THE CONTROL OF FERTILIZATION IN CLOVER

Thesis for the Degree of M. S.

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M. S. THESIS.

THE CONTROL OF FERTILIZATION IN CLOVER.

BY

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THESIS

CLOVER.

The clovers belong to the order Leguminosae, Family Papilionaceae, genus Trifolium. The number of species belonging to the genus Trifolium are about two hundred and fifty (250) in number. They are the most abundant in the North Temperate Zone. About sixty-five (65) species are known in North America, the great est number of these being found native to the Western states.

MECHANISM OF THE FLOWERS.

The flowers of the clover are composed of the FLORAL ENVELOPE (corolla and calyx), and the ESSENTIAL ORGANS (stamens and pistil). The corolla is GAMOPETALOUS. The calyx persists together with the corolla around the ripe fruit. The inflorescence is spike, capitulum or capitate umbel. The petal which overlaps the others in the bud is termed the BANNER or STANDARD, (vexellum). The two adjoining lateral petals are the WINGS, (alae), while the other two cohere at the top to form a hollow boat shaped body called the KEEL or CARINA. There are nine stamens. The upper ends of the stamens and style curve upwards in the pocket of the keel. They are all attached together at the base. When the keel is pressed down the essential organs protrude, but they return when the pressure is removed. The pollen is thus protected from the weather and from robbery. The flowers of the clovers contain honey which is secreted at the base of the cohering stamens. It surrounds the base of the ovary

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PLATE I

Fig. 3

Fig. 1

PLATE I

Fig 1

Fig. 2

Fi 3.

PLATE I

Fig. 2

Fig 1

Fi 3.

and can only be reached through two apertures, one on either side of the base of the superior free stamens. In order to reach the honey an insect must stand upon the wings. These and the keel are consequently pressed downwards. The banner is pressed upwards by the insect's head, and the stamens and the stigma protruding from the keel, are forced up against the ventral side of the insect. Cross fertilization takes place regularly since the stigma projects slightly beyond the anthers and therefore comes in contact with the insect before the stamens. When the insect leaves the flower, all organs return to their former place.

THE WHITE CLOVER, (Trifolium repens).

The color of the flowers are white or re dish. The corolla tube, which admits of the movements of the banner and keel are only 3 mm long, so that the less specialized bees can reach the honey.

Plate I, fig. 1 shows a Trifolium repens flower as seen from below.

Fig. 2 shows the same as seen from above after the removal of the calyx and banner.

Fig. 3 shows the right wing (ala) as seen from the i ner side

(a). Nectar passage.

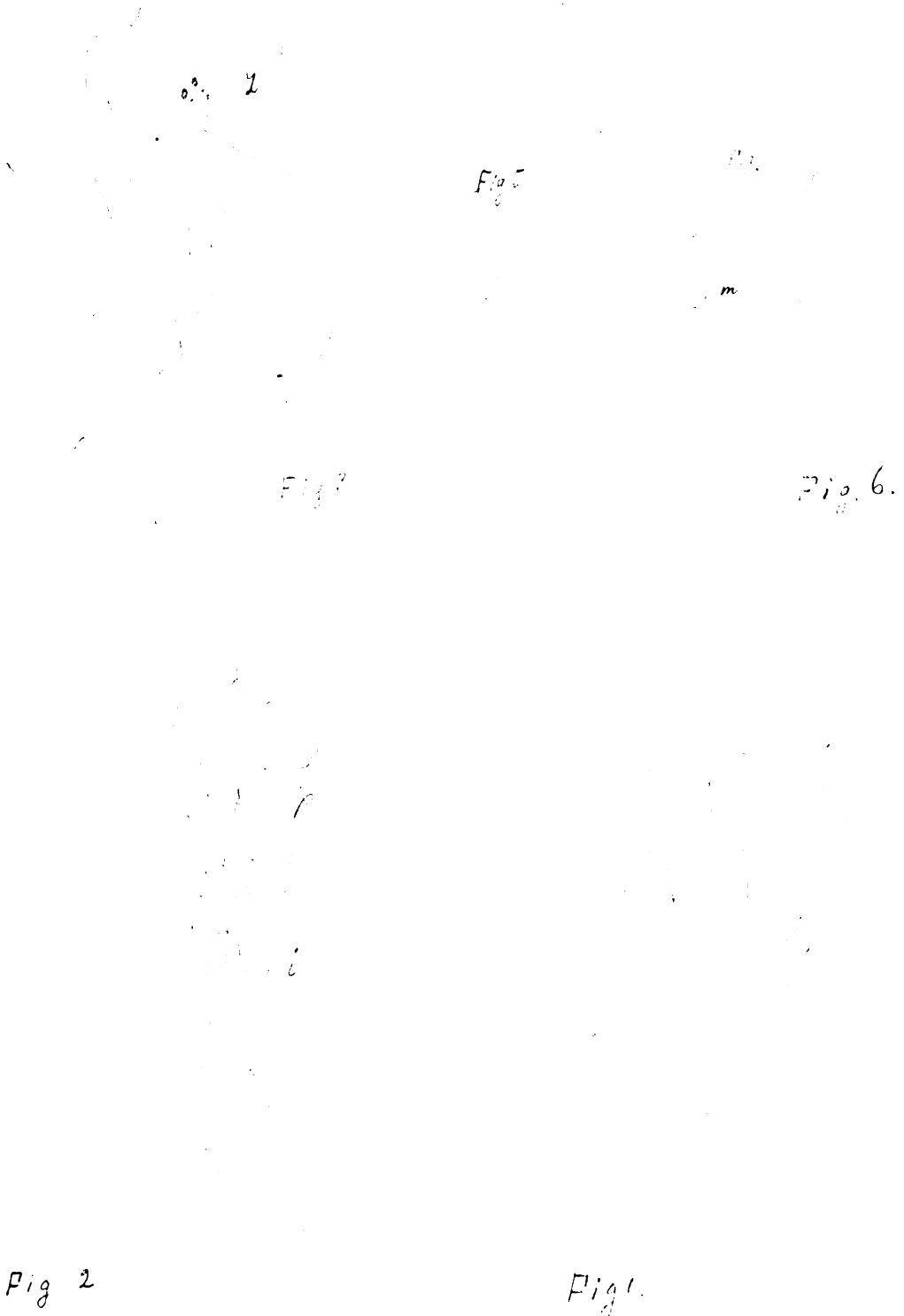
(cc). Pits in the keel petals into which the wing petals fit.

