A STUDY OF THE CAUSES OF STERILITY IN MEDICAGO SATIVA L. (ALFALFA) IN RELATION TO SEED-SETTING

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A THESIS

submitted to the University of Alberta in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE

This thesis represents 50% of the total graduate work

Edmonton, Alberta

April, 1936

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INTRODUCTION

The problem of sterility in alfalfa, *Medicago sativa*, has been of considerable interest to professional agriculturists during the last twenty-five or thirty years. The plant is one of the most valuable used for forage purposes, but its great variability in seed production presents difficulties of much economic importance. Consequently, a large amount of literature reporting investigations conducted under a wide diversity of environmental conditions has resulted.

Most of the reports have been concerned with ecological aspects or have involved entire populations of plants. It has been observed that individual plants exhibit the same relative seed-producing variability, and that these differences are maintained through consecutive years. Thus, it was decided to study, as carefully as possible, the processes involved in the

setting of seed in individual plants, known to vary widely in seed-setting capacity. It was hoped that an intensive study from this viewpoint would reveal important factors in the solution of the general problem.

At the outset it was desired to obtain a reliable fertility index for each plant so that the influence of specific variables might be accurately measured. To accomplish this, data were accumulated for each plant with reference to the number of pods set and the number of normal and abortive seeds per pod. Experiments were also outlined to obtain reliable information on the amount of pollen, and its germinating ability both on artificial media, and on the stigma. Rate of pollen-tube growth was investigated as well as the number of pollen tubes penetrating the ovary.

LITERATURE REVIEW

The Origin and General Variability of Alfalfa

The species *Medicago sativa* L. is generally recognized to be highly variable in its morphology. This variability has been emphasized by Fryer (16) and the validity of certain allied species and sub-species questioned. From his cytological study of the genus

Medicago he further concludes that, on the basis of chromosome number and morphology, there is no apparent reason why *M. media*, or cultivated alfalfa, should not have arisen from the hybridization of *M. sativa* and *M. falcata*. Sinskaia (29), after observing *M. sativa*, *M. hemicycla* and *M. falcata* in their native habitats in Russia, comments on the extreme variety of the three species. He decided that *M. sativa* has given rise, by ecologic-geographical differentiation, to *M. falcata* through *M. hemicycla*. The Grimm variety is the one which composes the greater proportion of cultivated alfalfa grown in Canada and the northern United States. McClymonds (24) believes it to be the product of a natural hybrid from a cross between *M. sativa* and *M. falcata*, and Kirk (20) cites it as an example of a collection of many lines developed under natural selection.

The Effect of Climate on Seed-setting

It is a common observation that alfalfa requires rather definite climatic conditions before satisfactory seed yields can be obtained, and reports from observers in various parts of the world are available. Alter (1), after an extensive survey of meteorological and yield data from the state of Utah, concludes that climate is

the controlling factor in seed production. He notes that the principal alfalfa seed exporting countries of the world have climates similar to the western plains and plateau regions of the United States. He remarks further, that satisfactory yields are obtained in humid areas only when drought conditions for corn prevail. A later report from Utah, by Carlson (6), stated that temperature was not found to be correlated with seed yield, but that some relationship between seed yield and relative atmospheric humidity was obtained. Spencer and Stewart (36), working in Colorado, decided that hot days, cool nights and dry air during both the day and the night resulted in heavy seed crops, but that hot dry weather during the blossoming period was particularly unfavorable. Under Kansas conditions, Throckmorton and Salmon (39) state that heavy rainfall is necessary for early growth but that, while dry weather is favorable at blossoming time, heavy rains, high temperatures and hot winds prevent fertilization and reduce the set of seed. Similar weather to that given for Kansas is favored by McClymonds (24) in Idaho. However, he believes that dry weather in the spring is best.

In Canada, Sigfusson (28) reports that the most profitable crops of seed are obtained in the drier parts of Manitoba, and Southworth (34) concludes that the more arid conditions prevailing at the Manitoba Agricultural

College were responsible for higher seed yields as compared to his former experience (33) at the Ontario Agricultural College. Dwyer (11) states that in Australia very dry and very humid areas are alike unfavorable to seed-setting. At Aberystwyth, Wales, Williams (44) reports a high yield during a hot dry summer as compared to a cold wet one, and Helmbold (18) considers that humid weather is unfavorable in Germany. Helmbold, however, believes that, while bad weather results in a reduction of bloom, its main effect is on the prevention of insect visits.

The Effect of Density of Stand and Soil Conditions

Another factor influencing seed yields is the density of the stand. Englebert (14) and Sigfusson (28) found that thin stands gave the best yields. McClymonds (24) obtained similar results and concluded that a maximum of the plant should be exposed to light and air. Spencer and Stewart (36) report that alfalfa in rows gave increased yields over solid stands, and spacing within the rows gave further increases. However, while thin seeding usually gave better yields, Carlson (6) states that it was equalled by thick seeding in good years.

Soil conditions have been discussed by Spencer and Stewart (36). They concluded that the soil should

not be highly fertile since extreme vegetative growth might occur at the expense of seed yield. Heavy applications of manure were unfavorable and soils deficient in lime were not suitable. Englebert (14) found up to 80% of brown or blasted seeds in certain areas, and decided that it was due to a sandy soil which allowed the water to escape too quickly. Martin (22) also has found that aborted seeds are usually associated with drought conditions.

Development of Floral Parts and Embryonic Growth

Considerable research has been done by certain writers on the development of the floral parts of alfalfa and the embryonic growth of the seed. Cooper (10) found that the embryo sac developed normally into an eight-nucleate, seven-celled structure. The fusion of the polar nuclei was not noted before fertilization, and it was found that the antipodal cells persisted until shortly before this event. Similar results were obtained by Reeves (27) except that he reports the disappearance of the antipodal cells long before the embryo sac reached maturity. These workers both found fertilization occurring between 24 and 32 hours after pollination, and the formation of two- or three-celled embryos at about 31 hours. Cooper (10), however, followed the process further and noted two- to four-celled embryos at

[illegible]

Development of floral parts and vegetative growth

[illegible]

48 hours; five- to six-celled embryos at 72 hours; and four- to twelve-celled embryos at 120 hours. Thus he demonstrated rather wide variations in embryonic growth rates. He also found, in unfertilized embryo sacs, that disintegration of the cytoplasm had commenced at the 72 hour period, and that it was complete, and the embryo sacs greatly shrunken, at 120 hours.

Martin (22) concluded that neither soil nor atmospheric moisture inhibited the development of ovules and that aborted seeds were due to arrested embryonic development. Aborted embryos have also been reported by Stevenson and Kirk (37) in self-fertilized lines of M. media.

Arrested bud development or bud-blasting has been reported by Carlson (6) and Sorenson (32). Both writers believe this to be caused by insect activity and particularly by thrips. Carlson considers that it may be a significant cause of yield decrease, especially in thick stands and rank growth. He also notes that the amount of bud-blasting in adjacent strains suggests differences in resistance to insect attacks.

Other observations by Carlson (4) concern the age of the flower and the survival of the pods set. He found that flowers one or two days in full bloom had the best chance of being fertilized. He also noted that 75%

of the pods set survive until harvest. His results further indicated that if 30 to 35% of the flowers set pods a good crop was obtained; 15 to 20% was not profitable, and 5 to 10% was a practical failure.

