Using Pollen Tube Growth Rates as a Screening Technique for Self-Compatibility in Apricots

Maxwell Norton

May 1980

TABLE OF CONTENTS

Page

LIST OF TABLES v

INTRODUCTION 1

LITERATURE REVIEW 3

The Nature and Mechanisms of Incompatibility and Expression in Pollen Tube Growth 3

Studies Done in Various Pomological Crops 11

Microscopic Techniques Used in Studying Pollen Tubes 14

MATERIALS AND METHODS 17

RESULTS AND DISCUSSION 20

BIBLIOGRAPHY 29

LIST OF TABLES

Table | Page

1. Distance pollen tubes observed down styles of hand pollinated apricot blooms (1978) 21

2. Distance pollen tubes observed down styles of hand pollinated apricot blooms (1979) 23

USING POLLEN TUBE GROWTH RATES AS A SCREENING TECHNIQUE FOR SELF-COMPATIBILITY IN APRICOTS

by

Maxwell Norton

A thesis

submitted in partial

fulfillment of the requirements for the degree of

Master of Arts in the School of Agriculture and Home Economics

California State University, Fresno

May 1980

INTRODUCTION

A common problem that exists in the attempt to breed certain tree fruits is that of self-incompatibility, the inability of an individual to pollinate itself and produce fruit or seed. The ramifications of such a condition are evident. If a variety is unable to pollinate itself, it requires a pollinator variety planted near enough for insects or wind to transfer pollen from the pollinator variety to effect pollination and fruit set.

Disadvantages of using pollinators are: (1) interplanted varieties have to be pruned and handled differently throughout the year, (2) fruit and nut harvesting must be accomplished without mixing varieties, (3) good pollination requires ample pollen carriers (bees), good weather in which the carriers can work and ample overlap of the bloom, which is not possible due to different temperatures every year. Weak beehives, bad weather, and insufficient bloom overlap often limit pollination enough to reduce the yield significantly.

Incompatibility is genetically controlled and can be expressed in several different ways and in different locations. Pollen grains can simply fail to germinate on the stigmatic surface or if they do, the pollen tubes may not penetrate the stigma. If the pollen tubes do penetrate the stigma, they may become arrested at some point down the style. Even if the ovary tissue is reached, the pollen tubes still may not be able to effect fertilization. In some situations the pollen tubes grow the entire distance down the style but grow so slowly that the ovule is no longer viable when the pollen tube reaches it (Ascher,

1966). If the incompatibility reaction is determined by the parent, it is sporophytically controlled (Hughes & Babcock, 1950). If the reaction is determined by the genetic makeup of the pollen grain, it is gametophytically controlled.

This study was undertaken to establish the feasibility of using pollen tube behavior as an indicator of compatible and incompatible crosses in breeding of fruit trees. If, after controlled crosses of known parent materials are made, pollen tubes do behave in a predictable manner, this method could conceivably be used as a screen for incompatibility.

The traditional method of testing for self-compatibility is to wait until the tree in question is old enough to produce blossoms and place bags over the blossoms to exclude foreign pollen and later observe for fruit set. This method has pitfalls. The movement of the bag on the branch can knock the delicate blossoms off and negate the test. If a frost occurs, the bloom can be damaged and will drop even though fertilization had taken place. Placing a bag over the blossoms creates an artificial microclimate around the pistil which can have an effect on the pollination or fertilization process. Using seed set as a criteria to determine compatibility can be unreliable because seed set can be induced or be apomictic (Emerson, 1938).

Thus, by this method, if fruit sets after bagging, we are fairly safe to assume that the selection is self-compatible. But, if no fruit sets, we do not know if the tree is self-incompatible or the bag simply knocked the blossom off. So not only do we want to speed up the screening process, we also want to make the test more reliable.

LITERATURE REVIEW

The Nature and Mechanisms of Incompatibility and Expression in Pollen Tube Growth

East and Mangelsdorf (1925), in their studies with species of Nicotiana, developed several correlations between the presence of self-incompatibility and pollen tube growth. They observed that in compatible matings, pollen tube growth was accelerated and affected fertilization, while in incompatible matings pollen tube growth was at a slow and steady rate which usually did not allow fertilization to occur while the egg was still viable. They suggested that this behavior was hereditarily controlled. To explain this phenomenon, East and Manglesdorf (1925) proposed the oppositional factor hypothesis. In this model there are alleles or sterility genes labelled S_1, S_2, S_3, etc., which are possessed by the two individuals in the cross. Any two specific S genes can function as a pair of allelomorphic genes. The actual mechanism was still not understood.

In a later paper, East and Mangelsdorf (1926) go on to explain that there was an interaction between the pollen tubes and the stylar tissue and this was due to their genetic makeup. But they did not know if having different genetic formulae induced, inhibited, or accelerated pollen tube growth. They did find that: (1) there were three distinct sterility factors, S_1, S_2, S_3, etc., (2) that pollen tubes carrying the same factor as those in the style were inhibited, (3) reciprocal crosses between plants having common factors yielded unlike progenies and the S group in which the female belongs was always absent in the

progeny, (4) populations produced by selfing gave expected segregations, (5) crosses between heterozygotes and homozygotes gave fertility reactions expected, and (6) materials being tested indicated the presence of additional S factors.

Through experiments performed by Crane and Lawrence (1929), it was learned the pollen tube could not function normally in a style carrying the same S factors and was arrested in crosses involving individuals possessing the same genetic composition. They also indicated that the S factors were acting in pairs and the factors were inherited independently. Crane and Lawrence indicated that polyploid plants can carry more than one factor of incompatibility. If this were true of the gametes, greater variation in pollen tube growth could be expected. This is due to the independent assortment of the S genes during meiosis.

In 1930 a study was made on incompatibility in polyploids by Lawrence. The pollen tube growth was found to be dependent upon the "ratio of like and unlike factors between the male gametophyte and the female sporophyte" (Lawrence, 1930, p. 273). This confirmed the earlier models of Crane and Lawrence. The model for polyploids is different from that of diploids in that only some of the pollen tubes present were inhibited. This depended upon the combination of S factors present. Lawrence summarized his findings by saying: "The polyploid theory is shown to imply that like factors in pollen and pistil positively inhibit and unlike factors positively promote pollen tube growth, but the potencies of these two opposite reactions are not equal." (Lawrence, 1930, p. 293)

In the diploid Prunus avium L., self-incompatibility and cross-

incompatibility are both common and are always reciprocally expressed (Crane & Lawrence, 1929). In the hexaploid Prunus domestica L., different degrees of compatibility as well as complete compatibility are observed (Afify, 1933). They are: (1) pollen grains did not germinate, (2) the pollen tubes grew a very short distance and bent upwards, (3) the pollen tubes grew about one fourth of the way down the style, (4) the pollen tubes grew approximately one half of the way down the style, and (5) the pollen tubes grew all the way down into the ovary and effected fertilization. Afify concluded that again selective processes were at work. Observations of Malus pumila Mill. were similar and his conclusions were the same.

