# AN INVESTIGATION INTO SOME PROBLEMS CONNECTED WITH INBREEDING IN LUCERNE

(MEDICAGO SATIVA)

BEING THE RESULTS FROM WORK FOR A THESIS FOR HONOURS IN FIELD HUSBANDRY (M. AGR. SC)

BY

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DURING THE PERIOD - AUGUST 1934 TO SEPTEMBER 1936.

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## SECTION 1.

### i. INTRODUCTION:

Lucerne as a crop has been known for thousands of years. As far back as the history of man goes it was used in Central Asia, being the oldest plant cultivated for forage alone. It was prized by the Medians, Grecians and Romans, the Romans carrying seed with them to establish at their military bases. Where known in England in the 15th century, it was highly prized but was not widely known till the 17th century. It was introduced to Germany in the 16th century and America in the 19th century.

Much improvement has taken place through the centuries with the result that a good variety, suited to the locality and well managed, will give a heavy yield of green herbage, when compared with the yield from pasture.
This herbage is produced in the main during summer periods when it is used for the supplementary feeding of cattle and sheep, and for the production of hay and silage.

Although lucerne has many advantages, it has not as yet been grown to the extent that it deserves in New Zealand. For instance in the 1935 season, just under 40,000 acres of lucerne were grown out of a total acreage of fodder crops of the 1½ million acres. Several reasons may be given for this low acreage, such as:-

(1). The low yield of many stands due to
   (a) wrong type of seed sown
   (b) lack of innoculation
   (c) bad management.

(2). The necessity for proper management of the area. On the average farm this often means increased labour, when such is not available.
Weed invasion - chiefly grass - is especially important in regions of high rainfall in the North Island, such as in Taranaki and North Auckland. Here special preparation of the seed bed is necessary, often meaning cropping for one or two years previously. Many farmers too, are not capable

of efficiently utilising the crop when grown.

(3). The disinclination of the grassland farmer to breaking up good permanent pasture, on which he can manage in most seasons.

(4). The expense for cultivation machinery on the grassland farm, which is not usually available.

(5). Lucerne will grow on many sandy areas, and stoney river banks, which will not support a permanent sward of grass. Here the yield of green feed or hay is not large, and farmers have come to regard this poor land as the natural habitat of lucerne, with the low yield as characteristic of it. Thus, although a good lucerne stand will produce as much as 6 - 8 tons per acre of hay per season, yet the Dominion average yield is under 3 tons per acre of hay and silage.

The type of seed sown is the main concern of this paper. It has been shown at Palmerston North, that Marlborough lucerne is the best generally for New Zealand conditions. Yet an analysis of imports shows that a comparatively large quantity of seed is imported every year, mainly from South Africa.

TABLE 1. showing imports of lucerne seed:-

| | Total | South Africa | |
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| | Cwt. | Cwt. | Value £. |
| 1933 | 224 | 200 | 667 |
| 1934 | 634 | 621 | 2,208 |
| 1935 | 343 | 300 | 1,318 |

The climate of New Zealand is good for seed production, and it would be reasonable to expect that an export trade should be possible for lucerne seed. But this has not been done, New Zealand being unable to supply her own seed requirements. The reason for this can be found in the uncertainty of the seed crop, farmers being loth to risk allowing the crop to seed.

This uncertainty in seed production, and the necessity for improving the general standard of lucerne plants in New Zealand caused the Plant Research Station at Palmerston North to commence breeding and selection work with lucerne. The problems considered here are ones that have arisen during this work, being problems of pollination and heterosis.

