutrients reach the endosperm.

Cooper and Brink (1940) stated that Bradbury's (1929) selfed sour cherries (Prunus cerasus) exhibited one or more of the

post-fertilization features of somatoplastic sterility. They felt that this might have been the result of inbreeding.

Biochemistry of Incompatibility

Linskens (1963) lists two types of self-incompatible plants from an experimental point of view:

1. Those in which the incompatible reaction occurs on the stigmatic surface.

2. Those in which the reaction occurs during growth of pollen tubes through the style.

In Type I the incompatibility barrier is probably the cuticle of the stigma (Christ, 1959, cited by Linskens, 1963). In all cases investigated, pollen from plants with a stigma cuticular layer also had a cutin-breaking enzyme system (Linskens, 1963). Kroh (1956, cited by Linskens, 1963) and Tatebe (1959, cited by Linskens, 1963) obtained normal seed set in incompatible pollinations of this type by bypassing the cuticular layer. Kroh also showed that pollen from this type plant placed on a cross-compatible plant for a short time then transferred to a self penetrated the self stigma. Linskens (1963) stated that this is evidence for a cutinase enzyme system linked to the incompatibility barrier and that the cutinase can be activated irreversibly.

In Type II incompatibility, pollen tube growth is inhibited within the style. Schlosser (1961, cited by Linskens, 1963) found abnormal pollen tube behavior in incompatible crosses when compared with compatible crosses. In about 50 percent of tubes studied the generative nucleus did not divide, and the vegetative nucleus disappeared just after germination without fulfilling its function. There was also a disturbance of the normal carbohydrate metabolism

evidenced by a large deposit of fibrils on thickened walls, branched tube tips, and an increased number of callose plugs (Linskens and Esser, 1957; Schlosser, 1961; Tupy, 1961; all cited by Linskens, 1963). Inhibited tubes also have a high respiration rate the first hours of growth (Linskens, 1955, cited by Linskens, 1963). And the fluorescence patterns of style extracts taken after incompatibility reactions show characteristic patterns after chromatographic separation; unidentified substances that inhibit tube growth appear (Linskens, 1963).

Linskens (1963) believes pollen tube inhibition may be caused by an antigen-antibody type reaction between a specific pollen protein and a stylar protein with homologous specificity. Direct evidence to support this conclusion comes from Lewis (1952) who found antigens that were specific to different pollen genotypes in Oenothera and by Linskens (1960) who found the same thing with protein in Petunia styles. Also Linskens (1958, 1959, cited by Linskens, 1963) showed that a complex is formed between pollen and stylar protein after an incompatibility reaction. Selection of specific antibodies occurs during pollen development as a result of intensive metabolic exchange between sporogen and female tissue in the same flower. After pollination, antigen of the pollen tube meets already selected antibodies in female tissue (Linskens, 1962, cited by Linskens, 1963).

Lewis (1960) showed that the S gene responsible for production of the protein causing incompatibility has two cistrons. One controls specific grouping of protein and the other controls the half-molecule carrier responsible for protein activity in pollen and style. According to Lewis mutations and recombinations are all changes in the carrier cistron.

Linskens (1963) believes that S protein action occurs on the pollen tube surface. Lewis' (1960) data support this hypothesis. He found that S protein diffused out of both macerated and intact pollen. This means that S protein either acts on the tube surface or outside in stylar tissue. But since incompatible and compatible tubes in the same style at the same time do not influence one another, it is likely that S protein acts on the tube surface (Linskens, 1963).

Incompatibility in Plums and Cherries

M. B. Crane (1925) first reported self- and cross-incompatibility in sweet cherry (Prunus avium). He showed that varieties involved could be assigned to groups within which all but a very small percent of self- and cross-pollinations failed. In one test over 18,000 selfed flowers produced only 21 fruits. Other workers including Wellington (1926), Tufts, Hendrickson, and Philp (1926), Sachoff (1931, cited by Crane and Brown, 1937), Schanderl (1932, cited by Crane and Brown, 1937), Gardner (1913), Schuster (1922), Florin (1924, cited by Crane and Brown, 1937), Einset (1932), Kobel and Steinegger (1933, cited by Crane and Brown, 1937), Wenholz (1936), Sprenger (1908, cited by Crane, 1925), and Hooper (1924) also showed that sweet cherry selfing is very rare.

Later Crane and Lawrence (1931) showed that incompatibility in sweet cherry was comparatively simple with self-incompatibility the rule and cross-incompatibility common, always reciprocally expressed. But in the plum (Prunus domestica) incompatibility occurring in one way of a cross and compatibility in the other was common. Darlington (1930) pointed out that the degrees of fertility and complex results in work with Prunus domestica were undoubtedly due to its hexaploid makeup

and manner of chromosome pairing. Bradbury (1929) found sour cherry varieties both self- and cross-compatible, but selfing produced a lower percent set than did crossing.

Cytological studies by Ewert (1922, cited by Crane and Brown, 1937) established the basic chromosome number in Prunus as 8 and that all varieties of the sweet cherry were diploids (2x = 16). Kobel's (1927, cited by Crane and Brown, 1937) work agreed with this. The sour cherries, P. cerasus and Dukes, P. cerasus x P. avium are tetraploid (Kobel, 1927; Darlington, 1928) as is P. cantabrigiensis (Faberge, unpublished, cited by Crane and Brown, 1937). Black cherry (P. serotina) is listed as tetraploid by Darlington and Ammal (1945).

Crane and Lawrence (1931) concluded that sweet cherry incompatibility was the oppositional factor type discovered in Nicotiana by East and Mangelsdorf (1925) and discussed earlier in this paper under incompatibility system III.

Roy (1938) compared (1) normal self-pollination, (2) self-pollination in which styles were treated with growth promoting phenylacetic acid before pollination, and (3) cross-pollination with a compatible variety in sweet cherry. No significant differences were observed between (1) and (2). Tubes which penetrated the style became arrested in stylar tissue, and many appeared to swell up at their ends. Tubes in (3) grew much more rapidly than in (1) and (2).

Earlier Afify (1933) studied pollen tube growth in cherries and plums and distinguished between different pollen genotypes by behavior of pollen upon germination and growth of tubes in the style. In sweet cherries he found five types of pollen, those that: (1) failed to

germinate, (2) produced very short tubes which finally bent upwards and ceased growth, (3) produced tubes that traveled about one-fourth the stylar length, (4) produced tubes that traveled about one-half the stylar length, and (5) traveled the length of the style and effected fertilization.

So in Prunus there is a system of incompatibility which is seemingly the same as that in several other genera. It is variable according to chromosome number but is characterized by viable pollen which germinates and produces tubes that penetrate the stigma, and by slowed growth of tubes carrying the same alleles as those found in stylar tissue.

Immature Fruit Drop in Cultivated Cherries

All cultivated varieties of cherry characteristically exhibit three waves of fruit drop. The first occurs shortly after peak flowering and is followed shortly by a second. The third drop is often called the June drop since it usually occurs in that month.

Considerable work has been done to uncover the reason or reasons for these drops including that of Detjen (1926) and Tukey (1933), but Bradbury (1929) gives the best discussion of the problem. In her work with sour cherry she made the following observations:

1. The first drop cannot be attributed to lack of pollination or to failure of pollen tubes to reach the ovarian cavities. From 82 to 99 percent of first drop fruits had been pollinated and, in 76 to 98 percent, pollen tubes had reached the ovarian cavities.

2. Degeneration probably begins in ovules of first drop fruits before normal fertilization time. Both ovules were shriveled and pollen tubes were growing at random in upper ovarian cavities.

3. Some unpollinated fruits were in the first drop, but the large number of pollinated fruits in the first drop and unpollinated fruits that were not in this drop indicate that pollination or lack of it does not fully account for fruit development past the first drop.

4. A large part of the second drop is not due to lack of fertilization. Embryos were present in 41 percent of dropped fruits and 95 percent either contained embryos or pollen tubes in ovarian cavities or ovules.

5. Partial embryo development has usually taken place in third drop fruits of sour cherry.

Bradbury concluded that unfavorable nutritional conditions probably play a major part in bringing about arrested development and dropping of sour cherry fruits. Tukey (1933) agreed since he successfully cultured embryos from dropped fruits. Stage of development is influenced not only by foods but also by pollination, fertilization, and embryo development. Finally, Bradbury concluded that physiological conditions in fruits leading to abscision layer formation possibly are the same whether brought about by unfavorable nutritional conditions or by lack of pollination or fertilization.

Techniques

Pollen Collection, Germination, and Storage

Schuster (1925) tried combing sweet cherry anthers off the flower and was successful, but found brushing flowers over a screen an

even better method. Anthers snapped off and fell through the screen. Schuster dried the collected anthers in open dishes until all pollen was liberated and dry.

All work with cherry pollen germination testing has been with sweet and sour horticultural varieties. Highest germination counts were observed in various sugar solutions. Pfundt (1910) used 20-30 percent, Crane and Brown (1937) used 8-12, and Raptopoulas (1939), 10-25 percent sugar. These workers all got 50 percent or better germination (Raptopoulas, 1939).

