STUDIES OF INCOMPATIBILITY IN THE SWEET POTATO AND OTHER *IPOMOEA* SPECIES

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INTRODUCTION

Sweet potato breeding is a major phase of the vegetable improvement work in the Department of Horticulture of the Oklahoma Agricultural Experiment Station.

Two difficult problems are confronting present day sweet potato breeders: (a) non-flowering characteristics of many sweet potato varieties and certain breeding lines and (b) incompatibilities and low fertility when flowering occurs.

The problem at the present time of securing flowering in sweet potatoes is of less importance since many lines flower freely under Oklahoma conditions. Developed methods of greenhouse management (12) and under outdoor conditions (24) were reported to have resulted in a satisfactory flowering of a number of varieties. Special grafting techniques on certain morning glory stocks, which presumably produce flowering hormones, were developed in a study at the Oklahoma Station by Lam and Cordner (20).

The problem of low fertility and the production of limited numbers of viable seeds impose certain major restrictions on the sweet potato breeding program; hence, all information that aids in circumventing these barriers to seed production is of special value.

The major objectives of this study were:

A. To test the hypothesis that incompatibility in sweet potatoes is a gene controlled physiological factor which prevents the growth and development of pollen tubes in the stigma or style.

B. To observe pollen tube behavior in certain self-incompatible, self-compatible, and cross-incompatible pollinations.

C. To develop techniques that may help circumvent incompatibility in stigma and style: (a) style grafting technique and (b) pollen injection technique.

D. To try to obtain fertile crosses between the sweet potato and a diploid morning glory, which would substantiate the theory that the sweet potato originated from the morning glory.

E. To test the value of the hormone para-chlorophenoxy acetic acid and also bud-pollination, in circumventing self-incompatibility in sweet potatoes, and cross-incompatibility between different species of Ipomoea.

REVIEW OF LITERATURE

Incompatibility in Plants

Gardner, Bradford, and Hooker (16) in a review of the internal factors associated with unfruitfulness in plants classified the causes of sterility as: (a) due to evolutionary tendencies; that is, heterostylism, differences in relative lengths of the styles and stamens; dichogamy, sexes maturing at different times; impotence from aborted pistils or ovules; impotence of pollen; (b) due to genetic influences; (c) due to physiological influences; that is, slow growth of pollen tube; premature or delayed pollination; nutritive conditions within the plant which may affect pollen viability or cause defectiveness of pistils.

Crane and Lawrence (9) made a distinction between incompatibility and sterility. Incompatibility is the result of some physiological hindrance to fertilization. The pollen and ovules, or at least a substantial portion of them, are functional, the failure to obtain seed being due to slow pollen-tube growth. Sterility was classified into (a) generational sterility, due to the failure of any of the processes concerned with the normal alternation of generations, namely, development of pollen, embryo-sac, embryo, and endosperm, and the relation of these to one another regardless of the cross made, and (b) morphological sterility due to suppression or abortion of the sex organs.

Hayes, Immer, and Smith (17) reported that East and co-workers provided the first evidence of a series of self-sterility alleles. According to the oppositional factor hypothesis, the genes responsible for self and cross-incompatibility belong to several different allelic series of factors, such as S_1, S_2....Sn, and Z_1, Z_2....Zn which affect pollen tube growth, and like other alleles two factors may be carried by a single diploid plant. A pollen tube is inhibited in a style which has in homozygous or heterozygous condition the same S factor or factors carried by the tube.

Kakizaki (18) proposed a theory of other series of stimulating factors such as F_1, F_2....Fn. A pollen tube carrying an S factor which is present in the style in heterozygous condition is compatible if it carries an F factor for which the style is homozygous.

Sears (29) classified incompatibility according to where the reaction occurs: (a) before the pollen germinates, a tendency toward localization of the incompatibility reaction in the stigma accompanied by a lack of stigmatic fluid; (b) when the tube reaches the ovule, the integuments of the ovule are concerned in the incompatibility reaction; and (c) while the pollen tube is growing in the style, the incompatibility reaction occurs in the stigma or in various parts of the style.

Hayes, Immer, and Smith (17) reported that in many species there is a rather clear demarcation between self-compatibility and self-incompatibility. In other cases the differences are not so clear-cut, and there may be a continuous graduation from self-fertility to self-sterility due to a series of causes that in some cases may be the result of polyploidy and the duplication of several series of sterility alleles.

East (11) suggested that substances in the stylar tissue of Nicotiana react with substances in the pollen tube to retard the rate of the pollen tube growth in self-incompatible combinations. He further suggested that, in most plants, these substances are not present in the young bud but appear during the twenty-four hour period preceding the opening of the flower. In self-sterile genotypes which were self-fertile when pollinated twenty-four to forty-eight hours before the flowers open, it was presumed that these substances were produced during the twenty-four hour period preceding flower opening. In another type in which self-sterile plants were self-fertile at the end of the flowering period it was presumed that these plants were unable to produce an adequate amount of the inhibiting substance late in the flowering period.

Yasuda (37) believed the inhibitory substances to originate in the ovule of the petunia, from which they may ascend to the stigma, depending on genetic differences in the plants and the environmental conditions under which they are grown. If they reach the stigma, they may inhibit germination of the pollen grains. If they only reach the style, they may inhibit pollen tube growth in the stylar region, and in some cases these substances remain in the ovary and inhibit pollen tube growth at this point. The suggestion is made also that weak self-fertility may be the result of a low production of these inhibitory substances.

Cooper and Brink (5) found that 14.6 per cent of self-pollinated ovules of seven alfalfa plants were fertile, whereas 66.2 per cent of cross-pollinated ovules led to fertilization. A low degree of fertilization under conditions of self-pollination was believed to be explained primarily on the basis of the oppositional-factor hypothesis. Of the ovules that became fertile, 34.4 per cent containing inbred embryos and endosperms collapsed within six days after fertilization; in the cross-pollinated plants, only 7.1 per cent containing hybrid endosperms and embryos collapsed. The collapse of ovules during the early development stages after fertilization has been called somatoplastic sterility.

Cooper and Brink further explained that like conditions in the ovule outside the embryo sac in these two cases makes it clear that the higher survival following crossing is the result of the more active growth of hybrid endosperm. Conversely, following self-fertilization, the rate of growth of the endosperm is frequently so low that the balance soon shifts in favor of the integuments.

Other cases of species crosses were also noted by Cooper and Brink where fertilization had taken place but the ovules collapsed soon after fertilization because of slow growth of the endosperm and lack of nutrients for the growing embryo. It seems probable that this may result from causes similar to those responsible for the reduction of vigor in selfed lines of plants normally cross-pollinated. If one accepts the Mendelian explanation of heterosis, it seems probable that the early collapse of ovules after fertilization is due primarily to genetic causes.

Incompatibility in Sweet Potato

Stout (30) reported that the sweet potato has been propagated by vegetative means for a period of at least 400 years. The varieties grown have not been subjected to selection for the production of flowers, fruit, or seed. These conditions have given full opportunity for the persistence of any type of sterility which may have existed in the original seedlings from which the clonal varieties now cultivated developed or of any type of sterility which may have developed later.

Stout further stated that when seed has been obtained, there usually has been opportunity for cross-pollination. He suggested some type of sterility operating which limits self-fertilization and which makes proper cross-pollination necessary for the formation of fruit. He also suggested that there is functional or physiological incompatibility between the two kinds of sex organs involved in the process of fertilization.

In further notes Stout (31) reported that the sweet potato is self-incompatible and to some extent cross-incompatible.

Investigations by Cordner and co-workers over a period of several years (7) have shown that approximately half of the parents investigated were completely self-incompatible. Parent 47 and its derivatives made up a good part of this group of self-incompatible lines. The remainder of the parents investigated showed different degrees of self-compatibility. Parent 38 and some of its derivatives ranked high in self-compatibility with a maximum fruit set of 60 per cent for parent 38.

Limited observations by LaVine (22) were made of pollen germination and pollen tube growth, following selfing of the self-incompatible sweet potato breeding lines 47 and 64. In these, few, if any, pollen tubes were observed to penetrate to the style. It was suggested further that this condition is possibly due to the presence of gene controlled chemical substances in the stigma and/or the style which strongly retarded the growth of pollen tubes.

Warmke and Cruzado (35) reported that the Jersey varieties of sweet potato, as a whole, set 11.21 seeds per 100 crosses when used as the female parent and 7.26 seeds per 100 crosses when used as the male parent. The moist-flesh varieties, as a group, set 15.46 seeds per 100 crosses when used as female parents and 29.99 seeds per 100 crosses when used as male parents. They also reported that the percentage of seed set at the beginning and end of the flowering season was low as compared to the set during the middle of the flowering season.