(F). Stigma.

RED CLOVER, (Trifolium pratense).

The inflorescence is ordinarily called a head, and consists of a round to elongated oval. The spike is usually single, but

PLATE II



is sometimes forked. It is one to one and one-half inches long and contains from one to two hundred (100-200) sessile flowers. In color they are light to dark pink and seldom white. They are abundantly visited by insects. The honey is at the base of the corolla which is from nine to ten mm. long. In order to reach the honey an insect must have a proboscis at least nine to ten mm. long. The pollen is accessible to all insects that can press down the keel. Bees or other insects having these requirements, cross-pollinate the flowers whether they are sucking the honeyor gathering the pollen.

Plate II, fig. 1 shows a Trifolium pratense flower as seen from below.

Fig. 2. shows the same as seen from above after the removal of the banner.

Fig. 3 shows the anterior part of the same with the edges of the keel pressed apart.

Fig. 4 shows the right wing (ala) with the claw torn off, as seen from the inner side.

Fig. 5 shows the right half of the keel, with the claw torn away, as seen from the outside.

Fig. 6 shows the essential organs emerging from the depressed keel.

(a). Calyx.

(b). Tube formed by the coalescence of the nine filaments with the claws of the banner, wings and keel.

(c). Banner.

(d). Hollow on the inner side of the wing.

(e). Lower border of the wing.

(f). Outside of the wing.

Heads of Crimson clover.

(g). Base of the wing dilated into a vesicular process.

(h). Keel.

(i). Style.

(k). Free upper stamens.

(l). Stigma.

(m). Anthers.

(n). Point of union between wings and keel.

(o). Point at which the keel is moved up and down.

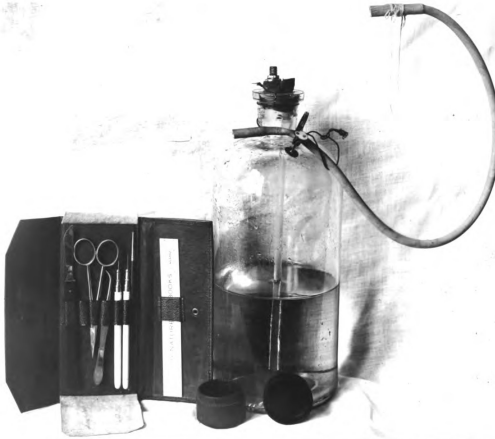
CRIMSON CLOVER, (Trifolium incarnatum).

The mechanism of the flowers is the same as in the Trifolium pratense. The length of the corolla tube is 8-9 mm and the calyx tube 5 mm. in length. The banner is folded up, and therefore serves to guide the proboscis of the longer-tongued insects to the honey.

ALSIKE CLOVER, (Trifolium hybridum).

The mechanism of the flowers is the same as that of Trifolium repens. The flowers are small and white or reddish in color.

In my clover pollination work these four species of the clovers were used, and the description of the flower mechanism is confined to these species solely. During the course of the work bumble bees, honey bees, and butterflies were observed working on these species of the clovers. Many other insects were observed visiting these plants and probably acted as agents in the cross-pollination.



Tools used in work.

TOOLS.

The following tools were used in the work;--

(1). A small pair of scissors for removing parts of the corolla, stamens, etc.

(2). A pair of forceps to hold the stamens while removing the anthers.

(3). A small sized camel's hair brush that was used to apply the pollen to the stigma.

(4). A small wooden box which was painted black inside and afterward covered with a solution of gum in alcohol to make the surface smooth. The black color enables one to see the pollen, while the smooth surface prevents the pollen from sticking to the box.

(5). A lens.

(6). Some good white blotting paper with which to dry the stigma after depollination by water.

(7). Some tags for keeping the records of the different experiments while the seeds are ripening.

(8). For depollination a wash bottle was used. It was an ordinary bottle of two gallon capacity, and was half filled with water. A two-holed stopper was put in the mouth of the bottle. A glass tube passes through one hole to near the bottom of the bottle. This tube is bent at right angles above the stopper and on it is attached a rubber tube. At the end of the rubber tube is another glass tube drawn out to a capillary tube at the end. A pinch valve closes the rubber tube. In the other hole is placed an ordinary bicycle valve. This enables one to pump up and retain air pressure within the bottle. By opening

e three flowers above are from white clover. The lower ones are from red
over. The first view at the left shows the natural flower in each case;
e second has floral envelope removed ; and the ones at the right are also
asculated.

Field operation in crossing alsike clover.

The three flowers above are from white clover. The lower ones are from red clover. The first view at the left shows the natural flower in each case; the second has floral envelope removed ; and the ones at the right are also emasculated.

Field operation in crossing alsike clover.

the pinch each one gets a fine jet of water.

PREPARATION OF THE HEADS.