No reference was found that gave a method of pollen storage for any species of Prunus, whether successful or not. Fresh pollen was evidently used in all work.

Artificial Pollination

East and Mangelsdorf (1926) disagreed with the old idea that stigmas are receptive only when they are secreting a substance assumed to promote pollen germination. They pollinated as early as three days before normal flower opening in Nicotiana without abnormal effect. Crane (1925) did the same with Prunus avium. Schuster (1925) normally pollinated cherry as soon as stigmas showed drops of liquid on their surfaces but pointed out that pollination was successful 48 hours or more before usual flower opening. Stigmas were either receptive before normal opening or some pollen remained on the stigma and germinated later when stigmas were receptive. He found that a flower past maturity showed a slight reddening of stylar tissue just above the ovary.

East (1919) showed that selfing could be successful in Nicotiana either by pollinating buds or by pollinating late in the flowering season. He concluded that an inhibitor was formed during the life of

the flower, but its production coincided with flower opening. Bud pollination avoided the inhibiting effect and allowed tubes to grow normally before being slowed by the inhibitor. The longer period for tube growth allowed fertilization to be accomplished. Smith (1926) showed that end-of-season fertility in Nicotiana is due primarily to the last few flowers remaining on the stem longer than usual.

The perfect flowers in species of Prunus make emasculation a consideration to remove any doubt about penetration of self-pollen tubes. Schuster (1925) emasculated as does Sharp (1964) with Prunus avium. Sharp simply pinches off stamens between his thumb and forefinger. This is done mainly to avoid mixing pollens since Prunus avium is highly self-incompatible. Schuster (1925) used a camel's-hair brush to apply pollen. Sharp (1964) uses a pencil eraser.

Schuster (1925) reported that emasculating and leaving flowers exposed was preferred to bagging. He found that when bagged, flower pistils often came in contact with the bag surface and were either heat- or frost-killed depending upon temperature. He also felt that insects would not be attracted to flowers without petals and did some work without bagging. But Schuster (1925) also showed that bagging added to flower longevity provided no contact with the bag itself occurred. So bagging, by prolonging flower life, could aid in obtaining fertilization from incompatible matings.

Time of Flower Removal

Roy (1938), in his histological study of pollen tube growth in Prunus, removed flowers four days after pollination. Whether in selfs or compatible crosses more than half of all pollen tubes penetrating

the stigma failed to grow more than 0.5 mm. A few did reach 5.0 mm in selfs and 6.5 mm in compatible crosses.

Flower Killing and Fixation

Roy (1938) used Flemming's and Karpechenko's fluids in a mixture of equal parts of 95 percent alcohol, glacial acetic acid, and lactic acid. For work where fine detail and lifelike appearance are not required, Jensen (1962) suggests the use of FAA--formaldehyde, alcohol, acetic acid--as a killing and fixing agent. Its action is fast and material can be stored in it some time before use.

Slide Preparation

Standard techniques for slide preparation are found in any one of several manuals or texts. Jensen's (1962), though much smaller than Johansen's (1940) standard text, adequately covers the process and gives more up-to-date information on new equipment. Jensen's suggested variation of replacing tertiary butyl with normal butyl alcohol in the dehydration phase is appropriate when temperatures may drop below 60° F, the freezing point of tertiary butyl.

Fine points of the process including use of new equipment were reviewed with C. Shell (personal communication, 1967) and L. Russell (personal communication, 1967) of the UT-AEC Laboratory in Oak Ridge, Tennessee. They gave the author instruction in the entire process from dehydration through staining and coverslipping.

MATERIALS AND METHODS

Plant Materials

A genetically controlled incompatibility system is more likely to show up in crosses among close relatives than among non-relatives. So a parent-offspring grouping was sought as test material. One of the most likely arrangements of this type is an old tree with young flowering trees under or very near it. A careful search around Gainesville, Florida, located such a situation in an area uncut for some time which had what appeared to be one or more offspring-parent groupings present. Two older trees--possibly siblings themselves--and six younger ones were selected as study trees. Locations of trees with respect to each other and Gainesville, Florida, are found in Figure 1.

Six companion trees were selected in the Norris, Tennessee, area to partially duplicate the Gainesville study. These were all young trees found in a single fence row. Locations of these trees with respect to each other and Norris, Tennessee, are found in Figure 2.

Principal Studies

One way that incompatibility has been shown in Prunus is to observe lengths and rates of growth of pollen tubes after different types of pollination. Another method is to make pollinations, then check results by observing fruit set. Both methods were used.

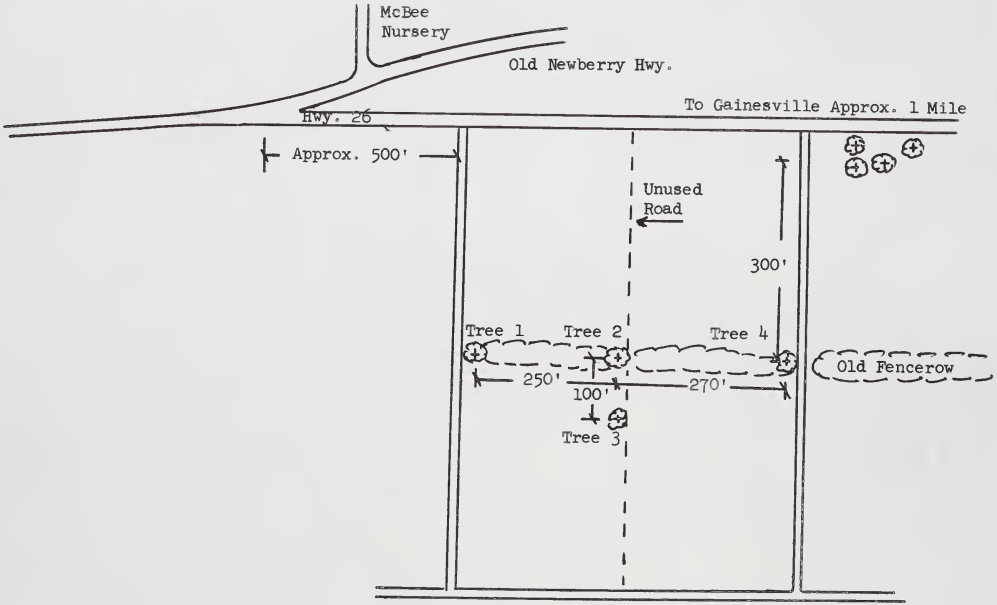


Figure 1. Location of Gainesville, Florida, test trees.

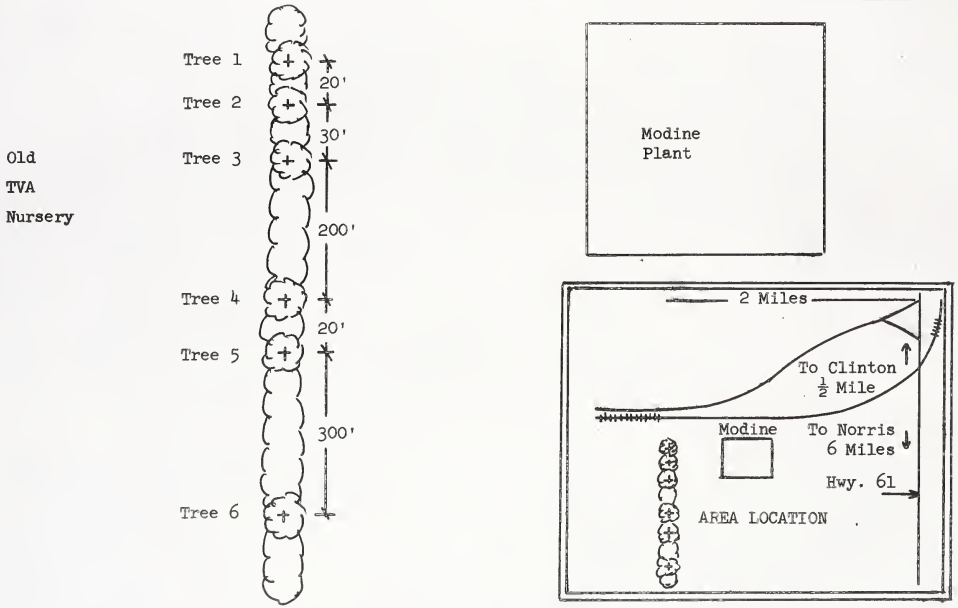


Figure 2. Location of Norris, Tennessee, test trees.

Pollen Tube Length Study--Gainesville

Two groups were made of the eight selected trees because of differences in flowering times. A breakdown of treatments for each Gainesville tree group is given in Table 2. On group A the entire scheme was reproduced three times to allow for removing flowers at 3-, 5-, and 7-day intervals after pollination. On group B two reproductions were used for 5- and 7-day removal intervals.

Pollination of young buds might be effective in avoiding an incompatibility reaction. Such was the theory of East (1934), who believed that an inhibitor was formed within 24 hours of flower opening in Nicotiana. Presumably, if germination occurred, tubes from such crosses would be well down the style before an inhibitor was formed and could possibly effect fertilization.