Lucerne is usually considered to be cross pollinated, although a considerable amount of self pollination can occur. (Piper 1914, Hayes & Garber 1921, Yarashevsky 1931, Jenkins 1931, Torssel 1931 etc.,). It had been observed on local material, and overseas experience supported the fact, that crossed plants generally were more vigorous than selfed plants, and had a greater chance of success in competing in the field, not only with other plants of the species, but also with weed growth. Such increased vigour is no doubt due to heterosis or hybrid vigour. Because of this manifestation of hybrid vigour, and the very mixed nature of the average lucerne stand, it is usual to consider lucerne as being very heterozygous, although Stewart (1934), suggests that lucerne is not as heterozygous as has been supposed. New Zealand experience shows Marlborough lucerne to be a mixture of types, some of which are very heterozygous and others less so. The usual test of heterozygosity is the progeny test, - being a measure of uniformity attained in one or two generations of inbreeding. Consequently, inbreeding is used extensively in lucerne breeding work for this purpose alone. It is also used to produce uniform lines, which of themselves are good and can be used in production of improved strains.

Problems have arisen in the practical application of inbreeding to this normally cross bred plant. Thus, although continued inbreeding tends to bring about uniformity, it is usually accompanied by a reduction in vigour, in yield, and in seed production after at the most two selfings. Many plants of the second inbred generation were low in yield, producing very few seed and presented problems in their utilisation. Obviously compared with the parents they were commercially valueless, and no information was available as to whether or

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It was on the suggestion of the Officer in charge of lucerne breeding that the work undertaken in this thesis was first commenced. The range of problems set was the determination of -

(1). How great was this loss of vigour (measured by yield of green material) on selfing lucerne plant families for one and two generations, i.e., how important was it in the field in keeping down yield from stands.

(2). Whether or not this loss in vigour could be recovered on subsequent crossing of selfed plants. Later on the scope of the thesis was widened to include the following related problems.

(1). A comparison of the relative efficiency of the several methods of crossing lucerne plants.

(2). A comparison of the seed setting capabilities of parent plants and their progeny from one and two years inbreeding.

(3). An attempt to find a reason for the apparent self-sterility of inbred lucerne plant families.

Since this work was commenced, plants have been selected by the Plant Research Bureau, which show little reduction in vigour when selfed, but as yet little is known as to the reason for this or whether such plants under inbreeding for longer periods will continue to exhibit such small loss in vigour, and so be valuable for breeding purposes.

ii. A GENERAL OUTLINE OF THE MAIN EXPERIMENTS.

(1). A measure of loss in vigour on selfing (See Section 111).

In this experiment an attempt was made to compare the yields from clones of parent plants with clones of plants

(a) representing the first selfed generation and

(b) representing the second selfed generation.

Two distinct families were used known as 91/10 and 99/3, by the Plant Research Station and representing two types selected by this station. Rows of parent plants, being clones from the original parent of each type were grown as controls. Four plants from the first selfed generation of each type were selected and propagated as clones and grown in between parent rows. Similarly 4 plants from the second selfed generation of each of the selected first selfed generation plants were propagated as clones and grown between the parent and L.1. plant rows. Yield weights of rows over the season were compared to respective parents, as, say 100 to give some idea as to the reduction in vigour on self-pollinating these plants. While any figures obtained could not apply directly to all the types of lucerne available, it was hoped to determine the relative importance of any reduction in yield.

(2). A measure of the Recovery in Vigour on crossing selfed plants. (See Section 1V).

Crosses were made between representative plants of the first and second selfed generations of the two selections 91/10 and 99/3. (i.e., first selfed generation 91/10 x first selfed generation 99/3, second by second selfed generation). It was decided that the best control would be the F1 generation from a cross between the two parent plants, as this would possess hybrid vigour, containing probably the maximum vigour factors of the two parents. Thus, if the crosses of selfings were as vigorous as the parent cross then we could say that the regain in vigour was complete. Care was taken to select only representative plants from each generation with no attempt at selection of the best progeny.

This experiment included plants of the first and second inbred generation as a basis of comparison, and also as a deliberate check upon the previous experiment, about which there was some doubt that the results would be satisfactory.

(3). A General Comparison of the yields from parent plants selfed and parent plants crossed with other parent plants (Section IV.a.)