The mechanism of the flowers in the clovers is such that the parts return to normal after an insect has visited the plant, and so one can not know but what pollination has taken place unless the flowers are covered before the banner unfolds from the top of the other parts. In the second series of these experiments the heads were thus covered by tying a piece of cheese cloth about each head. In the first series of these experiments this precaution was not taken and thus failure was the result. When flowers develop in the head that are possible to work this cover may be removed and then replaced after the work is done. The tag which records the experiment should be attached to the covering of the head and not separately to the stem. The stems are frequently broken at the time when the seeds are ripening, and thus heads are lost unless this is done.

METHODS OF DEPOLLINATION.

If the results of our crossings are to mean anything definite it is necessary to control the fertilization of the selected flowers, preventing self-fertilization. Different methods are recommended for this purpose.

(1). Emasculation, or the removing of the anthers before the pollen is deposited. The minute and delicate structure of the stamens in the clover flowers would seem to make the work difficult, but when one is experienced this work is easily done. Take a flower between the thumb and forefinger with the banner

Three stages in work with white clover.

resting against the finger. Using the forceps take hold of one of the wings. The wings and keel will be bent back together, and may be placed under the thumb. The essential organs are then exposed. Using the side of the forceps rub the organs upwards, or toward the banner. If the anthers have not yet begun to open they will be entirely broken off and removed. If the anthers are beginning to open they will be crushed by this process and one can easily know whether the flower selected is a safe one to use. The pistil should not be injured by this process as it bends toward the banner.

(2). A second method is recommended by Mr. George W. Oliver, plant propagator at Washington, especially in the case of clover. He recommends washing off the pollen by means of a small stream of water, just at the time when the anthers open and the pollen is deposited. This method is called depollination.

(3). In addition to these two methods I practiced a third one, the removal of the pollen by menas of a brush without washing the flower. Many authors such as Darwin, Knut, Hermann, and Müller write that clover is incapable of self pollination. Mr. Oliver says; "Many observations indicate that the flowers of the red clover are incapable of self fertilization when protected from insects, as plants which have been tested with this end in view have in no case produced seeds." (U. S. Dep't. of Agriculture, Bureau of Plant Industry, Bulletin No. 167, New Methods of Plant Breeding.). All this suggests the removal of the pollen by means of the brush without washing. This method is more easily done. It saves time as one cannot pollinate a wet stigma immediately after washing. The delicate parts of the flower

Three stages in work whit red clover.

are also in great danger of being injured by washing than by means of the brush.

In all of these operations all extra flowers which were not needed for fertilization or in which fertilization had already taken place, were removed, leaving just a few to operate on. In the beginning four or five flowers only were left. Later it was found possible to work with a greater number of flowers on each head. Practice indicated that the best plan was to leave the flowers in a single row from one side of the head across to the other. The flowers were easily handled by means of this plan.

GATHERING THE POLLEN.

To obtain the pollen of the clover very easily it is best to gather the flowers and put the stems in water the day before the pollen is needed. These heads were covered to protect them from insects while in the green stage. Before gathering the flowers have largely opened. They are gathered and taken indoors away from the wind, as the wind scatters the pollen. After a period of twenty-four hours an abundance of ripe pollen may be easily obtained. In extracting the pollen the flower is grasped between the thumb and forefinger just as in the case of depollination. The keel and wings are placed under the thumb, and the pollen is brushed into the box.

METHODS OF APPLYING POLLEN.

In some of the following experiments the floral envelope is removed, which facilitates the application of the pollen with the brush. In cases where the floral envelope is not cut the flower

is grasped much the same as in emasculation. The wings and keel are bent down and placed under the thumb; the banner is then bent down and placed under the finger; the pollination is by brush as before, and then the floral envelope is allowed to return to the normal.

THE FIRST SERIES OF EXPERIMENTS.

The following experiments can be divided into two general groups. In the first group belong all the experiments in which the flowers were not cross-pollinated. This group includes different methods of depollination. The second group includes all the experiments in which the flowers were cross-pollinated, as was mentioned above under the head "Preparation of Heads". In this series of experiments the flowers were not protected before the work of experimentation was begun. Later practice showed that this method was not practical.

GROUP I. POLLEN NOT APPLIED.

Division A, Depollination by water.

Division B, Depollination by brush.

Sub-division 1, Stamens and floral envelope were not cut.

Sub-division 2, Stamens were removed but floral envelope was not.

Division C, Control. Flowers were protected from insects.

Sub-division 1, Flowers were not pollinated.

Sub-division 2, Flowers were self-pollinated.

Experiment.	Number of flowers used.	Results.	
		Number of seeds obtained	Percent.
A 1	100	16	16
A 2	100	0	0
B 1	120	6	5
B 2	140	6	4.3
C 1	2247	19	.4
C 2	203	26	12.8

GROUP II. FLOWERS CROSS-POLLINATED.

Division A, Flowers depollinated by water.

Division B, Flowers emasculated

Sub-division1, Floral envelope removed.

Sub-division2, Floral envelope not removed.

Experiment	No. of flowers used	Results. No. of seeds obtained	%
A 1 Fresh pollen used at once	134	44	32.8
A 2 Fresh pollen used at once	159	5	38.7
A 2 " " " after 4 hours	154	31	20
A 2 Pollinated at once, pollen 5 days old	106	18	17
A 2 " " , " 10 " "	100	10	10
A 2 " " , " 15 " "	100	7	7
A 2 " " , " 20 " "	100	0	0
A 2 " " , " 30 " "	150	0	0
B 1 Fresh pollen used at once	144	7	4.8
B 2 Fresh pollen used at once	112	9	8
B 2 " " " after 4 hours	100	24	24
B 2 " " " " 24 "	141	24	17
B 2 " " " " 48 "	107	9	7

CONCLUSIONS FOR THE FIRST SERIES.