Montelaro and Miller (25) reported that there was an observed decline in sweet potato seed set during very hot or very cold periods, with the highest seed set during a period when the mean temperature was near 75° F. They further added that temperature did not influence seed set as seriously as did certain genetically controlled physiological disharmonies characteristic of some lines of sweet potatoes.

They also reported that all five of the clonal breeding lines of sweet potatoes tested functioned about equally well as pollen parents, but there were significant differences in their ability to function as seed parents. Varieties No. 201 and L-241 (Goldrush) when used as female parents showed a high degree of sexual incompatibility both when selfed and crossed. They suggested that this incompatibility appeared to be a varietal characteristic with no detectable relationship to the environment.

Ting and Kehr (33) reported that meiotic divisions were normal in the sweet potato varieties studied which function well as both seed and pollen parents. Varieties which do not function well as seed parents, but do as pollen parents, showed abnormal meiotic divisions in that laggards were found in both first and second metaphases.

They concluded from these observations that the problem of poor seed-setting ability of some varieties of sweet potatoes may result from some type of abnormal chromosome behavior during meiosis. They further suggested that in such varieties it would be expected that, despite a large percentage of pollen abortions, and abnormal pollen, enough normal pollen grains could be expected to be formed to allow such varieties to function satisfactorily as males. They added that abnormal meiosis in the mother cells would be expected to render most of the derived ovules non-functional, and hence the number of seed set on these varieties would be extremely low. They also suggested that this offers the best explanation for the poor seed setting reported above by Montelaro and Miller (25) in the varieties No. 201 and L-241.

Botanical Relationships of Sweet Potato to Other Species

The sweet potato in the genus Ipomoea is related closely to many domesticated ornamental plants, the morning glories, and other common weeds such as the wild morning glory and the wild sweet potato, although at this time no crosses between them have been reported.

King and Bamford (19) concluded from their cytogenetic research that the somatic chromosome number in the sweet potato was about 90 in comparison with 30 for species such as *I. tricolor*, *I. purpurea*, etc.

Further support of this hypothesis came through meiotic studies in sweet potatoes by Ting and Kehr (33). They suggested that the sweet potato originated from the (2N = 30) type of morning glory by first a doubling of the chromosomes to produce tetraploid (2N = 60) with a back-cross to the same diploid or to another diploid to produce a triploid (2N = 45), and in time this triploid doubled to produce the present (2N = 90) hexaploid sweet potato. A formula was proposed as follows:

Species A (2N = 60)

X ⟶ F_1 Sterile triploid (2N = 45) ⟶ (natural doubling) Fertile hexaploid (2N = 90)

Species B (2N = 30)

Special Techniques

Buchholz, Doak, and Blakeslee (3), in an attempt to control gametophytic selection, developed a technique for shortening and splicing of styles in Datura. They found that by splitting the style through to the mid-point, placing pollen grains in this wound in direct contact with the conducting tissue, and holding these sides together by means of a straw cut from a grass culm, the pollen germinated very satisfactorily. However, an examination showed that under these conditions the pollen tubes grew in both directions in the style.

They also found that when the parts of two different styles were joined perfectly in butt joints, properly protected, and held in this position by a grass culm, pollen tubes could pass from one style segment to the other. When the style segments were not joined perfectly, the ends of the pollen tubes would extend themselves slightly into space beyond the conducting tissue and enlarge considerably.

They further reported that their method should not be called style grafting because the tissues of a style are mature and do not unite by meristematic growth. The parts are merely brought together closely and held in place. The upper part of the joined style frequently dries out long before the lower portion, showing that no actual organic union of the stylar tissues occurred.

Hormones as an Aid to Fruit Set and Seed Production

The probable function of hormones in sexual reproduction was summarized briefly by Murneek (26,27). He reported that a rapid initiation of auxin production in the ovules after fertilization brings about food mobilization and movement into the fruit even at times to the disadvantage of the vegetative organs. Free auxin diffuses from the fertilized ovules, stimulating cell expansion of the fruit and preventing abscission. The conducting tissues of the pedicel are increased to facilitiate food and water uptake. Also, the growth of the whole plant is stimulated.

Murneek further reported that the endosperm produces a hormone identified as the ethyl ester of indoleacetic acid. This, or a derivative of it, seems to be an indispensable catalyst for the metabolism within the embryo and tissues making up the fruit.

He also concluded that growth regulators have been found to be effective tools in the control of the development of certain tissues and organs, from flower initiation to delay of fruit abscission during the pre-harvest period. The practical application of these materials, he stated, in several instances, has outrun our present scientific knowledge of how they actually function within plants.

One chief difficulty in cantaloupe breeding is the high frequency with which fruits drop from the plant at various stages of growth. Burrell and Whitaker (4) applied a 1 per cent indoleacetic acid in lanolin mixture to the stigma immediately after pollination. They made 164 cross-pollinations, treating 78 and leaving 86 untreated for comparison. Fifty-nine per cent of those treated with the plant hormone set fruit in contrast to 27 per cent fruit set of those untreated. Since all of the fruits contained about the same number of seeds, the treatment resulted in many more seeds because of the increase in the number of fruits set.

Wester and Marth (36) applied a mixture of two plant hormones, indolebutyric acid and para-chloraphenoxyacetic acid, to scratches made with a needle at the base of lima bean flowers at the time of pollination. A total of 438 flowers were pollinated; half were treated and half were left untreated for comparison. The treated pollinations set 63 pods containing 153 seeds, an average of 2.43 per pod. The untreated pollinations set 39 pods containing 76 seeds, an average of 1.95 per pod. These results indicate that the treatment also increased fertility since the number of seed obtained from crosses was approximately doubled.

Eyster (14) reported that self-sterile plants can be made self-fertile by spraying the flowering plants with a solution composed of ten parts of alpha naphthalene acetamide dissolved in one million parts of water. (Commercial preparation of this spray is known as Fruitone.)

He further stated that flowers of a self-sterile strain of petunia which were sprayed with this solution immediately before or shortly after they had been self-pollinated produced seed capsules filled with normal viable seeds. He concluded that alpha naphthalene acetamide neutralizes the effects of the ovarian secretion which diffuses into the style and inhibits or greatly retards the growth of the pollen tubes.

Eyster also reported that preliminary experiments indicate that the alpha naththalene acetamide greatly increases the self-fertility or self-compatability of highly inbred and highly sterile strains of African marigolds, cabbage, and red clover.

Crane and Marks (10) have reported hybrids between pears and apples following the use of a 40 ppm solution of beta-naphthoxyacetic acid brushed onto the ovaries at the time of pollination and again twenty-four hours later. They further suggested that, when distant plant species crosses are being attempted, the use of this hormone may be of value.

Emsweller and Stuart (13) have suggested that some self-incompatibilities are caused by the lack of sufficient hormonal activity at the time of pollination to set in motion the physiological activities necessary for ovary growth and fruit formation. They suggested that such types of incompatibilities may be overcome by hormones and that they have overcome the self-sterility in several Easter lily varieties by the use of naphthalene acetamide. A one per cent preparation in lanolin of this hormone was applied at the time of pollination to a wound formed by breaking a petal at its point of attachment at the base of the ovary.

This treatment was applied to 300 of 750 self-pollinated Creole lily flowers. All of the treated flowers formed pods, while the untreated flowers formed no pods. In 18 of the pods formed there were 117 good seeds. In the Croft lily variety 48 of 98 pollinations were treated, and 48 pods formed, six of which produced 119 seed.

The Ace variety of Easter lily occasionally sets some seed when self-pollinated. In this variety 90 flowers were treated, and 48 were left untreated. The treated flowers all set pods, containing 2,414 seeds; the untreated set nine pods with 207 seeds.

Emsweller and Stuart further reported that the hormone treatment was used successfully in making difficult crosses between different species of lilies. A total of 85 cross and self-pollinations was made with lily species other than the Easter lily. Seed was obtained from 47. In 16 of the successful pollinations, seed was produced only when the growth regulator was used. In 22 pollinations, seed was obtained from both treated and untreated flowers, but in all cases there were more seed in the pods produced from the treated flowers. The nine remaining successful crosses set about the same number of seed from both untreated and treated flowers. In those pollinations that failed to produce any seed, the treated flowers formed seed pods in 44 per cent of the crosses, and the untreated formed seed pods in 12 per cent.

EXPERIMENTAL PROCEDURES AND RESULTS

Style Grafting

Style Grafting Technique

To circumvent incompatibility in sweet potatoes, compatible stigmas and styles were substituted for incompatible ones by grafting. This was done to test the hypothesis that gene controlled physiological factors prevent the growth of pollen tubes in the stigma or style.

Buchholz, Doak, and Blakeslee (3) developed a technique for the shortening and splicing of styles in *Datura*, for the purpose of controlling gametophytic selection. They indicated that the styles were spliced and that a graft union did not take place since the tissues of a style are "mature and do not unite by meristematic growth."