The Nature and Viability of Pollen as Related to Seed-setting

In respect to the amount and viability of the pollen there is a fairly good agreement among various workers. Armstrong and White (2) noted a few plants which produced a medium or scarce quantity of pollen. They also noted pollen sterility resulting in a lower number of seeds per pod. Plants deficient in viable pollen were also deficient in the amount of pollen and in anther dehiscence. A definite relationship between the amount of pollen and the fertility of the plant was found, but they state that pollen was sufficiently abundant for fertilization in both high and low seed-setting types. Englebert (14) reports high percentages of non-viable pollen in sterile plants, and also plants with very little pollen or sterile anthers. She considered that vigorous and rapid germination of the pollen was associated with fertility, but that 22.5% of sterile pollen was unimportant if the amount was large. Clarke

and Fryer (8) found that the percentage of sterile pollen grains varied for different plants but remained constant for the same plant under widely different greenhouse conditions. When plants selected in the field on evidence of sterility were examined they found percentages of sterile grains running from 50% to 90%.

Following extensive experiments on pollen germination, Martin (22) concludes that germination of the pollen is dependent upon a proper relationship between atmospheric moisture and moisture supplied by the stigma. He obtained no germination at 100% humidity. Southworth (34) considers that the pollen should be mature but not too old.

The structure and content of the pollen of M. sativa has been described by Martin (23) and Reeves (26). It was found to contain much protein but no sugar, and starch was absent in mature grains. Fully developed grains contain a tube nucleus and a generative nucleus.

Pollen-tube Growth Within the Pistil of Alfalfa

Very little has been published on pollen-tube growth within the pistil of alfalfa. Armstrong and White (2) found, after a study of stigmas six to eight hours after pollination, that penetration was negligible

in untripped flowers, but had occurred in 84% of the tripped flowers. They conclude, however, that while penetration is somewhat better in fertile plants, the differences could not wholly account for discrepancies in seed-setting. Cooper (10) found an abundance of pollen-tubes in the ovaries of fertile plants between 31 and 48 hours after pollination, but states that only one-half or less of the ovules show pro-embryos at this time. Carlson (6) also reports that the average yield is only four to five seeds per pod, whereas the potential ovules are from three to four times that number.

Tripping as Related to Fertilization

Tripping, or the release of the staminal column is an adaptation of the alfalfa flower which is very probably intimately related to seed-setting capacity. The morphology of the flower in relation to the tripping mechanism has been fully described and discussed by Ufer (41) and Armstrong and White (2).

In regard to the ability of alfalfa to pollinate and set seed without tripping there is some disagreement in the literature. Coffman (9) found that practically all alfalfa flowers dehisce their anthers before the "hooded bud" stage, and Englebert (14) states that

untripped flowers, carefully examined in the field, always show dehisced anthers and the stigma more or less covered with pollen. Carlson (4, 5) found, under natural development, that tripping occurred only to the extent of 10.8% while 37% of the flowers set pods. Thus he concludes that under conditions in the Uintah basin of Utah, alfalfa tends to set seed rather freely in the absence of tripping. Kirk and White (21) report that pollination occurs in the early bud stage and that some plants, under greenhouse conditions, set seed freely without tripping. Piper et al (25), however, state that pollination is accomplished by a release of the staminal column, and Helmbold (18) and Ufer (41) report that fertilization never takes place without tripping.

A recent article by Armstrong and White (2) presents evidence which may assist in the interpretation of former results. In about 80% of the flowers examined in the erect standard stage, they found anthers dehisced and the stigma partly or wholly covered with pollen. However, they report a thin hyaline membrane covering the stigma of untripped flowers, and they believe that rupture of this film, by tripping or other means, is necessary before penetration of the pollen-tubes can take place. It was further noted that pollen tubes did not penetrate when they were germinated on untripped

stigmas at 100% humidity, and that flowers tripped following clipping of the standard set few pods. Unlike Piper et al (25) these writers found a marked improvement in seed-setting when stigmas were scarified with a tooth-pick or Camel's-hair brush.

The Effect of Artificial Tripping on Yield

It has been the general experience of investigators that artificial tripping gives greater seed yields over natural tripping. Hay (17) reports marked increases in a year when general seed-setting was poor. Clarke and Fryer (8) obtained 50% more seed and also note that enclosure in glassine bags did not affect the amount set, if the flowers had already been tripped. Southworth (35), after two years of tests, reports no seed from untripped flowers but an abundance was obtained after tripping. Piper et al (25) found about 100% increase following artificial tripping, and Tysdal and Clark (40) obtained approximately 50% more seed under greenhouse conditions. Ufer (41) states that untripped flowers eventually wither and fall off, and Fransden in Denmark, reported by Carlson (5), obtained 400% increases in pods set following artificial tripping. Carlson, from his own results, concludes that artificial

tripping not only improves yield but also tends to equalize the yields for different years.

The Effect of Climatic Factors on Tripping

Since tripping is so beneficial to seed yield it is interesting to consider the effect of climatic factors on this process in relation to the general weather conditions which have been found favorable to good crops of seed. It will be noted that, in general, factors associated with arid conditions are favorable to tripping, while factors tending to produce humidity have the opposite effect.

Southworth (35) and Piper et al (25) concluded that wind or rain caused little or no tripping. Dwyer and Allman (12) consider rain unimportant since they have shown pollen to be inviable under excessive moisture. Helmbold (18) reports that vigorous shaking of the branches had no effect, but Ufer (42) found that wind tended to increase the favorable effect of heat. However, he attributed this fact to the probable drying action.

The effect of heat has been extensively tested by Ufer (42). The general conclusion is that heat causes tripping and if combined with a drying action, the process is accelerated. Drying of itself did not

cause tripping. Dwyer and Allman (12) found that alfalfa flowers tripped instantaneously at temperatures ranging between 100°F. and 108°F. The effect was similar whether dry heat, steam, or hot water was used. Englebert (14), Piper et al (25), Ufer (42) and Dwyer and Allman (12) have all found that tripping is readily initiated by exposing the flowers to direct heat supplied by a lighted match, cigarette or burning-glass.

The effect of sunlight - undoubtedly its heating action - has been observed. Piper et al (25) state that automatic tripping is most frequent in bright sunshine, and that it was also very frequent when flowers were brought from the shade into direct sunlight. Dwyer (11) found that shaded flowers did not trip well. Armstrong and White (2) report that duration and intensity of sunlight are responsible for automatic tripping, and that artificial light did not compensate for sunlight under greenhouse conditions. Ufer (41) likewise considers that tripping is governed by the duration of hours of sunshine, and also by fluctuations in temperature. Helmbold (18), however, believes that neither sunlight nor wind, combined with pronounced dryness of the air, will cause tripping.