The no-pollen treatment, besides giving data on natural selfing, was also meant to serve as a yardstick by which other pollinations could be evaluated. This was required since flowers were not emasculated. Hopefully, self tubes could be differentiated from non-selfs in crosses based on their performance in natural-self checks. This was not considered too difficult since a cross would be made in an individual flower some time before pollen release in the same flower.

Some question arose as to whether results of the tube length portion of this work could be analyzed statistically. Unless most of the applied pollen which would eventually germinate did germinate within a short interval, tube length analysis would be confused. Tubes from applied pollen would be indistinguishable from those of self pollen from the same flower. Other factors which might affect an analysis

Table 2

Pollination Treatments, Gainesville Studies, by Female Parent

Pollen treatment	Group A Female parents 1	3	4	Group B Female parents 5	6	7	8
No pollen	+	+	+	-	-	-	-
Early self	+	+	+	-	-	-	-
Normal self	+	+	+	+	+	+	+
Pollen 1	-	+	+	+	+	+	+
Pollen 2	+	+	+	+	+	+	+
Pollen 3	+	-	+	-	-	-	-
Pollen 4	+	+	-	+	+	+	+
Pollen 5	-	-	-	-	+	+	+
Pollen 6	-	-	-	+	-	+	+
Pollen 7	-	-	-	+	+	-	+
Pollen 8	-	-	-	+	+	+	-

- No treatment

+ Treatment

Treatments:

1. No pollen: flowers bagged before opening, no pollen applied.
2. Early self: self pollen applied on flowers that would not normally open for at least 24 hours.
3. Normal self: self pollen applied on flowers that would open within one to two hours.
4. Crosses: cross-pollen applied to flowers that would open within one to two hours.

include stigma receptivity and loss of an exceptional number of observations. But the necessary elements of statistical design were included so that the material could be analyzed if conditions were favorable. Both tree groups were factorial experiments within a completely randomized design. Each treatment was replicated twice.

After the different day intervals had passed, flowers were removed, killed, and fixed in formalin-acetic-alcohol. Later, serial slides were prepared from longitudinally cut pistils, and data were taken on tube characteristics.

Since computer analyses would likely be used, all data were taken on IBM data sheets. Each flower from each treatment unit was identified as to its parent, pollen number, removal interval, location (bag) number, and flower number. Actual counts of tube sections were made on the upper one-third of the style and/or just above the stigmatic surface, number within the upper one-third of the style (Group I), number in the middle one-third of the style (Group II), number in lower one-third of the style (Group III), and number in the ovary. Additionally, numbers in Groups II and III were expressed as percentages of Group I to exert a standardizing influence on the data. So, a total of six variables appeared on each flower card.

Tube sections--pieces of tubes--were counted rather than actual tubes, because longitudinal microtoming of styles produced sectional pieces of the meandering tubes. These pieces were considered proportional to actual pollen tube members and were the only means of relating tube characteristics in one flower with those in other flowers.

Data from the six categories mentioned previously were transformed. The first four were transformed by adding 0.5 to each value, then taking its square root. The last two categories were expressed as percent values so were converted to arc sin values before analysis.

Each of the six variables for each tree was subjected to a separate analysis of variance using the Least Squares Analysis program of the Virginia Polytechnic Institute Computing Center. This program provides for unequal classes and will give unbiased estimates of main effects with or without interaction.

Effects showing significant differences were then subjected to Duncan's multiple range tests to pinpoint individual differences. However, results of the analyses will not be presented. Reasons are given in the results section; they pertain to unequal opportunity for tube penetration and lack of time for penetration.

Fruit Set Studies

The Gainesville fruit set study served as a check on the tube length study in that every pollen treatment in the latter was included in the former. The procedures for both tube length and fruit set studies were nearly identical except that some additions were made in the latter. For Tree Group A each pollen treatment was placed on flowers at three different locations rather than two. Also, both bagged and unbagged non-hand-pollinated flowers were included. The depetaled unbagged flowers served as a check on attraction of insects.

The divergence of the Gainesville fruit set and tube length studies came when flowers were removed from the tube length study.

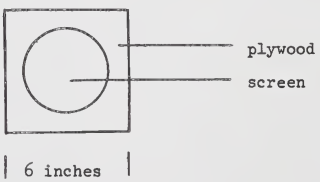
Fruit set flowers were left on the trees and collected and counted as fruit. Analysis was to be based on success or failure of pollen treatments to set fruit.

The Norris fruit set study was intended to strengthen its Florida counterpart and possibly uncover intraspecies differences due to latitude or other factors. A breakdown of treatments is given in Table 3.

Techniques and Procedures

Pollen collection.--To avoid the possibility of collecting insect-contaminated pollen in the Florida studies, cuttings were brought inside, flowers already opened removed, then pollen collected from those flowers which opened afterward. Anthers were dehisced and pollen dried in a refrigerated desiccator.

Trials proved that the best collection method involved gently rolling the cherry flower raceme between two pieces of ordinary window screen. With a little practice the collector can retain an almost pure batch of anthers. Actual equipment included several prepared screens made by mounting a piece of screen between two pieces of plywood with a circular hole in the center of each. They looked like this:



Pressure was applied by hand with a piece of screen shaped like a prism as follows:

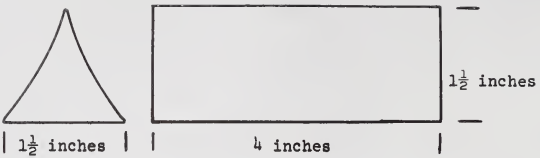
Table 3

Pollination Treatments, Norris Fruit Set Study,
by Female Parent and Pollen

	Pollen						
Female	No pollen self	1	2	3	4	5	6
1	+	-	+	+	+	+	+
2	+	+	-	+	+	+	+
3	+	+	+	-	+	+	+
4	+	+	+	+	-	+	+
5	+	+	+	+	+	-	+
6	+	+	+	+	+	+	-

- No treatment

+ Treatment



The collection sequence was:

1. Flowering branches collected.

2. Smaller twigs cut off and immediately placed in water.

3. Opened flowers removed and discarded.

4. Other flowers allowed to open.

5. Racemes with most flowers opened removed and anthers collected by the screen-on-screen pressure method.

6. Anthers placed in cotton-stoppered bottles in a refrigerated desiccator.

Pollen was not tested prior to use, because the period of the delay plus time required for pollinations might well have been longer than the flowering period of some or all of the trees. But a later check proved it viable.

In Norris whole racemes were bagged and flowers allowed to open. When needed, flower-bearing limbs were removed, still bagged, and placed in water. Racemes were then removed and used as applicators.

Pollination techniques.--Since black cherry has perfect flowers, emasculation was considered. However, two problems made this impractical. One is the small size and delicacy of the flowers; pinching or cutting off the anther-bearing portion is possible, but often the style is broken or cut. The second is that success leaves a weak, unprotected

style. Breakage of the style is the usual result either during the emasculation process or afterward due to jostling in a bag. This rules out emasculation as a practical device except possibly in greenhouse work. Here flowers would not be subjected to the frequent and sometimes extremely rough treatment common in unprotected trees.

In the Gainesville studies petals were removed with tweezers when they showed a lifting or swelling. This is about one to two hours before normal opening. At this time anthers are tucked under and around the stigma and style with the stigma usually easily accessible. Then it is a simple matter to touch the stigma with a pollen-laden applicator. In this case the applicator was a small dowel covered with sterile waxed paper. The matter of finding enough flowers at the same stage of opening offered no problem. Once the process was fully under way on a raceme, at least 8-10 flowers could be found at the same stage.

The pollination sequence in Gainesville was:

1. Opened flowers removed and discarded.

2. A treatment tag selected at random and attached to the limb.

3. Pollinator's hands and tools cleaned with ethyl alcohol.

4. Petals removed from flowers that would normally open within one to two hours (evidence for this was swollen petals).[1]

5. Appropriate pollen applied to the stigma of each flower.

6. Flowers bagged.

In Norris, pollinations were made on flowers that were previously bagged and allowed to open. Pollen was applied directly by brushing flowers with an opened raceme of flowers from the male parent.

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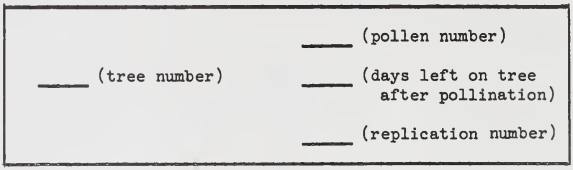
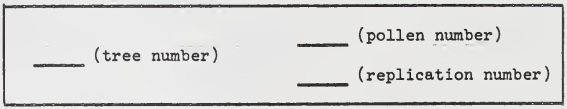
[1] A slight variation used here for one treatment is explained under principal studies.

Pollen was kept available by placing cut stems of bagged flowers in a rack of milk bottles filled with water. The pollination sequence was much the same as in Gainesville except that upon bag removal prior to pollen application, immature and overmature flowers were removed.