Material from 8 families was available, consisting of seed from these plants when self-pollinated, and also when they were crossed with from one to five other parent plants. A comparison of the yields from such seed demonstrated the effect of self pollination in reducing the yield of the lucerne plant, when compared with the desirable more vigorous plant resulting from cross pollination.

iii. <u>SOURCE OF MATERIAL</u>:

The original material used in crossings, and also later to provide cuttings, came from the Plant Research Station, Palmerston North, to the Officers of which the writer's thanks are due. Though it was not possible to transplant to my own area, all the material necessary for the first year's work on the experiment, permission was readily granted to make use of the plants on the Station area for crossing, and also for a supply of cuttings. As a result plant families were available containing the original parent selection, and progeny of the first and second inbred generations. Because of this assistance, it was possible to complete the experiment in two seasons instead of the four or five seasons that would otherwise have been necessary, had the work commenced with parent material alone. It was also possible to select plants of different types, with which to work, which would show up any results in the experiment better than where two similar types were used.

Two distinct lines were chosen, one a fairly erect type, termed 91/10, and the other a flat prostrate type termed 99/3. Both were promising selections made by the Station, being good parents and producing good progeny, yet showing a reduction in vigour on selfing, and some selfed plants showing a very marked self sterility.

## SECTION II.

### EXPERIMENTS AND OBSERVATIONS ON THE TECHNIQUE OF LUCERNE CROSSING.

i. "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 seed 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". (J. W. Hadfield; Proc. N.Z. Grassland Conference 1934).

In the field the honey bee does very little tripping (Englebert 1932, Southworth 1928, McClymonds 1926, Gray 1925, Hill 1924, Lovell 1924). In New Zealand the species doing most tripping is the bumble bee - Bombus terrestris, which unfortunately is not very common on lucerne plants, exhibiting host preference. Tripping is also affected by changes in temperature, by agitation from wind, and some seed may be formed in the absence of tripping. (Piper 1914, Carlson 1930, Clarke & Fryer 1930, Dwyer 1931, Dwyer & Allman 1932, Roberts & Freeman 1908).

The pollen sacs burst the flower is in the bed stage, and while the reproductive organs are still within the keel. No pollination occurs (as a rule), however, as the stigma is not receptive until its surface is ruptured. This occurs when it strikes the standard (Armstrong & White 1935). Emasculation aims at preventing rupture of the stigmatic surface and getting rid of adhering pollen, whereas pollination aims at the rupturing of the stigma surface, and deposition thereon of pollen from a desired parent.

ii. <u>REASON FOR THE EXPERIMENT</u>.

In any programme of lucerne improvement crossing of selected plants is necessarily important. Consequently the method of crossing used is also important. Lucerne crossing is very slow and tedious work. Any method shortening the time required in making a cross without sacrificing efficiency is indeed valuable, as the number of crosses made is usually limited by the time and labour available. When crossing inbred plants, e.g., Second inbred generation, a high degree of self sterility is present, and hence the method of emasculation is not so important as when crossing parent plants, where up to 40 - 50% or more of self pollination may occur when the flowers are artificially tripped. But, as much of the crossing work will be with parent plants the necessity for an efficient method of emasculation is apparent.

There are four methods of emasculation that could be used. designated as follows;

1. The Pin and Brush Method.
2. Blowing pollen off after using pin.
3. Blowing pollen off after cutting standard.
4. Washing pollen off after cutting standard.

Methods 1, 2, and 4, (without standard cutting in 4), have been used overseas, and method 1, was used extensively during the 1934-5 season at Palmerston North. Method 3, was developed by Mr. R. A. Calder of the Plant Research Bureau. It was easily the quickest method, but it was not known how it compared in efficiency with other methods. To obtain this knowledge the following experiment was designed -

A. To test the effeciency of each method of emasculation as measured by the percentage of emasculated florets setting pods, and the number of seeds per pod.