According to Anderson and Sax (1934), there are three means of testing for the oppositional factor hypothesis: (1) there should be inter-fertile and intra-sterile classes, (2) two levels of cross-fertile matings, fully compatible and semi-compatible should be observed, and (3) there ahould be two main classes of pollen in semi-compatible crosses, inhibited and uninhibited in equal numbers. Anderson and Sax did intra-sterile crosses. But due to poor microscopic technique, they were unable to tell just how far the pollen tubes travelled down the style and could not prove the existence of two distinct grades of cross-fertile matings. The data also did not indicate the presence of completely inhibited and uninhibited classes of pollen in equal numbers.

Sears (1937) wrote a 181-page doctoral thesis exploring the cytological phenomena of self-sterility of angiosperms. Sears explained that incompatibility can occur in the stigma, style or ovary. In the stigma, incompatibility is expressed as a decrease in pollen

germination. If the response is in the style, germination is normal, but pollen tube growth is inhibited in a variety of ways. The pollen tubes may grow all the way to the ovary but the egg may still not be fertilized as a result of incompatibility.

Since pollen grains will germinate on sugar or agar solutions, it can be assumed that pollen does not require a specific stimulating substance. Thus, if viable pollen does not germinate, the stigma must possess some inhibiting substance. If the stigma is removed, incompatible pollen will germinate, indicating that the reaction is restricted to the stigmatic surface. Likewise, Sears observed that if the style was excised as well, incompatible pollen can still contribute gametes that will fertilize the female sporophyte. So, in the first class of incompatible inhibition, pollen germination plays the major role in incompatible behavior.

In investigating the second class of incompatibility where pollen tubes are inhibited in the style, Sears (1937) made an S_1S_2 X S_1S_3 cross. Upon observing the styles, a normal distribution of pollen tubes was observed with many tubes growing all the way down and the rest of the tubes stopping at varying lengths, most ending near the stigma. Using the oppositional factor hypothesis, Sears assumed that the long tubes were ones carrying the compatible S_3 gene (unlike S_1 or S_2) and the short tubes were incompatible, carrying an S_1 gene that reacted against the S_1 gene in the stylar material. Morphological differences between compatible and incompatible pollen tubes were observed. Many of the incompatible pollen tubes had abnormally thick walls or the tips were swollen in a bulbous shape.

Using seed set as a criterion to determine compatibility can be unreliable because seed set can be induced or be apomictic (Emerson, 1938). Examination of pollen tube behavior directly in the style could be a more definitive test and eliminates the need for progeny tests.

Emerson, in 1940, did a very interesting experiment involving grafting styles of Oenothera organensis and made some enlightening observations. The response of pollen tubes with differing genetic makeup depended on the genetic makeup of the style only and was not influenced by the type of ovary present. Yasuda (1934) proposed that the ovary produced substances that diffused from the placental region and exerted an influence on the pollen tubes. However, from Emerson's observations, it appears that this is not the case.

To this point, most attention has been given to pollen tube behavior in self or intraspecific pollinations. What about interspecific pollinations? Roy (1939) made crosses between two species, Prunus divaricata var. Myrobalan yellow (a diploid) and Prunus domestica var. Victoria (a hexaploid). After 7 days, some interesting observations were made. When the diploid was pollinated by the hexaploid, about 6 percent of the blossoms matured to fruit. When the hexaploid species was pollinated by the diploid, 15 percent matured. Roy assumed that because the style length of the diploid was almost twice that of the hexaploid, the pollen tubes of the diploid must have travelled twice as fast as those of the diploid gametophyte. The causes of the slower growth rate of the hexaploid pollen tubes in the diploid style as compared to the diploid in a hexaploid were not explained.

Brewbaker (1957) classified incompatibility of plants in two

broad classes, heteromorphic and homomorphic. Heteromorphic incompatibility is simply expressed in terms of different forms of floral morphology which can physically impede pollen deposition on the stigma. Homomorphic incompatibility is an internal genetically controlled mechanism. The homomorphic class consists of gametophytic and sporophytic types, both of which are based on the action and interaction of multiple alleles for sterility.

In order to explain the "oppositional factor" scheme of inheritance, the following points must be considered. There is a single series of alleles that control compatibility in cross or self-pollinations. If a pollen grain has any one of these alleles in common with the style, inhibition takes place. This gives all plants the potential for being self-incompatible unless homozygotes are produced by some device for breaking down the incompatibility barrier (East & Mangelsdorf, 1926). Since the incompatibility reaction is determined by the allele carried in the pollen, its behavior is termed gametophytic. In the gametophytic system, the incompatibility reaction occurs during some stage of pollen tube growth or fertilization. Behavior is determined by the five alleles present or not present in the pollen grain. Thus, it is gametophytically determined.

Hughes and Babcock (1950) identified a system that is different from that described above. In Crepis, unlike Nicotiana species, all families do not react identically toward their parents. In Nicotiana, under the gametophytic system, most species are cross-incompatible within the group and compatible with species of other groups. In Crepis, species may be compatible with species of its own group as well as with

species of a variety of other groups. Crepis is similar to Nicotiana in that probably only one allele in common is sufficient to cause incompatibility, but the difference in behavior is due to the allele being different. Hughes and Babcock state that the reciprocal differences in Crepis cannot be accounted for by gametophytic determination. All of the pollen from one individual in this system acts the same in that all of the pollen either fails to germinate or will not penetrate the stigma. Thus, the pollen behavior is determined by the genotype of the parent and not according to its own genotype, and is sporophytically controlled.

Bateman (1955) explains that in the sporophytic system, the incompatibility system is determined before meiosis and resides in a structure formed at an early stage of development. He explains also that the pollen wall is made from the cytoplasm of the pollen mother cell and if the incompatible mechanism is in the wall, there would be inhibition immediately when it came in contact with the stigmatic surface. But if the compatibility is gametophytically controlled, the incompatibility mechanism is determined after meiosis and the effects are not expressed until the gametophyte is at a certain stage of development, e.g., pollen tube growth in the style.

Heslop-Harrison (1975) developed the following schematic flow chart to describe sporophytic control (in: de Nettancourt, 1977, p. 72):

Diploid sporophytic parent of male gametophyte	Diploid sporophytic parent of female gametophyte
Synthesis of "recognition proteins" in the tapetum	Synthesis of "recognition proteins" in microbodies in the stigmatic papillae
Transfer to the axine during pollen maturation	Transfer to the stigmatic surface to contribute to the outer pelicle

POLLINATION

Interaction of the exine and pellicle recognition factors--activation of the male gametophyte and stigma papilla leading either to....