Tagging for identification.--Tags were designed as follows:



Pollen Tube Length Study Tag



Fruit Set Study Tag

All were prepared before work on a given tree began. Treatments were assigned at random by drawing a tag from a bag after a flowering limb was selected. In the pollen tube length study, different day intervals were color coded with flags to permit easy identification and removal.

Bagging for isolation.--Crimped synthetic sausage casings were used for bags. One was placed over all flowers covered by a single treatment tag. Then cotton was placed around the limb near the base of and inside the bag and a tying wire, known as a "Twist-em," used to tie the bag around the cotton. A breathing quality of the bag material along with the cotton allowed some air into the bag but not insects.

Limbs which were not strong enough to support the bag--many were in this category--were supported by tying limbs to other limbs or in some cases by attaching a small dowel to the bag and limb, then attaching the dowel top and bottom to a higher limb. Theoretically, this second method would keep the bag from being pulled off. However, several were lost anyway.

Flower killing and fixation.--All flowers to be sectioned and mounted on slides were killed, fixed, and stored in formalin-acetic-alcohol (FAA) of the following formula:

70% ethyl alcohol - - - - - - - - -	90%
Formalin - - - - - - - - - - - - -	5%
Glacial acetic acid - - - - - - - -	5%

Slide preparation.--Material was processed as follows:

1. Pistils removed from flowers.
2. Groups of pistils run through alcohol series to paraffin.
3. Material placed under vacuum in warm paraffin.
4. Material embedded in paraffin.
5. Blocks cooled, then cut longitudinally at 20 microns on microtome.
6. Serial sections mounted.
7. Material dried thoroughly then run through xylene for paraffin removal then through alcohol series to water.
8. Material stained with lacmoid and martius yellow.
9. Material destained slightly in alcohol, then placed in xylene.
10. Slides coverslipped.

Under number 2 above, the alcohol series consisted of the following mixtures:

1. 70% alcohol -- 150 ml H_2O, 250 ml 95% alc., 100 ml butyl alcohol
2. 85% alcohol -- 75 ml H_2O, 250 ml 95% alc., 175 ml butyl alcohol
3. 95% alcohol -- 225 ml 95% alc., 275 ml butyl alcohol
4. 100% alcohol -- 125 ml 200% alc., 375 ml butyl alcohol
5. 50-50 paraffin and butyl alcohol
6. Paraffin - Paraplast mixture

Under 7, 8, and 9 of the process the series was the following:

1. xylene -- 3 minutes
2. xylene -- 2 minutes
3. absolute alcohol -- 5 minutes
4. 95% ethyl alcohol -- 5 minutes
5. 70% ethyl alcohol -- 5 minutes
6. water -- 5 minutes or more
7. lacmoid and martius yellow -- 10 minutes
8. water -- 15 seconds
9. absolute alcohol -- 30 seconds
10. xylene -- 3 minutes or more

The stain was a mixture of:

5 mg. martius yellow
5 mg. lacmoid
12 mg. water
enough NH_4 to rise pH to 8

RESULTS AND DISCUSSION

Preliminary Trials

Since very little breeding work, if any, had been done with black cherry prior to this study, especially with respect to controlled pollinations, new techniques often had to be worked out. Methods used by breeders of cultivated Prunus species were considered and used whenever possible. But familiarity with Prunus serotina came only after development and trials of new techniques. All trials except the chromosome count were carried out in the Gainesville, Florida, area.

Date and Duration of Flowering

Observations showed that both of these are largely dependent upon weather conditions, mainly temperature. In the Gainesville, Florida, area black cherry can be expected to flower sometime in late February or early March. Cherries in the Norris, Tennessee, area flower about one month later. Observations over a three-year period showed much variation among individual trees with some flowering several days or even one or two weeks before others in the same area. Normally a tree flowered over a two-week period. This interval was shortened to as little as five days when a hot period followed a cool-to-cold first part of February in Gainesville.

Flower Location on the Tree

Flowers occurred with near equal frequency from top to bottom and on all sides of a tree regardless of aspect. Careful observations of naturally set fruit showed heavy fruiting all over an open-grown tree. This indicated that the experimental design need not include replication in blocks to offset variations in flowers due to location on the tree.

Characteristics of Flowers and Racemes

Prunus serotina has perfect flowers which are about 1/4 inch in diameter when fully opened. The 1/8- to 1/4-inch pistil is surrounded by 15-18 stamens in a perigynous arrangement. Imminent flower opening is evidenced by swollen or lifted petals. At this stage about one to two hours in full sun remains to full opening.

Normally 50-70 flowers are borne on a raceme, but occasionally very long racemes containing 80-90 flowers are encountered. Flowers always begin to open at the base of the raceme, but some buds may be bypassed by more rapidly opening flowers above. An individual raceme may flower over a 2- to 5-day period.

Natural Crossing

Black cherry is insect pollinated. Although the principal pollinator is the honeybee in Gainesville, other insects, including other types of bees and several flies, were seen on flowers.

Temperature seems to highly influence insect activity, especially that of bees. One Florida cherry flowering season was cool, and bee activity was limited to a short period each day. The resulting crop was estimated at less than half that of years when temperatures

during flowering were in the 80° F to 90° F range during much of the day. A cool season, then, is good for controlled pollination because it spreads the flowering period out, but bad for natural pollination because of limited bee activity.

Stigmas are exposed to cross-pollination before anther dehiscence in single flowers. Often flowers are open for one to two hours before anthers show any sign of liberating pollen.

Natural Selfing

Nothing is known of what happens when a honeybee transfers self pollen within a black cherry tree. What is known, however, is that self pollen can easily be transferred from flower to flower or from anther to stigma on the same flower by contact alone. If such transfers produced compatible crosses, then black cherry crops would not vary directly with honeybee activity as is the case in the Gainesville, Florida, area.

The almost complete lack of selfing in black cherry in Gainesville was first uncovered during trials of pollination techniques. Unopened flowers, bagged and left, consistently failed to set fruit. Artificially selfed flowers produced the same result. But crosses, especially mixed-pollen crosses, set some fruit in almost every case. An exception to the natural selfing rule in black cherry occurred on one occasion during a second season of preliminary trials in Gainesville; three flowers on one tree did set fruit when isolated. This is the only instance of natural selfing within a bag that occurred in Gainesville studies over three years in thousands of flowers. Though a highly suspicious result, there was no reason to doubt that natural selfing had occurred.

Pollen Germination Tests

Tests on agar, agar with 10 percent sucrose, and distilled water showed that black cherry pollen germinated well on all three media. Germination on each one was 50 percent or better after three days of incubation. Tube growth was regular with most tubes of similar length. After three days, tubes of average length were long enough to have reached the ovary in normal pollinations. This regularity of tube growth and fairly short period of extension set the pattern for later testing.

Bagging

Bagging is not only a means of isolation but also a way to keep developing and mature fruits available and labeled, even though they may become detached for one reason or another. It avoids the need for constant attention over the ripening period to be sure that successes are not lost before being counted.

Individual flower life varied within bags, with temperature an important factor. Under cool conditions an individual flower would last as long as nine days when pollinated and bagged. At the six- or seven-day point pistils began to darken and wither. Very hot air temperatures reduced these figures to as low as five- and three-day periods, respectively, and prolonged contact with the bag usually meant death to individual flowers.

Since Schuster (1925) in his work with sweet cherry concluded that petal removal would deter insects, the possibility that isolation was not needed was considered. However, the probability that unbagged fruit would be eaten by birds or otherwise lost seemed high and bags were used.

Chromosome Count

Ploidy influences incompatibility in Prunus. Darlington and Ammal (1945) listed Prunus serotina as a tetraploid, and several tetraploids in Prunus are self-compatible. But trees in the Gainesville area, with the exception mentioned earlier, were all self-incompatible in early trials. For this reason chromosome counts were made. Seed for this study came from the Norris, Tennessee, area.

Root tip cells undergoing mitosis exhibited very long and fat chromosomes--much of the cell was filled with them. By focusing up and down on the microscope, each chromosome and/or broken parts were traced. Many squash preparations were examined, but in only five cells were chromosomes dispersed enough to make counting practical (Figures 3 and 4). Counts ranged from a low of 22 to a high of 25 chromosomes plus segments. One end of these short pieces was in almost every case very near what appeared to be the point of breakage of a much longer chromosome. Since there was no way to be sure of a count and continued squashing produced no better separation, these trees cannot categorically be classified as diploids. However, the arrangement of the short segments indicates that they are diploid. They are definitely not tetraploid.

Pollen Tube Length Study

Pollen Germination

The main purpose of this study was to supply a basis for comparison of pollen treatments with respect to tube penetration. Lack of germination was not expected for any pollen treatment including self, since early trials had shown black cherry pollen would germinate well

Figure 3. Black cherry chromosomes at metaphase, not well spread.

Figure 4. Well spread black cherry metaphase chromosomes.

on plain water. However, flowers on the same raceme showed highly different amounts of germination with the same pollen. This occurred even though techniques used were meant to give individual flowers an equal chance to respond to various pollen treatments. Some factors which might have influenced this result are:

1. Differences in receptivity among flowers treated the same.

2. Interference with liberation of self pollen because of direct damage caused by petal removal or indirect damage resulting from exposure of immature flower parts.