The Effect of Insects on Tripping

A large number of observations have been recorded on the activities of insects in relation to tripping. Southworth (33) concludes that they are the limiting factor in alfalfa seed production, since he obtained no seed when they were excluded. Certain insects, for instance bumble-bees and leaf-cutting bees, are regarded by Piper et al (25), McClymonds (24), Englebert (14) and Ufer (41) as being effective in tripping. Piper et al and Ufer also state that moths, butterflies and night-flying insects have a negligible effect. Helmbold (18) notes that honey-seeking insects are ineffective, but that the same insects when gathering pollen may cause self-pollination. The effect of the ordinary honey-bee is generally considered to be little or none. Ufer (41) made many observations of individual honey-bees and concludes that they are unimportant as a tripping agent. Southworth (34) and McClymonds (24) state that honey-bees were never noted to trip any flowers. Piper et al (25) and Englebert (14) consider their effect negligible. However, Dwyer and Allman (13) disagree with these conclusions in regard to honey-bees and are inclined to place them among the important agents which cause tripping. Their statements are based on general observations and caging experiments, and they

suggest that the failure of other observers to credit these insects with tripping has been caused by observations made when the flowers were not in a suitable physiological condition.

Plant Individuality and Flower Structure as Related to Tripping

Differences in individual plants is a factor which may have an important effect on tripping. Piper et al (25), McClymonds (24) and Ufer (42) report that plants vary widely in the ease with which their flowers are tripped. In a comparison of sterile and fertile plants, Armstrong and White (2) found that the fertile ones greatly excelled the others in the extent of self-tripping. They concluded that this factor largely determines the differentiation of fertile and sterile types.

Armstrong and White (2) also studied the floral anatomy in relation to tripping. Differences were found in the length of the anterior wing processes and their degree of adherence to the keel, as well as in the size and tension of the staminal column. They conclude that these variations are important factors in determining the degree of automatic tripping.

The Mode of Pollination in Alfalfa and the Effect of Selfing on Seed Yield

Very little appears to be known of the actual amount of self- and cross-pollination which occurs in alfalfa under field conditions. Kirk (20) states that, although it is largely cross-fertilized, self-fertilization takes place to a considerable extent. Williams (44) reports that alfalfa is largely cross-pollinated, and he found a few plants which were more or less completely self-incompatible. Dwyer (11), however, finds that alfalfa is mostly self-pollinated. He also noted delayed tripping and considers that this, together with wind, may be important in effecting cross-pollination.

If cross-fertilization is the normal mode of sexual behavior in alfalfa, it is to be expected that selfing would result in pronounced reduction in variability, and perhaps an accompanying loss in vigor. Tysdal and Clark (40) report a selfing programme up to the fourth generation. They found few lines in the first generation which were high seed producers, but segregation occurred later, and in the fourth generation some lines exceeded the original stock. Yield of forage tended to be maintained with seed yields in these tests. Stewart (38) found marked decreases in variability following selfing in many of the morphological characters studied. Due

to first generation selfing, approximately 50% decrease in yield is reported by Carlson (6), although little further reduction was evident in the second generation. Kirk (19) found 19% reduction of seed yield in the first; 28% in the second; and 47% in the third generation of selfing. However, he also noted some lines in the third generation which gave no significant decrease from the parent stock. In a later publication, (20), he reports 70% sterility in the second, and almost complete sterility in the fourth generation. Williams (43) reports a marked decrease in variability in the first generation of selfing and also a very marked decrease in vegetative vigor, which was most pronounced between the first and second generation selfings. He concludes (44) that, even after loss in vigor has been compensated, selfing results in a marked decrease of fertility in alfalfa. Spencer and Stewart (36) and Dwyer (11) also report a tendency for good seed-setting types to be poor forage producers.

The Effect of Selfing on Pollen Sterility

Inbreeding has also been noted to have an effect on pollen sterility. Armstrong and White (2) state that increases in pollen sterility were noted for each succeeding generation of inbreeding. Carlson (6)

reports the work of Torsell who concluded that pollen sterility was the chief cause of the reduction in fertility where selfing had been practised in alfalfa. Successive reduction was noted with inbreeding until a point was reached where no further loss of pollen fertility was noted.

The Effect of Cross-pollination on Alfalfa Seed Yields

Since inbreeding, in general, appears to cause marked decreases in seed-setting, it might be expected that in a partially cross-fertilized crop, such as alfalfa, artificial cross-pollination would give opposite results, and this has been the experience of all investigators. Carlson (6) obtained 44% pods set for artificial tripping (selfing) and 54% for artificial cross-pollination. When selfed lines were allowed to open-pollinate he recorded a gain of about 15% which he attributes to hybrid vigor. Where fertilization takes place without tripping, he suggests that thrips may effect cross-pollination. Carlson (5) also reports the work of Fransden who obtained ratios of 1:3.8 for natural tripping compared to artificial tripping, and 1:6.9 for natural tripping compared to cross-pollination. While natural and artificial tripping gave approximately the same number

towards the view of Coombs that abnormal [illegible]
sterility was the first cause of the production of
fertility where selfing had been practised to excess.
Alderman (?) concludes that [illegible]
plant was probably [illegible] lack of seeds
[illegible].

The Effect of Cross-pollination on
Alfalfa Seed Yields

Since Cross-pollination, in general, appeared to cause
marked increases in seed-setting, it might be expected
that in a particularly cross-fertilized crop, such as alfalfa,
artificial cross-pollination would give superior results,
and this has been the experience of all investigators.
Carlson [illegible] pointed out the artificial
tripping [illegible] and that for artificial cross-pollina-
tion. [illegible]
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tripping [illegible]. [illegible]
and artificial [illegible]

of seeds per pod, a gain of 246% was reported in favor of cross-pollination. Dwyer and Allman (13) obtained a better quality of seed when plants were caged with bees and they attribute this to more efficient cross-pollination. According to Englebert (14), two self-sterile plants set seed when cross-pollinated, although one other did not. Piper et al (25) found that cross-pollination increased the number of pods and the number of seeds per pod, and also that there were little differences in the results of cross-pollination between plants and between varieties. It has been found by Helmbold (18) that geitonogamy, or artificial cross-fertilization between flowers on the same plant, has the same effect as self-fertilization. Accordingly, he removed the pollen from a number of flowers and tested the effect of ordinary cross-pollination and geitonogamy. The latter gave only about 70 to 75% of the seed-setting obtained by artificial cross-pollination.

The Inheritance of Seed-setting in Alfalfa

Although no genetical data are available on the subject of seed-setting in alfalfa, it is evident from the results of inbreeding and cross-pollination that the character is inherited. Helmbold (18) states that it is an individual characteristic conditioned by internal of seeds per pod. A gain of 26% was recorded in favor of cross-pollination. Meyer and Hansen (18) found a better quality of seed when plants were caged with bees and they attribute this to more efficient cross-pollination, according to Williams (39). The self-fertile plants may seed when cross-pollinated, although some cross was not. Tiber et al (35) found that cross-pollination increased the number of pods and the number of seeds per pod, and also that there were large differences in the results of cross-pollination between plants and between varieties. It has been found by Lesins (14), that self-fertility, or artificial cross-pollination between flowers on the same plant, has the same effect as self-pollination. Accordingly, he compared the yields from a number of clones and tested the effect of the number of cross-pollinations and self-fertility. The latter gave only about 70 to 80% of the seed-setting obtained by artificial cross-pollination.

The Inheritance of Self-Sterility in Alfalfa

Although no particular data are available on the subject of self-sterility in alfalfa, it is evident from the results of inbreeding and cross-pollination that the character is hereditary. Kirk (10) states that it is an individual plant character conditioned by inherited generative causes, and he found marked differences in different plants. Southworth (35) found that the ability to set seed when tripped varied with individual plants and Kirk (19) found that seed-setting was not necessarily associated with forage yield. Similarly, Ufer (42) found that ease of tripping was not necessarily associated with fertility. Carlson (6) reports that seed production between varieties and strains varies widely and, as previously mentioned, he believed this evidence of hybrid vigor.