ACCEPTANCE REJECTION

A natural question to ask at this point is: How does the presence of the S gene actually inhibit pollen tube growth? Ascher (1966) explains that S genes can operate by controlling operons inside the pollen tube that regulate the metabolic rate. In gametophytic systems there are two operons controlling two different rates of metabolism and consequently pollen tube growth rates. The low velocity operon involves the metabolism of only pollen reserves and simple stylar components resulting in only slow constant growth typical to incompatible pollen tubes. The second, high velocity operon involves the activation of a different metabolic pathway that results in the typical rapid growth of compatible tubes.

It is believed that as a pollen tube grows down the style, stylar regulators controlled by S alleles diffuse into the pollen tube to interact with the products of the pollen regulators. If the two

regulators are the same, they combine to form a dimer represser which blocks, in some way, the high velocity system. The low velocity system continues, and the pollen tube grows at a slow rate until the pollen reserves are depleted and incompatibility results. Otherwise, as soon as the necessary stylar metabolites reach the tube, the high velocity system goes into effect (Heslop-Harrison, 1975).

Child (1966) indicates that whether or not fertilization takes place may well depend upon how long the ovule remains viable. The ovule tends to remain viable and receptive to fertilization for a limited time and if pollen tube growth is too slow due to incompatibility, temperature outside the optimum range, or other factors, it may not reach the ovule in time.

Studies Done in Various Pomological Crops

The type of self-incompatibility found in the Rosaceae family is the homomorphic type and gametophytic system (de Nettancourt, 1977, p. 16). There is variability in the behavior and fertilization capabilities depending upon the genetic factors involved in each species.

Fruit set in Malus sylvestris Mill. has been shown to depend on: (1) temperature, (2) ovule longevity (Child, 1966), (3) pollen viability, (4) the ability of the pollen tube to penetrate the loculus, and (5) the physiological condition of the ovule at the time of fertilization (Stott, 1972). Incompatibility in Pyrus communis L. as well as Malus sylvestris was determined by Modlibowska (1945) to be due to physiological reactions between the pollen tube and the stylar or ovarian tissues.

A common incompatibility response in Prunus salicina Landl. is the swelling of the tip of the pollen tube and the tube contents becoming separated from the pollen tube wall (Thiele & Strydom, 1964). Commercial varieties of Prunus avium L. are self-incompatible (Way, 1968) and as of 1968 there were 16 incompatibility groups based upon the S-gene combination being carried by the variety. Smole (1976) has assembled a large literature review on the genetics of incompatibility due to pollen tube inhibition as it relates to Prunus avium. In Prunus avium incompatible crosses and selfs usually have their pollen tubes inhibited in the loculus of many aborting embryos (Anvari & Stosser, 1978).

Raptopoulos (1941) indicates that induced tetraploid cherries are self-compatible, while triploids are not. The self-incompatibility in triploids was expressed as oppositional factors of incompatibility as well as high sterility of pollen and deformation of the styles.

Commercial varieties of Prunus amygdalus Batsch. are almost entirely self-incompatible. There have been some self-compatible cultivars discovered originating from the Apula region of Italy, but they have too many defects to be useful as commercial varieties. However, many are currently being used for breeding purposes (Grasselly & Olivier, 1976). After bloom the flower is only receptive for 3-4 days, so pollination must take place promptly. Exactly how long the flowers remain receptive depends upon the varieties involved (Griggs et al., 1975).

Both self-compatible and self-incompatible varieties of Prunus armeniaca L. are cultivated today. Many varieties are of high quality and would be used much more widely were it not for their

self-incompatible nature. The presence of self-compatibility can be traced back to the origin of the variety. Tunisian forms are universally self-compatible, while Spanish and North American forms show self-compatible features but still produce more fruit when cross-pollinated (Valdeyron & Crossa-Raynaud, 1956). In breeding trials by Kostina (1970), the self-unfruitfulness of European cultivars was confirmed, while cultivars of Middle-Asian and Irano-Caucasian groups tend to be self-incompatible. Kostina also indicates that hybrids of the two groups segregate out predictably. The rates were not given.

Of the more common varieties of Prunus armeniaca grown in North America, Perfection and Riland are self-incompatible, while Blenheim, Royal, Tilton, and Moorpark are self-compatible. All six are cross-compatible (Schultz, 1948). Schultz indicates that wind can cause the anthers to brush the stigma and result in good fruit set without insect vectors if the varieties are self-compatible.

Little has been published on pollen tube growth in Prunus armeniaca as it relates to incompatibility. Facteau and Rowe (1977) did a study on the effects of HF and HCl on pollen tube growth in vivo but did not relate it in any way to incompatibility. The only other study that this author knows of is a thesis done by Abdullah Farooq Lodhi (1962) at U.C., Davis, on the comparative cytology of self-compatible and self-incompatible apricots. In this study, Lodhi determined that there was evidence indicating that incompatibility is responsible for the higher degree of sterility in most members of F_1 seedlings of two apricot clones.

Microscopic Techniques Used in Studying Pollen Tubes

A variety of preparation sequences, stains, and light sources have been used in attempting to accurately determine how far pollen tubes have grown down the style. Early papers report using aceto-carmine stain in lactic acid (Chandler, 1931; Esser, 1955) which stains the pollen tubes a dark red, leaving the surrounding tissues a very light red. Buchholz (1931) used acid fuchsin in alcohol and cleared his material with lactic acid. Acidified aniline blue in combination with safranine has been used with questionable results (Dionne & Spicer, 1958; Nair & Narasimhan, 1963).

Datta and Naug (1967) compared cotton blue and carmine on a variety of plant materials. They found that with angiosperms, fixing the material in acetic-alcohol, clearing with lactic acid, and then staining with 1 percent cotton blue in lactophenol gave the best results. Apples, pears, and cherries have been observed successfully with resorcin blue in glycerin after killing and fixing the pistils by autoclaving in Na_2SO_3 (Ivanicka, 1977). The autoclaving also enables the tissue to be softened enough to be squashed under a cover slip, eliminating the need for dissecting out the center core of the style or sectioning with a microtome which is a very time-consuming process.