3. Interference due to a reaction between applied and self pollen.

4. Failure to distribute pollen uniformly.

Although there is no way to be sure, a combination of Factor 1 and Factor 2 seems the most likely reason for results obtained for the following reasons:

1. Flower sections showed no evidence of physical damage to the stigmatic surface; any damage must have been elsewhere.

2. Early trials using cross-pollens on unemasculated flowers with petals removed showed that crosses consistently produced fruit.

3. Some early-pollinated flowers failed to extend anthers.

4. Pollen applicators were always kept covered with fresh pollen and were checked regularly to be sure that pollen was being left on the treated flower.

If some treated flowers on each raceme were a day or more away from opening, applied pollen may not have germinated readily and self pollen would have been liberated later. Self pollen would not have had an opportunity to penetrate deeply if at all. Disregarding Factor 1, all

flower group means with the possible exception of early selfs should have been at least equal to no-pollen-self means since no flowers were emasculated. However, where the no-pollen treatment was included, it was either first or second among treatments in number of tubes present. There are only two possible explanations for this behavior: (1) the pollen application process interfered with release and/or growth of self pollen, or (2) flowers that received the no-pollen treatment were closer to the pollen-release stage as a group than were flowers in other treatments. The second alternative is a weak explanation except in the case of early self flowers which were selected on the basis of immaturity. However, there is the direct evidence for the first alternative listed under reason 3 above. All early self flowers extended anthers later than more mature flowers as expected, but some failed to extend anthers after seven days. Any flower selected should have opened within that period. The consistent last position of the early self treatment is probably the result of both non-receptivity and flower damage directly or indirectly (premature exposure) caused by the pollination process.

Ranks of pollen treatments are shown in Table 4 and Table 5. Statistical comparisons were run but are not considered valid since amount of pollen applied could not be controlled.

The fact that the no-pollen-self ranked high when used indicates that not only was pollen transferred within individual flowers successfully, but that it germinated well.

Cross and normal self treatment results are confused because of the likelihood that pollen from within the flower was also included in counts. But the rankings did produce some patterns that give

Table 4

Comparison of Pollen Treatments with Respect to Pollen Germination, Tree Group A

Tree	Rank* 1	2	3	4	5	6
1	P0	P2	P4	P3	NS	ES
3	NS	P0	P2	P1	P4	ES
4	P0	P2	P1	P3	NS	ES

* Rank 1 had highest germination.

ES Early self

NS Normal self

P0 No pollen

P1 Pollen 1

P2 Pollen 2

P3 Pollen 3

P4 Pollen 4

Table 5

Comparison of Pollen Treatments with Respect to Pollen Germination, Tree Group B

Tree	Rank* 1	2	3	4	5	6	7
5	P7	NS	P2	P1	P6	P8	P4
6	P7	P1	NS	P2	P8	P5	P4
7	P6	P8	P2	NS	P1	P5	-
8	NS	P7	P2	P6	P5	P1	P4

- No treatment

* Rank 1 had most germination.

NS Normal self

P1 Pollen 1

P2 Pollen 2

P4 Pollen 4

P5 Pollen 5

P6 Pollen 6

P7 Pollen 7

P8 Pollen 8

indications of performance by some treatments. In tree group A, pollen 2 ranked second or third, and on group B it was third three times and fourth once. Pollen 3 was only better than the applied selfs, and pollen 4 was last in three of five cases. Pollen 7 always ranked high and pollen 5 low.

Removal intervals were intended to give pollen tube growth rate data, but the receptivity problem voided this possibility. Comparative rankings are found in Table 6. The longer interval should have allowed maximum pollen germination. It should also have included more germinated pollen grains from flowers that became receptive later than expected. But five out of seven times the 5-day interval produced more germination. The 3-day interval was always last when included. Individual flowers were again unpredictable. Quite often single flowers from the 3-day interval showed more germination than others from longer intervals. No definite reason can be given at this time for these results. The following possibilities could well be responsible:

1. By chance, more flowers included in the 7-day interval were pollinated too early and failed to promote germination as did most of the early selfs.

2. A high percentage of flowers from the 5-day interval were close to or had reached peak receptivity, with the result that more pollen grains germinated.

3. Many flowers from the 3-day interval became receptive on the second or third day and had no chance to reach a high level of germination.

Table 6

Rank* of Removal Intervals in Tree Groups A and B with Respect to Pollen Germination

Tree	Removal intervals (days) 3	5	7
1	3	1	2
3	3	1	2
4	3	1	2
5	-	1	2
6	-	2	1
7	-	1	2
8	-	2	1

- No treatment

* Rank 1 had most germination.

The consistent last position of the 3-day interval is easy to understand and accept. But there seems to be no logical way to explain the average second position of the 7-day interval.

On tree 3, pollen 2 from the 3-day interval ranked second compared to seventeenth for pollen 2 from the 7-day interval, although the 3-day interval was the worst overall. On tree 6, pollen 8 from the 5-day interval was superior to the same pollen from the 7-day interval. And on tree 8, the normal self from the 5-day interval was superior to the same treatment from the 7-day interval. Clearly, some factor unaccounted for influenced the results.

Summary of results.--Among pollen treatments the no-pollen treatment ranked first twice and second once in the three cases it was used. Among removal intervals the 5-day period produced more germination than did the 3- or 7-day intervals, although the expected order was 7, 5, 3 (highest to lowest). Other noteworthy results included:

1. Early selfing proved unsuccessful. It ranked last whenever used.

2. Self pollen applied just prior to flower opening showed no consistent pattern. It ranked first on two trees, next to last on two others, and in varied positions on the other three trees.

3. Some cross pollens showed definite patterns. Pollens 2 and 7 always ranked high and pollens 4 and 5 always low.

4. Pollens varied in ability to germinate, but there was no barrier to germination.

Pollen Tube Extension

In this phase the style was divided roughly into three equal parts, and counts were made of tube sections in each part. Since no

control was possible over amount of pollen deposited on each stigma--and, consequently, the number of tubes possible--an equalizing effect was used; number of tubes in the middle and lower thirds of the style was expressed as a percent of the number in the upper third. These percentages were converted to arc sin values and analyzed statistically. However, these findings are not considered a fair estimate of pollen performance. Reasons are presented later.

Treatments for groups A and B are compared within and over all trees with respect to pollen tube penetration into the middle third of the style in Table 7. It is highly probable that self-pollen tubes were included in counts of cross-pollen treatments. Therefore, no-pollen treatment results could not be used to distinguish between self- and cross-pollen tubes. Results did indicate that no-pollen-self tubes failed to penetrate the lower third of the style. Because of these findings, data for middle-third penetration by cross pollens are not considered reliable, while the following lower-third data are thought to be more accurate.

Treatments for tree groups A and B are compared within and over all trees with respect to pollen tube penetration into the lower third of the style in Table 8. Tube penetration into the lower third of the style was much reduced over penetration into the middle third. Only 1.4 percent of the tubes reached this level. The most probable reason for this lack of tube extension is insufficient time for tube travel. Seven days was the maximum time allowed before flower removal and fixation. This interval was selected for three reasons: (1) tubes extended fully within three days in incubated pollen germination tests, (2) pollen tubes had extended into the ovary by this time in Prunus

Table 7

Percent of Pollen Tubes in the Upper Third of the Style that Reached the Middle Third, over All Trees and Removal Intervals, by Pollen Treatment and Tree

	Pollen treatment										
Tree	P0	P1	P2	P3	P4	P5	P6	P7	P8	ES	NS	Av*
1	9.1	-	32.8	7.1	0	-	-	-	-	0	0	10.6
3	0	12.8	5.3	-	8.0	-	-	-	-	2.3	3.5	5.3
4	0	1.8	3.0	8.5	-	-	-	-	-	0	0	2.2
5	-	4.9	5.5	-	2.2	-	0	3.7	37.0	-	4.2	6.4
6	-	53.5	9.8	-	46.1	31.1	-	28.4	47.8	-	27.0	35.9
7	-	15.4	0	-	0	0	12.3	-	4.6	-	0	7.1
8	-	18.7	0	-	0	0	50.0	0.5	-	-	0	10.2
Av.*	3.6	33.1	7.5	8.3	29.4	30.1	12.0	21.3	36.0	1.3	20.1	20.7

- No treatment
* Weighted average
ES Early self
NS Normal self
P0 No pollen
P1 Pollen 1
P2 Pollen 2
P3 Pollen 3
P4 Pollen 4
P5 Pollen 5
P6 Pollen 6
P7 Pollen 7
P8 Pollen 8

Table 8

Percent of Pollen Tubes in the Upper Third of the Style that Reached the Lower Third, over All Trees and Removal Intervals, by Pollen Treatment and Tree

Tree	Pollen treatment											Av.*
	P0	P1	P2	P3	P4	P5	P6	P7	P8	ES	NS	
1	0	-	5.2	0	0	-	-	-	-	0	0	0.5
3	0	0	1.1	-	0	-	-	-	-	0	0	0.3
4	0	0	0	0	-	-	-	-	-	0	0	0
5	-	3.3	0	-	0	-	0	0	0	-	0	0.9
6	-	2.7	0.4	-	1.1	3.7	-	1.8	0.6	-	1.2	1.8
7	-	0	0	-	0	0	9.1	-	0	-	0	3.5
8	-	4.7	0	-	0	0	50.0	0	-	-	0	3.6
Av.*	0	2.6	0.6	0	0.6	3.6	9.6	1.3	0.4	0	0.8	1.4

- No treatment

* Weighted average

ES Early self

NS Normal self

P0 No pollen

P1 Pollen 1

P2 Pollen 2

P3 Pollen 3

P4 Pollen 4

P5 Pollen 5

P6 Pollen 6

P7 Pollen 7

P8 Pollen 8

avium studies (Roy, 1938), and (3) flowers begin to deteriorate quickly after seven days. However, tube travel continued past this stage and probably was not completed until the style was well into decay. Support for this view comes from the fruit set phase of this work. Flowers in that phase treated exactly as those in the tube length phase produced some fruit from nearly every cross. So tube penetration must have continued in those flowers after flower removal in the tube length study. Because of the lack of time for tube extension, no statistical analysis is presented here.