The Use of Hybridization in Obtaining Higher Seed Yields

In view of the variability among alfalfa plants it has been felt by some investigators that a hybridization and selfing programme may be valuable in obtaining higher seed-setting strains. Southworth (33) concluded that interspecific hybridization between *M. sativa* and *M. lupulina* offered the best possibilities. After some years of selection he claimed (35) to have indications of a suitable strain derived from this cross. Stewart (38), however, believes that the great decreases which he obtained in variability through selfing, suggest good opportunities for hybridization and selection within the commonly cultivated varieties.

Summary of Literature Review

To summarize briefly the review of literature which has been presented, a few points may be emphasized.

It is evident that cultivated alfalfa is highly variable in seed-setting capacity, both in general and in regard to particular plants. The general variability may be due, at least partly, to mixtures in the original seed stocks of different ecological and morphological types. However, the particular differences between individual plants are very probably caused by differences in genetic factor combinations affecting fertility. The genes contributing to sterility would tend to be eliminated, or greatly reduced in numbers, in a self-fertilized plant, but not so in one, such as alfalfa, which shows evidence of considerable cross-pollination. Experiments on self-pollination support this belief - for example, the increase of pollen sterility in selfed lines.

The effect of climate is also very important. However, its influence on seed yield is, probably, mainly secondary and due to the action of climatic factors on tripping, and on the activity of insects which effect tripping.

Structural and anatomical differences between plants have also been demonstrated, and it is quite possible that they may have a vital bearing on the solution of the problem.

GENERAL MATERIALS AND METHODS

Under this heading, reference is made to materials and methods applicable to all, or almost all, of the tests. Those attending particular experiments are given in the sections to which they specifically apply.

Plants were selected with the object of obtaining two groups representing the extremes of seed-setting capacity. From a block of spaced progenies, twenty-one plants were chosen, which previously had been under observation for three or four years. Ten of these were rated as highly sterile, and the remainder were known to set seed abundantly under Edmonton conditions.

The information relating to the origin and the two main morphological characters of each plant is summarized in Table I. The classification of each as to fertility or sterility is also included. As this table might suggest, the selection was based on whether or not the plants were good seed setters.

In the fall of 1934 the plants were transplanted, each into a glazed earthenware crock with a hole at the base for drainage and containing about one cubic foot of soil. They were then stored in a root cellar to give them a dormant period. About the end of December they were removed to a greenhouse and kept at optimum moisture

TABLE I

Description of the individual plants

Plant designation	Pedigree No.	Origin	Flower color	Growth habit	Fertility classi-fication
8-28	I.28.18	Grimm, Disco	medium purple	erect	fertile
23-11	S_1.31.1	Grimm, Disco	light purple	erect	fertile
33-4	S_2.32.26	Grimm, Disco	light purple	erect	fertile
34-5	S_2.32.26	Grimm, Disco	light purple	erect	fertile
43-5	S_2.32.30	Grimm, Grafton's	greenish yellow	decumbent	fertile
43-29	S_2.32.30	Grimm, Grafton's	light purple	erect	fertile
40-10	S_2.32.29	Grimm, Lyman's	light purple	erect	fertile
21-23	I.31.9	Grimm	light purple	erect	fertile
21-35	I.31.9	Grimm	light purple	erect	fertile
10-34	S_2.32.7	Grimm, Kirk's	light purple	erect	fertile
47-5	S_1.32.32	Cossack	bluish	erect	fertile
37-15	S_1.32.28	Grimm, Disco	greenish yellow	decumbent	sterile
9-11*	S_1.28.3	Grimm, Grafton's	yellow	erect	sterile
6-33	S_3.33.9	Grimm, Lyman's	light purple	decumbent	sterile
19-7	S_2.32.11	Grimm	white	erect	sterile
8-33	S_3.33.10	Hansen's Hybrid	white	erect	sterile
9-38	S_2.32.5	Hansen's Hybrid	white	decumbent	sterile
10-20	S_2.32.5	Hansen's Hybrid	white	erect	sterile
1-12	S_3.33.1	Ontario Variegated	dark purple	erect	sterile
3-14	S_3.33.2	Ontario Variegated	dark purple	erect	sterile
4-5	S_3.33.3	Ontario Variegated	dark purple	erect	sterile

* Relatively difficult to trip.

conditions and at a temperature of approximately 75°F. Artificial light was used to supplement daylight until about the end of March.

On May 7, 1935, all plants were transplanted to the field and spaced at a distance of four feet each way. A screenhouse was constructed to enclose completely the plot. This latter measure was adopted as a precaution against insect pollination at any period when experiments were being carried out.

In all samples the flowers were fully opened and not more than three days old. Whenever yield was tested or pollen-tube growth studied, the flowers were artificially tripped in order to eliminate variation in fertility due to this factor. When a killing agent was required, a solution of 6 cc. of formalin added to 100 cc. of 70% alcohol was employed. This fixative may have had disadvantages in the subsequent staining of the material; however, it proved very convenient when many samples were taken over a fairly long interval, since the flowers could be left in it until it was desired to study them.

In the statistical analysis of results Fisher's analysis of variance (15) was used throughout. However, in computing significances the table of "F" values prepared by Snedecor (31) was used. A further modification was Snedecor's method of calculating variance when the

numbers of individuals in different samples are unequal. This was necessary for the analyses of data on the percentage of pods set and the number of seeds per pod. Wherever comparisons of any two means were made, differences of at least twice the standard error of the difference were considered significant.

PERCENTAGE OF PODS SET

Twenty plants were used in this test, nine of which had originally been classified in the sterile group. On each plant approximately three hundred flowers representing about thirty racemes were tested. One-third of these were tripped on July 29 and the remainder on August 9. After all buds and untripped flowers had been removed, and the remaining flowers artificially tripped, each raceme was tagged with the date and the number of flowers tripped. Both lots were harvested September 5, 1935. The pods set on each raceme were counted and the number expressed as a percentage of the original number of tripped flowers. A summary of mean data is given in Table II, and in Table III the statistical analysis of the variance is presented.