Seeds were often obtained in the first group of this series of experiments, where flowers were not pollinated. The flowers had been washed to rid them of the pollen, or were depollinated by means of the brush. This leads us to believe that fertilization had been accomplished by the insects before depollination, or else the depollination was not complete. Because of the results obtained in the first group we can come to no conclusion on the results in the second group. On account of these facts a second series of these experiments were undertaken, where all the flowers to be used were coverdd with cheese cloth while in the green stage.

[illegible] OF EXPERIMENTS.

Group I. Self-pollination experiments to compare methods.

No emasculation nor depollination.

Division A, [illegible]. Flowers not uncovered.

No. of Tag.	No. of Other Plant	No. of Flowers Used	Results
			No. of Seeds Obtained
1	13271	25	0
3	[illegible]	85	0
5	13274	90	0
7	13277	25	0
8	[illegible]	1 head	0
11	[illegible]	" "	0
13	13287	" "	0
1[illegible]	[illegible]	" "	0
22	[illegible]	" "	0
2[illegible]	13277	" "	0
26	13277	" "	0
[illegible]	[illegible]	" "	0
3[illegible]	[illegible]	" "	0
31	13293	" "	0
32	13293	" "	4
33	[illegible]	" "	0
34	[illegible]	" "	0
36	[illegible]	" "	0
37	13293	" "	0
38	[illegible]	" "	0
39	13291	" "	0
40	13295	" "	0
41	13249	" "	1
42	13249	" "	0
43	13249	" "	1
44	13249	" "	0
45	13249	" "	0
46	13249	" "	0
67	13295	" "	0
1[illegible]4	13174	" "	0
131	13174	" "	0
132	12971	" "	0
133	12971	" "	0
134	12971	" "	0
135	12971	" "	0
136	12972	" "	0
137	12972	" "	0
138	12972	" "	0
139	12972	" "	0

Table Continued;---

No. of Tag.	No. of Mother Plant	No. of Flowers Used	Results
			No. of Seeds Obtained
140	12[illegible]	1 head	0
141	12[illegible]51	" "	0
142	12[illegible]	" "	0
143	12[illegible]51	" "	0
149	11[illegible]7	" "	0
433	12[illegible]51	" "	0
434	12[illegible]79	" "	0
437	12[illegible]9	" "	0
443	12[illegible]9	" "	0
444	12[illegible]53	" "	0
450	12[illegible]51	" "	0
451	12[illegible]51	" "	0
465	13[illegible]50	" "	0
474	13[illegible]49	" "	0
475	13[illegible]49	" "	10
490	13[illegible]49	" "	0
491	13[illegible]49	" "	0
492	13[illegible]49	" "	0
493	13[illegible]49	" "	0
494	13[illegible]49	" "	0
495	13[illegible]49	" "	0
496	13[illegible]49	" "	0
504	13147	" "	0
505	13147	" "	0
506	13159	" "	0
507	13159	" "	0
508	13159	" "	5
509	13165	" "	0
510	13165	" "	0
511	13173	" "	0
512	13173	" "	0
513	13174	" "	0
514	13097	" "	0
515	13097	" "	2
516	13097	" "	12
517	13093	" "	0
518	13093	" "	0
519	13093	" "	0
520	13091	" "	0
521	13091	" "	0
522	12051	" "	0
523	12051	" "	0
524	12054	" "	0
525	12854	" "	0
526	12854	" "	0
527	12859	" "	0

Table Continued;---

No. of Tag	No. of Other Plant	No. of Flowers Used	Results
			No. of Seeds Obtained
528	12869	1 head	0
529	[illegible]	" "	0
530	[illegible]	" "	0
531	12871	" "	0
532	12872	" "	0
533	12872	" "	0
534	12873	" "	0
535	12777	" "	0
536	12773	" "	0
537	12[illegible]	" "	5
538	12553	" "	[illegible]
541	12[illegible]	" "	0
542	12557	" "	0
Also 7, heads on other plants			11
Total is 1[illegible] heads and 8[illegible]5 flowers			51

Considering a head as containing 150 flowers we have 8135 flowers under protection and 51 seeds .1%. The results of these experiments show that clover is sometimes fertilized by inside agents.

Messrs. Knuth and Müller both made the statements that as the stigma in clover projects beyond the stamens, clover needs outside agents for pollination. Some one may say that the cloth covering of the heads may cause pollination in some way. We put plants under a cage while all the flowers were in a green stage, and allowed them to bloom protected from insects. One of these caged plants produced a few seeds.

Group I. Division B. Self-pollination by pinching the flowers.

No. of Tag	No. of Mother Plant	No. of Flowers Used	Results
			No. of Seeds Obtained
16			
16	13267	8	0
18	13268	15	0
431	13269	3	0
432	13269	3	0
433	13270	3	1
434	13270	6	0
438	13269	3	0
439	13269	3	0
440	13269	3	2
441	13269	3	0
442	13269	3	0
445	13251	4	0
446	13251	3	1
447	13251	3	0
448	13257	3	0
449	13251	3	0
452	13251	3	0
453	13251	3	0
454	13251	3	0
Total		102	4 or 3.9%

Group I, Division C. Self-pollination by opening and closing the flowers.