The first visual evidence of possible incompatibility in black cherry was found in this phase. One pollen tube from the normal self on tree 1 produced a swollen tip or head (Figure 5). In studies of Prunus avium (Roy, 1938), this condition was considered good evidence for incompatibility. Another tube from the cross of tree 8 with tree 6 turned around and began to grow back up the style (Figure 6). This is also evidence of incompatibility in P. avium (Roy, 1938). No other examples of this type were found.

The consistently good overall performance of tree 6 as both male and female is worth discussion. Some tubes from every pollen used on this tree reached the lower style, even the normal self. No self on any other tree reached this depth. Good performance by all pollens on it indicates that tree 6 was highly receptive. It might even have a genetic advantage in this respect over the other trees. But the most important feature here is the penetration of self pollen. On P. avium, Roy (1938) found that self-pollen tubes usually became arrested in the style, and most of them appeared to swell up at their ends. Afify (1933) found that self tubes were short and often bent upward

Figure 5. Abnormal pollen tube, upper one-third style, Tree 1 normal self, 7 days.

Figure 6. Abnormal pollen tube, middle one-third style, Tree 6 x Tree 8, 5 days (lower tube).

before ceasing growth. But Roy (1938) also very rarely obtained one or two fruits after self pollination. On tree 6 with self pollen, tubes did not have swollen ends nor were any turned upward. However, both of these characteristics might have appeared had tubes been allowed more time to grow. On the other hand, one or more might also have penetrated the ovary. But fruit set study results indicate that fertilization probably would not have occurred.

Flower removal intervals for groups A and B are compared within trees and over all trees and pollen treatments with respect to pollen tube penetration into the middle third of the style in Table 9.

In tree group A, the pattern of tube penetration with respect to removal intervals was the same as that in pollen germination: the 3-day interval was low; the 5-day, high; and the 7-day, intermediate. But tubes penetrated the middle third on every tree in the 7-day interval, whereas tree 4 had no penetration in the 5-day interval, and trees 3 and 4 had none in the 3-day interval. The pattern here as in germination seems to reflect the stage of receptivity in the trees, although there is no explanation for the 7-day period being intermediate. Tree group B showed an overall advantage for the 7-day interval though it ranked first on only one tree. Over all trees in both groups the 7-day interval produced the highest percentage of tubes reaching the middle third of the style. This was caused by the very large number of penetrations observed on tree 6 in the 7-day interval.

Intervals for groups A and B are compared within trees and over all trees and pollen treatments with respect to pollen tube penetration into the lower third of the style in Table 10. Only the 5-day interval produced tubes at this depth in tree group A, and these were at very

Table 9

Percent of Pollen Tubes in the Upper Third of the Style that Reached the Middle Third, over All Trees and Pollen Treatments, by Removal Interval and Tree

Tree	Removal interval (days) 3	5	7	Av.*
1	3.6	14.0	5.4	10.4
3	0	10.8	6.1	5.1
4	0	0	3.5	2.1
5	-	6.3	5.0	6.1
6	-	30.6	38.2	34.5
7	-	7.1	6.5	6.9
8	-	15.4	4.1	9.8
Av.*	0.4	19.9	24.8	20.7

- No treatment

* Weighted average

Table 10

Percent of Pollen Tubes in the Upper Third of the Style that Reached the Lower Third, over All Trees and Pollen Treatments, by Removal Interval and Tree

Tree	Removal interval (days) 3	5	7	Av.*
1	0	0.8	0	0.5
3	0	1.0	0	0.3
4	0	0	0	0
5	-	0.9	0	0.8
6	-	3.1	0.4	1.7
7	-	3.9	1.9	3.4
8	-	4.0	3.0	3.5
Av.*	0	2.4	0.5	1.4

- No treatment

* Weighted average

low percentages. In group B, the trend set in the middle third of the style was reversed, and the 5-day interval ranked first. Percent penetration was much lower, but some penetration occurred in every tree-interval combination except tree 5, interval 7.

Summary of results.--The several noteworthy results included:

1. Pollen tubes from the no-pollen-self treatment, so highly ranked in pollen germination, penetrated to the middle third of the style only on tree 1, with no tubes reaching the lower third.

2. Early self tubes reached the middle third only on tree 3, with no further penetration.

3. Normal-self tubes reached the middle third on trees 3, 5, and 6, and the lower third on tree 6. Tree 6 pollen showed exceptional ability to penetrate tree 6 stylar tissue.

4. Cross-pollen results were clouded by the strong possibility that self pollen tubes were included in the counts. Based on no-pollen-self results it is highly probable that they were included in tube counts from the middle third of the style but unlikely in lower third counts.

5. The pattern of tube penetration with respect to time of flower removal after pollination showed less penetration in the shortest interval as expected, but also less extension in the longest than in the intermediate interval.

6. Two instances of abnormal pollen tube development, evidence for an incompatibility system similar to that in Prunus avium, were observed.

Fruit Set Studies

Prevention of Insect Pollination without Bagging--Gainesville

On each tree in group A, 60 flowers were depetaled. Half were bagged; half were not. No fruit set occurred among bagged flowers, but one unbagged flower did set on tree 3. If this low set could be duplicated over a large number of flowers, the results might be acceptable for most breeding work. However, the one fruit that did set causes some doubt as to the possible success of the procedure.

Selfing and Crossing--Gainesville

Both early and normal selfs were included on tree group A. Only normal selfs were included on group B. Not a single flower of any self set fruit. This paralleled findings in preliminary trials. After observing results of the pollen tube length phase of this work, lack of fruit set in the early self is understandable. Very little germination occurred, and in all but one instance tubes traveled no farther than mid-style. Since normal selfs produced many tubes with some penetrating into the lower style, the only reason for lack of fruit set is the incompatibility mechanism.

The overall fruit set among cross-pollinated flowers was 5.9 percent (Table 11). Pollens varied from 1.7 to 12.0 percent. Mother (female) trees varied from 1.7 to 14.2 percent. Some results followed trends set in the tube length study, but others did not. Tree 6 was a good pollen parent on each tree where it was used and a good mother tree for all pollens except 5 and 7, which failed on all trees. No tree crossed successfully with every other tree. Tree 1 crossed with every tree except tree 8. Tree 8 crossed only with tree 6.

Table 11

Percent Fruit Set among Cross-Pollinated Flowers, by Pollen and Mother Tree, Gainesville

Mother tree	Pollen parent (tree)								Av.*
	1	2	3	4	5	6	7	8	
1	-	6.7	3.3	13.3	-	-	-	-	7.8
3	0	0	-	10.0	-	-	-	-	3.3
4	6.7	0	0	-	-	-	-	-	2.2
5	10.0	0	-	5.0	-	10.0	0	0	4.0
6	40.0	10.0	-	25.0	0	-	0	10.0	14.2
7	10.0	15.0	-	0	0	20.0	-	0	7.0
8	0	0	-	0	0	10.0	0	-	1.7
Av.*	10.0	4.4	1.7	9.3	0	12.0	0	3.3	5.9

- No treatment

* Weighted average

With these data alone, only compatible crosses can be distinguished with accuracy. But there are some indications that tree 8 might be incompatible, or partially so, with one or more trees. It crossed successfully with tree 6 both as a male and female but with no other tree. However, it is not possible under the diploid system of incompatibility found in other species of Prunus for tree 8 to be completely cross-incompatible with all but one of the study trees. The reasoning here is that if tree 8 is completely incompatible with all of the others, then all must have the same alleles for incompatibility. If this were true, the others would also be completely incompatible among themselves. The logical deduction is that poor pollen germination or some other influence prevented fertilization or fruit set.