TABLE II

Number of pods set per raceme expressed as percentage of flowers tripped

Plant designation	July 29		August 9		Both dates			Differences between July 29 and August 9	
	No. of racemes	Mean % pods per raceme	No. of racemes	Mean % pods per raceme	No. of racemes	Percentage of pods per raceme			
						Mean	S.E.	Mean difference	S.E.
1-12	16	0.00	27	0.00	43	0.00	2.44	0.00	5.05
4-5	11	0.00	33	0.00	44	0.00	2.42	0.00	4.61
3-14	9	0.00	15	0.42	24	0.26	3.27	-0.42	6.75
8-33	9	0.00	28	0.89	37	0.68	2.63	-0.89	6.14
19-7	6	2.72	12	1.16	18	1.68	3.78	1.56	8.01
37-15	14	1.02	23	3.03	37	2.27	2.63	-2.01	5.43
6-33	10	9.43	17	6.71	27	7.72	3.08	2.72	6.38
9-38	15	3.77	24	13.94	39	10.03	2.57	-10.17	5.27
9-11	15	7.73	28	13.78	43	11.67	2.44	-6.05	5.13
40-10	8	30.41	23	19.38	31	22.23	2.53	11.03	6.58
10-34	8	30.05	21	22.94	29	24.90	2.98	7.11	6.66
34-5	11	73.47	19	26.72	30	43.86	2.93	46.75	6.07
23-11	6	61.67	16	43.88	22	48.73	3.42	17.79	7.67
21-35	8	63.33	17	53.01	25	56.31	3.20	10.32	6.87
21-23	11	90.06	18	35.81	29	56.37	2.98	54.25	6.13
43-5	12	52.36	23	67.01	35	61.99	2.71	-14.65	5.71
8-28	8	86.81	22	58.34	30	65.93	2.93	28.47	6.61
33-4	10	78.61	20	63.90	30	68.80	2.93	14.71	6.21
43-29	9	64.22	15	71.59	24	68.83	3.27	-7.37	6.76
47-5	8	85.68	18	82.32	26	83.35	3.14	3.36	6.81

TABLE III

Statistical analysis of data summarized in Table II

Variation due to	D.F.	Sum of squares	Mean square	"F" value	1% point
Plants	19	500,696.85	26,352.46	102.73	<2.22
Dates	1	5,920.01	5,920.01	23.08	<3.86
Plants x Dates	19	42,052.58	3,115.58	12.14	<2.22
Error	583	149,558.35	256.53		
Total	622	698,227.79			

Note: All "F" values are derived by mean square for variation/mean square for error.

Highly significant variation for all factors tested is indicated by the statistical analysis.

Due to the unequal sampling, a standard error applicable to any two means cannot be given. However, a range for this experiment may be stated which is derived as follows:

The S.E. of a single determination $= \sqrt{\text{mean square for error}}$

$$= \sqrt{256.53}$$

$$= 16.02\%$$

Considering the column in Table II representing the mean percentage of pods for both dates -

The S.E. of the difference of any two means is equal to $\sqrt{\left(\frac{16.02}{\sqrt{N_1}}\right)^2 + \left(\frac{16.02}{\sqrt{N_2}}\right)^2}$

where N_1 and N_2 are the number of racemes for any two different plants.

Thus, the S.E. of the difference between the two means containing the least values for N is

$$\sqrt{\left(\frac{16.02}{\sqrt{18}}\right)^2 + \left(\frac{16.02}{\sqrt{22}}\right)^2} = \sqrt{(3.78)^2 + (3.42)^2} = 5.09\%,$$ and

the standard error of the difference of any other two means cannot exceed this value.

Similarly, the S.E. of the difference between the two means containing the greatest values for N is

$$\sqrt{\left(\frac{16.02}{\sqrt{43}}\right)^2 + \left(\frac{16.02}{\sqrt{44}}\right)^2} = 3.44\%,$$ and the standard error

of the difference of any other two means cannot be less than this value.

Since twice the standard error is considered significant, then differences greater than 5.09 x 2 = 10.18% may be regarded as significant in all cases, and those less than 3.44 x 2 = 6.88% as not significant.

It is apparent that the greatest variable is the individual plant. It is also evident that these variations occur between plants within a group, as well as between the sterile and fertile classes. Since the material was selected to obtain extreme differences, it is very likely that all gradations in percentage of pods set would have been obtained had a larger, and random, sample been taken.

The variation due to dates is difficult to explain, especially since it occurs in either direction; that is, while most of the values for particular plants are higher for the first date, some of them are lower and vice versa. However, as indicated, the general

tendency is toward higher yields for the first date. It is possible that the weather subsequent to tripping offers a partial explanation. On July 31 a steady rain commenced and continued for over twenty-four hours; 1.61 inches of rain fell. This was followed by 0.32 inches, August 4; 1.08, August 13; 0.21, August 17, and only light showers from then on. Thus it may be concluded that the pods set following the first date would be in a more favorable position for soil moisture than those set after August 9. The warm dry weather predominating during the middle and latter part of August would likely be favorable for automatic tripping, but not necessarily so for the development of pods. It has been reported by Alter (1), Englebert (14) and others, that rain following fertilization helps to increase yields. It is also suggested that the age of the season's growth of the plant may have had some effect as, while the second date was less than two weeks after the first, it was very close to the end of the flowering period for the season.

The interaction between plants and dates is interesting. A study of mean differences shows it to be mainly caused by plants in the fertile class. This may be explained by the fact that plants in the sterile group set so few pods, on either date, that differences in their reactions would tend to be masked. It is

possible that the variability of plants in the fertile group may have been partially caused by the position of the racemes. On the second date, it was frequently difficult to secure enough exposed flowers to make up the required two hundred and occasionally racemes obscured by shading were employed. Thus differences in the amount of shading and consequent variations in temperature may have affected the set of pods to some extent.

THE NUMBER OF SEEDS PER POD

After the pods obtained in the previous experiment had been counted, they were decolorized in 70% alcohol. With the use of a strong transmitted light, the number of seeds in each pod was easily counted. Since the numbers of pods obtained from plants in the sterile group were not large enough to provide an adequate sample, they were not included in this study. A summary of the data obtained is given in Table IV.

The statistical analysis is presented in Table V. A highly significant variation for plants and also for dates is indicated, but plant and date interaction is not significant. As in the former experiment on the percentage of pods set, the significance of the difference between means representing the number of seeds per pod for both dates varies, according to the total number of pods analyzed for each plant. However, as previously stated,

a range may be given and differences of 0.217 seeds per pod are significant - and differences of 0.122 are not significant - for all comparisons.

TABLE IV

Number of seeds per pod

Plant designation	July 29		August 9		Both dates		
	Number of pods	Mean number of seeds per pod	Number of pods	Mean number of seeds per pod	Number of pods	Number of seeds per pod	
						Mean	S.E.
40-10	32	1.56	47	1.62	79	1.60	0.155
23-11	53	1.91	96	1.54	149	1.67	0.113
10-34	34	1.62	48	1.92	82	1.79	0.152
21-35	73	1.93	112	1.84	185	1.88	0.101
34-5	83	2.21	54	1.96	137	2.11	0.117
43-5	63	2.46	152	2.44	215	2.45	0.094
33-4	94	2.94	138	2.45	232	2.65	0.090
43-29	85	2.88	143	2.77	228	2.81	0.091
21-23	100	3.32	68	2.60	168	3.03	0.106
47-5	101	3.49	182	3.09	283	3.23	0.082
8-28	99	3.62	116	3.03	215	3.30	0.094

TABLE V

Statistical analysis of data summarized in Table IV

Variation due to	D.F.	Sum of squares	Mean square	"F" value	Levels of significance
Plants	10	654.72	65.472	34.67	$<2.53 = 1\%$
Dates	1	46.85	46.85	24.81	$<6.66 = 1\%$
Plants x Dates	10	25.43	2.543	1.35	$>1.75 = 5\%$ $>2.18 = 1\%$
Error	1951	3,684.53	1.8885		
Total	1972	4,411.53			

Note: - All "F" values are derived by mean square for variation/mean square for error.

In view of the results obtained for the percentages of pods set, it is probably to be expected that the number of seeds per pod should vary for both plants and dates. One might assume that factors tending to increase or decrease pod-setting would have a similar effect on the number of seeds per pod, since both pod-setting and seed-setting are necessarily dependent on fertilization.