No. of Tag	No. of Mother Plant	No. of Flowers Used	Results
			No. of Seeds Obtained
455	13251	6	2
456	13251	8	3
457	13250	5	0
458	13250	6	2
459	13250	8	2
460	13250	6	2
461	13250	5	0
462	13250	9	2
463	13250	9	1
464	13250	5	1
476	13248	7	0
477	13248	5	0
480	13248	8	0
481	13248	6	0
482	13248	6	0
483	13248	5	0
Total		100	17 or 17%

Group I. Division D. Self-pollination by brush.

No. of Tag.	No. of Mother Plant	No. of Flowers Used	Results
			No. of Seeds Obtained
468	13249	6	0
469	13249	6	2
470	13249	6	1
473	13248	6	0
484	13248	6	2
485	13248	6	2
486	13248	6	3
487	13248	3	1
488	13248	4	1
489	13248	4	2
498	13147	3	1
499	13147	4	1
500	13147	6	2
501	13147	2	0
502	13147	6	3
503	13147	3	1
Total		77	22 or 28.6%

GROUP II. Flowers depollinated by water at ripe stage when the pollen has been deposited. Not pollinated.

Division A. Control. Parts left normal.

No. of Tag.	No. of Mother Plant	No. of Flowers Used	Results
			No. of Seeds Obtained
2	13289	6	0
4	13281	12	0
6	13277	7	0
9	13272	7	0
10	13269	5	0
12	13268	7	0
14	13267	7	0
19	13264	6	0
20	13295	6	0
21	13295	8	0
24	13281	13	0
27	13277	6	0
28	13268	6	0
35	11651	5	0
63	13268	5	0
64	13268	5	0
181	13173	8	0
186	13269	15	0
200	13259	9	0
201	13259	14	0
Total		157	0

GROUP II. Depollination by water.

Division B. The floral envelope is removed, and the stamens are left.

No. of Tag.	No. of Mother Plant	No. of Flowers Used	Results
			No. of Seeds Obtained
47	13295	6	0
48	13295	6	0
49	13299	6	0
50	13299	6	0
51	13277	7	0
52	13277	8	0
53	13293	6	0
54	13273	8	0
55	13272	6	0
56	13272	8	0
57	13272	7	0
58	13269	7	0
59	13269	7	0
60	13269	5	0
62	13268	5	0
65	13267	5	0
66	13267	5	0
Total		108	0

GROUP II. Depollination by water .

Division C. The floral envelope and stamens are removed.

No. of Tag	No. of Mother Plant	No. of Flowers Used	Results
			No. of Seeds Obtained
84	13249	6	0
85	13249	6	0
86	13249	7	0
87	13249	3	0
88	13249	4	0
162	13297	4	0
163	13297	3	0
169	13297	4	0
170	13296	5	0
171	13296	6	0
172	13295	7	0
173	13295	7	0
174	13289	3	0
175	13289	4	0
176	13289	7	0
177	13288	4	0
178	13283	8	0
179	13283	8	0
180	13283	4	0
Total		100	0

GROUP III. Depollination by brush (without washing) at ripe stage. No pollination.

Division A. Control. Parts left natural.

No. of Tag	No. of Mother Plant	No. of Flowers Used	Results
			No. of Seeds Obtained
15	13267	9	0
393	13279	5	0
395	13279	6	0
396	13277	7	1
397	13277	7	0
398	13267	5	0
399	13267	4	0
400	13659	4	1
401	13659	5	0
402	13358	6	0
403	13358	5	0
404	13258	4	0
405	13258	5	0
406	13254	5	0
407	13254	5	0
408	13253	5	3
409	13253	13	0
Total		100	5 or 5%

GROUP III. Pollination by brush (without washing) at ripe stage. No. pollination.

Division B. The floral envelope is removed but the stamens are left.

No. of Tag.	No. of Mother Plant	No. of Flowers Used	Results
			No. of Seeds Obtained
299	13277	6	0
300	13277	6	0
301	13277	4	0
302	13277	9	0
303	13277	6	0
304	13277	6	0
305	13272	4	1
306	13272	5	1
307	13272	9	0
308	12756	5	3
309	12756	6	0
310	12756	4	0
311	12756	7	0
312	12754	5	1
313	12754	4	0
314	12754	3	0
315	12754	6	0
316	12754	6	0
Total		100	6 or 6%

GROUP III. Repollination by brush (without washing) at ripe stage. No pollination.

Division C. The floral envelope and stamens are removed.

No. of Bag.	No. of Mother Plant	No. of Flowers Used	Results
			No. of Seeds Obtained
317	13283	6	0
318	13283	3	0
319	13282	7	0
320	13265	6	0
321	13265	3	0
322	13265	4	0
323	13265	13	0
324	13283	9	0
325	13282	7	0
326	13259	4	0
327	13259	5	0
328	13259	3	0
329	13259	4	0
330	13259	2	0
331	13254	4	0
332	13254	4	0
333	13254	4	0
334	13254	10	0
394	13281	7	0
Total		105	0

GROUP IV. The flowers are emasculated in the green stage (anthers removed before opening).

Division A. Control. The parts are left normal.

No. of Tag.	No. of Mother Plant	No. of Flowers Used	Results
			No. of Seeds Obtained
356	12765	4	0
357	12765	5	0
358	12765	5	0
359	12765	6	0
360	12765	5	0
361	12765	7	0
362	12466	5	0
363	12466	7	0
364	12466	5	0
365	12466	5	0
366	12466	6	0
367	12466	7	0
368	12466	10	0
369	12466	6	0
370	12768	10	0
371	12768	12	0
Total		103	0

GROUP III. The flowers were emasculated in the green stage (anthers removed before opening).

Pavilion II. Flow 1 envelope removed.