Because the writer was interested in determining the possibility of obtaining successful transmission of pollen tubes by grafted styles, the first attempts were made by cutting and grafting styles of the tomato which is known to be self-compatible.

The method used in style grafting is illustrated by steps A - E in Figure 1. Two kinds of flowers were used: (a) those that were emasculated the day before but grafted

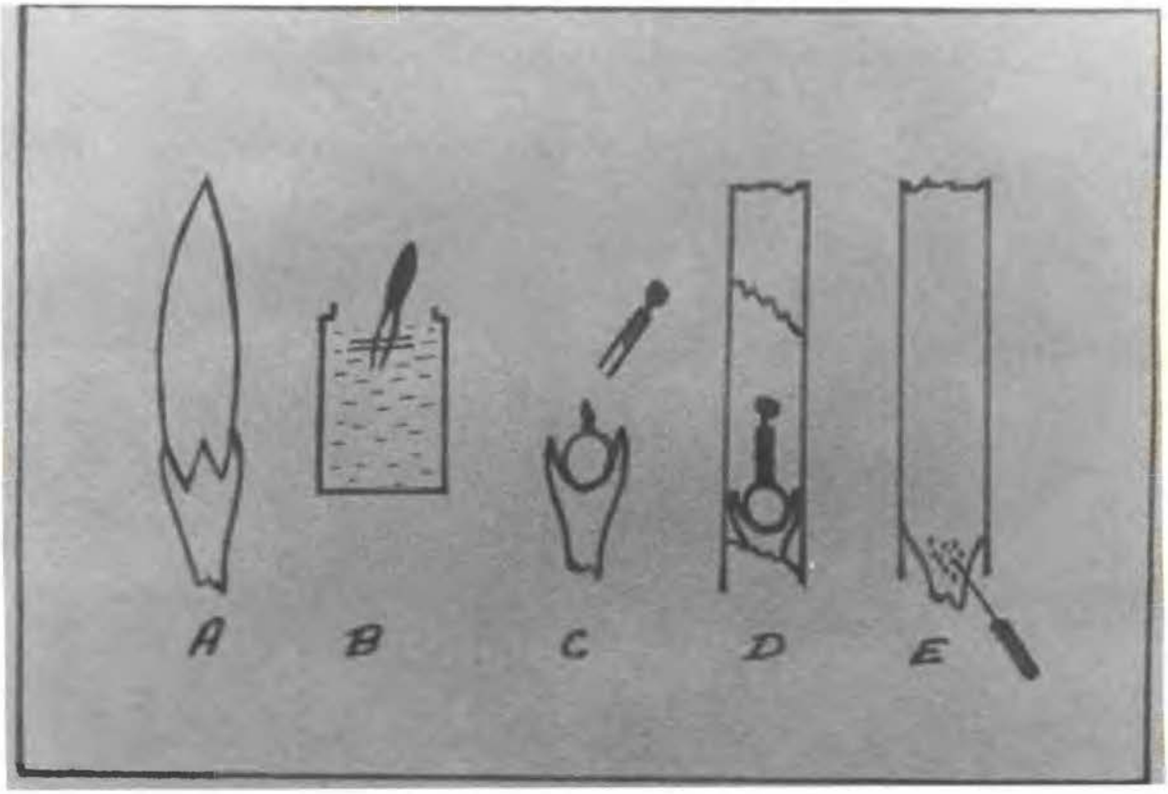

Figure 1. Showing successive steps used in obtaining style graft. A flower bud one day prior to anthesis (A) is emasculated and style removed (C). New style is inserted in closely-fitting glass capillary that previously was filled with sucrose solution (B) and (C). The capillary is then lowered over the cut end of the lower style, which remained on the ovary, until the two cut surfaces came in full contact (D). Finally, the flower is enclosed in soda straw moist chamber, and hormone is scratched into its base with a needle (D) and (E).

on the date of anthesis and (b) mature flower buds that were emasculated and grafted one day prior to anthesis as shown in step (A).

The upper portion of the style including the pollinated stigma was cut obliquely and inserted into a prepared closely-fitting glass capillary as shown in step (C). The glass capillary was filled previously with 5 per cent sucrose solution, by immersion as shown in step (B). It was believed that the sucrose solution would nourish the pistil and keep it favorably moist in similar fashion to Tukey's work with embryos (34).

The capillary was lowered carefully over the obliquely cut end of the lower style which remained on the ovary as shown in steps (C) and (D). It is very important that the two cut surfaces be brought into full contact, as can be observed through the glass capillary as the two are placed together.

To maintain high humidity, a piece of soda-straw with moistened cotton closing one end was used to cover the flower as shown in step (D). Finally the flower was tagged, and a hormone was scratched into its base with a needle as shown in step (E). The hormone used was para-chlorophenoxyacetic acid diluted 1 to 500 in lanolin. It was applied to guard against abscission of the flower, as a possible aid to the formation of callus and the union of the graft, and also, since only a few ovules are expected to be

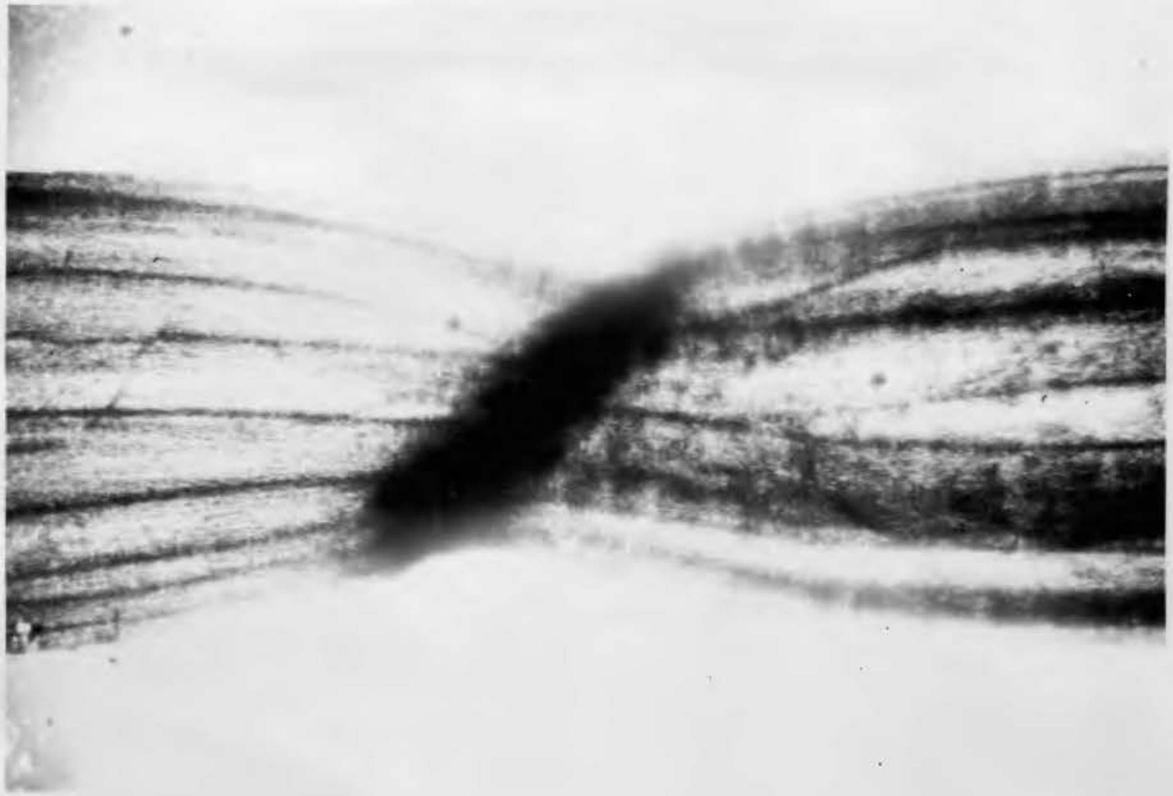

Figure 2. Grafted area of tomato style. Note callus cells at point of union in the darkened central area. This is a lactophenol blue preparation in which the entire style is flattened. The central constriction is in part due to greater rigidity in the grafted area and the less tendency for flattening under pressure.

Figure 3. A tomato fruit showing considerable enlargement following style grafting and hormone application. Note the closely-fitting glass capillary and the style that appears green throughout its entire length, which indicates that a good graft union was obtained.

Photo by W. R. Kays

Studies of Style Grafting as a Means of Circumventing Self-incompatibility

Sweet potato parent 47* and its derivatives made up a large part of the group of self-incompatible sweet potato lines investigated at Oklahoma A. and M. College. Parent 97 ranked high in self-compatibility. Reciprocal crosses between the two parents, 47 and 97, are highly fertile as indicated by data in Table I.