B. To measure the effect of the method of emasculation upon the subsequent pollination, measured as in 'A'.

iii. DESCRIPTION OF METHODS OF EMASCULATION:

A short summary of each method of emasculation follows:

1. The Pin and Brush Method.

In this method the flower is tripped by forcing a pin horizontally between the standard and the keel. The pressure on the keel edge causes it to open, releasing the stamens and style from the keel, the staminal column resting against the pin, preventing the stigma and stamens from striking the standard. The pollen sacs are gently brushed away with a fine camel hair brush, leaving the style by itself. Some pollen gets on the stigma of the flower, some when the flower trips (or even when untripped) and some is brushed over it.

"No Matter how gently the stigma is released from the keel, it is well covered with pollen". (Waldron 1919).

2. Blowing pollen off after using pin.

The flower is tripped as above, but the pollen is blown away, usually by means of a glass tube tapered to a nozzle, and held in the mouth. Care must be taken that saliva is not blown over the stigma. Some pollen rests on the sticky stigma surface, and is not dislodged by blowing, although most of the pollen is removed.

3. Blowing pollen off after cutting standard.

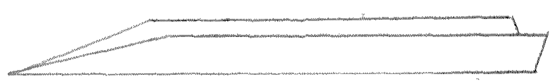
The standard is carefully cut off at such a level that when the style is released from the keel, the stamens and the stigma surface are above the remaining part of the standard, and consequently the stigma is not ruptured, but can be emasculated and pollinated. The pollen is then blown off as in (2). In this method all the florets on a raceme can be emasculated at once before pollination, e.g., the standards are all removed, all the flowers are tripped (by squeezing the keel between forceps), the pollen blown off, then all pollinated. Where the pin is used as in (1) and (2), each floret must be pollinated before the pin is withdrawn from the flower, otherwise the stigma becomes appressed against the standard and is exceedingly difficult to pollinate.

It is because of this advantage in method (3) that it takes less time.

4. Washing pollen off after cutting standard.

The standard is cut away as in (3), but a fine jet of water is used to wash away the pollen after the flowers are tripped. Washing the pollen away without cutting away the standard has been used overseas, but owing to the extreme difficulty in subsequent pollination, Mr. Calder's method of cutting away the standard was incorporated here. The surplus water may be removed with blotting paper, or the flower head shaken, and left to dry for a few hours before pollination. The latter course was taken in this experiment.

The method of pollination was the same in each case. A strip of thin cardboard about 3/8" wide was folded along its axis. Portion was cut away as in diagram to make a point on the line of fold

(a) 

The cardboard was then roughened along the inner side at 'a'. Pollen was collected on this point by so tripping the flowers that the staminal tube was ejected against it. This pollen was transferred to the stigma and rubbed in with sufficient force to rupture the surface of the stigma and cause it to be receptive.

Instruments are dipped into alcohol to kill adhering pollen before beginning a different cross, and alcohol is rubbed over the hands to kill pollen there.

iv. SOURCE OF MATERIAL.

The whole of this work was carried out in spare time on plants of the Plant Research Station, Palmerston North during 1936. The two plant families 91/10 and 99/3 were chosen for the experiment, being good seed producers, and bearing flowers which were easy to manipulate. Selfings and crosses were made on parent material, but the results should be applicable (allowing for self-sterility) to inbred plants.

v. A COMPARISON OF THE EFFICIENCY OF SEVERAL METHODS OF EMASCULATION OF LUCERNE FLOWERS.

A. The Method of the Experiment.

Parent plant 91/10 was used here. Racemes were selected in 4 groups, so as to give at least 100 florets in a group. On the 6th and 7th of February 1936, the florets were emasculated, each group being emasculated by one of the 4 methods described. The flower heads were covered with parchment paper packets, and the flowers left until such time as it was possible to count the seeds formed. The paper bags were removed as soon as all the florets had set or dropped off.

