

A STUDY OF THE PARTIAL STERILITY OF
CERTAIN ALFALFA STRAINS.

by

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A Thesis submitted for the Degree of
MASTER OF SCIENCE IN AGRICULTURE
in the department
of
BOTANY.

THE UNIVERSITY OF BRITISH COLUMBIA

October, 1936.

Acknowledgements

I wish to acknowledge with sincere appreciation my indebtedness to Dr. A.H. Hutchinson for his help, instruction, and encouragement throughout this work.

To Dr. G.G. Moe under whose suggestion and inspiration this work began, I give thanks. Appreciation is given to all those upon whom this study depended - Dr. G.G. Moe, Prof. P. Boving, and President Klinck. I am grateful to Dean F.M. Clement for the interest he has taken.

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Introduction

The purpose of this study was to investigate the variation in seed production, first noticed by Dr. Moe in his F₂ hybrid alfalfas. These plants were a result of a cross between the variegated alfalfa "*Medicago media*" and the yellow flowered "*Medicago falcata*". Cuttings from one plant of *falcata*, commonly known as Don, were brought to the University of British Columbia Farm, by Dr. Klinck formerly of Mac. Donald College and now President of the University of British Columbia. It is important to know that all Don material on the University farm came originally from one parent.

Professor Boving knowing the almost complete sterility of the Don, attempted in 1917 to obtain crosses by allowing the Grimm alfalfa, growing alongside it to bloom simultaneously and thus facilitate fertilization of the Don variety. Out of some 250 plants produced from seed gathered from the Don plants in 1917, 6 plants showed hybrid characters. In 1920 all other alfalfas were cut back except the six hybrid plants. These were left untended, but the flowers were manipulated. A heavy yield of seed was produced by these hybrids.

In 1921, a row of 33 plants was set out from the seed

of each hybrid. One hundred and seventy plants grew to maturity. All hybrid plants (first growth) were manipulated by squeezing with the hands, and the weight in grams of seed was taken. A heavy yield was produced from some plants, a medium from others, while again other plants produced only a few seeds or were completely sterile.

This cross has combined in one individual the spreading root system of the Don and the erect habit of the Grimm. Rhizomes of the Don develop approximately 2 inches below the surface of the ground. These break through the soil at varying distances from the centre of the plant, thus gradually increasing the circumference. The Grimm while being characteristically tap rooted, and therefore subject to heaving by frost, has long erect stems, making it suitable for hay. The combination in the hybrids, of spreading root system and tall erect stems produces a valuable hay crop, which is more resistant to heaving.

Since it has been shown that there is considerable variation in seed production as between the two parents Grimm and Don in the first place, and between the different hybrids in the second place, the problem arises as to the nature of incompatibility between these low seed producing forms, and whether this incompatibility is inherited or not, and if inherited upon what genes the incompatibility depends. The question arises as to whether low seed production is due to pollen-sterility, non-distribution of pollen, or incompatibility, the latter resulting in faulty development of pollen tubes, fusion of gametes, or development of the embryo and endosperm.

As a result of pollination studies including morphological studies of preserved pollen and pollen tube growth in sugar solution cultures, studies in tripping of plants exposed to the air, enclosed in cellophane sacs, and brought in the laboratory, as well as histological studies of carpels collected 3 days and 6 days after tripping, evidence indicated 6 conclusions;

(Helen M. Farley, A Study of the Partial Sterility of Certain Alfalfa Strains I, 1934 - 1935)

1. High seed production is not related to tripping.
2. High seed production is generally related to the rate of pollen tube growth.
3. Where there is low seed production there is poor development of gland cells lining the duct of the carpel.
4. When ovules degenerate, this begins immediately after fertilization would normally take place.
5. The ovules more remote from the style are most frequently abortive, or seed development takes place from the ovules situated in the upper part of the ovary in contrast to the less frequent development in the lower portion. This would seem to be dependent upon the rate of growth of the pollen tubes in relation to the maturity of the egg.
6. Forms with low seed production generally show poor endosperm development at least in the early stages, even in ovules where fusion occurs and the endosperm begins to develop normally.

Experimental methods

The F_2 material for this study was collected in July and August of 1935 on the University of British Columbia Farm. The nature of this hybrid material has already been given (Introduction). Eight stages of H 7-7, a high Grimm seed producer, were taken beginning before reduction division of the pollen mother cells to two days after tripping. The stage two days after tripping was also collected in the case of H 68-5, low Grimm seed producer, H 7-33, high Don seed producer, H 7I-26, low Don seed producer, and Parent Grimm, i.e. *Medicago media*. In the above cases where material was collected after tripping, or at time of tripping, tripping was artificially effected. In addition to the above artificially tripped stages of H 7-7, one stage was collected within twenty four hours following the time at which natural tripping occurred.

B.C. Fixing solution, a formal acetoalcohol fluid was used in killing and fixing. Longitudinal sections were cut ten microns in thickness from parafin preparations. Light green and safranin were used in staining.

Development of the male and female gametophytes of alfalfa.

Male gametophyte.

The microsporocytes as they lie in the anther fluid after rounding up, undergo two divisions to form quartets of microspores. Cytokinesis does not occur until after the second meiosis, when the cell is divided into four spores (Plate I, figs. 1 & 2). Each microspore develops a thick wall enclosing the male gametophyte (Plate I, fig. 3). At this stage some of the pollen grains begin to appear shrunken or aborted (Plate I, fig. 4). In the other pollen grains the microspore nucleus which has the reduced number of chromosomes divides into two, around one of which a membrane cuts out a generative cell (Plate I, fig. 3). The other nucleus and the rest of the microspore cytoplasm constitute the tube cell, which later grows out to form the pollen tube.

The generative cell divides to form two male gametes. This division occurs in the growing pollen tube after pollination, as it is making its way down the style (Plate I, fig. 5). Ordinarily only two sperms enter the embryo sac. One unites with the egg, the other with the fusion nucleus. In a number of embryo sacs however, spiral or vermiform sperms were seen after fertilization. (Plate II, fig. I4). Circumstances indicated that these were supernumerary sperms. According to Sharp the male nuclei in many species become vermiform, particularly after

entering the embro sac. This change may occur more rapidly in one nucleus than in the other, the two thus being unlike at late stages. The male gametophyte of alfalfa shows no marked modifications from the common development found in Angiosperms.