No. of Tag.	No. of Mother Plant	No. of Flowers Used	Results
			No. of Seeds Obtained
372	12768	5	0
373	12768	5	0
374	12768	10	0
375	12768	4	0
377	12768	10	0
378	12879	6	0
379	12879	9	0
380	12879	5	0
381	12879	6	0
382	12879	7	0
383	12879	5	0
384	12873	4	0
385	12873	4	0
386	12873	8	0
387	12873	6	0
388	12871	6	0
389	12871	6	0
390	12871	4	0
Total		110	0

GROUP V. Cross pollination, using fresh pollen.

Division A, the flowers were depollinated as in II A, (pollen washed off by water, parts left natural). Pollinated at once.

No. of Tag.	No. of other Plant	No. of plant from which pollen was taken	No. flowers Used	Results: No. of Seeds Obtained
108	13201	11661	6	0
110	13271	11661	12	6
113	13272	11661	5	3
114	13272	11661	4	2
115	13272	11661	5	3
116	13272	11661	11	4
118	13170	11015	9	7
120	13070	11015	4	1
121	13070	11015	4	2
122	13070	11015	9	4
125	13174	11015	5	0
126	13174	11015	4	0
127	12071	11015	6	5
128	12071	11015	6	5
129	12072	11015	5	4
130	12072	11015	5	4
Total			100	50 or 50%

GROUP V. Cross pollination, using fresh pollen.

Division 1, flowers depollinated as in II A. Pollinated after four hours.

No. of Tag.	No. Mother Plant	No. of Plant from which pollen was taken	No. Flowers Used	Results: No. of Seeds Obtained
89	13296	11661	7	5
90	13295	11661	11	6
91	13295	11661	7	5
92	13295	11661	7	1
93	13289	11661	13	5
96	13289	11661	4	2
97	13289	11661	3	3
98	13289	11661	3	3
99	13289	11661	4	2
100	13283	11661	5	2
101	13283	11661	5	2
102	13283	11661	5	1
103	13281	11661	4	2
104	13281	11661	6	3
105	13281	11661	4	2
106	13281	11661	8	4
107	13281	11661	4	2
Total			100	50 or 50%

GROUP V. Cross pollination by using fresh pollen.

Division C, Flowers depollinated as in II A. Pollinated after 24 hours.

No. of Bag	No. of Other Plant	No. of Plant from which pollen was taken	No. Flowers Used	Results: No. of Seeds Obtained
68	13250	11661	13	2
69	13254	11661	6	2
70	13250	11661	6	2
71	13253	11661	4	2
72	13253	11661	2	1
73	13253	11661	9	0
74	13253	11661	8	2
75	13253	11661	11	8
76	13253	11661	9	2
77	13253	11661	5	3
78	13254	11661	3	1
79	13252	11661	12	1
80	13251	11661	8	0
81	13250	11661	5	1
82	13250	11661	4	0
83	13254	11661	3	0
Total			110	33 o. 30%

GROUP V. Cross pollination by using fresh pollen.

Division D. Flowers depollinated as in II D. (pollen washed off by water also removing the floral envelope). Pollinated at once.

No of Tag.	No. Mother Plant	No. of Plant from which pollen was taken.	No. Flowers Used	Results: No of Seeds Obtained
182	13173	12355	4	0
183	13173	12355	4	0
184	13173	12355	5	0
185	13173	12355	4	2
187	13269	12555	5	1
188	13269	12555	5	1
189	13266	12555	5	0
190	13266	12555	6	0
191	13250	12555	6	1
192	13250	12555	6	0
193	13250	12555	1	0
194	13250	12555	6	2
195	13266	12555	11	3
196	13269	12555	10	0
197	13173	12555	9	0
198	13173	12555	3	0
Total			100	10 or 10%

GROUP VI. Cross pollination, using fresh pollen.

Division A, Flowers depollinated as in IIIA (pollen by the brush without washing, stamens and floral parts left Natural) and pollinated at once.

No. of Tag.	No. Mother Plant	No. of Plant from which pollen was taken.	No. Flowers Used	Results: No. of Seeds Obtained
1336	12871	11541	10	5
1337	12871	11541	10	5
1340	12871	11541	6	4
1342	12871	11541	5	2
1343	12871	11541	5	5
1345	12871	11541	5	3
1346	12870	11541	6	2
1347	12870	11541	6	3
1348	12870	11541	9	5
1350	12870	11541	10	9
1352	12866	11541	5	2
1353	12866	11541	5	2
1354	12866	11541	6	2
1355	12866	11541	6	2
Total			100	52 or 52%

GROUP VI. Cross pollination, using fresh pollen.

Division B, Flowers emasculated on in IIIA. Pollinated after four hours.

No. of Exp.	No. Mother Plant	No. of plant from which pollen was taken	No. Flowers Used	Results: No. of Seeds Obtained
261	13271	11465	18	4
263	13271	11465	11	4
265	13271	11465	9	2
266	13271	11465	10	4
269	13271	11465	7	4
270	13271	11465	7	4
271	13271	11465	4	2
272	13271	11465	5	2
273	13277	11465	8	6
274	13277	11465	1	4
275	13277	11465	8	5
276	13273	11465	5	3
277	13273	11465	6	3
278	13269	11465	3	2
279	13269	11465	4	2
280	13269	11465	5	2
Total			107	53 or 49.5%

GROUP VI. Cross pollination using fresh pollen.

Division 3, Flowers depollinated as in IIIA. Pollinated after 24 hours.

