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To the Graduate Council:

I am submitting herewith a thesis written by Chia-Lin Wang entitled "Relationships of Certain Pollen and Pistil Characteristics to Different Measures of Self-fertility in Alfalfa." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agronomy.

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RELATIONSHIPS OF CERTAIN POLLEN AND PISTIL CHARACTERISTICS TO DIFFERENT MEASURES OF SELF-FERTILITY IN ALFALFA

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Chia-Lin Wang

December 1965

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CHAPTER I

INTRODUCTION

Alfalfa (Medicago sativa L.) is one of the most important forage plants grown in the United States and many other countries. Many varieties of alfalfa have been developed which differ in adaptation, disease resistance, and other characteristics. Most of these varieties were developed by mass selection or by polycross progeny testing and maintained as synthetic varieties; therefore, such varieties do not make maximum use of heterosis.

Hybrid varieties have resulted in yield increases of 20 per cent in corn and several other crops. It has not been feasible to produce inbred lines for production of hybrid varieties in alfalfa because inbreeding results in severe decreases in both reproductive and vegetative vigor. The mechanisms responsible for the low self-fertility in alfalfa are complex and poorly understood. An understanding of the relationships of various pollen and pistil characteristics to different methods of estimating self-fertility would help elucidate self-sterility in alfalfa.

The first objective of this study was to compare three methods of estimating self-fertility. These estimates included the percentage of selfed flowers that produced pods, seeds per flower selfed and seeds per pod produced. The second objective was to determine the relationships of pollen viability, pollen tube length, pistil length, style length

and number of ovules per ovary to the three previously mentioned estimates of self-fertility in alfalfa.

CHAPTER II

REVIEW OF LITERATURE

I. DESCRIPTION OF ALFALFA

Alfalfa (Medicago sativa L., M. falcata L., and M. gaetula Urb.) is a widely grown perennial legume. Both diploid (n = 8) and tetraploid (n = 16) forms exist in these Medicago species (14).# Sprague (32) inter-crossed diploid plants of the three species and found both regular chromosome pairing and high fertility in the hybrids. He concluded that genomes of the three species were homologous and that M. sativa, M. falcata, and M. gaetula were varieties within one polymorphic species.

The most widely cultivated alfalfa is the tetraploid (n = 16) M. sativa. Cytogenetic and genetic evidence exists for both autoploidy and alloploidy in alfalfa (2, 21).

Alfalfa is highly cross-pollinated. Usually tripping of the flower is necessary for pollen germination and seed production. When the staminal column is released from the keel, the column strikes the standard petal and ruptures the surface of the stigma. The ruptured stigma releases a substance which is believed to be necessary for pollen germination (10). Occasionally autogamous flowers are found. Kirk and White (25) reported that some anthers dehisced before the flowers were tripped.

Numbers in parentheses refer to similarly numbered items in the Literature Cited.

II. EFFECT OF INBREEDING ALFALFA

Selfing usually results in a decrease in vegetative vigor and a greater decrease in reproductive vigor. Wilsie (37) selfed 437 alfalfa plants in the greenhouse and obtained 0.75 seeds per flower. Self-fertility was also studied on two groups of four clones each. For Group I, seeds per flower selfed averaged 1.32, 0.08, and 0.09 for clones, S_1 and S_2, respectively. For Group II, the corresponding values were 1.17, 0.11, and 0.10. He concluded that loss in self-fertility through one generation of selfing was 80 to 90 per cent. The loss in vegetative vigor from one generation of selfing was 20 to 30 per cent.

Koffman and Wilsie (26) studied self-fertility of eight 'DuPuits' plants and obtained a range of 0.32 to 4.84 seeds with a mean of 1.72 seeds per flower.

Armstrong (1) reported an average of 1.2 seeds per pod when selfed and 2.3 seeds per pod when crossed for six 'Ladak' plants. Bolton (8) studied one hundred highly self-fertile Ladak and 'Grimm' plants and found that self-fertility ranged from 0.02 to 5.22 with a mean of 1.58 seeds per flower. Cross-fertility (no emasculation) ranged from 2.67 to 8.26 with a mean of 5.54 seeds per pod. Sayers (31) reported data in which six 'Narragansett' clones averaged 1.11 seeds per flower selfed and 3.88 seeds per flower crossed.

Zaleski (38) studied self- and cross-fertility of five alfalfa varieties during two summers under field conditions. The percentage of flowers producing pods and seeds per pod were highest when the flowers

were pollinated by insects, intermediate when hand crossed, and lowest when hand selfed. The varieties did not appear to differ in self- or cross-fertility. Results for both self- and cross-fertility were similar for the two years.

Tysdal and Crandall (34) permitted natural crossing of three clones which had self-fertility estimates of 10, 50, and 85 per cent. The percentages of natural crossing for the three clones were 80.7, 71.7, and 52.0 per cent, respectively. They concluded that self-fertile clones were not good parents when a high degree of natural crossing was desired. Gartner and Davis (19) on the other hand found no relationship between estimates of self-fertility and the amount of natural crossing.

Pankiw and Bolton (28) selfed plants with purple-colored flowers and added pollen from yellow-flowered plants to these same flowers at intervals of time for 48 hours following self-pollination. They found that self- and cross-fertilized seeds were intermingled in the same pod but selfed seeds were not produced beyond the fourth position from the stylar end. Bradner and Frakes (9) obtained similar results using a recessive white flowered genotype.

Wilsie (36) reported that greenhouse conditions were more favorable than field conditions for producing selfed seeds. He found that average self-fertility for three clones was about twice as great in the greenhouse as in the field.

A correlation of 0.71 between self- and cross-fertility of nineteen alfalfa plants was observed by Wilsie (36). He suggested that

some of the genes that influence self-fertility also influence cross-fertility.

In a selfing study involving nineteen alfalfa plants, Gartner and Davis (19) found that pods set were correlated with seeds per pod ($r = 0.88$) and seeds per flower ($r = 0.96$).

Studies by Brink and Cooper (11, 16) showed that lower seed set following selfing was due to slower pollen tube growth, a tendency for pollen tubes to pass directly by the micropyles, and to differential endosperm development with subsequent ovule collapse. They also found more ovules aborted when selfed than when crossed and attributed this phenomenon to differential endosperm growth rates and the occurrence of hyperplasia. The growth rate of endosperm was found to be significantly higher after crossing than after selfing (12).

There are two general explanations of the deleterious effect of inbreeding. The dominance theory is based on the assumption that dominant genes are favorable and recessive genes are deleterious. Selfing results in an increase in homozygous recessive genes and consequently a detrimental effect. The overdominance theory is based on the supposition that the heterozygotes are superior to either homozygote. Inbreeding results in greater homozygosity and the detrimental effect (20). Both theories are reasonable and may be applicable to the explanation of the inbreeding effect in alfalfa.

Parsons (29) reported that, theoretically, the rate of approach to homozygosity with selfing is much slower for autotetraploids than for diploids. Tysdal _et al_. (35) found that inbreeding alfalfa resulted in

much greater losses in both vegetative and reproductive vigor than was expected for an autotetraploid. These workers and many others have found it difficult to maintain selfed lines after two or three generations of selfing because of the loss in vigor and fertility.

III. POLLEN CHARACTERISTICS AFFECTING SEED SET IN ALFALFA

Childers (13) analyzed percentage of aborted pollen in F_1 and F_2 progenies from the cross of male sterile x male fertile plants and found a range of 3 to 88 per cent aborted pollen. He concluded that partial male sterility occurs frequently, whereas complete male sterility or complete fertility are rare.

From studies involving ninety-six 'Ranger' alfalfa clones, Rotar and Kehr (30) reported that self-fertility was not correlated with percentage of pollen abortion, micronuclei per quartet or irregularities during meiosis. Pollen abortion counts made on the clones at different times were correlated.

Armstrong (1) studied pairing relationships of chromosomes at metaphase of six Ladak plants and observed that plants which had irregular meiotic behavior produced only 0 to 10 per cent viable pollen. He also suggested that nonviable pollen might be attributed to physiological abnormalities under genetic control.

Brink and Cooper (10) studied aborted pollen on 137 alfalfa plants and found that the correlation coefficients for percentage

aborted pollen with seeds per flower and seeds per pod were 0.13 and 0.03, respectively.