Selfing and Crossing--Norris

Percent set by male and female parent for selfs and crosses is found in Table 12. Of the 17,921 flowers allowed to self, 3,859 produced fruit for a 21.5 percent set. Every study tree produced selfed fruit. Individual trees ranged from a low of 9.9 to a high of 42.3 percent. Out of 1,181 cross-pollinated flowers, 516 or 43.7 percent set fruit. Male-female combinations varied from 0 to 80.0 percent set. Tree 1 produced a lower set from crosses than from selfs, the only tree to do so. It was pollinated just as the other trees began to flower. Racemes from other trees used to cross pollinate may not have liberated much pollen with the result that fewer flowers were pollinated. However, this does not explain why these flowers did not self.

Percentages in Table 12 are based on counts that were probably made just before what is called the third or June drop in cultivated

Table 12

Percent Fruit Set among Self- and Cross-pollinated Flowers, by Pollen and Mother Tree, Norris

Mother tree	Pollen (tree)							Av. for Crosses*
	Self	1	2	3	4	5	6	
1	9.8	-	17.4	0	8.0	2.5	0	5.0
2	11.5	40.0	-	13.5	20.5	36.7	65.6	34.3
3	42.3	63.2	63.4	-	22.5	80.0	63.6	58.8
4	28.5	48.4	55.4	54.3	-	70.0	55.9	57.0
5	29.8	37.9	56.4	47.6	67.7	-	36.0	50.0
6	16.8	74.4	14.7	30.2	38.5	32.3	-	38.7
Av.*	21.5	54.1	45.6	33.9	32.0	46.8	46.9	43.7

- No treatment

* Weighted average

cherry varieties. At that time--two weeks after peak flowering--fruit was well developed. Ten selfed fruits--two from trees 1, 3, 4, and 5; one from trees 2 and 6--were removed, opened, and inspected. Three of the ten--two from tree 4, one from tree 6--appeared normal. The others were either empty or had shriveled seed coats; five of these showed insect larvae damage. Normal appearing seeds were sectioned by hand and observed under the microscope. A typical picture of a developing embryo from this type seed is shown in Figure 7.

Of ten crosses examined, all were filled, but seven showed insect damage. Developing embryos were similar to that in Figure 7.

Five weeks after peak flowering only ten selfed fruits remained of the 3,859 that set, but 117 of the 516 crosses that set remained on the tree. There is little doubt that the insect infestation caused a large part of the fruit to drop, but the wide difference between selfs and crosses is unexplained. These results are similar in one respect to Bradbury's (1929) with sour cherry and Cooper's and Brink's (1940) with Nicotiana hybrids. In both cases selfs and interspecific hybrids showed restricted embryo development when compared to intraspecific crosses. What is important is that black cherry did self and that selfed fruit remained on the tree past the usual drop periods of other species of Prunus.

With regard to selfing, the Norris study produced completely opposite results from those in Gainesville. In Gainesville black cherry exhibited an incompatibility system similar to the one in sweet cherry. In Norris it was self-fertile, a characteristic also exhibited by the sour cherry, Prunus cerasus. The latter is a tetraploid.

Figure 7. Embryo from selfed fruit, Norris.

Chromosome counts will be made on Norris area cherry again and on those from Gainesville to accurately determine their ploidy, but there could be another reason for successful selfing in Norris cherry. It is possible that there are one or more self-compatible alleles in the Norris population. If this is the case, then there are at least two races of black cherry based on self-compatibility. The small amount of evidence now available supports this alternative.

Presence of selfing coupled with lack of emasculation of cross-pollinated flowers makes impossible the classification of crosses in the Norris study. The higher percent retention of crossed fruits does indicate that crossing was successful, however.

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary and Conclusions

Black cherry is one of our most valuable timber species and is now included in several tree improvement programs. Its breeding habits, however, had not been studied closely prior to this work. Consequently, this study is intended to supply black cherry breeders with not only a description of incompatibility in the species but also a working knowledge of breeding habits and techniques used in making controlled pollinations.

Lack of literature on breeding black cherry and techniques associated with the process made necessary preliminary trials. These were conducted prior to the major work and covered flowering habits, natural selfing and crossing, pollen germination tests, isolation of flowers, and a chromosome count. Techniques established for other species were adapted for black cherry when possible and new ones developed when necessary.

The major work was subdivided into studies of pollen tube length, or penetration into the style, and fruit set. Pollen tube characteristics, including amount of germination and depth of penetration, were observed for selfs and crosses among trees selected because of likelihood of close relationship. The objective was to obtain microscopic evidence for self- and/or cross-incompatibility among study trees based on behavior of pollen tubes in the style. Fruit setting

was used as a check on microscopic evidence and to uncover possible regional differences between the Norris, Tennessee, and Gainesville, Florida, areas. Pollinations that readily produced fruit were considered compatible.

Conclusions on the pollen germination phase of the tube length study accompany the following noteworthy results:

1. Pollen moved from anthers to pistil of the same flower without insect visitation and germinated well. Transfer occurred either by direct contact or through air currents. Both are possible. The former must have accounted for most of the transfer because of the close proximity of anther and stigma and lack of air movement in bags.

2. There was considerable variation in results among flowers receiving the same treatment. The best explanation for this is that flowers exhibiting external indications of opening (swollen petals) were not receptive to pollen of any type until shortly after the time of normal flower opening.

3. Selfing of young buds was unsuccessful. This failure resulted because of "2" above and also because petal removal had a detrimental effect on the maturation sequence as evidenced by the fact that anthers occasionally failed to extend.

4. Selfing just prior to flower opening varied from good to poor among mother trees. This, too, was caused by "2" above.

5. Cross-matings also produced variable results; some males were always good, some always poor, others intermediate in amount of pollen germinated. Part of this variation can be attributed to "2" above, but there was evidence that some pollens germinated better than others in general. Since all pollens were treated alike, some must

have had a genetic advantage; they probably had less stringent germination requirements.

6. There was no barrier to germination of any pollen. When a flower was receptive, any pollen including its own would germinate on the stigmatic surface.

Pollen tube extension results gave limited support to the case for self-incompatibility and some cross-incompatibility but did not exclude self-compatibility completely. Most interesting findings and conclusions based on them include:

1. Pollen tubes from self pollen distributed within individual flowers penetrated the middle third of the style on only one tree even though much pollen germinated. In trees of this phase of the overall study, self-pollen tubes got a late start and also grew more slowly than did those from cross pollen. These are the reasons for lack of natural selfing in the Gainesville trees. However, self tubes did penetrate more deeply than do self tubes in other Prunus species.

2. Pollen tubes from self pollen applied on young buds also reached the middle third of the style on one tree only. Selfing the young bud offers no advantage in obtaining longer tubes that might effect fertilization.

3. Pollen tubes from self pollen applied just prior to flower opening reached the middle third on three trees and the lower third on one. Self pollen, though not as fast growing as cross pollen, can penetrate the lower style and possibly farther on self-incompatible trees. Flowers were removed before these and tubes from crosses could penetrate the ovarian cavity. Styles were beginning to decay after

seven days, but tubes were still growing. This is supported by fruit set results.

4. Two instances of abnormal pollen tube development were observed; one was a self pollination and the other a cross. This lack of physical evidence of incompatibility plus the deeper penetration of self tubes in Prunus serotina than in other Prunus species are evidence that the incompatibility reaction is not as strong in Prunus serotina.

5. Natural self pollination between anthers and adjacent stigmas of individual flowers reduced the effectiveness of this work. In future pollen tube length studies, either emasculation must be practiced or a radioactive marker used to label applied pollen.

Fruit set study results varied greatly between the Gainesville, Florida, and Norris, Tennessee, test areas. The most important result in this phase and probably in the entire study is that all trees in the Norris phase selfed while no selfing occurred in Florida. Further, embryo development was normal in the three selfed fruits observed. Since chromosome counts ruled out the Norris trees being tetraploid, there might be one or more self-compatible alleles in the Norris population. If so, the Norris and Gainesville population could be different races. However, environmental effects must be studied before a firm conclusion is reached.

The overall fruit set percent among crosses in Gainesville was 5.9 compared to 43.7 in Norris. In Gainesville no tree crossed with every other tree; in Norris all crosses were successful at least one way. Selfing is a definite factor in these results, but since the percent of successful crosses was much higher than that of selfs (43.7 compared to 21.5) in Norris, either technique was better in Norris or Norris trees have a reproductive advantage over those in Gainesville.

One depetaled flower exposed to possible insect visitation set a fruit. Although this is not enough evidence to eliminate depetaling as a method of avoiding insect pollination, it causes some doubt as to its value.

Recommendations for Breeding and Tube Length Studies

1. Determine whether the particular trees to be worked are self-compatible or not. The easiest procedure is to bag several racemes on each tree prior to flower opening and observe them for fruit set. Self-fertile individuals should not be used as females in a cross-breeding program at this time; no satisfactory and, at the same time, practical emasculation method is available. Further, use of self-fertile trees as males will introduce self-compatibility genes into a self-incompatible breeding group.

2. If trees of a breeding group originated in several geographic regions, expect a wide range of flowering times. Even local trees will vary up to a week or ten days in initiation of flowering. Satisfactory long-storage procedures will be available soon from Robert Farmer,[1] but until that time do not count on keeping pollen more than one month. It can be successfully stored for that period in a refrigerated desiccator. This should be long enough to cross early trees with late ones.