Pollen tubes were more numerous in the styles and ovaries of high seed producers than of low. H 7-7 collected within 24 hours after natural tripping shows numerous pollen tubes in the upper portion of the style (Chart I, row 2). Plate VI, fig. I shows two pollen tubes passing down the style before reaching the ovules. Plate I, fig. 6 shows a pollen tube with its tube nucleus and two sperms, entering the micropyle of the third ovule. H 7-7 at time of artificial tripping, a stage a little earlier than the above, does not show pollen tubes (Chart I, row I). However it would seem that fertilization has taken place since a sperm appears to be fusing with the egg. If this is a sperm, then self fertilization has taken place. In H 7-7 at two days after artificial tripping, pollen tubes were numerous, and late pollen tubes were still making their way down the style (Chart I, row 5). Pollen tubes are fewer in the lower regions of the ovary and none were seen in the passages below the 9th ovule. It is evident however that they had reached the 14th ovule since a sperm is in the process of fertilizing the egg, and a second, near the micropylar region, is advancing towards the fusion nucleus with which it fuses to form endosperm (Plate III, figs. 21-23). No pollen tubes were seen later than 2 days after artificial tripping.

Pollen tubes in H 68-5, low seed producer of Grimm type, are few in number at two days after tripping (Chart I, row 4). Only around the first ovule were they observed, although glandular hairs were found to the fourth ovule. The carpel studied contained 8 ovules, the second to the last being decidedly shrunken. Pollen tubes had reached the last ovule, although too late to effect proper fertilization. The egg had become over mature, and the sperm maintained its membrane while entering the egg, whereas normally the membrane would disappear (Plate IV, figs. 24-26).

No pollen tubes were observed near any of the ten ovules of Parent Grimm, 2 days after artificial tripping. (Chart I, row 5). This is a result of the advanced development found here. Young embryos and endosperm had formed in the fourth and sixth ovules (Plate II, fig. 16). Apparently pollen tubes had not reached the tenth ovule where the egg still appears unfertilized.

Pollen tubes found in H 7-33, high seed producer of the Don type at two days after tripping, were considerably fewer than in H 7-7, and were again in the upper regions of the ovary only. None were seen in H 7I-26, low seed producer of Don type. It appears that in this hybrid development took place normally up until the time pollination would normally take place and then disintegration began. It is evident that the seed production of the plants concerned has decreased directly as the number of pollen tubes.

The pollen tubes are dependent upon glandular hairs for food supply, and in the material studied there was a definite correlation between them. The glandular hairs are several cells in length, and are attached to the wall of the ovary at the base of the funiculus as well as along the styler passage. The pollen tubes grow along the surface of the hairs, along the funiculus and into the micropyle (Plate VI, fig. 2).

Glandular hairs are most abundant in the upper regions of the ovary, and do not grow to any great extent below the point where pollen tubes were observed in the ovary. These hairs are more prevalent in some hybrids than others. In H 7-7 a high seed producer of Grimm type, they are much more vigorous, and are more abundant and larger in size than in H 68-5, a low seed producer of Grimm type, or H 7-33, a high seed producer of Don type. In H 7I-26 development of hairs is very poor. Hence there appears to be a double relationship between development of glandular hairs and pollen tubes - the relationship of their position in the ovary, and the relationship of their frequency in low and high seed producers respectively.

A second relationship is found between development of vascular bundles and pollen tubes, particularly in the style, where vascular bundles are invariably found in the vicinity of pollen tubes.

To sum up it may be said that pollen tubes are more numerous in the upper regions of the ovary than in the lower regions. Some have more rapid growth and fertilize the upper ovules, whereas those of slower growth fertilize the lower

ovules or fail to function in cases where the female gametophyte has passed maturity at the time of their arrival. In other cases where the pollen tubes have not been fully nourished, that is there is an insufficiency of glandular hairs and vascular bundles, the pollen tubes may discontinue growth before they arrive at an unfertilized gametophyte.

Female gametophyte

In dealing with the female gametophyte of the alfalfa studied for this thesis a comparison might be drawn between H 7-7, a result of the cross Medicago sativa X Medicago falcata, with the work done by Reeves, and that done by Cooper on Medicago sativa.

Each ovule of M. sativa as described by Reeves contains I to 3 primary sporogenous cells. Where more than one, these lie in a plane transverse to the longitudinal axis of the ovule in some instances, but occasionally they are in the plane of the longitudinal axis, while in H 7-7 not more than a single sporogenous cell in any ovule was found. As in sativa (Cooper & Reeves) the primary sporogenous cell of H 7-7 functions directly as the macrospore mother cell. In consequence of the two meiotic divisions of the single macrospore mother cell, four macrospores only are present in H 7-7, as compared with as many as 8 in sativa (Cooper & Reeves).

"In sativa the chalazal megaspore develops into an 8 nucleate, 7 celled embryo sac"; the other macrospores disintegrate, and the antipodals persist for some time after fertilization (Cooper). In some gametophytes an early 6 nucleate stage is followed by the fusion of two to form the fusion nucleus, the formation of three cells at the micropylar end, the egg and synergids, and the disappearance of the sixth which disintegrates early, long before the female gametophyte is mature. The occurrence in hybrid 7-7 is similar to that found by Reeves in

sativa. In other gametophytes of H 7-7 there were 5 or 7. Where 5 nuclei are formed, the egg, synergids and one cell of the fusion nucleus form at the micropylar end. The evidence indicates that the nucleus at the antipodal end does not divide, but fuses directly with the nucleus which migrates from the micropylar end. This delays the formation of endosperm.

Just prior to fertilization the micropylar ends of the synergids elongate and extend into the micropyle (Cooper). In H 7-7 the synergids are beaked, the beaks extending towards the micropyle. In sativa fertilization takes place between 24 and 27 hours after pollination. The synergids are not broken down by the pollen tube but persist for some time after fertilization (Cooper). In H 7-7 fertilization occurs at the same time but the synergids disappear rapidly. In sativa 2 celled proembryos are to be found 31 hours after pollination. The endosperm at this time contains 2-4 nuclei (Cooper). In H 7-7 at 48 hours, endosperm with lenticular cells had formed in the upper 3 ovules.

In sativa the male nuclei are not rounded but more elongate (Cooper). In H 7-7 they are also more elongate, some being spiral or vermiform. Circumstances indicated that the latter were supernumerary sperms which had arrived after fertilization had taken place.