Based on the number of pollen tubes in the style six to eight hours after pollination, Armstrong and White (3) concluded that pollen sterility had an important influence on pod setting and number of seeds per pod. Engelbert (18) found that as much as 22.5 per cent of the pollen being sterile was of little importance because a plant produces an abundance of pollen. He concluded that pollen sterility may be partly responsible for differences in seed setting by different plants.

Barnes and Cleveland (5) measured lengths of pollen tubes that were grown on agar medium. Average pollen tube lengths for individual plants varied from 1.6 to 4.1 mm. The short pollen tubes ceased to grow earlier than the long pollen tubes. Brink and Cooper (11) sampled pistils at 30, 48, 72, 96, 120, and 144 hours after self- and cross-pollination and found that few pollen tubes advanced beyond the mid-region of the ovary after selfing, whereas after crossing, the tubes usually reached the base of the ovary. Thirty hours after self-pollination the tubes had reached the eighth or ninth ovule from the stylar end. In a similar study, Cooper et al. (17) observed that following selfing more eggs were fertilized in the stylar end than at the base of the ovary. They attributed this phenomenon to the slow growth of the pollen tubes.

Some workers have found that age of pollen had some effect on viability. Pankiw and Bolton (28) reported that pollen germination on

agar medium did not decrease rapidly with age. Pollen from florets five days old or older produced shorter pollen tubes than pollen from younger florets. Through the use of a male sterile plant in a greenhouse study, Hanson (22) found that the longevity of pollen in situ was about eight to fifteen days. The percentage of flowers setting pods indicated that some deterioration began by the end of six to seven days.

Barnes and Cleveland (5) reported that larger pollen grains tended to produce longer tubes than smaller pollen grains.

IV. PISTIL CHARACTERISTICS AFFECTING SEED SET IN ALFALFA

In a study of comparative morphology of some Leguminosae, Martin (27) reported that the number of ovules in tetraploid alfalfa ovaries varies from twelve to eighteen. Cooper (15) examined one hundred alfalfa ovaries and observed a range of eight to fourteen ovules with most ovaries having ten to twelve. The ovary contains two rows of ovules, one on each side of the suture. Barnes and Cleveland (7) observed that flowers at the base of the racemes generally contain more ovules and produce more seeds than flowers farther from the base.

Gartner and Davis (19) found no correlation between ovules per ovary and either pods set or seeds per pod for selfed flowers. In the results obtained by Barnes and Cleveland (4), ovary length and number of ovules per ovary were significantly correlated ($r = .499$), ovary length versus style length and number of ovules per ovary versus style length were not correlated.

The results referred to previously by Brink and Cooper (11) and Cooper et al. (17) indicated that ovules fertilized by pollen from the same plant are located primarily in the stylar end of the ovary. This could mean that flowers having longer styles or longer ovaries would be less self-fertile than flowers having shorter styles and ovaries.

CHAPTER III

MATERIALS AND METHODS

I. ESTIMATES OF SELF-FERTILITY

In October, 1963, fifty plants of each of five varieties were taken at random from a two-year old field planting of alfalfa varieties and potted in the greenhouse. Through the use of supplementary lighting the plants were given a fourteen-hour light period per day. About ten flowers per raceme were selfed by tripping the flowers against a toothpick covered with sandpaper. Each plant was used as a female and crossed with several other plants which were taken at random from the five varieties. Crossing was accomplished by tripping flowers of the male parent against a toothpick covered with sandpaper and transferring the pollen to the stigma of the female parent. Flowers used as females in crosses were not emasculated because tubes from pollen grains produced by another plant are believed to grow faster and affect fertilization earlier than tubes from pollen produced by the same plant. After the seed had matured, each raceme was harvested and rubbed out individually. The number of pods and seeds produced by each raceme was counted and the percentage of flowers producing pods, seeds per flower, and seeds per pod were calculated for both selfs and crosses. Seeds per flower were determined by dividing the number of seeds per raceme by the number of flowers selfed or crossed on the raceme.

Descriptions of the five alfalfa varieties included in this study are given in Appendix A (23). The varieties were 'Atlantic,' 'Buffalo,' 'Delta,' 'DuPuits,' and 'Narragansett.'

II. MEASUREMENTS OF POLLEN VIABILITY, POLLEN TUBE LENGTH, PISTIL LENGTH, STYLE LENGTH, AND NUMBER OF OVULES PER OVARY

For this study, forty-two Buffalo alfalfa plants were taken at random from a one-year old field planting and potted in the greenhouse in December, 1964. These plants were selfed in the same manner as was previously described for plants used in comparisons of self-fertility estimates. The percentage of flowers producing pods, seeds per flower selfed, and seeds per pod were calculated.

Pollen Viability

Two methods were used to measure pollen viability.

In vitro germination. Preliminary studies involving different concentrations of sugar and agar showed that the medium used by Barnes and Cleveland (5) for pollen of diploid alfalfa was suitable for pollen of tetraploid alfalfa. About 1 ml of a medium which contained 2 g of Bacto-Agar and 15 g sucrose per 100 ml distilled water was added to a microscope slide. Pollen grains were collected on a camel hair brush and immediately spread on the medium. After one hour of incubation at 26 to 27°C, the pollen grains from which pollen tubes had emerged were

considered viable. A total of five hundred pollen grains were counted from three slide preparations for each plant using 120x magnification.

Hydrogen peroxide reaction. This method was similar to the one used by King (24) in testing pollen viability of several species. According to King (24),

> It is based on the oxidation of benzidine by peroxidase in the presence of hydrogen peroxide. The reaction in the pollen grain is accompanied by the emission of oxygen gas, which is freed from the hydrogen peroxide by catalase.

About 1 ml of a medium which contained 10 ml of 2 per cent Bacto-Agar, 5 ml of hydrogen peroxide, and 1.8 ml of 1 per cent benzidine (base) in 60 per cent ethanol was applied to a microscope slide. A small brush was used to spread the pollen grains on the medium. After thirty minutes of incubation at 26 to 27°C, five hundred pollen grains from three slide preparations were observed under 120x magnification and classified as viable or nonviable. Viable pollen grains were grown and nonviable pollen grains were light brown or colorless.

Pollen Tube Length

The agar medium used in the pollen germination study was also used in this study. About 1 ml of the medium was applied to a microscope slide. After pollen grains from four to six flowers were spread on three slides with a small brush, the slides were placed on wet filter paper in a covered Petri dish and incubated for thirty-two hours at 26 to 27°C. In order to stop tube growth, the slides were stored at about 0°C until measured. Pollen tubes were straightened by gently

pulling the pollen grain across the surface of the medium with a fine needle. Measurements of about seventy-five pollen tubes per plant were made with an ocular disc micrometer in which one ocular unit equaled 0.1 mm at 120x magnification.

Pistil Length, Style Length, and Number of Ovules Per Ovary

For these measurements, the first eight to ten flowers to open on the raceme were removed and stored in 50 per cent ethanol. Pistils were removed from the staminal columns with a dissecting needle and placed in a drop of water between two cover slips. Sufficient pressure was applied to split the ovaries longitudinally. Pistil length, style length, and number of ovules per ovary were determined at 30 to 40x on thirty-seven ovaries per plant.

Data for the estimates of self-fertility were analyzed statistically using the variation within plants of the varieties as error for testing differences among varieties. When more than a single observation was available for measures of pollen and pistil characteristics, within plant variation was used as error for testing differences among plants.

CHAPTER IV

RESULTS AND DISCUSSION

I. ESTIMATES OF SELF- AND CROSS-FERTILITY

Data on number of plants per variety, number of flowers selfed or crossed per plant, percentage of flowers producing pods, seeds per flower, and seeds per pod are presented in Appendix B and summarized by variety in Table I. From the data in Appendix B, it can be seen that plants within a variety had a wide range of self- and cross-fertility. The varieties did not differ in self-fertility when estimated by percentage of flowers producing pods, seeds per flower, or seeds per pod (Table II). Varieties differed in cross-fertility when estimated by percentage of flowers producing pods, but they did not differ for the other two estimates.

All varieties had a lower percentage of flowers producing pods, fewer seeds per flower, and fewer seeds per pod when selfed than when crossed (Table I). The average seeds per flower selfed of 1.28 is lower than the 1.58 average obtained by Bolton (8) for thirteen Ladak clones, but is higher than the 0.75 average reported by Wilsie (37). The average of 3.45 seeds per pod when crossed is lower than the 5.54 seeds per pod obtained by Bolton (8).

