LUCERNE POLLINATION.

Effect upon seed production and hybrid vigor.

Read by J.W. Hadfield, Agronomist, Plant Research Station, Palmerston North, before the New Zealand Grassland Association, October, 1934.

Mr. R.A. Calder and I are engaged in the breeding of lucerne, and because such a project necessitates an intimate knowledge of the pollination and fertilization of the crop under consideration, appropriate investigations have been undertaken.

The investigation may be divided into three divisions, viz.:-

(a) How pollination is effected.
(b) Pollination in relation to seed production.
(c) Pollination in relation to hybrid vigor.

The investigation is not complete, more particularly in that relating to hybrid vigor, and this paper must be regarded merely as a progress report.

(a) HOW POLLINATION IS EFFECTED.

The structure of the lucerne flower is such that the style and stamens are enclosed in a tense position within the keel and until freed are incapable of functioning normally although under such conditions a few seeds may develop. When this tension is released, the style and stamens spring forward, causing the anthers to burst and the stigma to strike with some force against the standard. This action allows the pollen to become available for, and the stigma subject to, cross- or self-pollination. The process is referred to as "tripping the flower," and on its occurrence, and method of occurrence, depends the ultimate production of seed.

During the flowering period the lucerne crop is visited by many forms of insects but unfortunately few of them seem to effect tripping. Members of many species have been listed as being of some value in this respect in other countries, but at Palmerston North only one type of bumble bee, probably Bombus hortorum, shows any inclination to gather nectar by first tripping the flower. Honey bees which are very prevalent, the large bumble bee which is rather more rare, and other insects, seem sufficiently content to collect their nectar by entering the tongue at the base of the coralla tube. On the rare occasions when the honey bee has been observed to effect tripping while taking nectar, the tongue has been trapped, much to the consternation of the bee, which finds difficulty in getting free. Continuous observation of one honey bee working on lucerne has revealed on an average one flower tripped every fifteen minutes. This is effected in various ways as the bee climbs around the racemes; but only on the rarest occasion's would the tripping be effected in such a manner as to bring about cross-pollination, unless foreign pollen were already on the standard of the tripped flower. An examination of honey bees has shown that they do carry pollen on various parts of the body, and the standards of untripped flowers have also revealed adhering pollen grains.

The small bumble bee on the other hand works very rapidly, pushes boldly into flower after flower, and is struck each time on the under part of the body by the stigma and anthers. This effects a double purpose+ Pollen is forced against the body and carried away by the bee, and the stigma comes into contact with pollen previously collected from other flowers on the same or different plant.

Tripping is also effected by changes in temperature, by agitation from wind, and some seed is also formed apparently in the absence of tripping.

Attempts to catch wind-borne pollen on greased slides exposed in the crop have given negative results, but they might conceivably bo very different in another locality.

Flowers can be artificially tripped quite readily by manipulating the racemes between the fingers. The observed increase in seed led to the inaugeration of a trial in Marlborough, the purpose of which was to devise some implement that would, when drawn over the field, trip a large number of flowers without causing undue injury. Results are not yet complete, but so far have proved negative. Such artificial tripping must, of necessity to a large extent effect self-pollination, and since self-fertilization as will be proved later, results in such a marked reduction in vigor, this means of increasing seed production is of doubtful ultimate value.

The first trials here were undertaken in 1931/32 and 1932/33, the object then being to evolve a satisfactory technique for crossing plants without resorting to hand pollination. The bumble bee was found very effective. Self-pollinated pods were small, cross-pollinated, pods large, so that it is possible to differentiate between self- and cross-pollinated pods with sufficient accuracy for ordinary breeding purposes.

(b) <u>POLLINATION IN RELATION TO SEED PRODUCTION</u>.

Seed production is extremely variable between unrelated plants. If in 8 series of treatments different plants were used the results might be a reflection of physiological differentiation not due to the treatment, and therefore be quite misleading. Therefore in any series of treatments one plant has been used, or what is virtually the same plant, since cuttings have been propagated and the clones used. In such a case, each treatment would contain one clone, all from the same mother plant, that is, the several treatments are brought to bear on several clones, all originating from the same parent.

On the other hand, clones from one parent vary in size to such an extent that total seed yields from clones would be nearly as misleading as from unrelated plants. Some other system must be employed.