Although there is an abundance of pollen tubes in the ovaries of sativa at the time of fertilization, only about half or less of the ovules show the presence of proembryos at 31 and 48 hours after pollination. The cytoplasm of an embryo sac in

which fertilization has not occurred remains apparently normal for a considerable time, the first evidences of disintegration being found in the ovules collected 72 hours after pollination. This disintegration continues and ultimately the whole ovule becomes involved, so that at 120 hours after pollination the unfertilized ovules are small and very much shrunken. In heavy seed-setting lines of alfalfa there is an average of 3-4 seeds per pod, whereas 10-12 ovules are present in each ovule. An examination of about 100 ovaries revealed a range in number of ovules per ovary from 8-14, and a range in number of fertilized ovules per ovary from 1-6, the average being 3-4 (Cooper).

In H 7-7 there are numerous pollen tubes, and examination of ovaries reveals a range in number of 12-16 ovules per ovary. The average number of fertilized ovaries per ovary in H7-7 is three. Seed development occurs most frequently in the fifth, sixth and seventh ovules from the stylar end of the ovary. Disintegration in ovules where fertilization has failed proceeds rapidly, some ovules disintegrating before tripping. At 96 hours the ovules have become completely shrunken.

As is characteristic of the Leguminosae, the ovules of sativa arise in two rows, one on each side of the ventral suture. The funiculus of the ovule becomes geniculate in shape and the ovule curved so that it becomes campylotropous, with the micropyle against the funiculus. The embryo sac is long and somewhat curved at maturity (Reeves). The ovules of H 7-7 are paired campylotropous, and the embryo sac is curved at maturity.

By way of introducing the work to follow it would be well to study further the development of the female gametophyte, particularly that of H 7-7. There are certain characteristics which are outstanding and indicate to a large extent whether an individual plant is high or low seed producing.

In general those ovules which lie in the upper regions of the ovary have their female gametophytes more advanced than those in the lower regions (Chart I, rows I & 3). Whereas the nuclei of the ovules in the lower portion of the ovary have not yet fused to form the fusion nucleus, in the upper regions of the ovary fusion has occurred and sperms have entered the embryo sac. (Chart I, row I). Similarly (Chart I, row 3) the gametes in the I4th. ovule are just ready to fuse, and several cells of the inner nutritive layer still remain, while in the uppermost ovules not only equally dimensioned endosperm cells, characteristic of the wandering and dividing condition, have formed, but lenticular endosperm cells are developing towards the periphery of the sac. In the I4th. ovule the presence of sperms, the absence of endosperm and the fact that fusion of the egg and sperm has not occurred, is good evidence that endosperm does not develop until fertilization has taken place, and there is evidence that after a certain stage of maturity fusion does not occur although late arriving sperms may be present.

The main supply of food within the female gametophyte after the tapetal layer has begun to disintegrate, is in the form of starch grains. They are very abundant around the female

gametophyte cells of low seed producers, and in high seed producers are more abundant in the lower ovules than in the upper ovules. Comparing H 7-7 with H 68-5 and Parent Grimm at 2 days after artificial tripping (Chart I, rows 3, 4, & 5) it is evident that while H 7-7, a high seed producer has little or no starch in the upper ovules, Parent Grimm has an intermediate number of starch grains and H 68-5, low seed producer, has numerous starch grains. It would appear that the starch present in low seed producers is in an unavailable form and cannot be utilized as food material.

In addition to the fact that the upper ovules are more advanced than the lower ovules, the ovules of different plants vary in degree of development. Parent Grimm is most advanced, showing young embryos in two of the ovules at two days after tripping (Chart I, row 5 & Plate II, fig. I6). The endosperm cells however are only in the wandering and dividing condition whereas in H 7-7 endosperm with lenticular cells has developed (Chart I, row 3). In Parent Grimm a few starch grains remain, and the inner nutritive layer is almost complete in the tenth or last ovule.

In the sac of the first ovule of H 7-7 endosperm with lenticular cells has formed and is beginning to develop towards the periphery of the sac. There are still a few disintegrating cells of the inner nutritive layer of the megasporangium, but no trace of starch grains. In the second and third ovules, some of the endosperm cells have become lenticular in shape, but the

remainder are rounded, characteristic of the wandering and dividing condition (Plate II, fig. 18). Ovules 6 and 7 show best development of the male and female gametophytes and contain supernumerary spiral sperms. In the 13th ovule there still remain a number of cells of the inner nutritive layer. There is a large representation of starch grains, as before mentioned, and several giant food bodies which are, by all appearances protein in nature. In the last ovule no endosperm has developed, starch grains are plentiful, and the inner nutritive layer still remains fairly intact (Plate III, fig. 21), although initial disintegration is evident.

H 7I-26 and H 7-33, low and intermediate seed producers of the Don type respectively, show no endosperm development, and abundant food material around the female gametophyte (Plate IV, figs. 24-26 & 27-30). Results in my last paper, "A study of the partial sterility of certain alfalfa strains I", indicated that in 1935, H 7-33 would again produce a low seed yield as it had done in 1923. Judging from the sections made this year the yield would again be low.

The egg, fusion nucleus and synergids of H 7I-26 have a very collapsed appearance (Plate IV, figs. 27-30). The fusion nucleus never could divide, and the synergids, apparently disintegrating, do not promise normal development. The lower portion of the ovary is distorted, and numerous long hairs are on the outside wall of the ovary - a Don characteristic. It would appear that in the majority of ovules some development took place, and then disintegration began.

Seed production as interpreted from data represented
in charts I and 2.

In the hybrid alfalfas 68-5 and 7-7, and Parent Grimm best seed production occurs in the fifth, sixth and seventh ovules from the stylar region. Above these the ovules develop in part, forming endosperm only in the majority of cases, whereas in other instances the embryo forms, but no endosperm as in H 7-7 at 6 days, or there is a poor development of both as in H 7-7 at 9 days. The first ovules mentioned above may be referred to as the seed-producing ovules. Those above as the upper non-seed-producing ovules.