All of the techniques employed above utilize direct or phase incandescent light. Although the pollen tubes could be followed in some tissue, usually they were barely visible or confused with xylem elements. As early as 1957, Currier and Strugger reported that pollen tubes stained with water-soluble aniline blue in a phosphate buffer and

viewed with fluorescent light showed brightly in contrast to the other stylar tissues. This phenomenon was later explained by Eschrich and Currier (1964). Callose, a plant material found in many parts of the plant, when stained with water-soluble aniline blue and irradiated with ultra-violet light, fluoresces brightly. Pollen tubes contain a large amount of callose in their walls in addition to thick callose plugs which are spaced irregularly in the pollen tube itself. The combination of these two phenomena results in this technique being an excellent tool for observing pollen tube growth. Only water-soluble aniline blue produces the desired fluorescing response, not the alcohol-soluble type.

The same year that Currier and Strugger were using fluorescent light microscopy with callose of Allium cells, Muller-Stoll and Lerch (1957) were using a similar technique for studying callose. Martin (1959) observed pollen tubes of Lycopersicon by fixing the styles in FAA for 24 hours, rinsing with tap water, clearing with NaOH, rinsing, and staining with 0.1 percent aniline blue in 0.1N K_3PO_4. Majumder (1964) used the same technique with pineapple. Pollen tubes of rhododendron have been observed successfully by using the above technique (Kho & Baer, 1968). Tomer and Gottreich (1975) observed the fertilization process by dissecting the ovules out, fixing in glacial acetic acid, and clearing in NaOH, and staining in 0.2 percent aniline blue. The pollen tubes were also easy to observe.

Hurter (1976) has developed the technique for staining and observing pollen tubes of plums. He fixed and softened the styles by boiling them in 5.0 percent Na_2SO_3, then washed in water and stained

with 0.1 percent aniline blue in 0.1 percent K_3PO_4. Hurter (year unknown) also describes a technique for observing pollen tubes of Marianna plum rootstock. The results were very good, with the pollen tubes fluorescing a bright yellow-green and the background material remaining a dark green-blue. Bowerman (1976) used a similar technique in observing pollen tubes in Vaccinium, where only the concentration of the Na_2SO_3 has been altered with good success.

MATERIALS AND METHODS

Materials for this study were obtained from eight selections of apricot grown at the USDA-SEA Fruit Production Research Station in Fresno, California. K53-57, Castlebrite, B60-12, B69-85, and Flaming Gold were known to be self-compatible. The self-incompatible selections were B67-10, K113-40, and K55-39. The experiment was performed in 1978 and repeated in 1979.

As each selection reached the red-bud stage, branches approximately 1 meter long were cut and taken indoors and placed in glass jars full of tap water. In 1978, the branches were placed in a laboratory where the temperature ranged from about 20 to 22°C and the relative humidity ranged from 59 to 72%. Large pans of water were set out in an attempt to maintain the upper range of humidity because the pistils, when emasculated, were prone to drying and dropping off, reducing the sample size. In 1979, the branches were kept in a greenhouse, where the temperature ranged from 22 to 35°C and the relative humidity ranged from 42 to 55%. If a selection was in a more advanced stage of bloom than the others, the branches were stored in a bucket of water at about 2°C from 2 to 4 days to allow the other selections to catch up.

As each selection reached the popcorn stage, it was emasculated and the anthers from each were rubbed out to obtain the pollen. In addition to the emasculated androeciums, popcorn stage blossoms were picked from the parent tree and rubbed out to assure a good supply of pollen. The process of emasculation and pollen preparation took 2 days.

On the third day the pistils on all of the branches were pollinated by coating the tip of the finger with pollen dust and dabbing the stigmas.

Each selection was selfed with its own pollen. The four types of crosses made were: self-compatible x self-compatible, self-compatible x self-incompatible, self-incompatible x self-compatible, and self-incompatible x self-incompatible. The self-compatible x self-compatible crosses were: K53-57 x Castlebrite, B60-12 x B69-85, B60-12 x Castlebrite, and B69-85 x Flaming Gold. The self-compatible x self-incompatible crosses were: B60-12 x B67-10, B69-85 x K113-40, and B60-12 x K113-40. The self-incompatible x self-compatible crosses were: K55-39 x Flaming Gold, and B67-10 x Flaming Gold. The self-incompatible x self-incompatible cross was B67-10 x K113-40. Sixty pistils for each cross were pollinated.

Ten pistils were collected from each cross 1, 2, 3, 4, 6, and 10 days after pollination (P+1, 2, 3, 4, 6, 10, respectively) and placed in a 5.0 percent solution of Na_2SO_3 and autoclaved for 5 minutes at 121°C. The pistils were then transferred to fresh Na_2SO_3 and frozen. The pistils were kept frozen from 1 to 12 months until examined.

The pistils were allowed to thaw to room temperature before examination. The pistils were rinsed in H_2O and placed on a glass slide in a few drops of stain. The stain solution was 0.1g water-soluble aniline blue + 0.71g K_3PO_4 in 100ml H_2O. Occasionally, the stain solution would lose its characteristic deep blue color but was easily restored by adding a few drops of 10 percent HCl. This loss in color, though, did not greatly affect the staining ability. The epidermal layers and hairs were stripped off with a teasing needle and tweezers.

The exposed central portions of the pistils were then transferred to a new slide with a few drops of stain solution. Several stain concentrations and formulas were tried and the one used by Bowerman (1976) was found to give the best results.

After arranging the pistils on the slide, a cover slip was gently lowered onto the pistils. The pistils were squashed by carefully pressing the cover slip with the wood end of the teaser. An oversized slide and cover slip were used to allow viewing several pistils at once. A few semi-permanent slides were made by sealing the edges of the cover slip with parawax.

The pollen tubes were observed using a Leitz Dialux microscope, ultraviolet light source, UG-1 and BG-12 excitation filters, K510 barrier filter and 125X magnification. The optimum combination of filters was arrived at through trial and error.

If stained and squashed properly, the pollen tubes fluoresced a bright yellow-green and could be followed fairly well through the stylar tissue down to the ovary. The ovary tissue fluoresced a bright red compared to the stylar tissue which appeared a dull to bright olive-green. Often the pollen tubes were obscured in the stylar tissue which sometimes fluoresced too brightly and the actual lengths of the pollen tubes could only be estimated. Estimates of the pollen tube lengths were made by counting the number of fields of vision it took to encompass the entire length of the pollen tube. By dividing that value by the number of fields of vision it took to observe the whole style, a figure for the relative length of pollen tube growth down the style was established.

RESULTS AND DISCUSSION

The results of the microscopic examinations are reported in Table 1 and Table 2. The pollen tubes in the pistils were measured in each sample, beginning at P+1, until the average length equaled 95 percent of the style length or more, or until P+10, whichever came first (except in five of the 1978 crosses). When a pollen tube reached the ovary tissue, its length was assigned a value of 100 percent of the style's length.