The fact that plant and date interaction is not significant indicates that each plant maintained relatively the same reaction for both dates. This suggests that the number of seeds per pod is less likely to be affected by environmental conditions than is the percentage of pods set. It has been noted in the literature review that Carlson (6) found that only about 75% of the pods set survive until harvest. It is likely that the other 25% were lost because of unfavorable environmental conditions. However, the number of seeds in the surviving pods would not necessarily be affected in the same way. Thus, if it can be established that the number of seeds per pod remains relatively stable, it may prove a more reliable index of inherent fertility than either the percentage of pods at harvest time, or the total seed yield.

THE PRODUCTION OF ABORTED SEEDS AND BLASTED BUDS

Aborted Seeds

In the data summarized in Table IV, all seeds which were recognizable as such are included. At the same time a record was kept of poorly developed or abortive seeds since they formed an important part of the total in some plants. The size of these seeds ranged from remnants, which could barely be seen with the naked eye, up to those which had attained about one-half normal size before growth had stopped.

The origin of these seeds is a problem which may be important in the development of high seed-producing strains. When only plants in the fertile group were considered it was found that about 4% of the total seeds were abortive. This proportion would be quite appreciable in a crop of alfalfa seed, and would tend to decrease quality as well as yield.

The full data are included in Table VI. They are not presented as conclusive results, but rather as an indication of what is likely to be encountered in selective breeding for seed production. The seeds obtained from the second date were not always fully mature, and it was rather difficult to classify them at

TABLE VI

Total number of aborted seeds for each plant

Plant designation	July 29		August 9		Totals of both dates	
	Number of normal seeds	Number of aborted seeds	Number of normal seeds	Number of aborted seeds	Number of normal seeds	Number of aborted seeds
1-12	0	0	0	0	0	0
3-14	0	0	1	0	1	0
4-5	0	0	0	0	0	0
6-33	20	0	15	7	35	7
8-33	0	0	2	0	2	0
9-11	12	1	35	4	47	5
9-38	4	4	54	5	58	9
19-7	1	7	0	1	1	8
37-15	1	0	5	0	6	0
8-28	358	0	351	0	709	0
10-34	52	3	85	7	137	10
21-23	313	19	175	2	488	21
21-35	140	1	206	0	346	1
23-11	95	6	134	14	229	20
33-4	219	57	288	50	507	107
34-5	173	10	101	5	274	15
40-10	50	0	76	0	126	0
43-5	154	1	371	0	525	1
43-29	223	22	395	1	618	23
47-5	351	1	561	1	912	2

times. However, it is noteworthy that abortive or blasted seeds are not confined to plants selected for evidence of sterility, and also that their presence cannot well be attributed to environmental effects. Martin (22) and Englebert (14) have suggested that they are caused by unfavorable soil or moisture conditions. But as this experiment shows, there are individual plant differences which are quite probably caused by inherent genetical defects. It seems possible that zygotic lethals or inhibitor factors may be largely responsible. Particular attention is drawn to plant 33-4. In this instance, the ratio of normal to abortive seeds fits very closely a 13:3 inhibitor-factor ratio. It will be interesting to test this plant further in regard to its genetical constitution for abortive seeds.

Bud Blasting

It may be noted in the description of plants used in this investigation (Table I) that one plant designated 10-20 is not included in any of the subsequent experiments. An abundance of buds were produced both in the greenhouse and in the field, but only about two racemes eventually developed flowers. The buds usually

attained the straight bud stage and then withered. Carlson (6) and Sorenson (32) have noted this condition in alfalfa and ascribe it to insect damage. However, insects were not noted to be numerous on this individual and, since it received the same treatment as the other plants, it is likely that, in this instance, bud blasting was caused by an inherent defect.

POLLEN VIABILITY

In the testing of pollen viability on stigmas, germination was allowed to proceed for two hours after tripping. The flowers were then fixed in the killing solution. The stigmas were dissected, placed on a microscope slide and then stained as described by Chandler (7) for pistils with a central canal. Pressure on the cover glass exposed the pollen tubes so that they could be counted. Conclusive data are not yet available from this study.

The viability of pollen as evidenced by its germination on an artificial medium was tested in the following manner. After trying various concentrations of sugar it was found that 12% cane sugar added to 1½% agar-agar gave the best results. The medium was poured into a Syracuse dish, allowed to cool, and a sample of

pollen from one plant was dusted upon it immediately. The dish was then covered and kept at room temperature for two hours. It was then placed in a cooling chamber at about 0°C. until it could be counted. A few drops of a weak solution of methylene blue placed on the medium just before a count was taken facilitated the process.

Since the amount of pollen in the anthers of different plants varied, no set number of flowers was used to obtain a sample. Usually from five to ten were sufficient. One hundred counts were taken from each dish using a low power objective and 10X oculars. Only grains showing unmistakable evidence of germination were classed as viable. For convenience in computation the one hundred counts taken from a sample were grouped at random into ten groups for each plant. The total number of pollen grains tested in this way ranged from four hundred to fourteen hundred according to the plant under test. The average was from eleven to twelve hundred per plant. Samples were taken on March 26, 1935, while the plants were in the greenhouse, and to represent field conditions, a further set of data was obtained on July 8, 1935. Unfortunately a method of measuring the amount of pollen produced was not devised and estimates only, of this factor, are presented. A summary of results is given in Table VII, and the statistical analysis is

presented in Table VIII.

TABLE VII

Percentage of pollen germination

Plant designation	Mean % germination March 26	Mean % germination July 8	Mean % germination for total of both dates	Differences between means for two dates	Estimated amount of pollen
3-14	17.43	9.23	13.33	8.20	Very low
1-12	28.84	27.04	27.94	1.80	Low
4-5	46.50	19.90	33.20	26.60	Low
9-38	53.23	28.00	40.62	25.23	Low
6-33	46.61	43.74	45.17	2.87	High
9-11	56.01	43.09	49.55	12.92	Fairly high
43-29	68.34	32.67	50.51	35.67	High
19-7	58.91	43.47	51.19	15.44	Fairly high
37-15	69.34	58.67	64.01	10.67	Low
34-5	72.98	69.44	71.21	3.54	High
33-4	78.01	66.71	72.36	11.30	High
47-5	78.86	71.28	75.07	7.58	High
21-35	77.84	74.78	76.31	3.06	High
10-34	84.78	70.70	77.74	14.08	High
8-28	85.26	76.00	80.63	9.26	High
21-23	89.32	79.30	84.31	10.02	High
40-10	89.63	79.24	84.44	10.39	High
23-11	85.59	79.50	84.55	10.09	High
43-5	91.55	84.26	87.91	7.29	High

TABLE VIII

Statistical analysis of data summarized in Table VII

Variation due to	D.F.	Sum of squares	Mean square	"F" value	1% point
Plants	18	176,933.12	9,829.62	268.91	<2.24
Dates	1	13,448.40	13,448.40	367.91	<3.87
Plants x Dates	18	6,895.04	383.06	10.48	<2.24
Error	342	12,501.46	36.5539		
Total	379	209,778.02			

<u>Note</u>: - All "F" values are derived by mean square for variation/mean square for error.