The method adopted has been to take several healthy branches, examine the racemes, and count the number of florets. The number of pods formed is then counted so that a percentage is determined of the number of pods set to the total number of florets that could have set pods. This gives the figure "Per cent. of Florets setting pods." Finally the number of seeds per pod is determined. By multiplying the percentage of pods set by the seeds per pod, a figure is obtained indicating the number of seeds produced. for every 100 florets.

Trial 1: A preliminary trial designed to determine the effects of different m&hods of pollination upon the production of seed 1931/32.

Treatment.	% of Florets setting pods.	No. of seeds per pod.	Seeds per 100 Florets.
1. Open pollination,	30	2	60
2. Enclosed but not tripped,	8	2	16
3. Enclosed and tripped,	53	2	106
4. Hand crossed with flower from same plant,	44	1.8	79
5. Hand crossed with flower from another plant,	80	6.0	480

The various treatments were undertaken with different plants and not on clones of the one plant. In all later trials clones were used. Nevertheless crossing with pollen from another plant has resulted in a remarkable increase in seed yield..

Trial 11: Designed to estimate the increased yield from tripping. Clones were used and the trial replicated 5 times. Season 1932/33.

Treatment.	% of Florets setting pods	No. of seeds per pod.	Seeds per 100 Florets.
1. Open pollinated,..	26.5	4.2	111
2. Open pollinated and tripped,	50.6	3.3	16'7
3. Covered and tripped,	41.0	2.1	86
4. Covered and not tripped,	16.0	1.9	30

The results of this trial indicate that:-

1. Open pollination aided by artificial tripping has increased the yield of seed by 50.4% over and above that produced by open pollination alone. This has been effected by nearly doubling the percentage of. florets that have set seed. One may suppose however that artificial tripping will increase the proportion of self-fertilized flowers and this seems to be reflected in this case by a reduction in the number of seeds per pod.

2. Tripping has also increased seed yield in the covered plant (3 and 4) in this instance by 187%, with no appreciable difference in the number of seeds per pod since both were self-pollinated.

3. Either the exclusion of insects in the covered plants, or the shelter and shade afforded by the covers has reduced seed production.

Trial 111: To determine the possibility of using the bumblebee as a pollinating agent under cages.

	TREATMENT.	
	Open Pollinated.	Bee Pollinated
Per cent. of pod-bearing racemes, . .	36.6	73.2
Per cent. of florets setting pods, .	19.7	49.c
Number of seeds per pod, . ..*.......	3.3	3.6
Number of seeds per 100 florets, . . .	65.0	176.4

Two adjacent plants were arranged so that a few branches of each were enclosed within a cage while the rest of each plant was exposed to open pollination. One bumble bee was maintained in the cage 'to effect pollination.

Under bee pollination double the number of racemes bore pods, and nearly three times the number of seeds were produced for each 100 florets. The increase in seed-production is therefore very great. It seemed possible to separate the crossed pods from the selfed pods according to size and colour, end seeds from large and small pods from trials 11 and 111 were sown and form the subject of trial V11.

Trial lV: season 1933/1934.

In this trini the Director of the Horticulture Division permitted Mr. Robinson, Apiary Instructor, Palmerston North, to assist, and he established and cared for the necessary hives of honey bees. The trial was designed to determine the relative performance of Bumble bee, Black Honey bee, Italian honey bee, Hybrid honey bee, as against open-pollination and self-pollination, in their effect upon seed setting and the vigor of the crop raised from this seed. Only the results of tho seed production are available now.

The cages were 12x6x6 ft high and constructed with string netting overlaid with mosquito netting.

In each of tha honey bee cages small two-frame hives were installed, the number of bees in each being about 4,000. The bumble bee cage was only half the size of the honey bee cages, and an endeavour was made to maintain an average of 5 to 6 bumble bees in their cage throughout the flowering period .

The open-pollinated block was situated on the sunny side of the trial in a well sheltered position. It was observed that far more bees were working on this plot than on any other exposed lucerne on the area, and there is no doubt the results obtained from open-pollination are higher than should have been the case.