Non seed production in the upper ovules may be attributed to either of two possible causes. It may be the result of cross pollination - the pollen tubes arriving at the ovules before the maturity of the egg and fusion nucleus resulting in poor development of both embryo and endosperm as shown in H 7-7 at 9 days, or it may be that the pollen tubes arrive before the maturity of the egg but at maturity of the fusion nucleus, resulting in development of endosperm but not of embryo as in H 7-7 at 2 days and H 7-7 at 9 days. This time relationship appears to lose significance if the development of the fusion nucleus is delayed in its early stages (see page II). It is not likely that the egg would become retarded in development in later stages, thus allowing the fusion nucleus to super-

cede it in development. This is a physiological explanation. A second theory to explain non-seed-production in the upper ovules is self pollination and a low degree of compatibility. The first evidence of self fertilization is found in H 7-7 at the time of natural tripping. Pollen tubes were seen passing down the style (Plate VI, fig. I), and sperms were seen in a pollen tube entering the micropyle of an ovule in the upper portion of the ovary (Plate I, fig. 6). No actual fusions were seen. The low degree of compatibility between the male and female gametes appears for the most part between the egg and sperm, resulting in ovules containing endosperm only, as shown in H 7-7 at 2-days, and Parent Grimm at 2-days. In other cases as in H 7-7 at 6 days where the ovule contains an embryo but no endosperm, apparent incompatibility is between the fusion nucleus and sperm. Where inferior embryos and endosperm develop in the same embryo incompatibility is between both the egg and sperm, and fusion nucleus and sperm.

In the fifth, sixth and seventh ovules, or the seed-producing ovules, seed production appears to be the result of cross pollination - the pollen tubes arriving at the ovules at the maturity of the egg and 24+ hours after tripping. This is believed to bring about best seed development resulting in the production of normal embryos and a large amount of endosperm.

In the lower non-seed-producing ovules examined, complete abortion has occurred in all ovules except ovule I2 of H 7-7, 6 days. As pollen tubes were seen to ovule 9 and sperms in ovule I4 of H 7-7 at 2 days (see charts I & 2), evidence

indicates that pollen tubes penetrate as far as the fourteenth ovule. Yet at 6 and 9 days after artificial tripping complete abortion has occurred below the seventh ovule in practically all cases (Chart II, rows IO-13). This is interpreted as the result of cross pollination followed by the arrival of sperms later than maturity of the egg and fusion nucleus.

An indication of the fact that self pollination occurs in the upper non-seed-producing ovules, is that there are different degrees and combination of development of embryo and endosperm in the upper ovules, whereas in the lower non-seed-producing ovules complete abortion occurs.

East and Yarnell (1929, Studies on self-sterility VIII. Self-sterility allelomorphs. Genetics 14, 455-487) explain the difference between self-fertile and self-sterile plants by rapidity of pollen tube growth. Own pollen germinates on own stigmas just as readily as does foreign, and the first increment of growth of the two types takes place at the same rate. After that own pollen tubes continue to grow steadily and normally at the same rate they started, but the rate of growth of foreign tubes is continuously accelerated as though they were receiving some stimulus which was ineffective on own pollen tubes. Own pollen tubes fail to reach the ovary before the stigma and style have decayed while foreign tubes with their accelerated growth reach their goal before the way has become blocked.

In alfalfa own pollen tubes are interpreted as slow

in growth, and as fertilizing the upper ovules thus incapacitating the latter to become fertilized by foreign pollen. After tripping the accelerated foreign tubes fertilize the fifth, sixth and seventh ovules but below that region the egg has become too mature and fertilization fails.

The twelfth ovule of H 7-7 at 6 days may be regarded as an exception in that development has occurred where aborted ovules are usually found. This probably illustrates the fact that rapid pollen tubes in H 7-7 grow to that point. This is again shown in H 7-7 at 2-days where sperms were seen in the fourteenth ovule. Ordinarily however the egg has passed maturity before pollen tubes penetrate below the tenth ovule, and ordinarily fusion does not take place, as in the case of the fourteenth ovule (Plate III, figs. 21-23).

Comparison of H 7-7, a high seed-producer,
and H 68-5, a low seed-producer, at 2-, 6-, and 9-days
after tripping.

1. There is a smaller number of ovules in H 68-5 - a maximum of 11 as compared with 18.
2. Pollen tubes are seen to the first ovule only at 2-days in H 68-5; and finally, sperms were found in the 8th ovule as compared with the 14 in H 7-7. No supernumerary sperms were seen in H 68-5 whereas several were found in H 7-7.
3. Embryos develop in ovules from 2-6 in H 68-5. In H 7-7 they are found from 2-12.
4. Quite a large amount of starch is present in the embryo sacs of H 68-5 at 6 and 9-days but none was observed in H 7-7, and at two days starch was present in all ovules of H 68-5 but only in the lower ovules of H 7-7.
5. The best developed ovules of H 68-5 at 9-days, are not as well developed as those of H 7-7 at 9-days, particularly in respect to endosperm.
6. In H 68-5 the disintegration and shrinkage of ovules is more rapid and is complete by the 6th or 9th day, while in H 7-7 all stages of development from the many celled embryo

and endosperm to the completely disorganized ovule are present at this time.

Comparison of H 7-7, H 68-5 and Parent Grimm
at 2-days after tripping.

1. H 68-5 is distinctly retarded in development as compared with H 7-7 and Parent Grimm.
2. H 68-5 has fewer ovules than H 7-7 and Parent Grimm.
3. No embryos had formed in H 68-5 whereas they had in H 7-7 and Parent Grimm.
4. In ovule I4 of H 7-7 the gametes are ready to fuse, and in ovules 8 of H 68-5 they are a little past maturity for effective fusion whereas ovules 8-10 of Parent Grimm appear to be disintegrating.
5. Pollen tubes were seen only as far as the 1st ovule in H 68-5 whereas they were seen down as far as the 9th in H 7-7.
6. The upper ovules of H 68-5 seem to be at the same stage of development as the lower ovules whereas in H 7-7 and Parent Grimm there is a distinct difference, the upper ovules developing earlier.
7. Disintegration of eggs to some extent occurs in all three.
8. Starch is abundant in all ovules of H 68-5 but only in the lower ovules of H 7-7.

Review of literature.

Sterility is classified into three fundamentally different kinds (Crane and Lawrence). Generational sterility, is sterility due to the failure of any of the processes concerned with the normal alternation of generations, namely, development of pollen, embryo sac, embryo and endosperm, and the relations of these with one another and with their parents regardless of the cross made. Morphological sterility is suppression or abortion of the reproductive organs. The third kind is incompatibility.

Incompatibility best explains the sterility found in the hybrid alfalfas studied in this work. Of the three theories of incompatibility - the genetical, the chromosome, and the physiological, the last most nearly parallels the situation.