Since the number of samples for each plant was the same, it is possible to give a significance value for any two means. If differences between plants for the mean percentage germination for the totals of both dates is considered, 3.82% may be regarded as significant. For differences between means of the two dates, values of 7.64% are significant.

The variability of plants with regard to pollen viability is very striking. It is noticeable that plants in the fertile group are higher in percentage germination than those in the sterile class and that, in many instances, these differences are very wide. The only exception is plant 43-29. It appears that a low percentage of viable pollen is related to sterility - at least in some plants. However, the relationship is not very pronounced and certainly cannot account for the wide difference exhibited between the two groups in the percentage of pods set. Where plants have been estimated to have a low amount of pollen they also tend to be low in the percentage of viable pollen, and it is possible that these two factors may be related.

If the results of this experiment are compared with those given in Table II, it will be found that a fairly close agreement exists between the percentage of viable pollen and the percentage of pods set - if plants

in the sterile group are considered. However, there appears to be no relationship between the two ratings if one considers plants in the fertile class alone. Thus, it is very likely that, after a certain level has been reached, the percentage of viable pollen has little or no effect on fertility. These conclusions agree with those of other investigators. Armstrong and White (2) state that pollen was usually abundant enough for fertilization in both high and low seed-setting types, and Englebert (14) concluded that 22.5% of sterile pollen was unimportant if the amount was large.

The lower pollen germination secured from samples taken under field conditions is difficult to explain, and this is also true of the plant and date interaction. However, it is believed that one class of non-germinating pollen may afford an explanation. Two types were noted. One of these consisted of small, clear grains. As previously noted, this class of pollen has been studied by Clarke and Fryer (8) and found to remain constant in amount for a given plant under wide environmental variations. The other type was composed of normal-appearing grains. It is possible that this latter type might have germinated if a different medium had been used. However, it is also reasonable to assume that it may have been more sensitive to changes in conditions and consequently its viability more or less adversely affected by unfavorable variations in the

environment.

POLLEN-TUBE GROWTH WITHIN THE PISTIL

For the study of pollen-tube growth within the pistil, material was collected at two different periods. The procedure at each period was to trip sufficient flowers on each plant for the experiment. Different lots of about ten flowers were taken at successive intervals after pollination, and killed and fixed in the formalin-alcohol solution. The intervals for the first period were 2½, 5, 8, 13, 19, 25, 31, 37, 43 and 49 hours - and for the second period 3, 5, 7, 9, 12, 16, 20, 24, 27, 30, 33, 36, 40 and 48 hours after pollination. Variations of temperature and of relative humidity were recorded by means of a thermo-hydrograph.

After the material had been collected, it was embedded in paraffin, sectioned, stained and mounted in Canada balsam. The sections were then examined with a low power objective and 10X oculars. Due to the hard tissue composing the outer part of the style, fresh material could not be examined by the staining-and-crushing method. It was also found that dehydration with n-butyl alcohol, recommended by Zirkle (45), improved the staining properties of the material, as

compared with staining after ordinary ethyl alcohol.

Much difficulty was met in finding a suitable stain. This may have been due to the killing solution since formalin-alcohol is not usually considered a satisfactory fixing agent to be followed by the best stains. However, it was found that acid fuchsin gave very good differentiation in the stigma and ovary, although it was seldom possible to trace pollen tubes within the central and lower parts of the style. The following schedule for staining and mounting was devised:

1% aqueous acid fuchsin	-	1 to 2 hours.
Distilled water	-	6 changes.
70% alcohol	-	1 minute.
95% alcohol	-	1 minute.
Absolute alcohol	-	5 minutes.

Clear in xylol and mount in Canada balsam.

It was necessary to use distilled water (pH 4.7) throughout as tap water (pH 7.7) removed the stain in the water changes.

Because of the labor involved in the use of the paraffin method only three plants were studied. These differed widely in their seed-setting capacity. Plant 8-28 was one of the most fertile on test; 6-33 set a very low percentage of pods, and 1-12 had set no pods. The relative humidity and temperature records for both

periods are given in Figure 1, and the remainder of the data are presented in Tables IX and X. It should be indicated that all numbers of pollen tubes are minimum values, since it was often difficult or impossible to be sure of the total number present.

TABLE IX

Summary of data relating to the penetration of pollen tubes into the stigma

Plant designation	Total number of stigmas examined		Total number of stigmas penetrated		Total number of pollen tubes penetrating		Mean number of pollen tubes penetrating	
	July 31	Aug.6	July 31	Aug.6	July 31	Aug.6	July 31	Aug.6
8-28	36	36	34	35	270	275	7.9	7.9
6-33	36	36	28	32	90	178	3.2	5.6
1-12	35	34	14	3	38	3	2.7	1.0

Note: - Mean values do not include stigmas which showed no evidence of penetration.

In obtaining the data summarized in Table IX only material collected at the first four intervals of each period was used. An examination of this table shows that the figures for plant 8-28 are higher than for plant 6-33, both in the number of stigmas penetrated and the number of pollen tubes penetrating. However, the differences do not seem sufficient to account for the marked spread in fertility between the two. Plant 1-12 is very low throughout but, on the basis of penetration

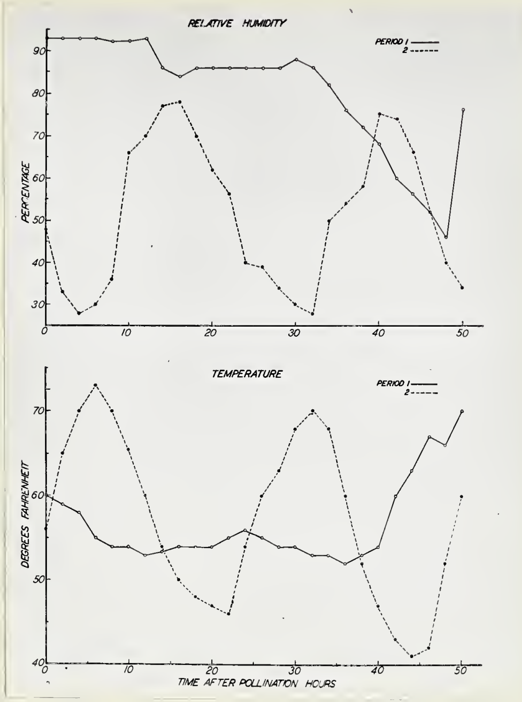

Figure 1. Temperature and relative humidity records for two periods during which samples were taken for the analysis of pollen-tube penetration into the stigma and ovary. Period 1 commenced at 4.30 p.m. July 31st and ended 5.30 p.m. August 2nd. Period 2 commenced at 8.30 a.m. August 6th and ended at 8.30 a.m. August 8th.

into the stigma, one would expect some fertilization to occur.

TABLE X

Summary of data relating to the penetration of pollen tubes into the ovary

Plant designation	Total number of ovaries examined		Total number of ovaries penetrated		Total number of pollen tubes penetrating		Mean number of pollen tubes penetrating		Time of first penetration into ovary		Number of pollen tubes present at time of first penetration into ovary	
	July 31	Aug. 6	July 31	Aug. 6	July 31	Aug. 6	July 31	Aug. 6	July 31	Aug. 6	July 31	Aug. 6
8-28	74	113	67	112	314	445	4.7	4.0	8 hr.	7 hr.	1	30
6-33	75	116	47	99	63	174	1.3	1.8	13 hr.	7 hr.	2	2
1-12	73	112	25	9	35	10	1.4	1.1	25 hr.	9 hr.	5	1

Note: - Mean values do not include ovaries which showed no evidence of penetration.