Since no absolute measure of self-fertility was available, the methods of estimating self-fertility were compared with one another and

TABLE I

SUMMARY OF SELFED AND CROSSED SEED SET ON PLANTS OF FIVE VARIETIES OF ALFALFA

	Variety						
	Atlantic	Buffalo	Delta	DuPuits	Narragansett	Total	Average
Number of plants							
Selfed	40	38	41	34	40	193	
Crossed	37	33	38	35	37	180	
Number of flowers							
Selfed	16,227	7,164	7,735	10,157	18,044	59,327	
Crossed	5,714	2,494	3,398	4,614	3,833	20,053	
Percentage of flowers producing pods							
Selfed	51.8±6.0#	39.7±7.8	41.2±6.6	48.7±6.4	46.5±6.3		45.6±2.9
Crossed	66.4±4.8	57.0±8.0	55.2±6.1	52.6±8.1	57.3±5.7		57.7±2.9
Seeds per flower							
Selfed	1.52±0.26	1.22±0.35	1.03±0.20	1.31±0.27	1.33±0.28		1.28±0.11
Crossed	2.28±0.35	1.77±0.43	1.87±0.37	2.07±0.33	1.79±0.28		1.95±0.15
Seeds per pod							
Selfed	2.78±0.35	2.55±0.47	2.31±0.30	2.83±0.35	2.88±0.43		2.67±0.17
Crossed	3.63±0.39	3.35±0.43	3.20±0.45	3.72±0.45	3.34±0.35		3.45±0.19

Confidence intervals based on $t_{(.05)} \frac{S}{\bar{x}}$.

TABLE II

ANALYSIS OF VARIANCE OF THREE ESTIMATES OF SELF- AND CROSS-FERTILITY IN FIVE VARIETIES OF ALFALFA

Character	Source	DF	Mean Square	
Self-fertility				
Percentage of flowers producing pods	Among varieties	4	557.75	N. S.
	Within varieties	188	283.88	
Seeds per flower	Among varieties	4	1.22	N. S.
	Within varieties	188	.59	
Seeds per pod	Among varieties	4	2.20	N. S.
	Within varieties	188	.97	
Cross-fertility				
Percentage of flowers producing pods	Among varieties	4	916.88**	
	Within varieties	175	364.77	
Seeds per flower	Among varieties	4	1.64	N. S.
	Within varieties	175	1.18	
Seeds per pod	Among varieties	4	1.59	N. S.
	Within varieties	175	1.41	

**Significant at the .01 level of probability.

with similar methods of estimating cross-fertility. The three possible simple correlation coefficients among the three estimates of self-fertility were calculated for each variety and over-all varieties (Table III).

Positive and highly significant correlations between percentage of flowers producing pods and seeds per flower were found for each variety. The correlation for all varieties was also highly significant. No pods without seed were observed; thus, as the number of pods increased the number of seeds per flower also increased.

The positive correlation between percentage of flowers producing pods and seeds per pod was highly significant for plants of all varieties and for plants of each variety, except Narragansett (Table III). This relationship means that as the number of pods increased there was an increase in number of seeds per pod. The self-fertility mechanism in alfalfa may have an effect on both the retention of flowers following pollination and number of seeds produced per pod. Another interpretation is that flower retention was affected by the number of eggs that were fertilized and developed into seeds. This must be considered in view of the fact mentioned previously that pods apparently were not produced unless at least one seed was produced. The second interpretation is not independent of the first, but it implies a more indirect effect of the self-fertility mechanism on the percentage of flowers that produce pods. Selfing decreased both the percentage of flowers producing pods and seeds set per pod (Table I, page 16). However, this observation fails to favor one interpretation over the other.

TABLE III

SIMPLE CORRELATION COEFFICIENTS BETWEEN MEASURES OF SELF-FERTILITY IN FIVE ALFALFA VARIETIES

	Variety					Over-all varieties
	Atlantic	Buffalo	Delta	DuPuits	Narragansett	
Percentage of flowers producing pods vs. Seeds per flower	.781**	.793**	.748**	.822**	.863**	.795**
Percentage of flowers producing pods vs. Seeds per pod	.364*	.586**	.361*	.511**	.280	.416**
Seeds per flower vs. Seeds per pod	.713**	.849**	.735**	.871**	.384*	.705**
Degrees of Freedom	38	36	39	32	38	191

*Significant at the .05 level of probability.
**Significant at the .01 level of probability.

Seeds per flower and seeds per pod were positively correlated. Since pods are produced from flowers, an increase in seeds per pod would be expected to give an increase in seeds per flower.

The three methods of determining self-fertility were compared for cross-fertility (Table IV). Each plant was used as a female and crossed without emasculation with a random group of males from all the varieties.

Percentage of flowers producing pods and seeds per pod were significantly correlated for Delta and Buffalo but were not significantly correlated for the other varieties. When plants of all varieties were considered the correlation was highly significant. This suggests that more fertile plants not only produce more pods but also produce more seeds per pod. A similar and perhaps stronger relationship was found between percentage of flowers producing pods and seeds set per pod for the selfed flowers. These results are in agreement with those reported by Wilsie (36). He found a positive correlation between cross- and self-fertility in alfalfa and suggested that self- and cross-fertility are affected by some of the same genes.

The relationship between seeds per flower and seeds per pod for crossed flowers was positive and of the same magnitude as for selfed flowers.

The effect of one generation of selfing was measured by expressing the ratio of self- to cross-fertility for the three estimates (Table V). Considering plants from all varieties the ratios were 0.78, 0.66, and 0.77 for percentage of flowers producing pods, seeds per flower, and seeds per pod, respectively. One generation of selfing apparently had

TABLE IV

SIMPLE CORRELATION COEFFICIENTS BETWEEN MEASURES OF CROSS-FERTILITY IN FIVE ALFALFA VARIETIES

	Variety					Over-all varieties
	Atlantic	Buffalo	Delta	DuPuits	Narragansett	
Percentage of flowers producing pods vs. Seeds per flower	.548**	.879**	.658**	.810**	.482**	.696**
Percentage of flowers producing pods vs. Seeds per pod	.175	.404*	.392*	.182	-.038	.248**
Seeds per flower vs. Seeds per pod	.582**	.711**	.847**	.693**	.828**	.735**
Degrees of Freedom	35	31	36	33	35	178

*Significant at the .05 level of probability.

**Significant at the .01 level of probability.

TABLE V

RATIO OF SELF- TO CROSS-FERTILITY FOR THREE ESTIMATES OF FERTILITY[#]

Variety	Percentage of flowers producing pods Selfed/Crossed	Seeds per flower Selfed/Crossed	Seeds per pod Selfed/Crossed
Atlantic	0.78	0.67	0.77
Buffalo	0.70	0.69	0.76
Delta	0.72	0.55	0.72
DuPuits	0.94	0.63	0.76
Narragansett	0.82	0.74	0.86
All Plants	0.78	0.66	0.77

[#]Data for ratios were taken from Table I.

a slightly greater effect on seeds per flower than on either of the other two estimates.

The three estimates of self-fertility were interrelated. Selfing resulted in lower fertility regardless of the estimate used. Less effort would be required in a selfing program to determine the percentage of flowers producing pods than to make either of the other two estimates. The inbreeding effect may be slightly more pronounced when seeds per flower is used rather than percentage of flowers producing pods or seeds per pod.

II. POLLEN VIABILITY, POLLEN TUBE LENGTH, PISTIL LENGTH, STYLE LENGTH AND NUMBER OF OVULES PER OVARY

These data were obtained from forty-two Buffalo plants selfed during the winter of 1964-65. Individual plant data are presented in Appendix C and summarized in Table VI. For these plants, 44.3 (range 21.1 - 71.1) per cent of the selfed flowers produced pods, an average of 1.33 (range 0.32 - 3.42) seeds were produced per flower selfed and an average of 2.84 (range 0.92 - 4.96) seeds were produced per pod. Corresponding values for the set of Buffalo plants used in the estimates of self-fertility study were 42.2 (range 5.0 - 84.5), 1.22 (range 0.05 - 5.22), and 2.55 (range 0.05 - 6.55), respectively.