3. Leafing out is an excellent indicator of the approach of flowering in black cherry; the two are closely correlated. Flowers will not open until full leafout. Therefore, no close examination is needed until a tree is in full leaf.

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[1]Physiologist, TVA Forestry and Fisheries Laboratory, Norris, Tennessee.

4. Temperature is closely correlated with both initiation and period of flowering. In 85° F and over weather, expect only five to seven working days per tree. Daytime temperatures under 65° F will hold up flowering; between 65° and 85° F, the flowering period will last at least two weeks on each tree.

5. Use fresh pollen when parents are close together and are flowering at the same time. Small branches containing bagged flowers can be kept in water and the pollen used up to three days. Pollen collection and short storage will be required in other situations.

6. Select stout upright limbs of at least 3/16-inch diameter for bagging. Often a larger limb can be trimmed so that several racemes close to the base will fit into a bag. The synthetic sausage casings used in this study are cheap and easy to use, but a better type is made of a special paper. It is large, light weight, and has a plastic viewing window. Forestry Suppliers, Jackson, Mississippi, handles it.

7. Use a camel's hair brush to apply collected pollen and also fresh pollen, if desired. The brush will hold more pollen than stiff applicators and offers little chance for flower damage. Have plenty of them available since each pollen treatment on each tree will require one. Also place only small amounts of pollen to be applied in an application bottle because of the transfer of pollen from unemasculated flowers. Discard this pollen after use on a single tree or clone.

8. Pollinate only those flowers that have recently opened. On a raceme there are usually two or three distinct classes of flowers. Early during flowering only freshly opened flowers and buds are present. Within two or three days there will be overmature, freshly opened, and buds. Five or six days after initial flowering, only overmature and a

few freshly opened flowers remain. Pollination is best when the three classes are present; more flowers are at peak receptivity. Strip buds and overmature flowers from the raceme; 15 to 25 flowers should be left. Overmature flowers have brownish-green sepals compared to the bright green receptive flowers. Also, look for shriveled petals on overmature flowers. Beginning discoloration--reddish brown spot--on the style is a sure sign of overmaturity.

9. Remove synthetic sausage casing bags as soon as pollinated flowers show signs of overmaturity. Slit the bag longitudinally and carefully open it to avoid dislodging flowers. Some flowers will fall at this time and afterward on all racemes, even though pollinated. Paper bags can be left on the tree without heat buildup and will protect developing fruit from birds.

10. Fruit will turn a bright red, then dark red, then black. Begin harvesting at the dark red stage.

11. In tube length studies labelled pollen or some form of emasculation must be used because of presence of self pollen. Complete removal of calyx and corolla is unsatisfactory because it leaves an unprotected style. For a small-scale project anthers can be clipped off with small scissors. This is a very tedious and time-consuming job, but it will work. Labelling applied pollen with a radioactive tracer will allow rapid pollination procedures. The more detailed exposure to film and subsequent processing to get tube tracings can be done in the laboratory when convenient. Satisfactory procedures are listed in Jensen's (1962) Botanical Histochemistry under autoradiography.

12. Plan to remove flowers for killing and fixation after several periods with the longest at least 10 days. Styles will begin to dry up and wither six to seven days after opening and possibly sooner in very hot weather, but tubes will not penetrate the ovary until seven or more days after pollination. Flower removal after different intervals will allow study of fresh tissue at all stages of tube penetration.

Recommendations for Future Work

Future work can be subdivided into two classes: work with (1) self-compatible, and (2) self-incompatible types.

Since no seedlings known to be the result of self pollination are available, there is no way to estimate the extent of inbreeding depression likely to be encountered. This should be studied first under class 1. If depression is moderate to low or negligible, production of pure lines should be relatively easy for two reasons: (1) only isolation is necessary to obtain selfs and (2) the interval from seed to flowering tree is about five years, a short period for a forest tree. Once lines are developed that exhibit desired characteristics, they could be crossed to combine traits and hopefully obtain hybrid vigor.

A large crossing program dealing with self-compatibles would require either a new and acceptable emasculation method or development of male-sterile lines. Both of these approaches should be investigated.

For self-incompatible trees the most needed work is in the area of cross pollination techniques. Such innovations as controlled insect pollination, adaptation of normally insect carried pollen to a type dispensed by air currents, and better means of isolation all should be studied.

Work on pollen and seed storage is being done now, but acceptable methods are not yet available. There is room for more of this work.

APPENDIX

Figure 8. Fence row that included study trees, Norris.

Figure 9. Heavily fruiting tree, Norris.

Figure 10. Typical tree, Norris.

Figure 11. Fruit set from a cross, Norris.

Figure 12. Fruit set from a self, Norris.

Figure 13. Fruit set from a self, Norris.

Figure 14. Typical bag, Gainesville.

Figure 15. Petal removal prior to pollination, Gainesville.

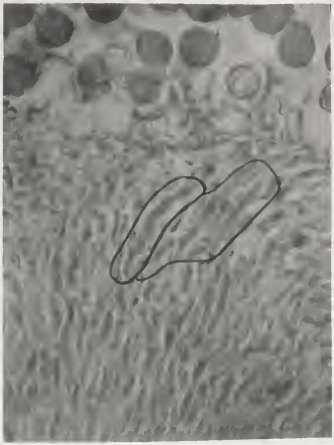

Figure 16. Pollen tubes, upper one-third style, Tree 4 no pollen, 3 days.

Figure 17. Pollen tubes, stigmatic surface, Tree 1 no pollen, 5 days.

Figure 18. Pollen tubes, stigmatic surface, Tree 4 no pollen, 5 days.

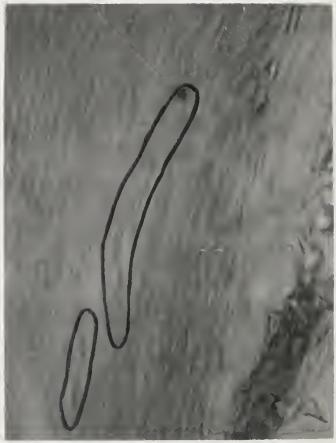

Figure 19. Pollen tubes, lower one-third style, Tree 6 normal self, 5 days.

Figure 20. Pollen tubes, lower one-third style, Tree 6 x Tree 2, 5 days.

Figure 21. Pollen tubes, lower one-third style, Tree 6 x Tree 4, 5 days.

Figure 22. Pollen tubes, lower one-third style, Tree 6 x Tree 5, 5 days.

Figure 23. Pollen tubes, lower one-third style, Tree 6 x Tree 5, 7 days.

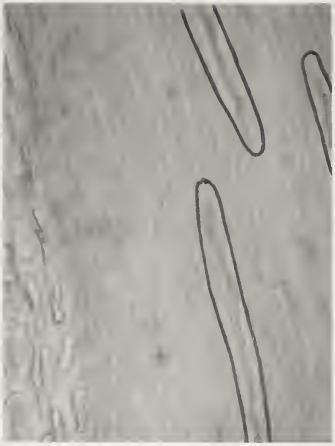

Figure 24. Pollen tubes, lower one-third style, Tree 6 x Tree 7, 5 days.

Figure 25. Pollen tubes, lower one-third style, Tree 6 x Tree 7, 5 days.

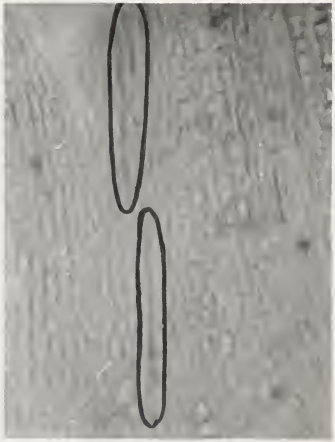

Figure 26. Pollen tubes, junction ovary and style, Tree 7 x Tree 6, 7 days.

Figure 27. Pollen tubes, upper one-third style, Tree 7 x Tree 8, 5 days.

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BIOGRAPHICAL SKETCH

Donovan Charles Forbes was born August 4, 1933, at Hammond, Louisiana. In May 1951, he was graduated from Hammond High School. That same year he enrolled at Southeastern Louisiana College and, after a tour of duty with the U. S. Army from 1953 to 1956, received the degree of Bachelor of Science with a major in Forestry in 1958. He then enrolled in the Graduate School of Louisiana State University where he worked as a graduate assistant in the School of Forestry. In 1959 he took leave and served as interim instructor in Agriculture at Southeastern Louisiana College. In 1960 he returned to Louisiana State University and in 1961 received the degree of Master of Forestry. He then took a position with the Tennessee Valley Authority in Norris, Tennessee. From 1963 to 1966 he was in residence at the University of Florida and has continued to work toward the degree of Doctor of Philosophy to the present time.

Donovan Charles Forbes is married to the former Susan Elaine Hyde and is the father of three sons. He is a member of Xi Sigma Pi, Alpha Zeta, and Phi Sigma.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1969

Dean, College of Agriculture

Dean, Graduate School

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