No. of Tag.	No. Pollen Plants	No. of Plant from which Pollen was taken	No. Flowers Used	Results: No. of Seeds Obtained
241	13197	11465	12	2
243	13199	11465	11	1
245	13172	11465	10	1
247	13181	11465	4	1
248	13181	11465	5	2
249	13174	11147	9	2
251	13173	11147	7	2
252	13173	11147	4	0
253	13173	11147	5	0
254	13250	11147	8	4
255	13252	11147	7	6
257	13248	11147	10	6
259	13248	11147	4	2
260	13248	11147	4	3
Total			100	32 or 32%

GROUP VI. Cross pollination, using fresh pollen.

Division [illegible], Flowers pollinated as in IIIB (pollen removed by brush without washing. Floral envelope was removed.) Pollinated at once.

No. of Tag	No. Mother Plant	No. of Plant From which pollen was taken	No. Flowers used	Results: No. of Seeds Obtained
281	12[illegible]9	12775	6	3
282	12769	12775	12	0
284	12769	12775	11	3
290	12769	12775	10	8
290	12772	12775	10	0
[illegible]	12772	12775	6	0
291	12772	12775	5	0
292	12772	12775	4	0
293	12772	12775	5	0
294	12771	12775	3	0
295	12771	12775	4	0
296	12771	12775	6	0
297	12768	12775	5	0
298	12768	12775	10	0
Total			100	14 or 14%

GROUP VIII. Cross pollination, using fresh pollen.

Division A, Flowers emasculated as in IV A. Floral envelope left natural. Pollinated at once.

No. of Tag	No. Mother Plant	No. of Plant from which pollen was taken	No. Flowers Used	Results: No. of Seeds Obtained
411	12067	11557	6	3
412	12067	11559	7	2
413	12054	12454	10	2
415	12064	12454	3	2
416	12072	12454	9	0
418	12077	12454	10	0
420	12072	12454	7	0
421	12072	12454	12	0
423	12051	12454	12	4
425	12059	11557	4	0
426	12059	11557	4	0
427	12059	11557	4	1
428	12059	11557	4	0
[illegible]	12059	11557	3	0
430	12059	11557	4	0
Total			100	14 or 14%

GROUP VII. Cross pollination, using fresh pollen.

Division B, Flowers emasculated as in IVA. Pollinated after four hours.

No. of Tag	No. Mother Plant.	No. of Plant from which pollen was taken.	No. Flowers used	Results: No. of Seeds Obtained
202	12260	11457	5	0
203	12260	11457	7	0
205	11567	11457	10	5
207	11567	11457	9	3
209	11567	11457	7	0
210	11066	11457	6	0
211	11066	11457	6	0
212	11066	11670	8	0
214	11761	11670	5	2
215	11761	11670	6	4
216	11761	11670	5	0
217	11761	11670	5	0
218	11761	11670	6	2
219	11761	11670	6	2
220	16110	11670	4	2
221	16110	11670	5	3
Total			100	26 or 26%

GROUP VII. Cross pollination, using fresh pollen.

Parentage 3, Flowers emasculated as in IV A. Pollinated after 24 hours.

No. of Tag	No. Mother Plant	No. of Plant from which pollen was taken	No. Flowers Used	Results: No. of Seeds Obtained
222	12063	11450	10	0
224	12063	11450	13	0
226	12063	11450	4	0
227	12063	11450	7	2
228	12063	11450	7	0
229	11755	11450	2	0
230	11755	11450	9	0
232	11755	11450	7	0
233	11755	11450	6	2
234	11755	11450	3	0
235	12454	11450	12	0
237	12454	11450	4	2
238	12454	11450	4	2
239	12454	11450	5	1
240	12454	11450	4	0
Total			100	20 or 20%

GROUP .II. Cross pollination, using fresh pollen.

Division D, Flowers emasculated as in IV A. Pollinated after 48 hours.

No. of Tag	No. Mother Plant	No. of plant from which pollen was taken	No. Flowers Used	Results: No. of Seeds Obtained
144	11957	11959	2	0
145	11957	11959	3	0
146	11957	11959	6	4
147	11957	11959	9	0
150	11959	11456	9	0
151	12060	11456	10	0
153	12060	11456	4	0
154	12060	11456	5	0
155	12060	11456	8	0
156	12060	11456	10	0
157	12063	11456	5	2
159	12063	11456	4	2
160	12063	11456	5	2
161	12170	11456	6	3
162	12170	11456	5	0
Total			100	13 or 13%

GROUP VII. Cross pollination, using fresh pollen.

Division A. Flowers emasculated as in IV D. (Removed anthers and floral envelope). Flowers were pollinated at once.

No. of Tag	No. Mother Plant	No. of Plant from which pollen was taken	No. Flowers Used	Results: No. of Seeds Obtained
550	13291	11661	10	1
551	13291	11661	6	0
552	13291	11661	7	0
553	13291	11661	7	0
556	13181	12454	6	1
557	13181	12454	6	0
558	13181	12454	4	0
559	13181	12454	5	2
561	13181	12454	4	0
562	13269	12454	5	0
563	13269	11465	4	0
565	13269	11465	7	1
566	13269	11465	5	0
567	10971	11465	7	0
568	10971	11465	8	1
569	10971	11465	10	1
Total			102	7 or 6.86

GROUP III. VIABILITY OF POLLEN.

The flowers were cross pollinated at once with pollen which had been stored in a little gelatine capsule in a cool dry place.

Division A. Flowers depollinated as in II A (pollen washed off by water. The floral envelope was left natural) Flowers pollinated with pollen that was five days old.