TABLE I

FRUIT SET FOR SELF-POLLINATIONS AND RECIPROCAL CROSSES OF SWEET POTATO PARENTS 47 AND 97 DURING THE PERIOD 1951-1956*

Kind of pollination	Blooming Seasons 51 - 52 No. Fls.	No. Set	53 - 54 No. Fls.	No. Set	55 - 56 No. Fls.	No. Set	Total No. Fls.	No. Set	% Set
47 selfed	907	4	388	2	13	0	1308	6	0.4
97 selfed	498	181	193	63	439	184	1130	428	37.8
47 x 97	52	5	9	1	4	2	65	8	12.0
97 x 47	133	39	38	18	2	2	173	59	34.0

*Data provided by the Oklahoma Agricultural Experiment Station.

*Selected groups of varieties and breeding lines are used in the sweet potato breeding program at the Oklahoma Experiment Station. They are identified by numbers. Those designated as parent 47, parent 97, etc., have been under observation for several years in the greenhouse and were selected for this study on the basis of their reaction when self-pollinated. (See Table I for performance data.)

In an attempt to obtain selfed seeds from the self-incompatible parent 47, stigmas and styles from previously emasculated 97 flowers were employed. These were pollinated with 47 pollen and grafted on 47 flowers where they were expected to function in transmitting the pollen tubes down to the ovary.

Thirty-one grafted flowers from parent 47 were selfed by this technique. No fruits set although the ovaries became enlarged and remained in good condition for more than one week. This was due to the effect of the hormone applied, as was previously illustrated in the presentation of the style grafting technique.

Four flowers from the self-incompatible sweet potato parent 90 also were selfed in the same way by employing stigmas and styles from 97. The results were similar to those obtained when 47 was selfed.

Employing Style Grafting in Aiding Inter-species Crosses

An effort was made to obtain fertile crosses between the sweet potato and a diploid morning glory in order to enlighten the theory that the sweet potato originated from a morning glory.

Four crosses were made by grafting stigmas and styles from the sweet potato parent 108 on flowers of the morning glory I. tricolor. These were pollinated with pollen from parent 108. The ovaries enlarged somewhat and remained in good condition over a considerable period indicating fruit set. This probably was due to the effect of the hormone used, since no seeds were obtained.

Six crosses were made by grafting stigmas and styles from the sweet potato parent 97 on morning glory I. purpurea flowers and pollinating them with pollen from parent 97. No seeds were formed although, as in the previous crosses, indications of fruit set due to the influence of the hormone were apparent.

Three stigmas and styles from the sweet potato parent 15 were grafted onto I. purpurea flowers and were pollinated with pollen from parent 15. Here also no seeds were formed although fruits were set and considerable enlargement occurred in the pedicels, as shown in Figure 4, due to the effect of the hormone.

Figure 4. An _I._ _purpurea_ fruit that set following grafting with a sweet potato style (parent 15). Pollen of parent 15 also was used. The pedicel as well as the ovary show considerable enlargement due to the application of hormone. The scar indicates the point of hormone application. No seeds were developed in this fruit.

Photo by Bruce Steddum

Pollen Injection

Pollen Injection Technique

Pollen injection was also tried for the purpose of circumventing incompatibility in the stigma and style. In this case the direct placement of pollen in the carpel or the base of style was carried out.

Here, also, the first study involved an attempt to prove the possibility of obtaining successful fertilization by pollen injection. Tomato flowers with stigmas free of pollen were used for this purpose. Pollen from the dwarf small fruited Tom Thumb variety was injected into the base of the style, or directly into the carpel of the variety Sioux.

The technique is illustrated by steps A - D in Figure 5. The pollen was transferred from the anthers into a five per cent sucrose solution on a porcelain spot plate - Step (A). The solution was expected to help germination. The pollen suspension was then forced by means of an eye-dropper with fine capillary end into a hole in the ovary or base of the style which was punctured previously by a sharp needle as shown in step (B). In another method a small drop of pollen suspension was put on the ovary which was then punctured within the drop by means of a sharp needle as shown in step (C).

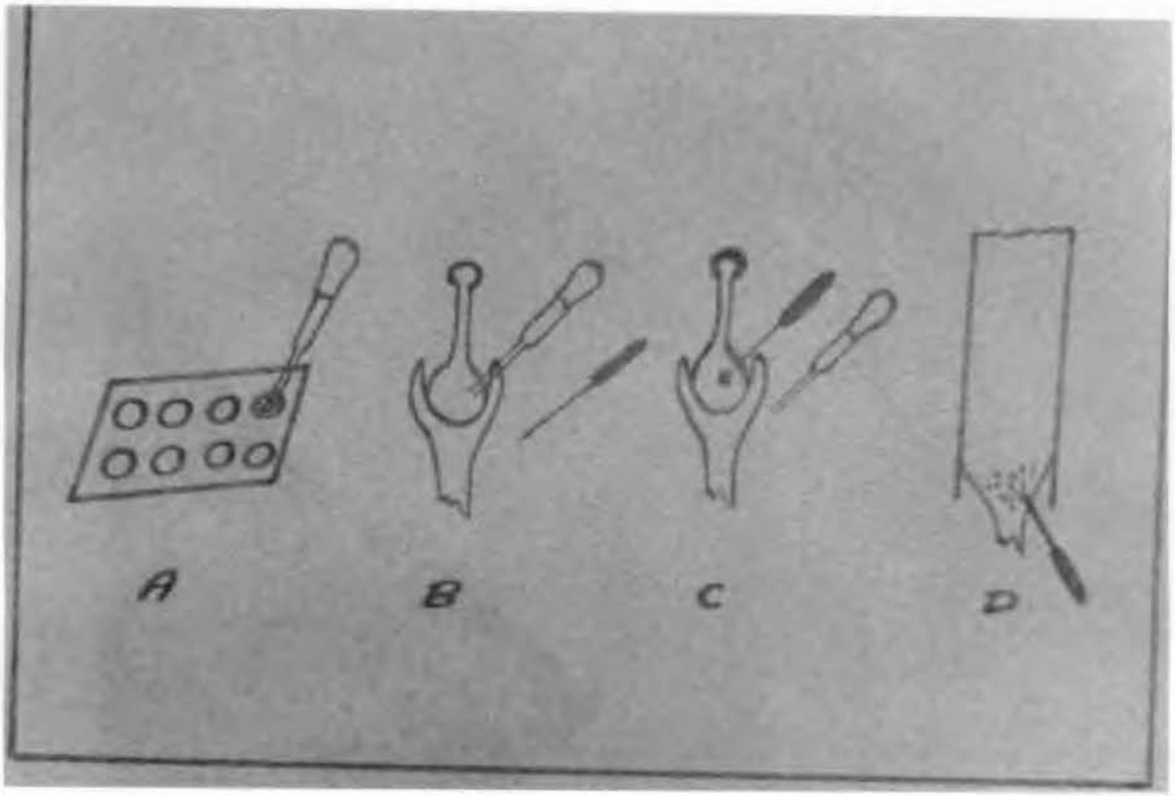

Figure 5. Illustrating pollen injection technique. Pollen is taken from anthers into 5 per cent sucrose solution in depression of a spot plate (A). Pollen suspension is then drawn into fine capillary-tipped dropper, and injected into base of style or into carpel (B). In other cases, a drop of the pollen suspension is placed on the ovary, which is then punctured with a sharp needle. The withdrawal of the needle is expected to create enough suction to pull the suspended pollen into the tissue of the pistil (C). Finally, a hormone in lanolin is scratched into the base of the flower (D).

Figure 6. Tomato fruits developed from ovaries which received pollen injection and hormone treatment. Fruit at left was injected near the base of the style. In the fruits at right the pollen suspension was injected in the carpels near the stem ends. Note enlarged peduncles (arrows) resulting from hormone treatments.

Photos by Bruce Steddum

Studies of Pollen Injection as a Means of Circumventing Self-incompatibility

Pollen injection to circumvent self-incompatibility was tried in the sweet potato parent 47.

Twenty-two flowers of parent 47 were selfed by employing the pollen injection technique. No fruits were set although the ovaries became enlarged and were maintained in good condition over a considerable period, which was due probably to the effect of the hormone treatment.

Pollen Injection in Inter-species Crosses

Pollen injection was tried as an aid to obtain fertility in several different inter-species crosses.

Table II shows the different crosses that were attempted. No seeds were obtained from any of these crosses, although six parthenocarpic fruits were set in response to the hormone treatment.

TABLE II

INTER-SPECIES CROSSES ATTEMPTED BY MEANS OF POLLEN INJECTION

Female P.	Pollen P.	No. Fl.	No. Set	No. Seeds
I. tricolor	*I. Nil*	1	0	0
I. tricolor	*I. batatas* (P136)	4	3	0
I. purpurea	*I. batatas* (P136)	4	2	0
I. purpurea	*I. Nil*	1	1	0
I. purpurea	*I. batatas* (P97)	1	0	0

Hormones

Use of Para-chlorophenoxy Acetic Acid in Inter-species Crosses

In an attempt to obtain successful inter-species crosses, the growth regulator para-chloyophenoxy acetic acid diluted 1 to 500 in lanolin paste was employed by scratching it into the tissues at the base of the flower.