Pollen Viability

Two methods were used to measure pollen viability.

TABLE VI

SUMMARY OF PERCENTAGE OF FLOWERS PRODUCING PODS, SEEDS PER FLOWER, SEEDS PER POD, POLLEN VIABILITY, POLLEN TUBE LENGTH, PISTIL LENGTH, STYLE LENGTH, AND NUMBER OF OVULES PER OVARY OF FORTY-TWO BUFFALO ALFALFA PLANTS

Character	Mean	Range
Percentage of flowers producing pods selfed	44.3 $\pm$ 8.3 ##	21.1 - 71.1
Seeds per flower	1.33 $\pm$ 0.21	0.32 - 3.42
Seeds per pod	2.84 $\pm$ 0.26	0.92 - 4.96
Pollen viability #		
(a)	55.1 $\pm$ 4.6	20.1 - 77.2
(b)	68.9 $\pm$ 4.5	34.0 - 95.7
Pollen tube length, mm	.87 $\pm$ 0.06	.59 - 1.32
Pistil length, mm	5.24 $\pm$ 0.10	4.33 - 5.95
Style length, mm	2.53 $\pm$ 0.06	2.08 - 2.87
Number of ovules per ovary	10.18 $\pm$ 0.28	8.71 - 12.64

(a) In vitro germination.

(b) Hydrogen peroxide reaction.

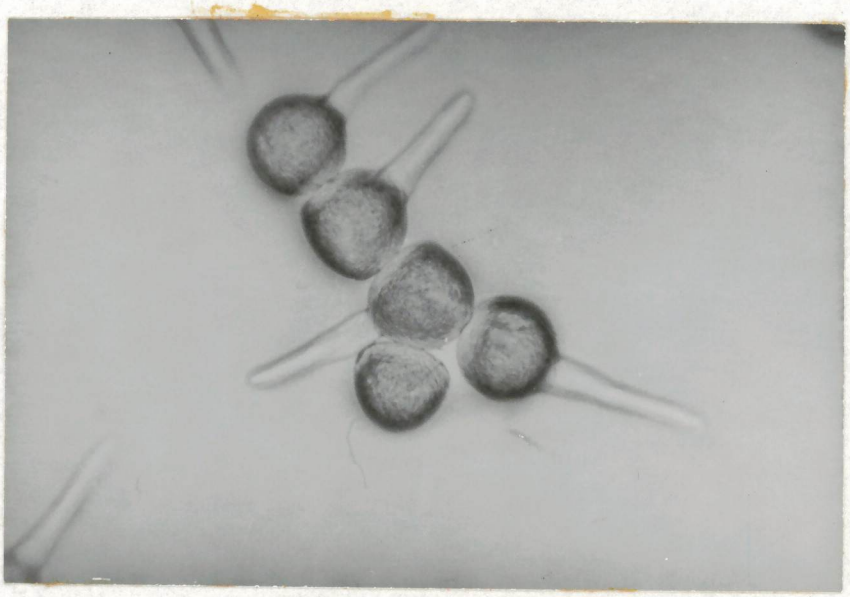
Confidence intervals based on $t_{(.05)} S_{\bar{x}}$.

In vitro germination. Even though most of the pollen grains germinated in vitro within fifteen minutes, some had not germinated two hours after being placed on the medium. Since the mass of pollen tube growth made it difficult to distinguish between germinated and non-germinated pollen grains, all counts were made one hour after the pollen was placed on the medium (Figure 1a). The percentages of germinated pollen, based on five hundred pollen grains per plant, ranged from 20.1 to 77.2 and averaged 55.1 per plant (Appendix C and Table VI, page 24).

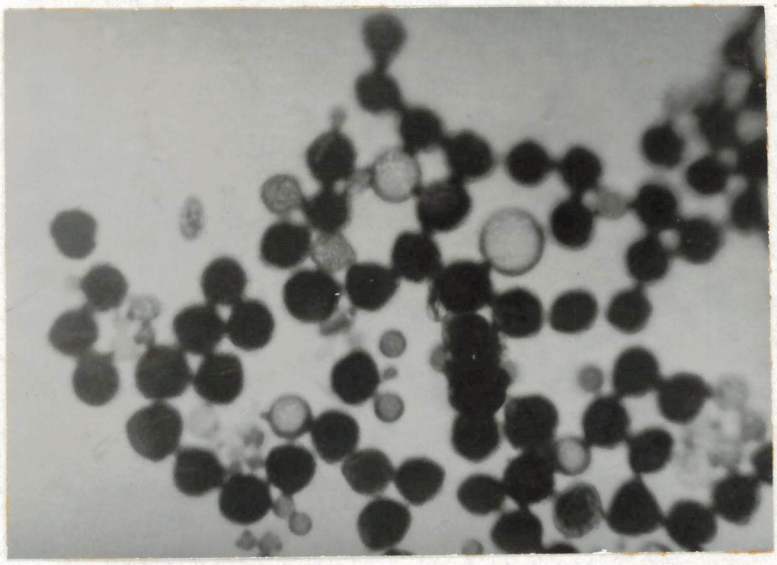
Hydrogen peroxide reaction. Percentages of viable pollen grains, based on five hundred pollen grains per plant, ranged from 34.0 to 95.7 with an average of 68.9 per plant for the hydrogen peroxide reaction (Appendix C and Table VI). Pollen viability estimated by this method was higher than the estimates based on in vitro germination. Some viable pollen grains probably had not germinated on the agar medium at the end of one hour. Estimates by the two methods were significantly correlated ($r = 0.782$).

Pollen Tube Length

Average length (Figure 2) of at least seventy-five pollen tubes from each of forty-two plants are presented in Appendix C and summarized in Table VI. The range in average length of pollen tubes for the plants was 0.59 to 1.32 with an average of 0.87 mm. Significant differences were found among the plants for average pollen tube length (Table VII). Results obtained by Barnes and Cleveland (5) indicated that pollen tube length was genetically controlled.



a. In vitro germination, 200x.



b. Hydrogen peroxide reaction, dark brown is viable and colorless is nonviable, 120x.

Figure 1. Pollen viability.



Figure 2. Pollen tubes after one hour *in vitro*, 120x.

TABLE VII

ANALYSIS OF VARIANCE OF POLLEN TUBE LENGTH, PISTIL LENGTH, STYLE LENGTH, AND NUMBER OF OVULES PER OVARY IN FORTY-TWO BUFFALO ALFALFA PLANTS

Characters	Source	DF	Mean square
Pollen tube length	Among plants	41	383.82**
	Within plants	3366	11.26
Pistil length	Among plants	41	3.18**
	Within plants	1521	1.63
Style length	Among lengths	41	0.53**
	Within plants	1521	0.07
Number of ovules per ovary	Among plants	41	47.73**
	Within plants	1521	1.71

**Significant at the .01 level of probability.

Pistil Length

The distance between the stigma and the last ovule in the base of the ovary (Figure 3) was measured on thirty-seven pistils from each of the forty-two plants. The range in pistil length was 3.9 to 6.7 with a mean of 5.24 mm (Appendix C and Table VI, page 24). Plants differed significantly for average pistil length (Table VII, page 28).

Style Length

The distance between the stigma and the first ovule from the stylar end (Figure 3) was measured on thirty-seven styles from each of the forty-two plants. Style length ranged from 1.7 to 3.7 with a mean length of 2.53 mm. Plant differences in average style length were significant (Table VII).

Number of Ovules Per Ovary

Ovule counts were made on thirty-seven ovaries from each of the forty-two plants (Figure 3). Ovaries were selected from the first ten flowers that opened on the raceme. The range for ovule numbers was five to fifteen with a mean of 10.18 per ovary. Martin (27) reported twelve to eighteen and Cooper (15) found eight to fourteen ovules per ovary in alfalfa. Analysis of variance showed significant differences in the number of ovules per ovary among the plants (Table VII).