Eighteen parent plants were used, and each cage contained one clone from each of the parents, making 18 clones per cage. Owing to the lay-out of the experiment the results have to be presented in two sections, and the figures in one cannot be compared directly with those of the other. A summary of the results is presented.

Trial lVa:

Pollinating Agent.	% of florets to set pods.	No. of seeds per pod	Seeds per 100 florets.
Insects excluded (selfed)	1.27	2.2	2.8
Open pollinated, ...	16.8	3.8	63.8
Black honey bee, ...	25.7	4.4	113.1
Italian honey bee, ...	23.6	4.8	113.3
Hybrid honey bee, ...	23.9	4.2	100.4

There is no significant difference between the three honey bees. The fact is proved that honey bees will trip flowers and induce saed setting, but in this instance the concentration of some 4,000 honey bees within such a small space no doubt accounts for the large setting of seed. Despite this ooncontration of honey bees, they tripped and caused seed to set on not more than 25 per cent. of the florets available.

The bumble bee on the other hand set over 40 per cent.

Trial lVb:

Pollinating Agent.	%age of florets to set pods.	No. of seeds per pod	Seeds per 100 florets.
Bumble bee, ...	40.8	3.7	151.0
Italian bee, ...	36.7	3.5	128.4
Open pollinated, ...	18.2	3.3	60.1

Reasons have already been given why the results of the open-pollinated plots are deemed to be higher than normal. Counts were therefore made in a plot of lucerne about 150 yards away and this revealed the following: 130 racemes produced 2743 florets. Of these florets 377 set pods, so that the percentage to set seed amounted to 13.7. The pods contained an average of 2.7 seeds so that ths final result is 37 seeds per 100 florets.

It may be concluded that five or six bumble bees have caused far more seed to set than 2,000 honey bees. As a pollinating agent the bumble bee is entirely efficient, the honey bee relatively inefficient.

Just how much of this seed is cross-pollinated and how much self-pollinated will be revealed in the relative vigor exhibited when the seed from the different treatments is grown.

Owing to the somewhat variable size of the clones, the yield of seed from this trial is not reliable.

(c). POLLINATION IN RELATION TO HYBRID VIGOR.

These trials are designed to determine the relative productivity of seed that has been produced under different methods of pollination. The main trial in this connection will be undertaken next season, with seed derived from trials IV.a and IV.B. In the meantime the following results are very significant.

While many workers have drawn attention to the importance of cross-pollination in the setting of seed, they have not stressed, so far as the speaker is aware, the productivity of such seed as revealed by weight of the resultant progeny. That it is of significant economic importance seems amply proved, and any insect that induces cross-pollination serves a double purpose in that it increases the yield of seed and such seed is of greater potential value than that produced when partially self-fertilized, as appears to occur under present field conditions.

Trial V: Season 1933/34: Designed to determine the green weight yields obtained from seed that has been self-pollinated as against thnt produced from seed on the same plants which has resulted from open pollination (this in effect is partly self- and partly cross pollination.)

Green weight yield of progeny in pounds per plant.

Parent Plant.	1st Cut. Selfed	1st Cut. Open	2nd cut, Selfed	2nd cut, Open	3rd Cut. Selfed	3rd Cut. Open.
45/24	.59	1.13	.32	1.3	0.0	.16
47/23	1.30	1.62	1.31	1.34	.14	.18
28/17	.67	1.17	1.07	1.36	.47	.65
54/17	.73	1.39	.83	1.35	.28	.47
56/18	.96	1.32	1.29	1.69	.35	.41
60/23	.77	1.44	1.01	1.87	.32	.67
70/13	1.77	1.95	1.88	1.96	.47	.67
72/23	1.15	1.60	1.38	1.65	.47	.62
84/5	.50	.97	.96	1.27	.43	.53
88/20	.87	1.18	1.82	1.63	.75	.85
120/14	.77	1.43	1.36	1.38	.48	.83
Totals,	10.08	15.20	13.23	17.40	4.16	6.04

Significance is established. The increase of open-pollinated over self-pollinated amounts to 40.66%. Every result is consistent except the second cut in 88/20.

Trial VI: 1933/34.

In this trial a stage further is reached but unfortunately insufficient material was available to prove the point in this past season.

Open pollination may be regarded as resulting partially in self- and partly in cross-fertilization.