As noted in the materials and methods section of this study, the pistils of some of the crosses were prone to drying up and falling off the branches. When this happened, fewer living pistils were left intact to dissect and examine. This was especially a problem in 1978 where the sample size of some crosses was severely reduced. In 1979 more pistils were pollinated than in 1978 in an attempt to compensate for this. Future users of this technique should consider adding a substance to the jars of water that is known to prolong the life of cut flowers (which is, in a sense, what these cut branches were).

The distance down the style that a pollen tube had grown was determined by the most proximal point where a pollen tube could be seen. This does not imply that there were not pollen tubes that went farther. If the pollen tubes fluoresced dimly, or the background stylar tissue showed too brightly, it was difficult to ascertain exactly where the pollen tubes ended. Often only the callose plugs fluoresced and sometimes the thin strands of pollen tube tissue that connected them. After viewing several styles with pollen tubes, the observer became

Table 1. Distance pollen tubes observed down styles of hand pollinated apricot blooms (1978).

Cross	Type		Time after pollination[z] P+1	P+2	P+3	P+4	P+6	P+10
B60-12 X[y]	SF[x] X	#[w]	4	7	8			
		d[v]	65	74	55			
		%[u]	25	57	88			
Flaming Gold X	SF X	#	8	8	14	7		
		d	41	92	87	93		
		%	0	60	69	86		
Castlebrite X	SF X	#	9	6	8	10	5	
		d	35	68	59	73	100	
		%	0	17	25	50	100	
K53-57 X	SF X	#	7	4	7			
		d	56	36	82			
		%	0	0	71			
B69-85 X	SF X	#	7	10				
		d	54	95				
		%	0	90				
B67-10 X	SIF[t] X	#	10	8	7	9	8	
		d	23	33	34	61	61	
		%	0	0	0	0	13	
K113-40 X	SIF X	#	3	6	3	8	5	8
		d	0	33	44	55	63	32
		%	0	0	0	13	20	0
K55-39 X	SIF X	#	7	8	10	8	8	11
		d	55	44	62	50	42	49
		%	0	0	20	0	13	0
B67-10 x K113-40	SIF x SIF	#	11	10				
		d	65	100				
		%	0	100				
K53-57 x Castlebrite	SF x SF	#	2	6				
		d	79	100				
		%	0	100				
B60-12 x Castlebrite	SF x SF	#	4	6				
		d	20	58				
		%	0	50				

Table 1. (Continued)

Cross	Type		Time after pollination P+1	P+2	P+3	P+4	P+6	P+10
B60-12 x B69-85	SF x SF	#	5	9				
		d	56	86				
		%	0	86				
B69-85 x Flaming Gold	SF x SF	#	8	8				
		d	52	100				
		%	0	100				
B60-12 x B67-10	SF x SIF	#	6	8	9			
		d	35	73	100			
		%	0	63	100			
B69-85 x K113-40	SF x SIF	#	9	10				
		d	55	100				
		%	0	100				
B60-12 x K113-40	SF x SIF	#	11	7				
		d	67	100				
		%	36	100				
K55-39 x Flaming Gold	SIF x SF	#	9	10	11			
		d	57	93	100			
		%	0	90	100			
B67-10 x Flaming Gold	SIF x SF	#	10	10				
		d	33	100				
		%	0	100				

[z]P+1, P+2, etc. represents 1 day, 2 days, etc. after pollen was applied to the pistils.

[y] X = selfed.

[x]SF = self-fertile.

[w]# = number of pistils examined in the sample.

[v]d = average length of pollen tube growth reported as percent of style length that the longest pollen tube was observed to reach.

[u]% = percentage of the pistils in the sample to have pollen tubes reach the ovary tissue.

[t]SIF = self-infertile.

Table 2. Distance pollen tubes observed down styles of hand pollinated apricot blooms (1979).

Cross	Type		Time after pollination[z] P+1	P+2	P+3	P+4	P+6	P+10
B60-12 X[y]	SF[x] X	#[w]	5	7	5			
		d[v]	69	94	100			
		%[u]	0	88	100			
Flaming Gold X	SF X	#	10	10				
		d	69	99				
		%	10	90				
Castlebrite X	SF X	#	10	9				
		d	74	98				
		%	70	89				
K53-57 X	SF X	#	4	5	4			
		d	60	59	100			
		%	0	40	100			
B69-85 X	SF X	#	7	10				
		d	70	100				
		%	57	100				
B67-10 X	SIF[t] X	#	10	10	8	9	5	3
		d	55	78	74	77	47	65
		%	0	0	25	22	0	0
K113-40 X	SIF X	#	4	8	8	7	6	1
		d	35	84	47	69	45	
		%	0	63	13	14	17	
K55-39 X	SIF X	#	7	9	9	9	9	9
		d	42	54	57	63	45	64
		%	0	22	0	0	10	10
B67-10 x K113-40	SIF x SIF	#	5	6	8			
		d	83	94	97			
		%	20	83	89			
K53-57 x Castlebrite	SF x SF	#	10	8	7	7		
		d	30	87	93	100		
		%	30	67	71	100		
B60-12 x Castlebrite	SF x SF	#	7	9				
		d	96	97				
		%	71	89				

Table 2. (Continued)

Cross	Type		Time after pollination P+1	P+2	P+3	P+4	P+6	P+10
B60-12 x B69-85	SF x SF	#	4	8				
		d	79	100				
		%	25	100				
B69-85 x Flaming Gold	SF x SF	#	9	5				
		d	90	100				
		%	13	100				
B60-12 x B67-10	SF x SIF	#	2	2	7			
		d	70	100				
		%	50	100				
B69-85 x K113-40	SF x SIF	#	10	9				
		d	92	100				
		%	20	100				
B60-12 x K113-40	SF x SIF	#	7	11				
		d	66	100				
		%	14	100				
K55-39 x Flaming Gold	SIF x SF	#	7	7				
		d	76	100				
		%	13	100				
B67-10 x Flaming Gold	SIF x SF	#	5	7				
		d	90	99				
		%	40	89				

[z]P+1, P+2, etc. represents 1 day, 2 days, etc. after pollen was applied to the pistils.

[y] X = selfed.

[x]SF = self-fertile.

[w]# = number of pistils examined in the sample.

[v]d = average length of pollen tube growth reported as percent of style length that the longest pollen tube was observed to reach.

[u]% = percentage of the pistils in the sample to have pollen tubes reach the ovary tissue.

[t]SIF = self-infertile.