Physiologically, self- and cross-incompatibility may be brought about by failure of pollen tubes to reach the egg, by failure of zygote formation after the male nucleus has entered the embryo sac, or abortion of embryos during the early stages. In alfalfa incompatibility is brought about by failure of the pollen tubes to reach the egg in time for effective fusion of the gametes. Jost (1907) finds that in *Lilium* the pollen tubes of self-incompatible plants grow so slowly that fertilization is not achieved. High seed production is therefore related to rate of growth of pollen tubes. Martin (1913) found that pollen tubes in self-pollinated red clover grew much more slowly than

pollen tubes in a cross pollinated plant. Coe and Martin (1920), and Williams say this fact may be correlated with the slow growth of its own pollen tubes. Sansome studied the pollen tubes in Crane's apple material and found on style dissection that there was a great difference in growth rate in the style, between compatible and incompatible pollen. Other investigators, among whom are Correns (1913) for *Cardamine pratense*, Compton (1913) for *Roseda*, Moore (1917) for *Tradescantia*, Crane and Lawrence for cherries, plums and apples, East and Mangelsdorf for *Nicotiana*, hold that the pollen tube growth in the style is the cause of compatibility or incompatibility of the matings in these plants.

Whether incompatible pollen tubes are inhibited in growth, or whether both inhibition and acceleration may be present in these plants is as yet undecided. The success of pollination in the bud, or incompatible matings in *Nicotiana*, red clover and cabbage does not decide the matter since the development of inhibitory substances may be restricted to a certain period in stylar degeneration. End-season fertility, by which normally, self-sterile plants may give some seeds late in the season of growth, has been reported in *Nicotiana*, East (1923) and Anderson (1924), and in *Lythrum*, Stout (1922). It has been used by Kakizaki (1930) to support the view that there is an inhibitory action which wanes at the end of the season. This hypothesis, however, is open to the objection that the flower does not wither so quickly at that time, therefore the

slower growing tubes achieve fertilization owing to the time factor alone.

Discussion and conclusions

The development of the female gametophyte, at the time the pollen reaches the ovule, is a very important factor in seed production, not only in different flowers but in ovules of the same flower. Consequently the varieties which show a high degree of self tripping at the time 24+ hours before ovule maturity are in most cases high seed producing. Varieties which are not readily self tripped may be facilitated in seed production by the visits of insects. That is the insects seem to have 2 beneficial effects;

- I. A greater proportion of tripping before the time of maturity of the egg.
2. Transfer of pollen since cross pollen seems to show greater compatibility (Carlson; Piper; Frandsen; Kirk; Torsell; Dwyer);

In other words there seem to be 2 important factors in alfalfa seed production; (1) compatibility of the pollen (Carlson; Piper; Frandsen; Kirk; Torsell; Dwyer) and (2) the time of arrival of the pollen tube in relation to the development of the ovule. This time factor may be satisfied by well regulated self tripping or by the frequent visits of suitable insects. It would appear that in extreme cases the greater weight of humble bees as compared with honey bees is a factor in this time period of tripping.

The work recorded here gives some very definite evidence as to the importance of the time of pollination and fertilization and the number and condition of the ovules at that period.

Conclusions

1. Self fertilization occurs in alfalfa, at least in certain forms. Fertilization sometimes occurs before tripping.
2. Development of the male gametophyte follows the usual type found in Angiosperms.
3. Development of the female gametophyte is unusual in some respects. The usual 8 nuclei found in the female gametophyte is reduced in number to 6 in most cases: 5 and 7 were also observed.
4. Where there is low seed production there is poor development of glandular hairs and pollen tubes.
5. In the style vascular bundles are invariably found in the vicinity of pollen tubes.
6. Development of glandular hairs and vascular bundles are related to rate of growth of pollen tubes.
7. Fertilization occurs between 24 and 27 hours after pollination and rapid disappearance of the synergids occurs.
8. A small number of ovules as in Parent Grimm and H 68-5 gives a relatively small margin of development time and ordinarily a correspondingly small number of seeds.
9. Those ovules which lie in the upper regions of the ovary

have female gametophytes more advanced than those of the ovules in the lower regions.

10. The food store and general condition of the female gametophyte, which does not seem to follow any regular sequence within the ovules of any given ovary, may determine seed development.

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Description of plates.

Plate I.

- Fig. 1 H 7-7: Stage 3 Rounded up microsporocytes showing one of the 2 divisions in the formation of the quartet of microspores. (X I350)
- Fig. 2 H 7-7: Stage 3 A tetrad of microspores. (X I350)
- Fig. 3 H 7-7: Stage 4 A mature microspore containing tube and generative cells. (X I350)
- Fig. 4 H 7-7: Stage 4 A shrunken pollen grain which has not germinated. (X I350)
- Fig. 5 H 7-7: 2 days after artificial tripping A pollen tube making its way down the style showing the tube nucleus and 2 sperms. (X 2400)
- Fig. 6 H 7-7: Within 24 hours after natural tripping. Two pollen tubes, one of which shows the tube nucleus and two sperms, about to enter the micropyle. (X 778)
- Fig. 7 H 7-7: Stage 2 Result of the first division of the megaspore mother cell. (X I350)
- Fig. 9 H 7-7: Stage 3 Result of the second division of the megaspore mother cell. (X I350)

- Fig. IO H 7-7: Stage 4. Later stage showing one megaspore enlarging and the others disintegrating. (X I350)
- Fig. II H 7-7: Stage 4. Shows a similar stage except that the megaspore has divided to form the first 2 nuclei of the female gametophyte. (X I350)
- Fig. 8 H 7-7: Stage 4. Megaspore has divided to form 4 nuclei of the female gametophyte (X I350)
- Fig. I2 H 7-7: Stage 4. Divisions in the megaspore mother cell resulting in 6 nuclei. (X I350)

PLATE 1.