A total of 547 cross pollinations were made, and in 371 of these the flowers were treated with hormone at the time of pollination. The other 176 flowers were left untreated for comparison. (See Table III.)

None of the untreated flowers set fruit. The treated flowers in many cases set parthenocarpic fruits, and all the ovaries became enlarged and were maintained in good condition for more than one week.

Out of 62 crosses attempted (*I. purpurea* x *I. tricolor*), 21 set fruit. Of these only one had viable seeds. These were planted, and one of the three plants produced appeared to have some hybrid vigor but seemed to be within the range noted in the *I. purpurea* stock. When it bloomed it was crossed as shown in Table III. Some of these crosses were aided by hormones, some were untreated, and the remainder were bud pollinated. It was expected to be more likely to obtain fertile inter-species crosses when one or both of the parents involved are hybrids. But from all these crosses no seeds were obtained.

TABLE III

THE EFFECT OF PARA-CHLOROPHENOXY ACETIC ACID ON INTER-SPECIES CROSSES OF IPOMOEA

Male Parent	Female Parent	Time of Pollination	Plus Hormone No. Fls.	Plus Hormone No. Set	Plus Hormone No. Seeds	Minus Hormone No. Fls.	Minus Hormone No. Set
I. species[1]	I. Nil	1/14-3/24	8	3	0	1	0
Iran[2]	I. Nil	1/24	1	1	0	-	-
I. tricolor	I. Nil	3/20-3/29	8	3	0	1	0
I. quinquifolia	I. Nil	3/20	2	1	0	-	-
S.P. 86	I. Nil	1/18	2	0	0	-	-
S.P. 97	I. Nil	1/27	1	1	0	-	-
S.P. 134	I. Nil	1/28	4	3	0	-	-
S.P. 90	I. Nil	2/14	3	3	0	-	-
I. Nil.	I. pur.[3]	1/12	1	1	0	-	-
I. species[1]	I. pur.	1/16-3/24	49	14	4	11	0
I. tricolor	I. pur.	1/26-3/30	62	21	3	44	0
I. quinquifolia	I. pur.	3/15-3/20	7	1	0	4	0
S.P. D-1-62	I. pur.	2/7	5	3	0	-	-
S.P. 97	I. pur.	1/5-1/27	3	1	0	-	-
S.P. 136	I. pur.	1/16-2/28	6	1	0	3	0
S.P. 90	I. pur.	2/14	4	4	0	-	-
I. species[1]	I. tri.	1/13-3/24	6	1	0	-	-

[1]P. I. No. 207820

[2]P. I. 227365 (Iran)

[3]pur. for purpurea

TABLE III--Continued

Male Parent	Female Parent	Time of Pollination	Plus Hormone No. Fls.	Plus Hormone No. Set	Plus Hormone No. Seeds	Minus Hormone No. Fls.	Minus Hormone No. Set
I. purpurea	I. tri.**	1/24-4/2	22	7	0	3	0
S.P. D-1-62	I. tri.	2/7	1	1	0	-	-
S.P. 97	I. tri.	1/5-1/27	5	2	0	-	-
S.P. 90	I. tri.	2/14	6	4	0	3	0
S.P. 136	I. tri.	2/18	4	1	0	-	-
I. species[1]	S.P.136	2/20-3/6	9	3	0	-	-
I. species[1]	S.P.47	1/25-3/6	19	0	0	4	0
I. species[1]	S.P.90	2/20-3/28	10	3	0	1	0
I. species[1]	S.P.129	3/6	2	1	0	1	0
S.P. 97	I. pur.x I. tri.	3/28-3/30	5	3	0	-	-
S.P. 97	I. pur.x I. tri.*	3/28-4/2	15	10	0	-	-
I. tricolor	I. pur.x I. tri.	3/29	3	1	0	2	0
I. pur.x I. tri.	S.P.97	4/6-4/17	35	35	0	34	0
I. pur.x I. tri.	S.P.47	4/6-4/17	60	60	0	59	0
I. pur.x I. tri.	S.P.90	4/6	3	3	0	5	0
Total			371	196	7	176	0

Data taken from 1/5/56 to 4/17/56

[1]P. I. No. 207820

*Bud pollinations

**tri. for tricolor

Out of 49 crosses attempted (*I. purpurea* x *I.* species*), 14 set fruit, but only one had four viable seeds. These were planted, and two of the four plants produced appeared to have hybrid vigor but again seemed to be of *I. purpurea* derivation.

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*P. I. No. 207820

Use of Para-chlorophenoxy Acetic Acid in Relation to Self-incompatibility in Sweet Potatoes

The several roles that hormones play in the growth and development of fruits and seeds have been described by Murneek, Eyster, and Crane and Marks, as reported in the review of literature. They also stated several cases where hormones increased fertility and overcame self-sterility.

In the attempt to overcome self-incompatibility in sweet potatoes, it appeared reasonable that hormones probably would aid the growth of the pollen tubes in the stigma and style and perhaps in the ovary. It also appeared probable that these chemical stimuli would help maintain the ovary, by delaying abscission and establishing a suitable condition for the pollen tubes to reach the ovules and effect fertilization.

A total of 124 flowers from different self-incompatible parents of the sweet potato were selfed, 64 of which were treated with hormone. The remainder were left untreated for comparison.

Table IV shows the different parents that were selfed. In all pollinations the untreated flowers set no fruits. The ovaries of the treated flowers became enlarged and remained succulent over a considerable period. In many cases parthenocarpic fruits were set, but no seeds were obtained.

TABLE IV

A HORMONE TREATMENT TO OVERCOME SELF-INCOMPATIBILITY IN FOUR SWEET POTATO PARENTS

Parent Selfed	Time of Pollination	Plus Hormone No. Fl.	No. Set	No. Seed	Minus Hormone No. Fl.	No. Set	No. Seed
136	2/28 - 3/3	5	2	0	4	0	0
90	2/28 - 3/15	4	3	0	2	0	0
128	3/3 - 3/9	4	1	0	3	0	0
47	2/28 - 3/19	51	19	0	51	0	0
Total		64	25	0	60	0	0

From these results it appears that para-chlorophenoxy acetic acid as used was not effective in overcoming self-incompatibility. To complete this plase of the study, other hormones should be investigated, such as: a mixture of indolebutyric acid and para-chlorophenoxy acetic acid (36); indoleacetic acid (4); alpha-naphthalene acetamide (14); beta-naphthoxy acetic acid (10); naphthalene acetamide (13); and others.

Employing Para-chlorophenoxy Acetic Acid to Increase Fertility in Self-Compatible Species

To study the effect of this hormone in increasing the fertility of certain self-compatible lines, a total of 160 flowers of I. purpurea were selfed. Half were treated with the hormone, and half were left untreated for comparison as shown in Table V. The 80 treated flowers set 74 fruits containing 284 seeds. The untreated flowers set 70 fruits containing 278 seeds, with no significant difference between the two.

TABLE V

THE EFFECT OF PARA-CHLOROPHENOXY ACETIC ACID IN INCREASING FERTILITY IN SELF-COMPATIBLE I. PURPUREA

Anthesis Date	Plus Hormone No. Fl.	Plus Hormone No. Set	Plus Hormone No. Seed	Plus Hormone Ovules Set(%)	Minus Hormone No. Fl.	Minus Hormone No. Set	Minus Hormone No. Seed	Minus Hormone Ovules Set(%)
4/5	20	19	81	67.5	20	20	83	69.1
4/10	20	19	80	66.7	20	20	77	64.1
4/11	40	36	123	51.2	40	30	118	49.1
Total	80	74	284	59.1	80	70	278	57.9

No important increase in fertility was obtained from the hormone treatment in this study. The hormone caused considerable enlargement of the pedicels of the treated flowers. Other untreated flowers on the same plant also showed enlarged pedicels, which was attributed to a systemic effect of the hormone.

Table VI, column one, shows the diameters of pedicels of hormone treated flowers; column two shows the diameters of flowers on these same plants but not treated with hormone; and column three shows diameters of flowers on plants on which hormone was not applied to any flower.

According to the paired observation method that was used in the analysis of these data, there was a very significant difference in the diameter of pedicels between both those of the treated flowers and the flowers with the systemic effect on one side, and those of the untreated flowers on the other side. There was no significant difference between those of the treated flowers and those of the flowers exhibiting the systemic effect.

Figure 7 shows the differences in pedicel diameters under the three different conditions.