"trained" to discern between callose plugs and other artifacts that glowed similarly. The path of the pollen tubes could often be determined by following the callose plugs. Since the ovary tissue fluoresced a bright red and is granular in appearance, the pollen tubes contrasted sharply. It was obvious if a pollen tube had entered the ovary tissue or not, but the same cannot be said for how far a pollen tube went through the style. That is why both values are given for each cross.

An examination of the tables shows that pollen tubes resulting from self-compatible selves and all of the crosses reached the base of the style by P+2, 3, or 4. The self-incompatible self, however, either never reached the base of the style, or reached the base after 6 days. Occasionally, a pistil would have a pollen tube that reached the base but none in succeeding days. Consideration of the number of pistils examined should be concomitant to examining the percentage to reach the base. If the number of pistils examined is small, a single pistil showing a 100 percent value can give a deceivingly high percentage value.

During the pollination process, it is very easy to contaminate a cross with the pollen from previous crosses, especially if one does not wash his hands between crosses. When a self-incompatible self showed pollen tubes reaching the base while the average length was still low or when none was observed in following days, it was judged to be due to either contamination or a phenomenon of self-incompatible selves. If we were to assume that all of the selections were cross compatible, any stray pollen from another selection would grow down the style and fertilize a selfed self-incompatible selection, making it appear to be self-compatible. During the pollination process, the branches being

treated should be isolated from the rest to reduce the chance of contamination.

Due to bad weather, attempts to bag blossoms on the parent trees to see if they behaved normally that year failed. In an attempt to see if self-compatibility or self-incompatibility is transmittable, 40-100 of the progenies of the crosses were also bagged in the field, with similarly disappointing results. The paper bags were either torn off, torn open, or knocked around by the wind so much that the delicate flowers were knocked off to give a no-set value.

There are bold differences between the self-incompatible selves, self-compatible selves, and all of the crosses. Therefore, I believe that when considering pollination, apricots do behave predictably. The fact that self-incompatible x self-incompatible crosses behave similarly to self-compatible selves suggests that crossing in apricot removes the self-incompatibility reaction and that there is very little cross-incompatibility. This is only of academic interest since our goal is to screen for self-compatibility as an existing trait.

In evaluating this experimental procedure as a screening technique, a few problems are evident and need to be worked out. The microscopic technique needs to be developed further to make viewing the pollen tubes easier and more definite. This would eliminate much of the guesswork in estimating the position of the pollen tubes and enable a person with less training to do the screening. The conditions under which the branches were kept needs to be maintained at a moderate temperature and fairly high humidity.

The advantages of this system could be summarized thus:

though we cannot accelerate much when the trees first bear enough flowers, once the tree does reach popcorn stage, the whole process can be initiated and data collected within 15 days. Using the bagging system, a few weeks must pass before fruit set can be evaluated. Since we have eliminated the problems associated with tying a bag over the blossoms, the lab technique is in this respect more reliable. Since the crosses are done inside, we are not subject to weather variation. If the selection fails to set fruit in the field, we did not discover exactly why. It may be due to the bag knocking the flower off. But if the pollen tubes are behaving in an incompatible manner, the range of causes are narrowed considerably. And, finally, the whole system requires only a moderate amount of training.

The disadvantages of this system are easy to identify. Careful timing is required to assure that everything is done in accordance with the developmental age of the flower. A high level of sanitation to guard against stray pollen grains must be maintained. Adequate greenhouse or lab space must be available where the branches can be undisturbed. The microscopic technique requires a few days of practice to develop a proficiency in preparing the pistils and in viewing the pollen tubes. Also, the microscope used is rather complicated and very expensive, thereby limiting the number of people who can use this system. If an alternate staining technique could be developed to use incandescent light successfully, a conventional microscope could be used.

In summary, microscopically testing for self-compatibility shows great promise. Most of the problems and disadvantages can be

worked out in time. Since breeding tree crops is a slow and expensive process, anything that would speed the process and make the screening process more reliable would be of great value.

BIBLIOGRAPHY

Afify, A. 1933. Pollen tube growth in diploid and polyploid fruits. J. Pomol. & Hort. Sci. 11:113-119.

Anderson, E. and K. Sax. 1934. A cytological analysis of self-sterility in Tradescantia. Bot. Gaz. 96:607-621.

Anvari, S. F. and R. Stosser. 1978. Study of pollen tube growth and state of ovules in sour cherries under the fluorescent microscope. Mitteilungen Klosterneuburg Rebe und Wein, Obstbau und Fruchteverwertung 28(1):23-30.

Ascher, P. D. 1966. A gene action model to explain gametophytic self-incompatibility. Euphytica 15:179-183.

_____. 1975. Special stylar property required for compatible pollen tube growth in Lilium longiflorum Thunb. Bot. Gaz. 136:317-321.

_____. 1977. Localization of the self and the interspecific-incompatibility reactions in style sections of Lilium longiflorum. Plant Science Letters 10:199-203.

_____ and S. J. Peloquin. 1968. Pollen tube growth and incompatibility following intra- and inter-specific pollination in Lilium longiflorum. Amer. J. Bot. 55:1230-1234.

_____ and _____. 1970. Temperature and the self-incompatibility reaction in Lilium longiflorum Thunb. J. Amer. Soc. Hort. Sci. 95(5):586-588.

Bateman, A. J. 1955. Self-incompatibility systems in angiosperms. III. Cruciferae. Heredity 48:53-68.

Bowerman, E. H. 1976. The development of the male gametophyte in situ under selfing and cross-pollination in six genotypes of tetraploid Vaccinium. Dissertation Abstracts International 39:4778-B.

_____ and G. Jelenkovic. 1976. The development of the male gametophyte in situ under selfing and cross-pollination in six genotypes of tetraploid Vaccinium. Fruit Var. J. 30:31.

Brewbaker, J. L. 1957. Pollen cytology and self-incompatibility systems in plants. J. Hered. 48:271-277.

Brown, A. G. 1955. Incompatibility. John Innes Inst. Ann. Rept. 1954: 7-8.

Buchholz, J. T. 1931. The dissection, staining and mounting of styles in the study of pollen tube distribution. Stain Tech. 6:13-24.

_____ and A. F. Blakeslee. 1922. Studies of the pollen tubes and abortive ovules of the globe mutant of Datura. Science 55:597-599.

_____, L. F. Williams, and A. F. Blakeslee. 1935. Pollen tube growth of ten species of Datura in interspecific pollinations. Proc. Nat. Acad. Sci. 21:651-656.

Burden, M. 1979. Univ. Calif., Davis. Personal communication.

Chandler, C. 1931. A method for staining pollen tubes within the pistil. Stain Tech. 6:25-26.

Child, R. D. 1966. Pollen tube growth in relation to temperature and ovule longevity in the cider apple Michelin. A. R. Long Ashton Research Station, Univ. of Bristol, pp. 115-134.