No. of Tag	No. Mother Plant	No. of Plant from which pollen was taken	No. Flowers Used	Results: No. of Seeds Obtained
570	13272	12555	9	2
571	13272	12555	9	4
572	13272	12555	9	0
574	13269	12555	9	3
575	13269	12555	7	1
576	13269	12555	6	0
577	13269	12555	4	0
578	13269	12555	6	0
580	13253	12555	4	2
581	13250	12555	7	2
582	13253	12555	9	4
583	13253	12555	7	2
584	13266	12555	9	2
585	13266	12555	10	2
Total			110	24 or 24%

GROUP VIII. Flowers were cross pollinated at once.

Division B. The flowers were depollinated as in II A. The pollen used was ten days old.

No. of Tag	No. Mother Plant	No. of Plant from which pollen was taken	No. Flowers Used	Results: No. of Seeds Obtained
587	11959	12454	10	2
588	11959	12454	6	0
589	11959	12454	5	0
590	12452	12454	6	0
591	12452	12454	9	1
592	12452	12454	9	0
593	12452	12454	8	1
594	12059	12454	7	0
595	12059	12454	9	3
596	12059	12454	7	1
597	12260	12454	8	0
598	12260	12454	8	0
599	12260	12454	7	2
600	12260	12454	3	2
601	12260	12454	5	0
Total			110	13 or 11.8%

GROUP VIII. The flowers were cross pollinated at once.

Division B. Flowers were depollinated as in II A. The pollen used was 15 days old.

No. of Tag	No. Mother Plant	No. of Plant from which pollen was taken	No. Flowers Used	Results: No. of Seeds Obtained
602	13000	11661	8	1
603	13000	11661	5	0
604	13000	11661	7	0
605	13000	11661	4	2
606	13293	11661	5	0
607	13293	11661	5	0
608	13293	11661	7	1
609	13296	11661	8	0
610	13296	11661	8	0
611	13296	11661	7	0
612	13296	11661	5	0
613	12872	11661	4	0
614	12872	11661	10	1
615	12872	11661	8	1
616	12059	11661	8	0
Total			100	6 or 6%

SUMMARY.

Bearing in mind the fact that all the flowers were covered while green to protect them from the insects, the results of the second series of experiments may be summarized as follows;--

Experiment	No. of Flowers Used	Result: No. of Seeds Obtained	Result: Percent
Group I. Self-pollination			
A. Flowers not treated	28135	51	.18
B. " " pinched	102	4	3.90
C. " " opened and closed	100	17	17.00
D. " " brushed	77	22	28.60
Group II. No pollination. Flowers depollinated by water			
A. Control-Parts left natural	157	0	0
B. Floral envelope removed	102	0	0
C. Floral envelope and stamens removed	100	0	0
Group III. No pollination. Flowers depollinated by brush			
A. Control--Parts left natural	100	5	5.
B. Floral envelope removed	100	6	6.
C. Floral envelope and stamens removed	105	0	0
Group IV. No pollination. Flowers emasculated green			
A. Control--Parts left natural	103	0	0.
B. Floral envelope removed	110	0	0
Group V. Cross pollination. Fresh pollen, depollinated as in II A.			
A. Flowers depollinated as in IIA, pollinated at once	100	50	50.
B. Pollinated after 4 hours	100	50	50.
C. " " 24 "	110	33	30.
D. Depollinated as in II B. Pollinated at once	100	10	10.
Group VI. Cross pollination			
A. Flowers depollinated as in III A. Pollinated at once	100	52	52.
B. Pollinated after 4 hours	107	53	49.5
C. " " 24 "	100	32	32.
D. Depollinated as in III B, and pollinated at once	100	14	14.

Table continued;---

Experiment	No. of Flowers Used	No. of Seeds Obtained	Result Percent
Group VII. Cross pollination.			
A. Emasculated as in IV A. Pollinated at once	100	14	14.
B. Pollinated after 4 hours	100	26	26.
C. "" " 24 "	100	20	20.
D. "" " 48 "	100	13	13/
E. "" as"in IV.E. Pollinated at once	100	7	6.8
Group VIII. Cross pollination using old pollen. Depollinated as in II. A. Pollinated at once			
A. Pollen 5 days old	100	24	24.
B. "" 10 " "	111	13	11.8
C. "" 15 " "	100	6	6.

The results for group I show plainly that clover can be self-pollinated even when the flowers are simply covered to protect them from outside agents. The amount of self-pollination is increasingly greater, when pinched, when opened and closed, and when brushed. Therefore when working in clover pollination it is necessary to carefully remove all flowers which are not needed to operate on. When it is desirable to close fertilize clover, the brushing gives the greatest results, but the opening and closing is far more easily done.

The results of Groups II and IV show that depollination by means of water and emasculation have given no seeds. They are reliable methods and with a little practice can be easily accomplished, when carefully done. On the other hand, the results of Group III show that depollination by means of the brush is unreliable unless we also emasculate the flower (III.C.).

When we come to consider cross pollination, we must lay aside the results of Group VI, because as we have seen, depollination by means of the brush is unreliable or incomplete. The results of Groups V, VII, and VIII are reliable because based on depollination by water or emasculation. Among these we find that the best results have been obtained in Group V, under depollination by water when the pollination is at once or within four hours. About half as good results have been obtained in Group VII under emasculation, but not when pollinated at once. Here we find better results when pollinated between four and twenty-four hours later. The flowers used in Group V are at least twenty-four hours older than those used in Group VII. This shows that the stigma ripens a few hours ahead of the anthers, and is receptive for at least two days. V.D. and VII.E. show us that the removal of the floral envelope during depollination or emasculation reduces the yield of seed to about one-fifth what it would have been with the floral envelope. The stigma is probably protected by the floral envelope.

The results of Group VIII show that pollen can be kept at least two weeks and still obtain seeds. However, each five days reduces its value about one half. That is, about one-fourth remains at ten days and one-eighth at fifteen days.

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