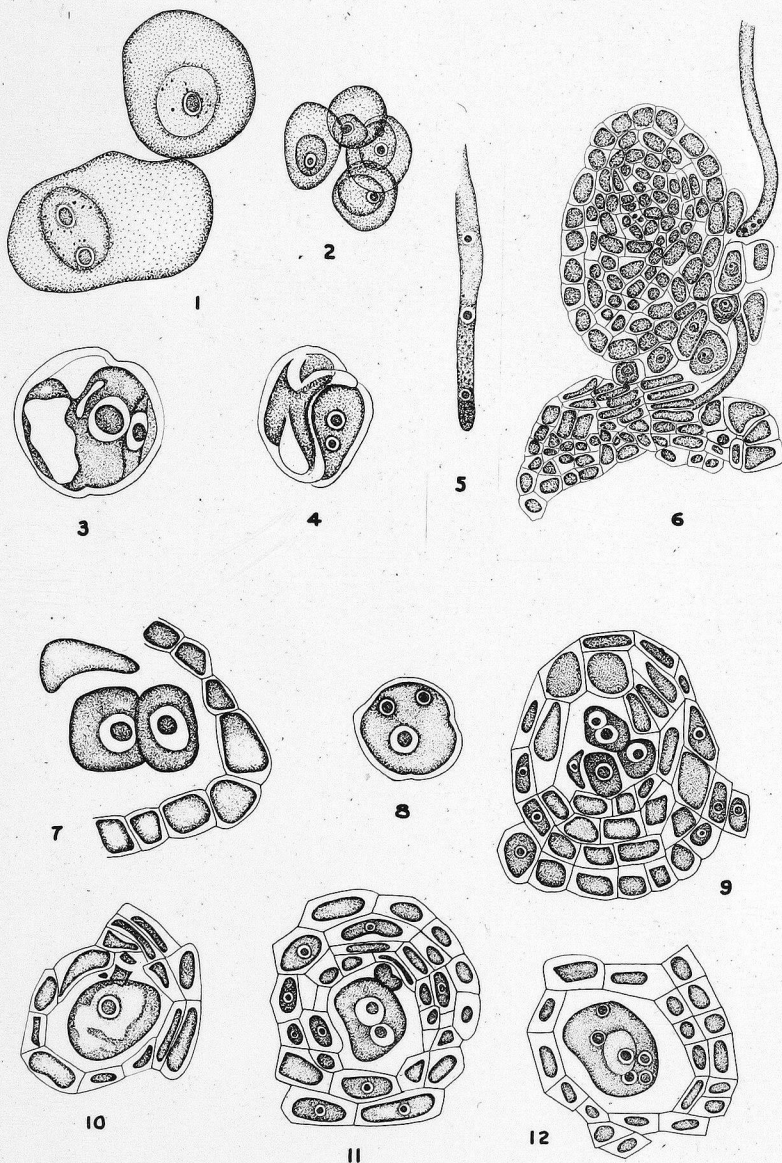


Plate II

Fig. I3, I4 & I5

Fig. I3 H 7-7: 2 days after artificial tripping showing
 the egg and 2 synergids. (X I350)

Fig. I4 Same ovule showing the egg and 2 supernumerary
 sperms. (X I350)

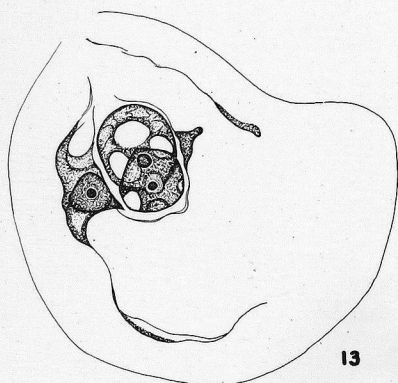
Fig. I5 Same ovule showing the fusion nucleus. (X I350)

Fig. I6 Parent Grimm: 2 days after artificial tripping show-
 ing a young embryo and several wandering endosperm
 cells. (X I350)

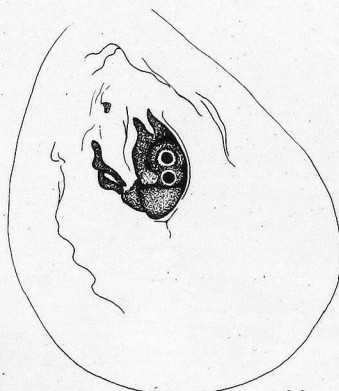
Figs. I7 and I8

 H 7-7: 2 days after artificial tripping showing
 remnants of the inner nutritive layer and formation
 of endosperm but no development of an embryo. (X I350)

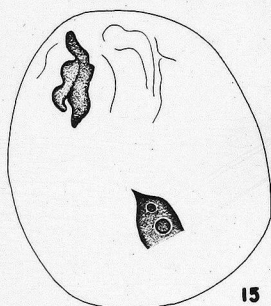
PLATE 11.



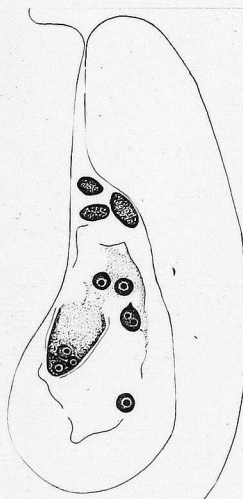
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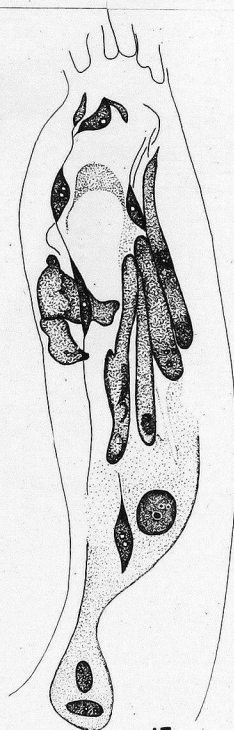
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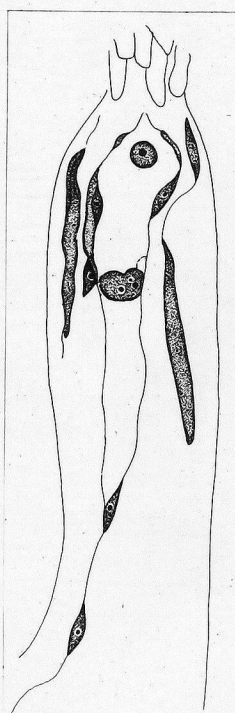
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16



17



18

Plate III

Figs. I9 & 20

Fig. I9 H 7-7: Time of artificial tripping showing the egg and a nucleolus which has become dislodged from the synergid - also a portion of a sperm. (X I350)

Fig. 20 Same ovule showing the two synergids, the fusion nucleus and the remainder of the sperm shown in fig. I9. (X I350)

Fig. 2I, 22 & 23.

Fig. 2I H 7-7: 2 days after artificial tripping showing the fusion nucleus and a sperm - also starch grains. (X 778)

Fig. 22 Same ovule showing the two synergids and the second sperm entering the egg - also starch grains and remnants of the inner nutritive layer. (X 778)

Fig. 23 Same ovule showing a glandular hair, the micropyle of the ovule and the embryo sac containing the two synergids, and a mass of giant food bodies. There are no antipodal cells. (X 778)

PLATE III.