Company, R. S. I., D. E. Kester, and M. V. Bradley. 1976. Effects of temperature and genotype on pollen tube growth in some self-incompatible and self-compatible almond cultivars. J. Amer. Soc. Hort. Sci. 101(5):490-493.

Crane, M. B. 1931. Sterility and incompatibility in diploid and polyploid fruits. J. Genet. 24:97-107.

_____ and A. G. Brown. 1937. Incompatibility and sterility in sweet cherry, Prunus avium L. J. Pomol & Hort. Sci. 15:86-116.

_____ and W. J. C. Lawrence. 1929. Genetical and cytological aspects of incompatibility and sterility in cultivated fruits. J. Pom. & Hort. Sci. 7:276-301.

Crossa-Raynaud, P. 1957. New observations on self-compatibility in the apricot. Annl. Serv. Bot. Agron. Tunisia 30:33-43.

Crowe, L. K. 1964. The evolution of outbreeding in plants. I. The angiosperms. Heredity 19:435-457.

Currier, H. B. 1957. Callose substances in plant cells. Amer. J. Bot. 44:478-488.

_____. 1979. Personal communication.

_____ and S. Strugger. 1957. Aniline blue and fluorescence microscopy of callose in bulb scales of Allium cepa L. Protoplasma 44: 552-559.

Datta, P. C. and A. Naug. 1967. Staining pollen tubes in the style; cotton blue with carmine for general use. Stain Tech. 42:81-85.

de Nettancourt, D. 1972. Self-incompatibility in basic and applied researches with higher plants. Genetica Agraria 26:163-216.

_____. 1977. Incompatibility in angiosperms. Springer-Verlag, New York, p. 72.

_____, R. Ecochard, M. D. G. Perquin, T. van der Drift, and M. Westerhof. 1971. The generation of new S alleles at the incompatibility locus of L. peruvianum Mill. Theoret. Appl. Genetics 41: 120-129.

Dionne, L. A. and P. B. Spicer. 1958. Staining germinating pollen and pollen tubes. Stain Tech. 33:15-17.

East, E. M. 1934. The reaction of the stigmatic tissue against pollen tube growth in selfed self-sterile plants. Proc. Nat. Acad. Sci. 20:364-368.

_____ and A. J. Mangelsdorf. 1925. A new interpretation of the hereditary behavior of self-sterile plants. Genetics 11:166-171, or Proc. Nat. Acad. Sci. 11:166-171.

_____ and _____. 1926. Studies on self-sterility. VII. Heredity and selective pollen tube growth. Genetics 11:460-481.

Eaton, G. W. and A. M. Jamont. Embryo sac development in the apricot, Prunus Armeniaca L. cv Constant. Proc. Amer. Soc. Hort. Sci. 87: 95-101.

Einset, J. 1944. Cytological basis for sterility in induced autotetraploid lettuce, Lactuca sativa L. Amer. J. Bot. 31:336-342.

Emerson, S. 1938. The genetics of self-incompatibility in Oenothera organensis. Genetics 23:190-202.

_____. 1940. Growth of incompatible pollen tubes in Oenothera organensis. Bot. Gaz. 101:890-911.

Eschrich, E. and H. B. Currier. 1964. Identification of callose by its diachrome and fluorochrome reactions. Stain Tech. 39:303-307.

Esser, K. 1955. Pollen tubes in the style after chloral hydrate and aceto-carmine. Stain Tech. 30:195-197.

Facteau, T. J. and R. E. Rowe. 1977. Effect of hydrogen fluoride and hydrogen chloride on pollen tube growth and sodium fluoride on pollen germination in Tilton apricot. J. Amer. Soc. Hort. Sci. 102(1): 95-96.

Fassuliotis, G. 1977. Self-fertilization of Cucumis metuliferus Naud. and its cross-compatibility with C. melo L. J. Amer. Soc. Hort. Sci. 102(3):336-339.

Flaschnriem, D. R. and P. D. Ascher. 1979. Pollen tube expression of pseudo-self-compatibility (PSC) in Petunia hybrida. Theor. Appl. Genet. 54:97-101.

Frankel, R. and E. Galun. 1977. Pollination mechanisms, reproduction and plant breeding. Springer-Verlag, New York.

Gerstel, D. U. 1950. Self-incompatibility studies in guayule. II. Inheritance. Genetics 35:482-505.

_____ and M. E. Riner. 1950. Self-incompatibility studies in guayule. I. Pollen tube behavior. J. Heredity 41:49-55.

Grasselly, C. and G. Olivier. 1976. Mise en evidence de quelques types autocompatibles parmi les cultivars d'amandier (P. amygdalus Batsch) de la population des pouilles. (Demonstration of some compatible types amongst the almond tree varieties.) Ann. Amelior. Plantes 26(1):107-113.

Griggs, W. H., B. T. Iwakiri, and M. V. Bradley. 1975. Pollen tube growth in almond flowers. Calif. Agric. 29(7):4-7.

Grun, P. and M. Aubertin. 1966. The inheritance and expression of unilateral incompatibility in Solanum. J. Heredity 21:131-138.

Haasbroek, F. J. and N. Hurter. 1969. The use of ^{32}P-labelled pollen for studies of pollen tube growth in plum. Agroplantae 1:195-198.

Hall, A. D. and M. B. Crane. 1933. The apple. Chap. IV, Fertilization, sterility, and incompatibility. Hopkinson, Martin, Ltd., London.

Henry, R. J. and P. D. Ascher. 1973. Effect of auxin (3-indoleacetic acid) on in vivo compatible and incompatible pollen tube growth in detached styles of Lilium longiflorum Thunb. Incompatibility Newsletter 3:14-17.

_____. 1975. Kinetin removes self-incompatibility reaction in detached styles of Lilium longiflorum Thunb. HortSci. 10:25-26.

_____. 1976. The inheritance of pseudo-self-compatibility in Nemesia strumosa Benth. Theoret. & Appl. Genetics 48:185-195.

_____. 1977. Sporophytic recognition of pollen S alleles in the gametophytic self-incompatibility system of Nemesia strumosa Benth. Theoret. & Appl. Genetics 49:15-19.

Heslop-Harrison, J. 1975. Incompatibility and the pollen stigma interaction. Ann. Rev. Plant Physiol. 26:403-425.

Hughes, M. B. and E. B. Babcock. 1950. Self-incompatibility in Crepis foetida (L.) subsp. rhoeadifolia (Bieb) Schiz et Keller. Genetics 35:570-584.

Hurter, N. 1976. Technique for observing pollen tubes in squashed styles from plums under fluorescent light. Personal letter to Dr. David Ramming, USDA-SEA, Fresno.