B. The Results of the Experiment.

TABLE II A comparison of the Methods of emasculation showing the percentage of florets setting pods, average seeds per pod and seeds per 100 florets.

| Method of Emasc: | Flts. Emasc. | Pods Set | Pods 100 flts emasc. | No. seeds. | Seeds per pod. | Seeds per 100 flts. emasc. |
|---|---|---|---|---|---|---|
| 1.Pin & Brush | 111 | 41 | 37.0 | 89 | 2.17 | 80.2 |
| 2.Blow after pin | 111 | 50 | 45.05 | 95 | 1.9 | 85.6 |
| 3.Blow & cut | 112 | 16 | 14.3 | 22 | 1.4 | 19.6 |
| 4.Cut & Wash. | 131 | 11 | 8.4 | 21 | 1.9 | 16.0 |

(C). DISCUSSION ON RESULTS.

(a) A Comparison of Methods (1) and (2).

Here we have pods set per 100 florets emasculated - 37.0 in method (1), and 45.0 in methods (2), a result generally speaking favouring method (1), but not sufficient to be significant. In seeds per pod formed, 2.17 in (1) and 1.9 in (2), the results again are not significantly different.

Comparing seeds per 100 flts. emasculated, we find that there is no significant difference between the two methods. As the only difference in treatment is in the manner of depollination, brushing the pollen away as compared to blowing it away, it is evident that neither of these two methods has any advantage over the other. Actually both methods effect very poor removal of pollen, as the results in Table II, show seed setting very nearly that obtained by artificially tripping the flowers to obtain self-pollination (cf. Hadfield & Calder 1936).

(b). A comparison of methods (2) and (3).

Method (3) gives a significantly lower number of seeds per 100 florets emasculated - 14.3 to 45.05, seeds per pod - 1.4 to 1.9, and seeds per 100 florets - 19.6 to 85.6 - than method (2). These two methods differ only in the removal of the standard in (3). Consequently, it is reasonable to suppose that this increase in efficiency of method (3) over method (2) is due to the removal of the standard. The reason for this is apparent from a study of the work of Armstrong and White in Canada (1935). According to them, the pollen grains cannot germinate, and penetrate the stigma, unless the stigma cells have been ruptured. This rupture breaks the tough surface, which pollen tubes cannot penetrate, and also the ruptured cells provide a suitable medium for pollen tube growth. It might be suggested that this is an adaption to secure cross pollination, self pollen being unable to germinate before the flower is tripped, although present on the stigma. If outside pollen is available on the stigma when tripping occurs, previous work would indicate that the outside pollen would compete with home pollen and effect the majority of fertilisations.

Removing the standard in method (3), prevents the natural rupturing of the stigma cells, which usually occurs when the stigma strikes the firm surface of the standard. Consequently, many stigmas fail to be ruptured at all and allow no fertilisation, while others are only slightly ruptured allowing a small number of pollen grains to germinate with poor fertilisation reflected in the reduced number of seeds per pod when compared with method (2).

It might be suggested that brushing the pollen off after removing the standard would be less efficient than blowing it off, because the mechanical friction of the brush on the stigma surface would cause a considerable rupturing of the surface cells, assisting fertilisation. Thus if the brush is used no advantage is gained by removing the standard except the shortening of the time required to cross a raceme of flowers.

(c) Method (4) was actually no better than method (3) although lower in the number of pods setting, it gave a higher value for the number of seeds per pod, with no significant difference in the number of seeds per 100 florets emasculated. Generally speaking the method is slow and tedious and makes subsequent pollination difficult and uncertain. A jet of water will no doubt remove most of the pollen grains on the stigma and the remainder may be drowned, or burst by the intake of an excess of water. It cannot be advocated as a useful method for field work.

D. CONCLUSIONS FROM THE EXPERIMENT.

Method (3) is more efficient than methods (1) and (2) as a method of emasculation. It is as efficient as method (4) and possesses many advantages over (4) in ease of working, time taken, and as will be seen later, does not effect pollination so much as method (4). Consequently method (3) - blowing the pollen off after cutting away the standard - can be recommended in field work. As far as the writer knows, this method has not been used overseas, but originated at the Plant Research Station at Palmerston North.