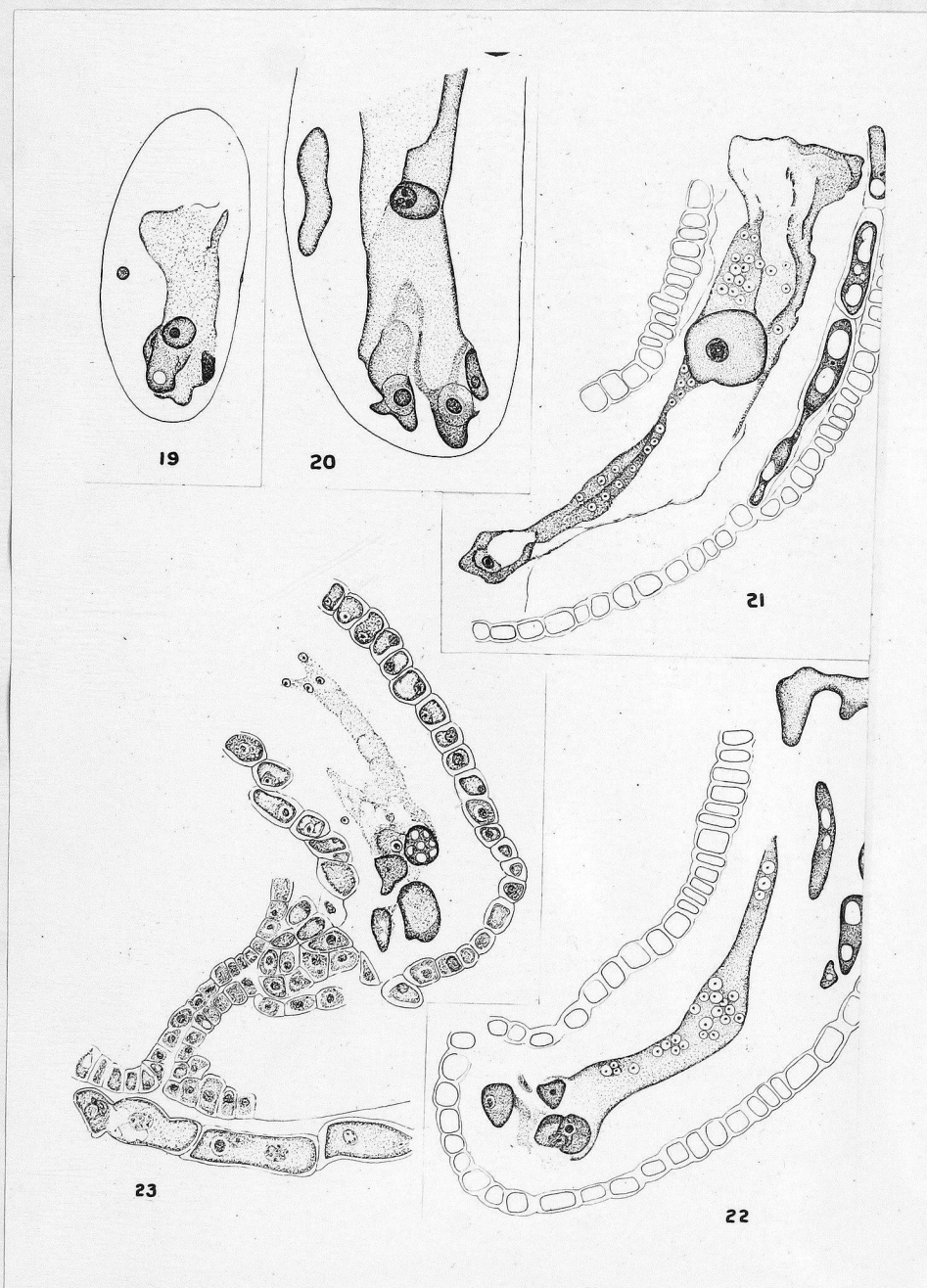


Plate IV.

Figs. 24, 25 & 26

Fig. 24 H 68-5: 2 days after artificial tripping showing in the eighth ovule, the greater part of the egg, abundant starch grains and remnants of the inner nutritive layer. (X 778)

Fig. 25. Same ovule showing one beaked synergid and a sperm which has arrived too late for effective fusion of the gametes and numerous starch grains. (X 778)

Fig. 26 Same ovule showing the fusion nucleus alongside of which is a sperm which has arrived too late for effective fusion of the gametes. (X 778)

Figs. 27, 28, 29 & 30.

Fig. 27 H 7I-26: 2 days after artificial tripping showing a shrunken egg and a very large number of starch grains. (X 778)

Figs. 28-30

Other cells of the same sac showing the shrunken condition of the female gametophyte. (X 778)

PLATE IV.