_____. Year unknown. Pollen tube development in pistils of the Marianna plum rootstock. Portion of a dissertation submitted to the Univ. of Stellenbosch for Ph.D.

Ivanicka, J. 1977. Staining pollen tubes in the styles of fruit species by resorcin blue. Biologia 32:289-292.

Jefferies, C. J. and A. R. Belcher. 1974. A fluorescent brightener used for pollen tube identification in vivo. Stain Tech. 49: 199-202.

Jensen, W. A. 1962. Botanical Histochemistry. Chap. IX. Carbohydrates and cell wall constituents (callose-aniline blue-fluorescence method), pp. 203-204. W. H. Freeman, San Francisco.

Kho, Y. O. and J. Baer. 1968. Observing pollen tubes by means of fluorescence. Euphytica 17:298-302.

_____ and _____. 1970. A microscopical research on the incompatibility in the cross Rhododendron impeditum x R. williamsianum. Euphytica 19:303-309.

Kostina, K. F. 1970. Investigations on apricot self-pollination. Ialta, Crimea, Gosudarstvennyi Nikitskii Opytnyi Botanicheskii 45:7-17.

Lawrence, W. J. C. 1930. Incompatibility in polyploids. Genetica 12:269-294.

Lewis, D. 1943. Physiology of incompatibility in plants. III. Autopolyploids. J. Genetics 45:171-185.

Lin, J., W. J. Uwate, and V. Stallman. 1977. Ultrastructural localization of acid phosphatase in the pollen tube of Prunus avium L. (sweet cherry). Planta 135:183-190.

Lodhi, A. F. 1962. Comparative cytology of self-compatible and self-incompatible apricots. Master's Thesis. Univ. Calif., Davis.

Majumder, S. K. and J. L. Brewbaker. 1964. Fluorescence microscopy with aniline dye in studies of fertilization and infection. Amer. J. Bot. 51:663 (Abstr.)

Martin, F. W. 1959. Staining and observing pollen tubes in the style by means of fluorescence. Stain Tech. 34:125-128.

_____. 1961. The inheritance of self-incompatibility in hybrids of Lycopersicum esculentum Mill x L. chilense Dun. Genetics 46:1443-1454.

Mellenthin, W. M., C. Y. Wang, and S. Y. Wang. 1972. Influence of temperature on pollen tube growth and initial fruit development in 'd'Anjou' pear. HortSci. 7:557-559.

Modlibowska, I. 1945. Pollen tube growth and embryo sac development in apples and pears. J. Hort. Sci. 21:57-89.

Muller-Stoll, W. R. and G. Lerch. 1957. Uber Nachweis, Entstehung und Eigenschoften der Kallosebildugen usw. (Untranslated) Flora 144: 297-334.

Nair, M. K. and R. Narasimhan. 1963. Acidified safranin analine blue; a selective stain for pollen tubes of sugar cane. Stain Tech. 38: 341-342.

Ńebel, B. R. 1931. Lacmoid-martius-yellow for staining pollen tubes in the style. Stain Tech. 6:27-29.

Pandey, K. K. 1959. Mutations of the self-incompatibility gene (S) and pseudo-compatibility in angiosperms. Lloydia 22:222-234.

_____. 1962. A theory of S gene structure. Nature 196:236-238.

_____. 1965. Centric chromosome fragments and pollen-part mutation of the incompatibility gene in Nicotiana alata. Nature 206:792-795.

_____. 1967. Origin of genetic variability: Combinations of peroxidase isozymes determine multiple allelism of the S gene. Nature 213:669-672.

_____ and R. D. Henry. 1959. Use of pectinase in pollen tube and pollen mother cell smears. Stain Tech. 34:19-22.

Peterson, C. A. and R. A. Fletcher. 1973. Lactic acid clearing and fluorescent staining for demonstration of sieve tubes. Stain Tech. 48(1):23-27.

Ramanna, M. S. 1973. Euparal as a mounting medium for preserving fluorescence of aniline blue in plant material. Stain Tech. 48(3): 103-105.

Ramming, David W. 1972. Pollen tube growth and development in relation to chemical thinning of pecan nuts. Master's thesis, Oklahoma State University.

_____, H. A. Hinrichs, and P. E. Richardson. 1973. Sequential staining of callose by aniline blue and lacmoid for fluorescence and regular microscopy on a durable preparation of the same specimen. Stain Tech. 48(3):133-134.

Raptopoulos, T. 1941. Pollen tube growth studies in cherries. J. Genetics 42:73-90.

Roy, B. 1939. Studies on pollen tube growth in Prunus. J. Pomol. 16:320-328.

Schultz, J. H. 1948. Self-incompatibility in apricots. J. Amer. Soc. Hort. Sci. 51:171-174.

Sears, E. R. 1937. Cytological phenomena connected with self-sterility in the flowering plants. Genetics 22:130-181.

Smole, J. 1976. Self-sterility and intersterility among sweet cherries (Prunus avium) as a consequence of incompatibility--A review. Pl. Breed. Abstr. 48(3).

Stott, K. G. 1972. Pollen germination and pollen tube characteristics in a range of apple cultivars. J. Hort. Sci. 47:191-198.

Stout, A. B. 1931. Pollen tube behavior in Brassica pehinensis with reference to self-incompatibility in fertilization. Amer. J. Bot. 18:686-695.

_____ and C. Chandler. 1933. Pollen tube behavior in Hemerocallis with special reference to incompatibilities. Bull. Torrey Bot. Club 60:397-416.

Suss, J. and J. Tupy. 1976. On the nature of RNA synthesized in pollen tubes of Nicotiana alata. Biologia Plantarum 18(2):140-146.

Thiele, I. and D. K. Strydom. 1964. Incompatibility studies in some Japanese plum cultivars (Prunus salicina, Lindl.) grown in South Africa. S. Afr. J. Agric. Sci. 7:165-168.

Tomer, E. and M. Gottreich. 1975. Observations on the fertilization process in avocado with fluorescent light. Euphytica 24:531-535.

Valdeyron, G. and P. Crossa-Raynaud. 1956. Contributions to the study of the system of fertilization in the apricot. Aml. Inst. Natn. Rech. Agron., Ser. B. 6:217-238.

Watson, D. P. 1951. Staining pollen tubes of apple. Stain Tech. 26: 273.

Way, R. D. 1968. Pollen incompatibility groups of sweet cherry clones. J. Amer. Soc. Hort. Sci. 92:119-123.

Yasuda, S. 1934. Physiological research on self-incompatibility in Petunia violacea. Bull. Imp. Coll. Agric. For., Morioka, Japan. 20:1-95.