In addition to the enlargement of the pedicels, the hormone seemed also to cause a malformation and distortion of the leaves and to have some dwarfing effect on the plant.

TABLE VI

PEDICEL DIAMETERS IN MILLIMETERS SHOWING THE EFFECT OF DIRECT HORMONE APPLICATION TO PEDICELS AND THE SYSTEMIC EFFECT ON UNTREATED PEDICELS ON THE SAME PLANT

	Hormone Treated	Systemic Effect	Untreated
	3.1	2	1.2
	2.6	2.5	1.5
	2.4	2	1.6
	2.6	1.9	1.1
	2.2	2.3	1.3
	2.8	2	1.2
	2	2.4	1.6
	1.9	1.9	1.4
	2.4	2.2	1.7
	2.1		1.4
	2		1.8
	2.1		1.5
			1.7
			1.5
			1.4
			1.3
			1.5
			1.3
Total	28.2	19.2	26.0
Average	2.35*	2.13*	1.44

*Significantly greater than average for untreated check

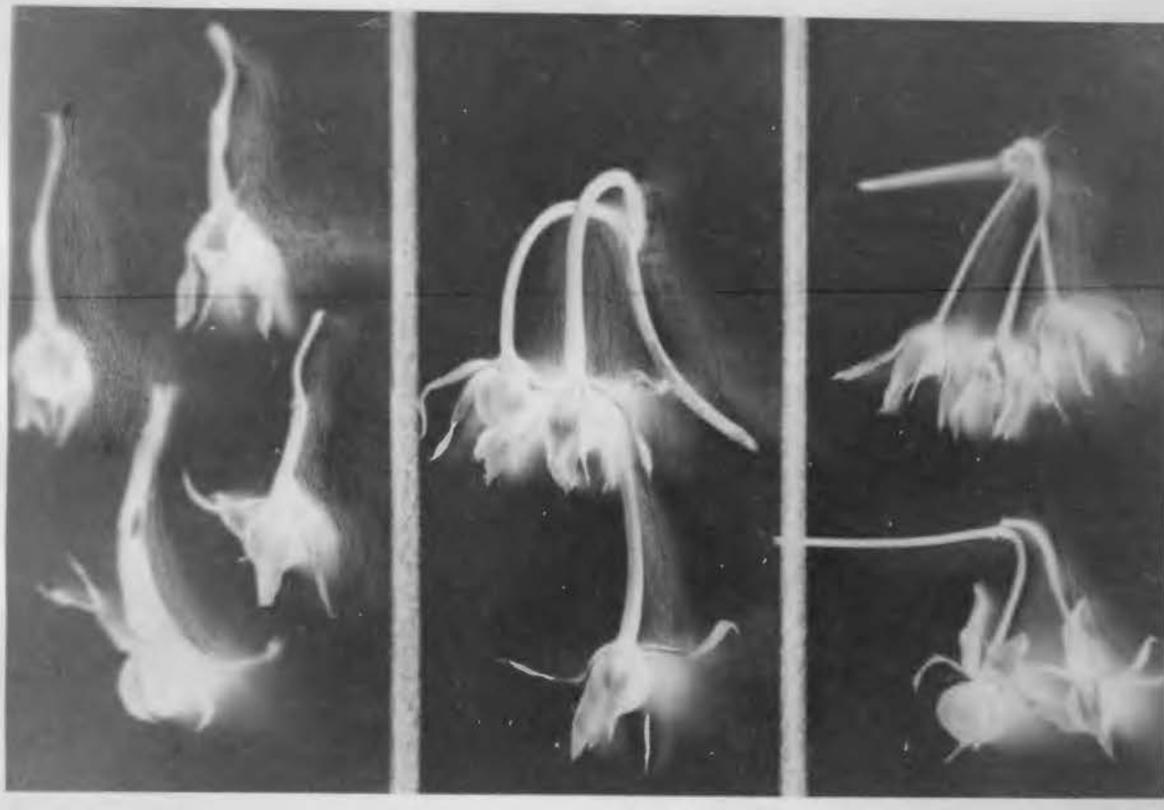

Figure 7. Pedicels at the left were directly treated with hormone. Those in the center were untreated but growing on the same plants as those at the left. Normal pedicels from plants on which no hormone was used are shown at the right. (Table VI includes diameter measurements of these pedicels.) Note: Photo taken from color film which resulted in negative effect in this picture.

Pollen Tube Observations

Pollen germination and pollen tube growth were studied in certain self-incompatible lines of sweet potato and certain inter-species crosses of *Ipomoea*.

The plants used in these experiments were all grown under fairly uniform greenhouse conditions with comparable pollinations being made at the same time.

The lactophenol staining technique (23,28) was employed in these studies. The formula for the lactophenol preparation is: phenol crystals, 20 gm. or cc.; lactic acid, 20 cc.; glycerin, 40 cc.; distilled water, 20 cc. The crystals were dissolved by gently heating the mixture.

The tissue to be studied was killed in the lactophenol solution for 30 minutes at 140° F. Then it was mounted on a slide and a drop or two of lactophenol blue (prepared by adding 0.05 gm. cotton blue to the above formula) was applied, and a cover slip was placed on carefully. The cover slip was pressed down gently but firmly to reduce the tissues (stigmas and styles) to a relatively thin section in order that the pollen tubes or other structures might be clearly visible. The slide then was kept flat for several days prior to microscopic examination. It was found that the details of pollen germination and pollen tubes become more clearly visible in preparations one month or more old. In new preparations the pollen tubes usually are not so readily distinguished from the surrounding tissues.

When pollen from the sweet potato parent 97 was used on stigmas of *I. quinquifolia* and *I. purpurea*, the pollen grains burst and no penetration of pollen tubes in the style was noticed as shown in Figures 8 and 9.

When pollen from the sweet potato parent 97 was used on *I. tricolor* stigmas, very good germination of the pollen was observed, and some of the pollen tubes penetrated into the style, as illustrated by Figure 10.

The sweet potato parent 63 (Goldrush) was known to be unfruitful. When pollenized by parent 81, a good penetration of pollen tubes in the style was observed, but still no fruit set occurred. Figures 11 and 12 show a stigma and style of parent 63 with pollen tubes from parent 81 penetrating to four-fifths of the length of style.

In selfing 97, which is highly self-compatible, the pollen germination was poor, but some pollen tubes showed good growth and penetration through the style as shown in Figure 13.

When the self-incompatible sweet potato parent 47 was selfed, the germination of pollen in the stigma was good, and the pollen tubes made very good penetrations through the style. Figures 14 and 15 show some of the 47 pollen tubes in their own style and upper end of ovary.

The pollinations studied include pertinent data shown in Table VII.

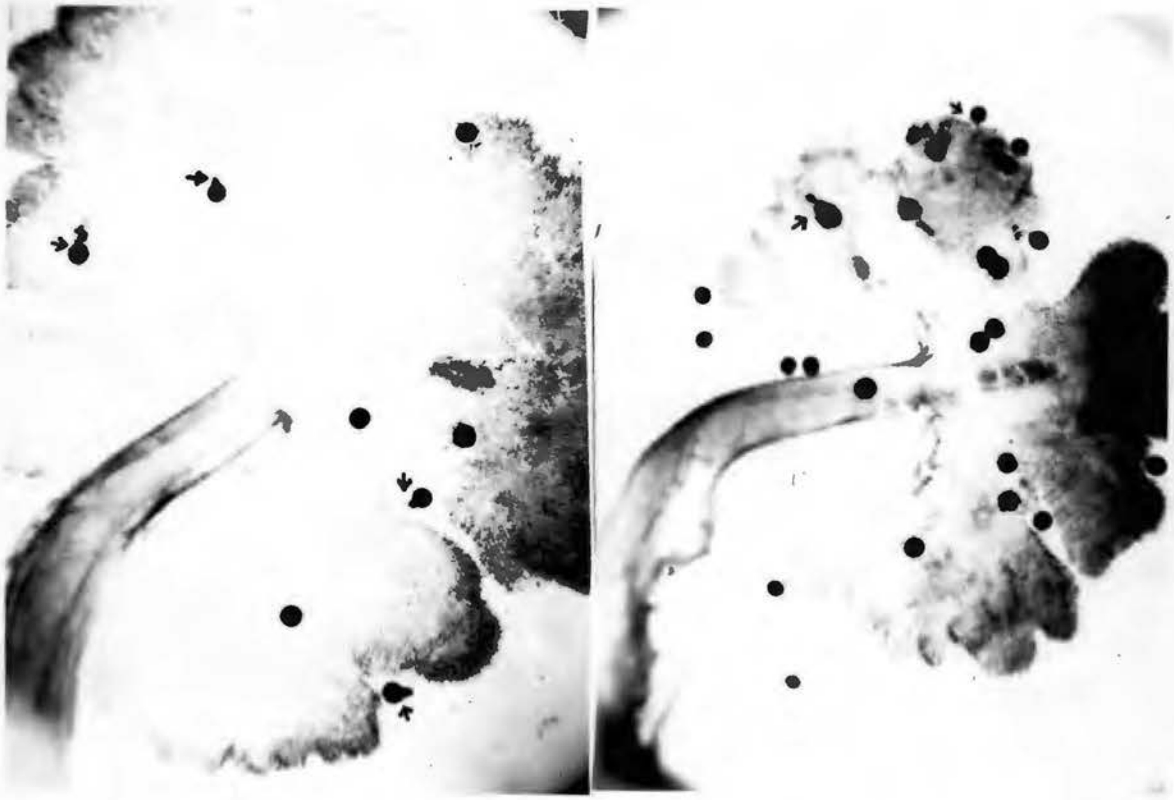

Figure 8. (left) Good portion of pollen grains burst, as indicated by the arrows, and the remainder did not germinate when pollen from the sweet potato parent 97 was used on I. quinquifolia stigma.