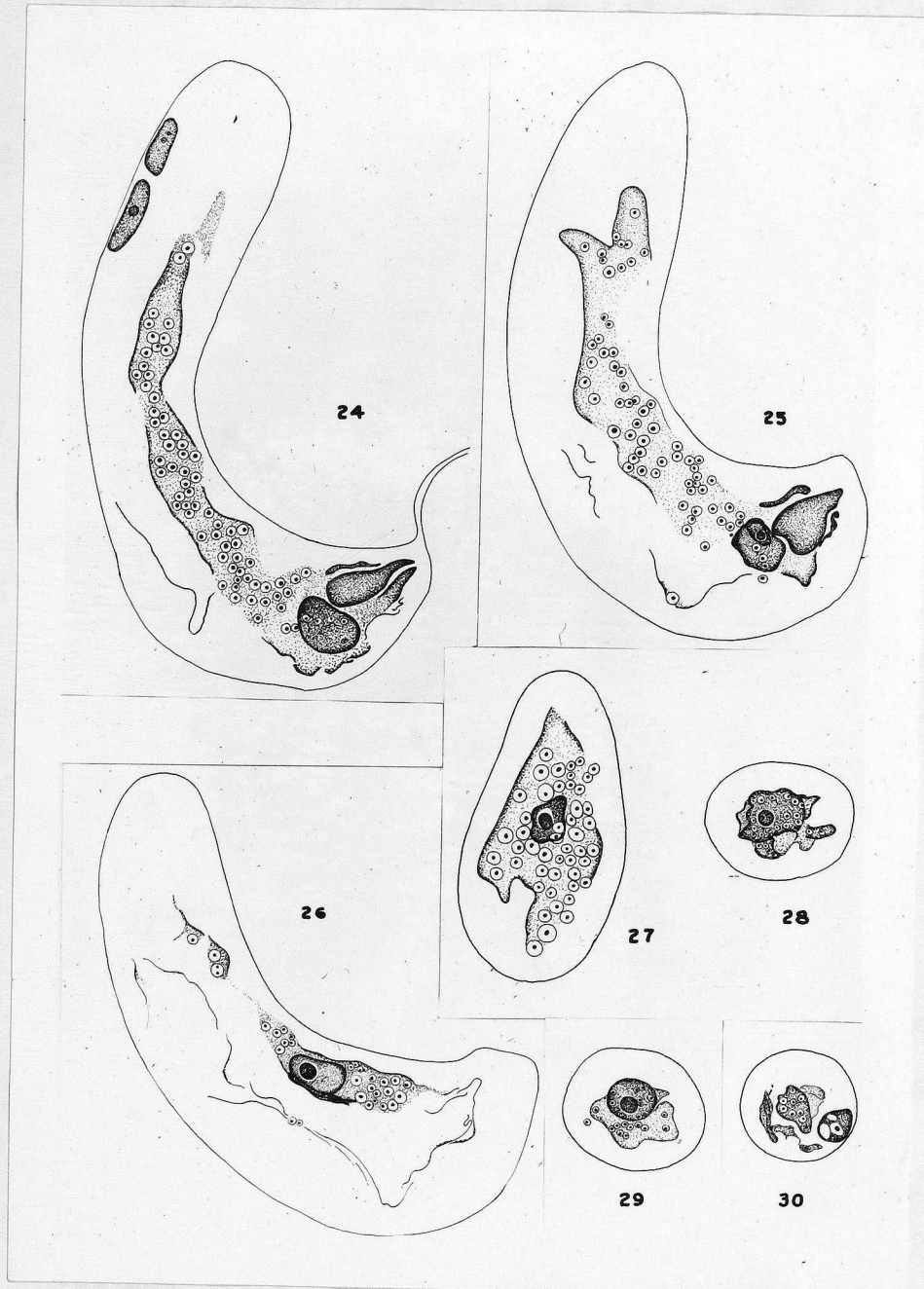


Plate V.

- Fig. 1 H 7-7: Stage 4. Shows the paired campylotropous condition of the ovules, the left ovule of which shows a megaspore which has divided to form 4 nuclei of the female gametophyte (See plate I, fig. 8). (X 260)
- Fig. 2 H 7-7: 2 days after artificial tripping showing paired campylotropous. (X 160)

Plate VI.

- Fig. 1 H 7-7: Within 24 hours after natural tripping showing two pollen tubes passing down the style. (X 726)
- Fig. 2 H 7-7: 2 days after artificial tripping showing a pollen tube growing along a gland hair and a portion of an ovule. (X 726)

Plate VII.

- Fig. 1 H 7-7: 1 day after artificial tripping. Shows an embryo sac containing one antipodal cell and the egg full of food material and surrounded by starch grains. (X 270)
- Fig. 2 H 7-7: Time of artificial tripping showing fusion of the nuclei in the formation of the fusion nucleus, the egg and one synergid. (X 160)

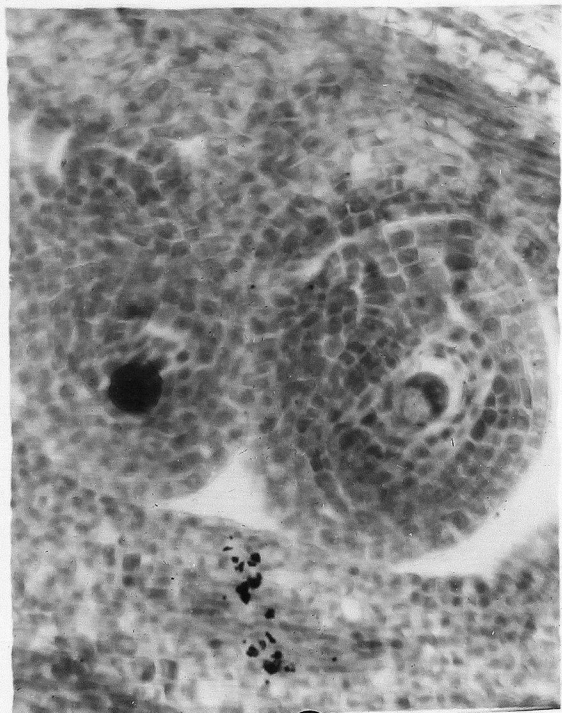


PLATE V

Fig. 1



Fig. 2

PLATE VI

Fig. 1



Fig. 2

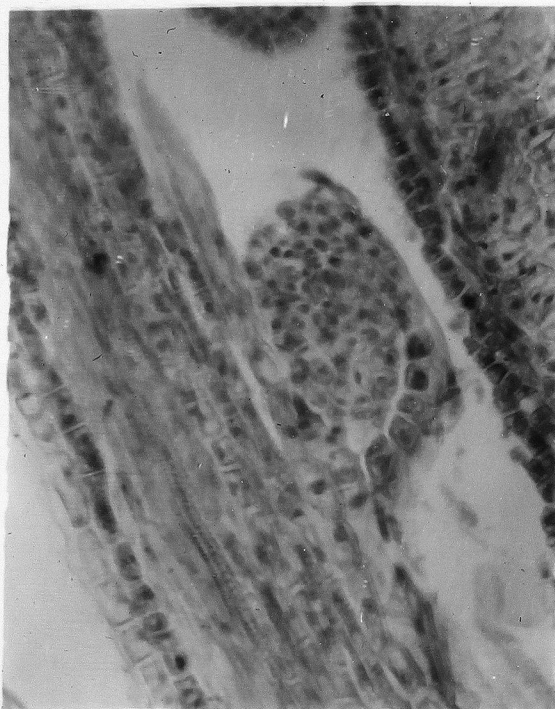


PLATE VII

Fig. 1



Fig. 2



CHART 1.












































































	H 7-7 ARTIFICIAL TRIPPING	H 7-7 24 HOURS-	H 7-7 2 DAYS	H 66-5 2 DAYS	PARENT GRIMM 2 DAYS
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16	1	2	3	4	5

CHART 11.

	H 7-7 3 DAYS	H 7-7 3 DAYS	H 7-7 4 DAYS	H 7-7 4 DAYS	H 7-7 6 DAYS	H 7-7 9 DAYS	H 68-5 6 DAYS	H 68-5 9 DAYS
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16	6	7	8	9	10	11	12	13