To secure the data presented in Table X the ovaries collected at each interval were examined and the minimum numbers of pollen tubes present were noted. The differences in the numbers of pollen tubes penetrating the ovary are relatively the same as those given for the penetration of the stigma and serve to verify the former set of data. Additional data were obtained on the rate

of growth of the pollen tubes as measured by the time at which they penetrated the ovary. The differences are quite marked for the July 31st period and if the number of pollen tubes present, at the interval when penetration was first noted, is considered, there are also apparent differences between the plants during the August 6th period. However, these latter variations may not be very significant. At any rate, it appears that under the more favorable conditions prevailing at the August 6th period, difference in rate of growth of pollen tubes, between plants, tended to lessen.

It is apparent that, while the variation between plants is the most noticeable, there are also definite differences in the same plant between the two periods. Examination of the temperature and relative humidity records given in Figure 1 offers an explanation. The material collected during the first period was pollinated at 4.30 p.m., July 31st, and was followed by about two days of wet weather. On the other hand, the material secured during the August 6th period, was pollinated at 8.00 a.m. Wide fluctuations in temperature and humidity followed pollination, and these differences are particularly noticeable if attention is confined to the first four intervals when most of the penetration of the stigmas and ovaries took place. It is probable that either temperature or humidity conditions contributed

favorably to the rate of pollen-tube growth during the second period.

Buchholz and Blakeslee (3) found a steady increase in the rate of pollen-tube growth in Datura Stramonium from 52°F. up to a maximum at 92°F. Smith and Cochrane (30) found 70°F. to 85°F. to be the optimum range for pollen-tube growth in the tomato. In view of these results it seems probable that temperatures of slightly over 70°F. may have accelerated the rate of pollen-tube growth in alfalfa as compared to a temperature of approximately 55°F.

The opposite reactions of plants 1-12 and 6-33 during the two periods are interesting. It will be noted that the numbers of pollen tubes penetrating both the stigmas and ovaries of plant 1-12 were much higher during the July 31st period. The opposite reaction is evident in plant 6-33. It is possible that a combination of temperature and humidity conditions may account for these differences. Martin (22) and Armstrong and White (2) have shown high humidity to be favorable for pollen germination in alfalfa. Smith and Cochrane (30) have demonstrated that a high temperature, 85°F., was optimum for pollen germination in the tomato. Plant 6-33 is decumbent, and it is probable that at the time of the second pollination, a high humidity would be maintained

in the flowers, and this, combined with the higher temperatures prevailing at the second period, would likely be very favorable for pollen germination. On the other hand, plant 1-12 is very erect in its growth habit and consequently its flowers would be exposed to the drying action of the sunlight and wind to a greater degree than those of plant 6-33. Thus the lower moisture conditions in certain flowers may have overcome the favorable effect of higher temperatures.

GENERAL DISCUSSION AND CONCLUSIONS

In this investigation dealing with sterility in alfalfa two main objects have been kept in mind. The first has been to discover, if possible, more precisely the causes of sterility. The second has been to apply later the knowledge gained to a practical plant breeding programme.

Perhaps the most striking general result is the wide degree to which different plants vary in fertility under the same conditions. This variability has been demonstrated in all the experiments conducted and in all the observations made. It has also been shown that, under differing conditions, the same plant may react differently, or it may tend to remain stable.

These two results point strongly to inherent genetical factors, as affecting fertility, which are more or less favorably accumulated in particular plants.

The adverse effects of selfing may not be too important. It is noteworthy that some of the most fertile plants used in the investigation are the product of first or second generation selfing and that, as previously stated, other workers have found very fertile plants in the fourth generation of inbreeding.

The studies on the amount and viability of the pollen and on pollen-tube growth suggest that, in plants varying widely in seed-setting capacity, these factors may be important, and that they may also be important between plants in the sterile group. Where the amount of viable pollen is above a certain level variations in this may possibly be disregarded as a cause of sterility - provided, of course, it remains constant under limited, varying environmental conditions. However, it appears that no single feature of the pollen can wholly account for the extreme differences between sterile and fertile plants. It is also fairly evident that, in fertile plants, these effects may be relatively unimportant in differentiating small degrees of seed-setting ability.

The application of temperature and humidity records to the interpretation of the results obtained indicate that in certain plants, these factors may influence both pollen viability and pollen-tube penetration. This suggests that environmental factors may be very useful in the detection of unstable plants with constitutions unsuitable for breeding purposes.

In view of the results obtained in these experiments it seems probable that the seed-setting variability of alfalfa stands may be caused by a large proportion of unstable individuals which require favorable external conditions in order to set seed freely. Thus, where adverse conditions are likely to occur, it would seem important to analyze carefully foundation stocks for their stability under different environmental factors.

SUMMARY

1. A review of the important literature relating to sterility in alfalfa has been presented.

2. Plants classified as sterile or fertile varied widely in their capacity to set pods when the flowers had been artificially tripped. These

differences were maintained both within and between the two classes.

3. Significant differences in the number of seeds per pod were found between plants in the fertile group.

4. When the results for two dates of tripping were compared, the percentage of pods set and the number of seeds per pod were lower on the second date. It is suggested that soil moisture and age of the plant may have contributed somewhat to these differences.

5. From the analysis of single plant reactions on different dates, evidence was obtained to show that, for very fertile plants, the number of seeds per pod may be a more reliable index of inherent seed-setting ability, than the percentage of pods set.

6. Blasted buds and abortive seeds were found to characterize particular plants.

7. The test of pollen germination on an artificial medium gave wide variations between different plants. Much higher germination was secured from plants in the greenhouse than from the same plants later growing in the field. Differences in plant reaction to the two conditions were also highly significant.

8. Two classes of sterile pollen were noted. One consisted of clear, empty-appearing grains, and the other was apparently normal except that it did not germinate. It is suggested that the latter type may have been normal pollen adversely affected by environmental factors - thus causing differences in plant reactions, and in the lower germination obtained under field conditions.

9. Pollen-tube penetration of the stigma and ovary was studied. It was found to vary widely during two different periods when temperature and relative humidity conditions were markedly different. Differences in single plant reactions were also noted in this experiment.

10. The effect of temperature and relative humidity on pollen germination and pollen-tube growth is discussed. It is believed that these two factors have important effects on the processes leading to fertilization.

11. It is suggested that an accumulation of genes which contribute to fertility, may tend to maintain the seed-setting stability of a plant even when environmental conditions may be somewhat unfavorable.

ACKNOWLEDGEMENTS

The writer of this thesis wishes to express his sincere thanks to Dr. J. R. Fryer, of the Department of Field Crops, University of Alberta, who suggested the subject, and who has offered valuable advice and criticism throughout the investigation, and in the preparation of this report. Dr. E. H. Moss, of the Department of Botany, University of Alberta, also gave useful suggestions in the use of stains and confirmed the identification of pollen tubes. Mr. J. J. Sexsmith contributed technical assistance which was of considerable value at various stages in the project. Finally, it is desired to express appreciation for the grant received from the Carnegie Corporation Research Fund which was of financial assistance in carrying out the investigation.

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