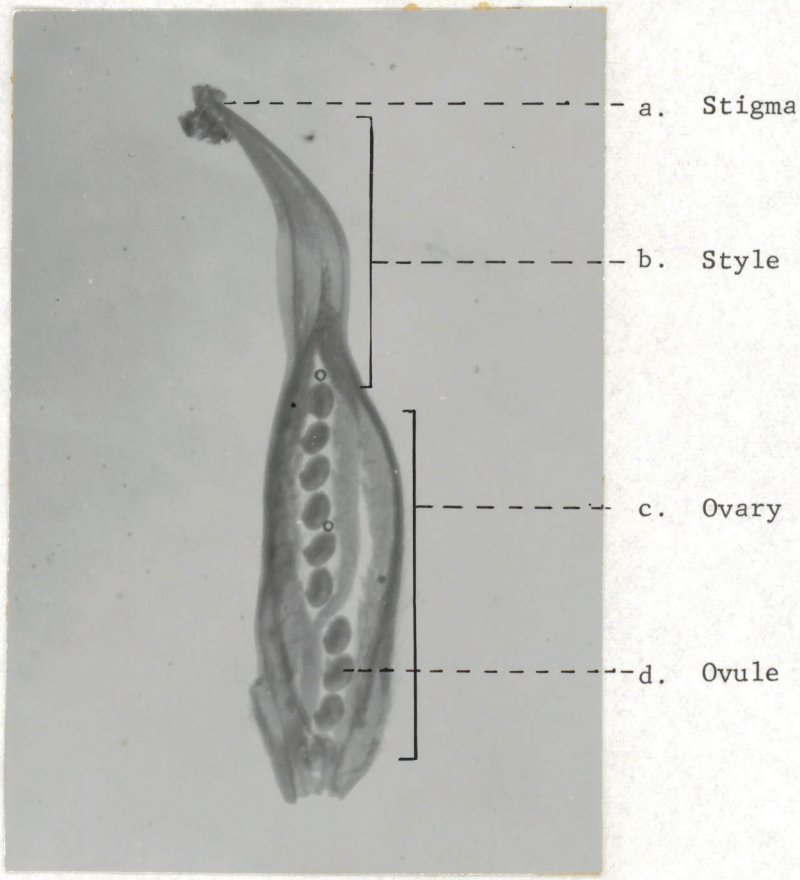


Figure 3. Alfalfa pistil, 18x.

III. RELATIONSHIPS OF POLLEN VIABILITY, POLLEN TUBE LENGTH, PISTIL LENGTH, STYLE LENGTH, AND NUMBER OF OVULES PER OVARY TO ESTIMATES OF SELF-FERTILITY

Relationships among the estimates of self-fertility for this second group of Buffalo plants were similar to the relationships among the first group used in the self-fertility study. For the first group, the correlation between percentage of selfed flowers producing pods and seeds per flower was 0.793, between percentage of flowers producing pods and seeds per pod the correlation was 0.586, and between seeds per flower and seeds per pod the correlation was 0.849. Corresponding correlation values for the second group were 0.797, 0.497, and 0.827, respectively. Correlation coefficients for measures of pollen and pistil characteristics with estimates of self-fertility are presented in Table VIII.

Pollen viability. There was no significant correlation between pollen germination and level of self-fertility of the plants. Assuming that germination on agar medium was indicative of ability to fertilize, a viability of only 20.1 per cent did not affect seed production. Brink and Cooper (10) reported data for alfalfa crosses in which the correlations for percentage aborted pollen with seeds per flower and seeds per pod were 0.13 and 0.03, respectively. Engelbert (18) concluded that 22.5 per cent sterile pollen was not sufficient to affect cross-fertility. Armstrong and White (3) found that pollen germination on the flower

TABLE VIII

SIMPLE CORRELATION COEFFICIENTS FOR CERTAIN POLLEN AND PISTIL CHARACTERISTICS WITH THREE ESTIMATES OF SELF-FERTILITY

	Percentage of flowers producing pods	Seeds per flower	Seeds per pod
Pollen viability			
a. *In vitro* germination	.187	.102	-.117
b. Hydrogen peroxide reaction	.445**	.282	.174
Pollen tube length	.014	.279	.356*
Pistil length	.324*	.153	.112
Style length	.079	.222	-.094
Number of ovules per ovary	.329*	.211	.245

*Significant at the .05 level of probability.

**Significant at the .01 level of probability.

stigma was related to pod setting and number of seeds per pod, whereas, in the present study no relationship was found with pollen germinated on artificial medium.

Pollen viability as measured by the hydrogen peroxide reaction was significantly positively correlated with percentage of flowers producing pods, but the correlation with seeds per flower or seeds per pod was not significant (Table VIII). In view of the relationship among the self-fertility estimates, it would appear that, if the hydrogen peroxide reaction measures pollen viability, all of the estimates would have been affected. The explanation of this discrepancy is not obvious.

The alfalfa flower produces many pollen grains and only a few are needed in fertilization; therefore, a very high percentage of the grains would have to be nonviable to cause a reduction in seed set.

Pollen tube length. Average pollen tube length was not correlated with percentage of flowers producing pods (Table VIII). The correlation coefficients for pollen tube length with seeds per flower and seeds per pod were higher and the latter was significant.

This implies that plants producing pollen which developed longer tubes *in vitro* tended to produce more seeds per pod. Actual length of pollen tubes produced *in vitro* apparently were shorter than those produced *in vivo* because average length of the measured pollen tubes was 0.87 mm, whereas, average lengths of the pistils and styles were 5.24 and 2.53 mm, respectively. Barnes and Cleveland (5) reported an *in vitro* average pollen tube length of 4.0 mm for one plant.

Pollen tube growth in vitro does not include the effect of the style. They tend to grow faster in styles of other plants than in styles of the plant producing the pollen. A method is needed which will permit pollen tube length to be measured in the style. Such an attempt was made in the present study, but it was difficult to section the style in such a manner that a tube could be followed and measured.

Pistil length. Brink and Cooper (11) and Pankiw and Bolton (28) reported that basal ovules were fertilized less frequently than ovules closer to the stylar end when alfalfa was selfed. Based on this observation, it was expected that a negative correlation would exist between pistil length and self-fertility (Table VIII, page 32). In these data there was no relationship between pistil length and self-fertility, except for percentage of flowers producing pods, which was positively correlated with pistil length. Further investigation revealed that pistil length and ovule number were positively correlated (0.624), and ovule number and percentage of flowers producing pods were correlated (Table VIII). Therefore, the positive relationship between pistil length and percentage of flowers producing pods may be due to the relationship of pistil length and percentage of flowers producing pods to number of ovules per ovary.

Style length. Style length and self-fertility estimates were not correlated. This observation along with those of Pankiw and Bolton (28) and Brink and Cooper (11), in which ovules in the stylar end were fertilized more frequently than basal ovules, suggests that sufficient pollen

tubes grow through the style but generally do not extend beyond the fourth ovule.

Number of ovules per ovary. The number of ovules per ovary was correlated with percentage of flowers producing pods but not significantly correlated with seeds per flower or per pod. Since the flowers produced 10.18 ovules per ovary and seeds per pod averaged 2.84 (Table VI, page 24), number of ovules should not have limited the number of seeds per pod. If the pollen tubes failed to grow beyond about the fourth ovule as was suggested by previous work (28), additional ovules would have no effect.

Although estimates of self-fertility obtained by different methods were interrelated, they were not equally related to the measured pollen and pistil characteristics. The percentage of flowers producing pods was significantly correlated with pollen viability, as determined by the hydrogen peroxide reaction, with pistil length, and with ovules per ovary. Seeds per pod were significantly correlated with pollen tube length, and seeds per flower were not significantly correlated with any of the pollen or pistil characteristics (Table VIII, page 32).

Most of the significant correlations between the self-fertility estimates and the pollen and pistil characteristics were between 0.3 and 0.4 (Table VIII). Thus, only 9 to 16 per cent of the variation in self-fertility was associated with variation in any one of the pollen or pistil characteristics. Several reasons may be responsible for these low relationships. Apparently the self-incompatability mechanism was

not expressed through these physical characteristics of the pollen and pistil. All the estimates of self-fertility were ratios rather than simple measurements. Based on range and distribution of the measurements, the pollen and pistil characteristics appeared to be quantitative and subject to environmental influence. Wide genetic differences would be expected in gametes produced by a heterozygous tetraploid plant. In this study an average of 1.33 seeds were produced per flower, but the flowers produced numerous pollen grains and averaged 10.18 ovules (Table VI, page 24). The gametes which united to form the seed were a small sample of the total gametes. More reliable results could be obtained by studying individual pollen grains and ovules.