When bumble bees are introduced the bulk of the pollination may be regarded as effecting cross-fertilization, This trial was designed then to compare normal open pollination with what may bo regarded as dominantly cross-pollination. Only two plant s were available affording one cross.

Green weight yield of progeny in pounds per plant.

	Open pollin. parent 52/24.	Dominantly cross pollin. 52/24x53/24	Open Pollin Parent 53/24	Dominantly cross-poll. 53/24x52/24
1st out 30.1.34	.88	1.08	1.17	1.30
2nd out 26.3.34	1.34	1.53	1.40	1.5'7
3rd cut 30.5.34	.81	.73	.74	.74
	3.03	3.34	3.31	3.61

Significance cannot be established with so few replications, but the increased yield of dominantly cross-pollinated over open-pollinated amounts to 9.6%.

Trial Vll: It has already been mentioned that moss-fertilization appears to be correlated with the production of large pods. Therefore large pods and small pods from the same plant s were threshed and sown separately. Thi result has been a statistically significant increase yield from large pods over that *from* small pods as follows:

Green weight yield of progeny in pounds per plant.

Parent	1st Cut Large Pods	1st Cut Small Pods	2nd Cut Large Pods	2nd Cut Small Pods	3rd Cut Large Pods	3rd Cut Small Pods
54/17	1.65	1.06	1.29	.96	.5	.32
56/18	1.38	1.25	1.60	1.48	.41	.39
88/20	1.06	.97	1.84	1.78	.83	.82
120/14	1.33	1.28	1.95	1.78'	.91	.68
47/16(a)	1.42	1.23	1.50	1.56//	.50	[illegible]
47/16(b)	1.32	1.38//	1.56	1.63//	.53	.50
48/7 (a)	1.55	1.18	1.82	1.42	.67	.44
48/7 (b)	1.50	1.03	1.78	1.17	.68	.40
50/3 (a)	1.30	.85	1.92	1.39	.88	.73
50/3 (b)	1.13	1.00	1.47	1.37	.64	.73//
50/7 (a)	1.43	1.16	1.92	1.78	.93	.83
50/7 (b)	1.53	1.37	2.12	1.73	.88	.78
56/13(a)	1.43	.88	1.69	1.34	.65	.56
56/13(b)	1.51	.95	1.58	1.06	.63	.41
'Totals:	19.54	15.59	24.04	20.45	9.64	8.14

All results are consistant except five marked //. The increase in yield from the use of seed from large pods over the use of that from small pods amounts to 20.46% and is statistically significant.

Trial Vlll: Under normal field conditions open pollination may result in both self- and cross-fertilization. If artificial tripping is used as an aid to open pollination the result is an increase in the yield of seed, (see trial ll) but the seed so formed is likely to be largely self-fertilized and therefore not so productive as when artifioial tripping is excluded.

Trials have been made both here and in other parts of the world to increase seed production by artificial tripping, but the wisdom of such a method is questioned on account of the amount of self-fertilization thus induced.

It was hoped to demonstrate this supposition but the trial was badly designed, and although there is a slight decrease in yield owing to tripping, this decrease is not statistically significant. As this information would be of considerable practical value, anothar attempt to obtain it will be made.

SUMMARY.

1. How pollination is effected. The observation at Palmerston North indicates that the honey bee is relatively inefficient, but that Bombus hortorum is extremely efficisnt 8s a pollinating agent. Observations in other parts of New Zealand are highly desirable. The observations so far made do not, however, explain the proportion of cross-pollination that apparently takes place under natural conditions.

2. Pollination in relation to seed production.

Cross-pollination results in haavy yields of seed, self-pollination in relatively low yields. Artificial tripping will augment automatic tripping and increase the setting of seed.

3. Pollination in relation to hybrid vigor.

Seed resulting from self-pollination produces a markedly less vigorous crap than seed resulting from cross-pollination. Cross-pollination is therefore desirable in respect of both seed production and subsequent vigor.

Steps are being taken by the Entomologist to investigate the possibility of increasing the incidence of those insects capable of effecting cross-pollination in Lucerne.

In conclusion I would like to acknowledge the careful and patient work of my associate, Mr. Calder, who is responsible in a large measure for such success as has attended these investigations.