Figure 9. (right) Pollen grains of 97 on I. purpurea show also the incompatibility behavior of no germination and few bursting. In both figures penetration of pollen tubes was not noticed through the style.

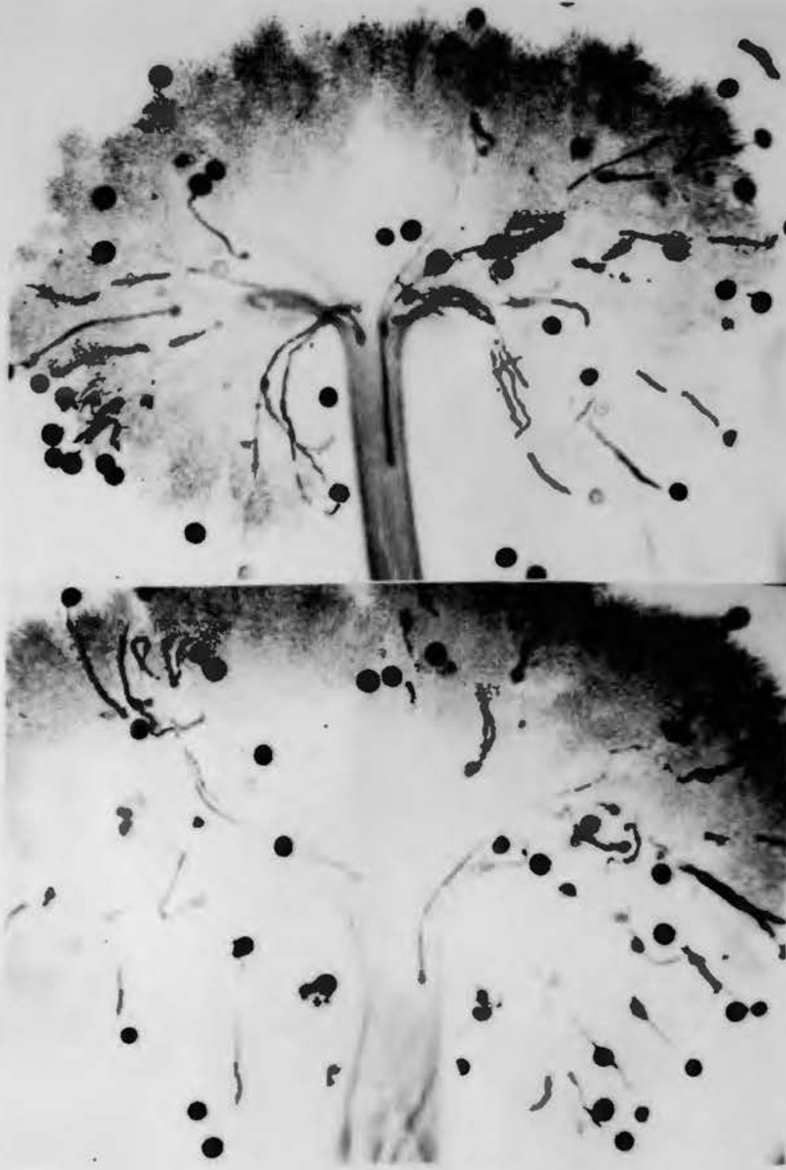

Figure 10. Pollen from the sweet potato parent 97 on *I. tricolor* stigmas show the compatible behavior of high germination with some pollen tubes penetrating the style.

Figure 11. (left) Showing some germinated pollen of 81 on a stigma of the female sterile sweet potato parent 63 (Goldrush) as indicated by arrows.

Figure 12. (right) Shows three tubes of pollen 81 penetrating into the style (arrows).

Figure 13. Self-pollinated stigma of the highly self-compatible sweet potato parent 97. Note that a good portion of the pollen grains did not germinate and some burst (arrows), although sufficient pollen tubes made good penetration into the style (arrows).

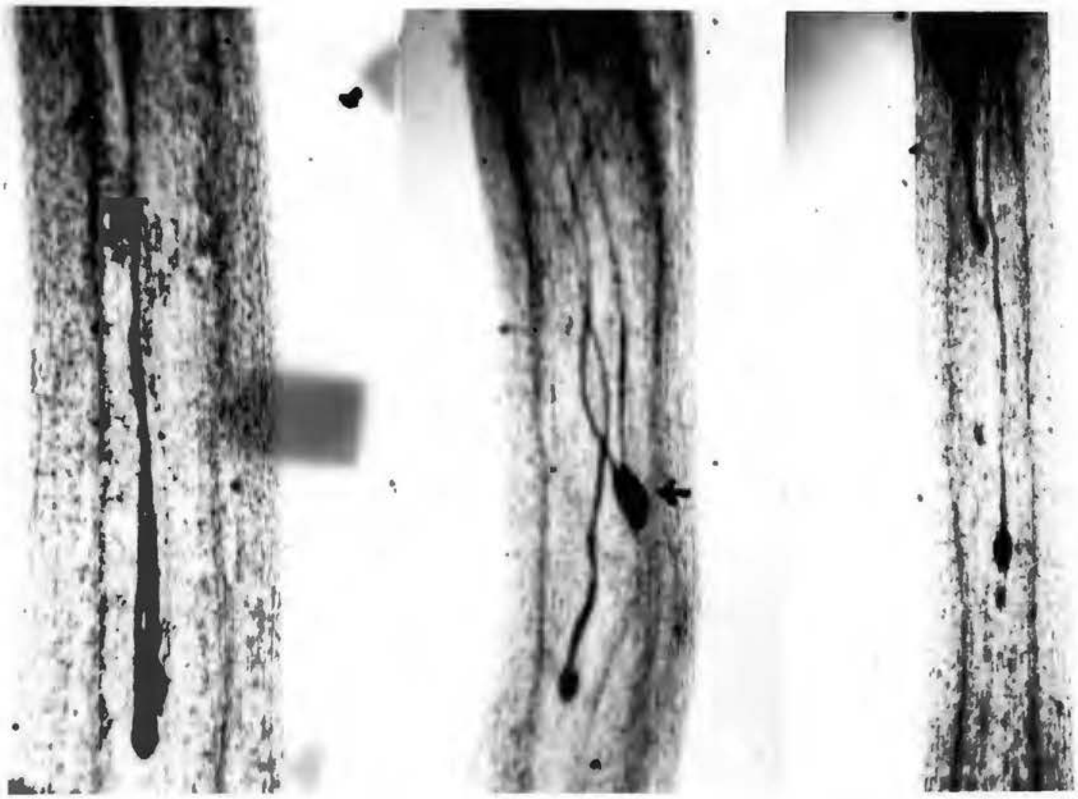

Figure 14. Showing some of the pollen tubes of the self-incompatible sweet potato parent 47 penetrating through the style in a self pollinated flower. The figure shows three areas of style. Note in the center two heads of pollen tubes at the point indicated by arrow.

Figure 15. Showing pollen tubes of the self-incompatible sweet potato parent 47 at the upper ends of two ovaries, as indicated by the arrows. The tearing and displacement of the tissues in these preparations are in part due to the resistance of the thick base of style to flattening under pressure.

TABLE VII

GERMINATION OF POLLEN AND PENETRATION OF POLLEN TUBES THROUGH STYLES OF CERTAIN *IPOMOEA* SPECIES

Kind of Pollination	No. Stig. and Styles Studied	Max. No. germ. in a single stigma	Max. No. found in a single style	Max. penetration at 24-28 hr.	Remarks
I. quinquifolia x S.P.97**	3	none	none	----	Pollen either burst or not germinated
I. purpurea x S.P.97	5	none	none	----	Pollen either burst or not germinated
I. tricolor x S.P.97	5	40	3	1/10S.L.*	Very good pollen germination
S.P.63 x S.P.81	6	10	3	4/5S.L.	Good penetration of pollen tubes
S.P.97 selfed	10	6	7	8/10S.L.	Germination poor, germinated ones penetrated good through style
S.P.47 Selfed	16	10	10	Entire S.L.	good germination and very good penetration through style

*S.L. for style length

**S.P. for sweet potato

DISCUSSION AND CONCLUSIONS

In starting these studies, it was assumed that incompatibility in sweet potatoes is a gene controlled physiological factor which prevents the growth and development of pollen tubes in the stigma or style. It was hoped that some means of circumventing this incompatibility would be found.