The comparisons of methods of estimating self-fertility and studies of the relationships of pollen and pistil characteristics with self-fertility were based on one generation of selfing. Results reported by Wilsie (37) and other workers indicate that self-fertility decreases rapidly from the first to the second generation of selfing. These relationships and methods need to be studied in later generations.

CHAPTER V

SUMMARY

Studies were conducted to compare three estimates of self-fertility and to determine the relationship between certain pollen and pistil characteristics and self-fertility in alfalfa (Medicago sativa L.).

Approximately forty plants from each of five varieties--Atlantic, Buffalo, Delta, DuPuits, and Narragansett--were selfed and crossed in the greenhouse during the winter of 1963-64. The varieties did not differ for the three estimates of self-fertility which included percentage of flowers producing pods, seeds per flower, and seeds per pod.

The estimates of self-fertility were interrelated. Correlation coefficients for plants of all varieties were 0.795 between percentage of flowers producing pods and seeds per flower, 0.416 between percentage of flowers producing pods and seeds produced per pod, and 0.705 between seeds produced per flower and seeds produced per pod.

The significant positive correlation between percentage of flowers producing pods and seeds per pod implies that the self-fertility mechanism in alfalfa affects both retention of flowers following selfing and number of seeds produced per pod.

Selfing resulted in lower fertility in all varieties regardless of the estimate used. Using plants of all varieties the ratios of

self- to cross-fertility were 0.78, 0.66, and 0.77 for percentage of flowers producing pods, seeds per flower, and seeds per pod, respectively.

The relationship of the three estimates of self-fertility with certain pollen and pistil characteristics was studied on a second group of forty-two Buffalo plants during the winter of 1964-65.

Pollen viability as measured by germination on an agar medium was not correlated with any of the self-fertility estimates. Pollen viability determined by the hydrogen peroxide reaction was positively associated with percentage of flowers producing pods but was not correlated with seeds per flower or seeds per pod. Length of pollen tubes produced on an agar medium was positively related to seeds per pod, but was not related to the other two estimates.

Pistil length was positively correlated with percentage of flowers producing pod, but was not correlated with seeds per flower or seeds per pod. Style length was not associated with any of the estimates of self-fertility. The number of ovules per ovary was positively related to percentage of flowers producing pods. The correlations for number of ovules with seeds per flower and seeds per pod were positive but not significant.

Variation in measured pollen and pistil characteristics was not highly correlated with variation in self-fertility of Buffalo alfalfa plants.

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LITERATURE CITED

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APPENDICES

APPENDIX A

TABLE IX

DESCRIPTIONS OF FIVE ALFALFA VARIETIES USED IN ESTIMATES OF SELF-FERTILITY STUDY

Variety	Origin	Characteristics
Atlantic[a]	Developed by New Jersey Agricultural Experiment Station, released in 1940.	Variable growth habit, dark green foliage and light purple flowers.
Buffalo[a]	Developed by Kansas Agricultural Experiment Station and USDA, released in 1943.	Purple flowers, quicker to recover than Kansas Common, bacterial wilt resistant.
Delta[b]	Developed by USDA and Mississippi Agricultural Experiment Station, released in 1965.	Purple flowers, dark green foliage.
DuPuits[a]	Developed in France, received for testing in United States in 1947.	Dark purple flowers, quick recovery, coarse stems.
Narragansett[a]	Developed by Rhode Island Agricultural Experiment Station from crosses between M. sativa and M. falcata, released in 1949.	Blue flowers, variable growth habit and dark green foliage.

[a]Descriptions by Hanson et al. (23).

[b]Unpublished description taken from notice of naming and release of Delta alfalfa by the Miss. Agr. Exp. Sta. and USDA.

APPENDIX B

TABLE X

SELFED AND CROSSED SEED SET ON PLANTS OF FIVE VARIETIES OF ALFALFA

Plant number	Number of flowers		Percentage of flowers producing pods		Seeds per flower		Seeds per pod	
	Selfed	Crossed	Selfed	Crossed	Selfed	Crossed	Selfed	Crossed
			I. ATLANTIC					
1	453	265	50.6	80.4	1.14	3.03	2.26	3.74
2	534	170	63.5	77.6	1.66	1.42	2.62	1.83
3	347	125	32.6	39.2	1.06	1.14	3.25	2.92
4	203		15.8		0.18		1.16	
5	376	149	54.4	49.7	1.59	1.22	2.91	2.46
6	560	172	35.2	51.2	0.83	1.64	2.35	3.20
7	513	81	65.7	63.0	2.07	4.26	3.15	6.76
8	238	256	31.9	27.3	0.63	0.78	1.96	2.86
9	298	223	52.3	59.6	1.12	1.58	2.15	2.64
10	493	108	63.1	60.2	1.78	2.26	2.83	3.75
11	120		68.3		2.17		3.17	
12	384	187	29.7	55.6	0.73	2.06	1.91	3.70
13	138	107	44.2	72.9	2.16	2.47	4.89	3.38
14	195	79	36.9	93.7	0.73	3.89	1.97	4.15
15	296	222	64.9	67.6	1.33	2.11	2.05	3.12
16	370	176	33.2	79.5	1.15	2.38	3.47	2.99
17	458	234	50.9	85.9	1.09	2.41	2.13	2.81
18	311	116	46.9	68.8	1.67	2.88	3.56	4.12
19	43	87	32.6	57.5	1.44	2.33	4.43	4.06
20	853	367	45.4	65.1	0.91	2.33	2.01	3.58
21	507	118	67.3	45.8	1.58	1.85	2.35	4.04
22	719	268	49.8	71.3	1.39	2.62	2.80	3.68
23	381	204	49.1	70.6	1.61	2.06	3.28	2.92
24	750	63	72.4	74.6	3.26	2.81	4.50	3.77
25	37		64.9		2.43		3.75	

TABLE X (continued)

Plant number	Number of flowers		Percentage of flowers producing pods		Seeds per flower		Seeds per pod	
	Selfed	Crossed	Selfed	Crossed	Selfed	Crossed	Selfed	Crossed
26	608	131	74.8	89.3	2.16	3.62	2.88	4.05
27	344	135	61.0	93.3	1.38	2.21	2.26	2.37
28	357	78	52.4	50.0	1.38	0.88	2.64	1.77
29	442	192	49.1	60.4	1.22	1.81	2.49	3.00
30	668	104	46.1	62.5	1.12	1.55	2.44	2.48
31	42	5	61.9	80.0	2.81	3.20	4.54	4.00
32	305	144	81.0	63.2	4.46	4.27	5.51	6.76
33	548	22	56.8	59.1	1.76	2.23	3.09	3.77
34	377	100	51.2	68.0	1.37	2.58	2.68	3.79
35	319	158	68.3	66.5	2.03	2.93	2.96	4.41
36	614	154	41.9	68.8	0.78	2.36	1.88	3.42
37	401	181	43.6	79.0	1.13	2.91	2.59	3.69
38	589	200	46.5	67.5	0.98	1.96	2.11	2.90
39	791	254	41.3	65.0	1.07	2.88	2.60	4.43
40	245	79	49.0	92.4	1.26	6.38	2.57	6.90
				II. BUFFALO				
1	177	20	58.2	40.0	2.36	2.20	4.06	5.50
2	123	66	35.0	47.0	1.13	2.12	3.23	4.52
3	264	109	53.0	57.8	0.69	1.70	1.35	2.94
4	98	82	32.7	39.0	0.96	0.66	2.94	1.69
5	149	6	60.4	83.3	1.72	4.17	2.84	5.00
6	109	10	33.9	90.0	0.80	2.90	2.35	3.22
7	110	55	21.8	50.9	0.71	1.16	3.25	2.29
8	97	67	84.5	97.0	2.93	4.66	3.46	4.80
9	261	153	21.1	48.4	0.59	1.10	2.78	2.28
10	20	88	20.0	87.5	0.45	3.26	2.25	3.73

TABLE X (continued)