Although the grafted styles appeared to function in effecting fruit and seed development in the self-compatible tomato, they were not effective in overcoming self-incompatibility in the sweet potato parents investigated. The style grafting technique was also employed in species crosses, and again negative results were obtained.

Similarly, the pollen injection technique, which appeared to be successful with tomatoes, did not prove effective in overcoming these incompatibilities.

It seemed probable that the use of a hormone would delay abscission, maintain the ovary in a functional condition, and provide for the pollen tubes to reach the ovules and effect fertilization (26,27,14,13). However, in this study, the application of para-chlorophenoxy acetic acid did not help to obtain seeds, nor did it prove effective in neutralizing the inhibitory substances in stigma and style of the parents investigated (14).

In many cases the ovaries became enlarged and were maintained for more than one week, and as a result parthenocarpic fruits often were obtained. This probably was due to a partial substituting effect that the applied hormone had to the natural hormones usually produced by the ovules for the development of the ovary.

The hormone used also proved to have a systemic effect, which appeared in the form of enlarged pedicels. In addition, the hormone when applied to the first flowers on young plants seemed to be associated with malformation and distortion of the leaves and to cause some dwarfing.

In studying the pollen germination and the pollen tube behavior in the stigma and style, it was found that in selfing the sweet potato parent 97, which is highly self-compatible, the germination of pollen on the stigma was poor; however, a few pollen tubes showed good penetration through the style. It also was found that, in selfing the self-incompatible sweet potato parent 47, the pollen germination on the stigma was good, and many pollen tubes penetrated through the style. This contrasts strongly with the pollen tube behavior in the highly self-compatible parent 97.

Because of the good penetration of pollen tubes through the style in the self-incompatible sweet potato parent investigated, it seems logical to assume that the incompatibility factor in this case is not present in the stigma or style, at least to the extent of preventing pollen tubes from passing through these tissues. Incompatibility in parent 47 is, therefore, of another nature, and further cytological experimentation is required to complete this study, since the present experiments show only that the pollen tubes reach the ovary. On this fact two hypothetical explanations could be made, the validity of which will depend on further experimentation.

The first hypothesis is that the inhibitory substances in sweet potato originate in the ovule (37). In cases like that of the highly self-compatible parent 97, the inhibitory substances ascend to the stigma from the ovule, and they become diluted in all, the ovary, the style, and the stigma. This explanation fully agrees with the poor germination of pollen when parent 97 is selfed. However, in this case, because of genetic differences, a few pollen grains germinate and produce pollen tubes which penetrate to the ovule and effect fertilization.

In cases like that of the self-incompatible parent 47, the inhibitory substances are localized in the ovule or ovary where they inhibit pollen tube growth. This also agrees with the good germination behavior of pollen grains on the stigma and the very good penetration of pollen tubes through the style when selfing 47, since the inhibitory substances are localized in the ovary and do not reach the stigma or style.

The gradation from self-compatibility to self-incompatibility among the various parents of sweet potato may be due to a gradation from low to high in the production of inhibitory substances in the ovules, which is probably the result of polyploidy and the duplication of several series of sterility alleles (17).

The second hypothesis is that the pollen tubes reach the ovules, but in self-fertilization the endosperm is not activated by hybridization, as is usually necessary for normally cross-pollinated plants, and the nutrient balance soon shifts in favor of the integuments (5). This may be the major cause of a lack of nutrients and the starvation of the young embryo. If this immature embryo could be caused to develop in a nutrient culture (34,27) this hypothesis would be substantiated.

The different degrees of fertility, among the various combinations of sweet potato parents, may be partially interpreted according to the amount of hybrid vigor in endosperm for these various combinations (5). Also, the amount and localization of inhibitory substances, as influenced by genetic differences, may contribute to the variations in fertility (37).

When pollen from parent 97 was applied to _I. tricolor_ stigmas, very good germination of pollen grains was observed, and some pollen tubes penetrated through the style, but no seeds were obtained from such pollinations.

In this case fertilization might have taken place and the ovules collapsed soon after fertilization, due to a slow growth of the endosperm and the starvation of the young embryo, as sometimes happens in plants when species crosses are attempted (15).

When pollen from parent 97 was used on stigmas of I. purpurea and I. quinquifolia, the pollen grains burst and no penetration of pollen tubes in style was found. This may indicate a difference of osmotic pressures which has been noted by other investigators in species crosses, or this may have been due to some other inhibitory factor present in the stigma and style. Even if fertilization might have taken place when the style grafting and the pollen injection techniques were used in these crosses, the ovules probably collapsed soon after fertilization because of the slow growth of endosperm. Embryo culture may prove helpful under these conditions in further perpetuating this study.

The unfruitful characteristic of the sweet potato parent 63, although the pollen tubes show good penetration through the style, is probably due to abnormal meiosis in the embryo sac mother cells (33), which causes this parent to be female sterile, although it functions satisfactorily as a male parent, since enough functional pollen grains are formed.

SUMMARY

The objectives of this study were: (A) To test the hypothesis that incompatibility in sweet potatoes is a gene controlled physiological factor which prevents the growth and development of pollen tubes in the stigma and style. (B) To observe pollen tube behavior in certain self-incompatible, self-compatible, and cross-incompatible pollinations. (C) To develop techniques that may help circumvent incompatibility in the stigma and style. (D) To obtain fertile crosses between the sweet potato and a diploid morning glory in order to test the theory that the sweet potato originated from the morning glory. (E) To evaluate the hormone para-chlorophenoxy acetic acid and also to employ bud-pollination as means of overcoming self-incompatibility in sweet potato and cross-incompatibility between different species of *Ipomoea*.

A style grafting technique was developed, using tomato flowers, to make it possible to substitute an incompatible stigma and style with compatible ones. Although the grafted styles appeared to be functional in effecting fruit and seed development in the self-compatible tomato, they were not effective in overcoming self-incompatibility in the sweet potato parents investigated, nor were they helpful in circumventing cross-incompatibility in inter-species crosses.

A technique for pollen injection in the carpel or the base of style was also developed. This method also failed to overcome these incompatibilities.

The hormone para-chlorophenoxy acetic acid similarly was not effective in overcoming these self and cross-incompatibilities. However, it was effective in inducing parthenocarpic fruit set, and it also had a systemic effect on untreated flowers of treated plants.

When the self-incompatible sweet potato parent 47 was selfed, it was observed that the pollen germination on the stigma was good and many pollen tubes penetrated readily through the stigma and style. Contrasting with this, the highly self-compatible sweet potato parent 97, when selfed, appeared to be less compatible as indicated by the behavior of the pollen tubes in the stigma and style.

It was demonstrated that the incompatibility factor in the sweet potato parent 47 is not present in the stigma or style, while in parent 97 the incompatibility factor is present to some degree in the stigma and style.

Two possible explanations are suggested, the validity of which requires further experimentation.

The first explanation is that the inhibitory substances originate in the ovules. In the self-incompatible parent 47 these materials are localized and concentrated at the ovules or ovary and are effective in preventing fertilization. Dilution of the inhibitory substances, by diffusion into the style and stigma, permits the passage of some pollen tubes to effect fertilization in the self-compatible parent 97.

The gradation from self-compatibility to self-incompatibility among the various parents of sweet potato is due to a gradation from low to high in the production of inhibitory substances, and also due to differences in the localization of these materials.

The second explanation is that pollen tubes reach the ovules of self-incompatible lines, whereas following self-fertilization, the endosperms are not activated by hybridization as is usually necessary for normally cross-pollinated plants; therefore the nutrient balance soon shifts in favor of the integuments. This may be the major cause of a lack of nutrients and the starvation of the young embryos.

The different degrees of fertility among the various combinations of sweet potato parents may be partially interpreted according to the amount of hybrid vigor present in endosperm of these various combinations.

Failure of inter-species crosses was attributed either to the presence of inhibitory substances in the stigma and style or to their localization in the ovary or ovules. In some cases fertilization may have been effected but the ovules collapsed due to a slow growth of the endosperms and the starvation of the young embryos. In some species combinations differences in osmotic pressues resulted in the bursting of the pollen grains.

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Master of Science

Thesis: STUDIES OF INCOMPATIBILITY IN THE SWEET POTATO AND OTHER IPOMOEA SPECIES

Major Field: Horticulture

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Date of Final Examination: November, 1956.

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