Plant number	Number of flowers		Percentage of flowers producing pods		Seeds per flower		Seeds per pod	
	Selfed	Crossed	Selfed	Crossed	Selfed	Crossed	Selfed	Crossed
11	36		30.6		0.86		2.82	
12	65		33.8		0.65		1.91	
13	20	17	5.0	23.5	0.05	1.53	0.05	6.50
14	400	63	64.5	87.3	1.59	3.03	3.41	3.47
15	121	60	24.0	68.3	0.34	1.68	1.41	2.46
16	40		25.0		0.43		1.70	
17	544	151	13.8	25.8	0.13	0.42	0.96	1.62
18	288		36.7		0.76		2.07	
19	184	156	50.0	53.2	2.57	0.94	3.13	1.77
20	134	79	69.4	53.2	2.35	1.09	3.39	2.05
21	277	22	51.6	13.6	1.53	0.32	2.97	2.33
22	135	90	63.0	85.6	1.39	4.72	2.20	5.52
23	228	60	45.2	85.0	1.15	3.18	2.53	3.75
24	396	56	22.7	26.8	0.31	0.36	1.37	1.33
25	372	134	44.9	71.6	1.10	2.28	2.45	3.19
26	96	47	44.8	83.0	1.54	3.02	3.44	3.64
27	183	52	28.4	61.5	0.64	1.54	2.27	2.50
28	59	133	79.7	31.6	5.22	0.88	6.55	2.79
29	135	70	40.0	67.1	1.08	3.21	2.70	4.79
30	439	88	21.0	15.9	0.43	0.27	2.03	1.72
31	190	81	36.3	60.5	0.88	1.70	2.42	2.82
32	77		83.1		3.35		4.03	
33	402	80	65.9	63.8	1.50	2.65	2.28	4.16
34	332	147	45.2	75.5	1.39	2.84	3.07	3.77
35	255	7	24.3	71.4	0.40	3.71	1.63	5.20
36	170	50	18.8	30.0	0.26	0.62	1.41	2.07
37	10	30	70.0	20.0	0.80	0.70	1.14	3.50
38	167	165	46.7	67.9	1.45	2.47	3.10	3.63

TABLE X (continued)

Plant number	Number of flowers		Percentage of flowers producing pods		Seeds per flower		Seeds per pod	
	Selfed	Crossed	Selfed	Crossed	Selfed	Crossed	Selfed	Crossed
			III. DELTA					
1	80	79	33.8	31.0	0.79	0.48	2.33	1.27
2	130	111	51.5	71.2	1.25	3.54	2.42	4.97
3	319	54	37.3	59.3	0.79	2.30	2.12	3.88
5	283	89	57.2	56.2	2.04	2.34	3.56	1.78
6	153	14	15.0	71.4	0.33	3.50	2.17	4.90
7	274	82	32.1	18.3	0.63	0.27	1.97	1.47
8	285	128	58.9	72.7	1.81	1.71	3.07	2.35
9	46	15	28.3	80.0	0.81	2.80	2.85	3.50
10	145	8	18.6	87.5	0.43	5.88	2.33	6.71
11	251	45	51.0	62.2	1.16	2.22	2.27	3.57
12	101	9	38.6	77.8	0.74	2.22	1.92	2.86
13	242	246	60.3	43.5	1.54	1.29	2.55	2.97
14	91		34.1		0.57		1.68	
15	282	109	68.8	72.5	1.24	2.87	1.81	3.96
16	163	113	71.8	93.8	1.67	2.47	2.33	2.63
17	64	40	75.0	37.5	1.35	0.70	1.79	1.86
18	47	36	25.0	91.7	0.58	1.25	2.33	1.36
19	186	94	51.1	66.0	1.03	1.54	2.02	2.34
20	261	187	45.6	69.5	1.49	3.26	3.28	4.69
21	314	153	15.3	40.5	0.37	1.46	2.40	3.60
22	219	86	66.2	62.8	2.44	3.22	3.69	5.13
23	370	155	24.3	40.0	0.36	0.83	1.49	2.06
25	570	169	15.6	34.9	0.36	1.01	2.28	2.90
26	190	47	48.9	53.2	2.10	2.77	4.28	5.20
27	294	96	47.6	59.4	1.25	1.58	2.62	2.67
28	329	16	30.1	50.0	0.30	1.94	1.00	3.87

TABLE X (continued)

Plant Number	Number of flowers		Percentage of flowers producing pods		Seeds per flower		Seeds per pod	
	Selfed	Crossed	Selfed	Crossed	Selfed	Crossed	Selfed	Crossed
30	210	61	46.7	50.8	1.11	0.77	2.38	1.52
32	193	170	32.6	34.1	0.92	0.94	2.83	2.76
33	62	35	38.7	85.7	0.77	3.09	2.00	3.60
34	70	78	20.0	57.7	0.30	1.20	1.50	2.09
35	78	123	62.5	39.8	1.60	2.11	2.60	5.29
36	94	104	43.6	59.6	1.53	2.49	3.51	4.18
37	54	51	20.4	17.6	0.02	0.00	0.09	0.00
38	108	62	28.7	64.5	1.02	1.19	3.55	1.85
39	131	199	50.4	50.2	1.23	1.62	2.44	3.22
40	147	113	45.6	56.6	1.03	2.61	2.27	4.61
41	160		27.5		0.43		1.54	
42	189	59	32.8	55.9	0.95	1.98	1.98	3.55
43	153	102	50.3	82.4	1.38	2.78	2.74	3.38
44	40		40.0		0.53		1.31	
45	354	61	77.0	77.0	2.66	2.66	3.45	3.45
			IV. DU PUITS					
1	343	101	70.3	85.1	1.91	2.56	2.72	3.01
2	442	99	35.7	47.5	1.53	2.06	4.28	4.34
3	646	85	56.5	56.5	0.93	2.53	0.94	4.48
4	46		30.5		0.87		2.86	
5	219	143	1.8	13.3	0.02	0.24	1.25	1.84
6	266	85	51.1	68.2	1.26	1.95	2.46	2.86
7	45	134	35.6	38.1	0.80	0.80	2.25	2.32
8	324	115	62.3	71.3	1.52	2.50	2.45	3.15
9	348	152	48.6	59.9	1.64	2.67	3.37	4.46
10	433	119	38.3	50.4	1.23	1.47	3.21	2.92

TABLE X (continued)

Plant number	Number of flowers		Percentage of flowers producing pods		Seeds per flower		Seeds per pod	
	Selfed	Crossed	Selfed	Crossed	Selfed	Crossed	Selfed	Crossed
11	390	107	80.8	86.0	3.84	3.55	5.75	4.13
12	289	168	38.1	32.7	0.95	1.14	2.49	3.49
13	493	298	31.2	32.9	0.80	1.40	2.56	4.26
14	367	116	54.0	55.2	1.40	1.99	2.60	3.61
15	12	66	25.0	24.2	0.42	1.18	1.67	4.88
16	294	103	62.9	46.6	1.95	1.88	3.10	4.04
17	166	327	80.2	57.5	1.85	2.00	3.30	3.48
18	363	156	46.0	69.2	1.33	2.36	2.89	3.41
19	481	130	33.5	64.6	0.66	2.49	1.98	3.86
20	151	175	33.1	28.6	0.91	0.84	2.74	2.94
21	75	73	49.3	63.0	1.91	1.95	3.86	3.09
22	425	200	29.4	34.0	0.80	1.12	2.74	3.28
23	374	160	31.8	40.6	0.82	1.03	2.59	2.53
24	173	57	35.8	84.2	0.87	3.65	2.42	4.33
25	216	157	50.5	63.1	1.12	1.86	2.38	2.95
26	99	112	55.6	73.2	1.79	3.53	3.22	4.82
27		77		74.0		3.43		4.61
28	168	158	57.1	70.9	2.21	3.17	3.88	4.47
29		10		20.0		0.70		3.50
30	196	61	63.3	90.2	2.04	2.56	3.23	2.84
31	418	206	29.4	24.2	0.67	1.66	2.27	3.38
32	288	60	46.2	50.0	1.36	3.17	2.95	6.33
34	561	203	32.6	45.3	0.78	2.20	2.40	4.86
35	679	119	52.7	58.8	1.36	2.91	2.58	4.94
36	149	141	57.0	66.0	1.82	2.24	3.19	3.40
37	218	143	47.0	44.8	1.75	1.55	3.75	3.45

TABLE X (continued)

Plant number	Number of flowers		Percentage of flowers producing pods		Seeds per flower		Seeds per pod	
	Selfed	Crossed	Selfed	Crossed	Selfed	Crossed	Selfed	Crossed
			V. NARRAGANSETT					
1	756	168	43.5	60.7	0.80	2.01	1.83	3.30
2	667	124	36.9	29.0	0.94	0.61	2.54	2.11
3	658	41	36.2	34.2	0.86	0.78	2.38	2.29
4	512	138	41.0	55.8	1.15	1.40	2.80	2.51
5	631	164	70.2	64.6	2.26	2.41	3.24	3.73
6	345	83	36.8	53.0	0.99	1.57	1.89	2.95
7	662	20	46.4	100.0	1.25	1.45	2.69	1.45
8	222	98	47.7	58.1	1.58	3.97	3.30	6.83
9	283	106	48.8	64.2	1.25	1.88	2.57	2.93
10	496		35.7		1.36		2.68	
11	640	241	65.3	51.5	2.03	1.84	3.11	3.58
12	343	116	57.4	58.6	1.71	1.23	2.97	2.10
13	702	120	23.5	36.7	0.39	1.11	1.65	3.02
14	490	119	46.5	46.2	0.94	1.74	8.40	3.76
15	631	59	25.4	20.9	0.48	3.29	2.30	5.54
16	128		26.6		0.94		3.33	
17	606	116	32.3	51.7	0.73	1.28	2.27	2.48
18	546	96	58.1	70.8	1.71	2.02	2.94	2.85
19	412	79	39.1	40.5	1.04	1.77	2.67	4.38
20	509	127	64.2	64.6	1.80	1.90	2.81	2.94
21	276	52	49.3	69.2	1.28	2.62	2.60	3.78
22	441	136	50.4	73.5	1.61	3.71	3.19	5.05
23	509	85	36.9	48.2	0.62	0.92	1.69	1.90
24	725	93	47.0	71.0	1.30	2.68	2.77	3.77
25	126	98	12.7	7.1	0.28	0.23	2.19	3.29
26	490	146	37.3	52.1	0.99	2.13	2.50	4.09

TABLE X (continued)

Plant number	Number of flowers		Percentage of flowers producing pods		Seeds per flower		Seeds per pod	
	Selfed	Crossed	Selfed	Crossed	Selfed	Crossed	Selfed	Crossed
27	487	90	60.4	51.1	1.75	1.42	2.90	2.78
28	541	49	44.5	40.8	1.45	0.96	3.25	2.35
29	417	127	35.7	53.5	0.96	1.82	2.69	3.40
30	465	98	49.7	73.5	1.16	2.28	2.40	3.10
31	287	72	42.5	47.2	0.86	0.83	2.02	1.76
32	328	151	73.5	81.5	3.43	3.69	4.66	4.53
33	162	93	79.0	69.9	2.09	1.84	2.64	2.63
34	229	9	46.8	66.7	1.36	2.56	2.91	3.83
35	632	77	16.8	52.0	0.31	1.94	1.82	3.73
36	302	46	53.0	67.4	1.86	2.72	3.52	4.03
37	532	107	73.7	68.2	1.97	2.01	2.67	2.95
38	133	28	44.4	53.6	2.14	2.75	4.81	5.13
39	331		52.9		1.45		2.74	
40	392	261	73.7	80.8	2.17	2.21	2.95	2.73

APPENDIX C

TABLE XI

PERCENTAGE OF FLOWERS PRODUCING PODS, SEEDS PER FLOWER, SEEDS PER POD, POLLEN VIABILITY, POLLEN TUBE LENGTH, PISTIL LENGTH, STYLE LENGTH, AND OVULE NUMBERS PER OVARY OF FORTY-TWO BUFFALO ALFALFA PLANTS

Plant number	Number of flowers Selfed	Percentage of flowers producing pods	Seeds per flower	Seeds per pod	Pollen viability[#] (a)	Pollen viability[#] (b)	Length (mm) Pollen tube	Length (mm) Pistil	Length (mm) Style	Number of ovules per ovary
2	327	27.2	0.33	0.92	54.1	48.2	0.93	5.17	2.65	8.96
3	583	69.8	2.38	3.40	74.4	83.7	0.87	5.61	2.60	10.64
4	390	67.4	1.84	2.74	42.9	90.3	0.59	5.04	2.23	10.00
5	730	44.3	1.37	3.12	52.4	71.0	0.99	5.37	2.64	10.36
6	673	39.7	1.33	3.37	50.4	64.1	1.15	5.07	2.51	9.48
7	625	38.4	1.73	4.52	53.3	63.1	1.22	4.97	2.46	9.81
8	534	55.6	1.44	2.60	70.0	87.5	1.13	5.78	2.87	10.89
9	538	33.8	0.84	2.63	44.8	65.7	0.82	5.34	2.47	10.80
10	390	38.0	1.20	3.16	53.0	73.5	0.85	4.98	2.41	8.71
11	1205	44.3	2.00	4.54	50.3	80.5	0.95	5.58	2.70	11.09
12	719	40.1	1.01	2.55	62.7	78.1	0.74	5.42	2.81	11.29
13	160	57.5	1.08	1.87	71.4	76.3	0.75	5.52	2.86	10.91
14	443	62.8	2.07	3.29	72.1	90.3	0.63	5.42	2.50	9.54
16	217	45.2	1.44	3.18	40.2	79.0	0.75	5.21	2.50	10.33
17	623	58.4	1.66	2.84	70.1	62.0	0.73	5.63	2.65	10.57
18	300	40.0	1.07	2.68	73.6	70.1	0.61	5.22	2.65	10.15
19	259	29.3	0.68	2.30	73.4	65.5	1.07	4.92	2.50	9.00
20	439	56.3	2.41	4.28	48.2	95.7	1.05	5.16	2.67	9.00
22	418	44.3	1.36	3.08	38.7	80.4	0.83	5.48	2.48	10.32
23	300	52.3	1.90	3.61	74.9	84.8	0.79	5.61	2.68	10.89
24	562	31.3	0.74	2.35	57.1	58.3	0.70	5.34	2.76	10.83
25	448	56.3	1.95	3.46	75.6	78.8	1.00	5.25	2.69	9.21
26	583	63.3	2.23	3.54	60.7	73.9	1.19	5.34	2.57	11.43
28	349	59.6	1.71	2.87	56.6	63.0	1.21	5.69	2.58	10.09

TABLE XI (continued)

Plant number	Number of flowers Selfed	Percentage of flowers producing pods	Seeds per flower	Seeds per pod	Pollen viability# (a)	Pollen viability# (b)	Length (mm) Pollen tube	Length (mm) Pistil	Length (mm) Style	Number of ovules per ovary
29	544	53.9	2.00	3.71	49.9	55.2	0.96	5.30	2.51	10.85
30	489	51.3	1.79	3.45	77.2	76.1	0.81	5.55	2.42	12.64
32	570	30.2	0.72	2.38	24.6	34.0	0.73	4.99	2.49	8.91
33	485	71.1	2.76	3.89	76.3	86.0	0.92	4.94	2.43	9.61
34	516	27.7	0.53	1.97	68.2	65.6	0.70	4.84	2.48	8.82
35	336	21.1	0.32	1.52	60.5	89.9	0.98	5.64	2.36	10.49
36	608	32.4	0.86	2.66	23.2	38.7	0.84	5.20	2.46	10.60
38	352	68.5	3.21	4.69	64.4	74.6	0.79	5.53	2.40	11.08
39	399	58.7	3.42	4.96	52.9	55.5	1.18	5.51	2.74	9.85
40	441	46.9	1.67	3.57	42.1	75.2	0.68	4.40	2.08	9.31
41	361	47.4	1.69	3.57	50.4	63.7	1.15	4.33	2.12	9.76
42	299	57.5	2.08	3.85	20.1	68.6	0.88	5.23	2.49	9.91
43	360	68.3	1.89	2.76	74.2	79.6	0.73	5.30	2.69	10.97
44	450	48.4	1.18	2.44	55.6	70.0	0.78	5.11	2.23	11.10
45	279	67.5	1.69	3.34	65.9	84.7	0.93	5.95	2.65	12.40
47	499	53.7	2.31	4.31	31.5	59.9	1.32	5.16	2.33	10.80
48	404	54.5	1.62	2.98	62.8	75.5	1.18	5.20	2.51	10.62
49	625	54.7	1.33	2.43	47.7	63.9	0.67	5.33	2.39	9.66

(a) = In vitro germination.

(b) = Hydrogen peroxide reaction.