## vi. THE EFFECT OF THE METHOD OF EMASCULATION UPON SUBSEQUENT POLLINATION.

A. The Purpose of the Experiment.

Observations and theoretical considerations tended to suggest that the method used to emasculate lucerne florets might affect the pollination and fertilisation of such florets. Consequently, it was no use conducting an experiment to decide which method of emasculation was the most efficient, unless a corresponding experiment was conducted comparing the effect of the various methods of emasculation used upon the subsequent pollination and fertilisation of the flower, as the aim in emasculating flowers is to so treat the flower that one has control over the pollination occurring.

B. The Method of the Experiment.

Another plant from the clone 91/10 was used as the female parent in this experiment, and a plant of 99/3/P, was used as the male parent. On 12. 2.36, florets were selected in 4 groups, as in the previous experiment to give at least 100 florets per group, and emasculated by the four methods used. Pollination was carried out according to the requirements of each method of emasculation, for instance, with methods (1) and (2) each floret was pollinated as emasculated, and then pollinated, while in method (4), the florets were emasculated and then left for 1½ - 2 hrs. before pollination.

C. The Results of the Experiment.

These can be summarised in the following tables.

TABLE III: A Comparison of the effects of the methods of emasculation upon subsequent pollination, as measured by percentage of pods set, seeds per pod and seeds per 100 florets.

| Method of Emasc: | Florets Pollinated. | Pods. Set. | Pods per 100 flts. pol: | No.Seeds. | Seeds per pod. | Seeds per 100flts Polltd: |
|---|---|---|---|---|---|---|
| 1.Pin & Brush | 109 | 96 | 88.1 | 634 | 6.6 | 581.6 |
| 2.Pin & Blowg: | 114 | 103 | 90.35 | 728 | 7.07 | 638.6 |
| 3.Cuttg & Blowg | 120 | 81 | 67.5 | 410 | 5.06 | 341.6 |
| 4.Cuttg & Washg | 108 | 42 | 38.9 | 157 | 3.74 | 145.4 |

TABLE IV. The theoretical nett setting due to cross pollination with each method.

| Method of Emasculation. | Per.Cent pods settg. When Emasc. only (a) | Emasc. & pollinated. (b). | % due to crossing $\left(\frac{b-a}{a} \times \frac{100}{1}\right)$ | Seeds per 100 flts. Emasc. only | Emasc.& pollinated | % due to cros |
|---|---|---|---|---|---|---|
| 1.Pin & Brush | 37 | 88.1 | 58.0 | 80.2 | 581.6 | 86.2 |
| 2.Pin & Blowg: | 45 | 90.35 | 50.2 | 85.6 | 638.6 | 86.6 |
| 3.Cuttg:& Blowg: | 14.3 | 67.5 | 78.8 | 19.6 | 341.6 | 94.2 |
| 4.Cuttg:& Washg: | 8.4 | 38.9 | 78.4 | 16.0 | 145.4 | 89.0 |

While the above table cannot be considered accurate because actually when "outside" pollen has to compete with "home" pollen the latter is less successful than when alone, and under conditions of competition would hence effect a less number of fertilisations. Yet it is useful in giving an indication of the general effect that we can expect.

D. Discussion on Results.

(a). A Comparison of Methods (1) and (2).

An analysis of the two preceeding tables shows that both these methods allow round 90% of total pollinations, about 7 seeds per pod, and round about 600 seeds per 100 florets emasculated, 86% of which could be ascribed as due to cross pollination. Neither method has any advantage over the other. Armstrong & White (1935) suggest "that the natural means of scarification of the stigma is more effective than the artificial methods, probably because the contact of the standard and the stigma maintains a moisture condition which provides a better medium for pollen germination". It is indeed likely, that the high number of pollinations, seeds per pod and seed per 100 florets with these methods is in great measure due to these facts.

(b). Method (3) does not give such a high percentage of pods setting seed as methods (1) and (2) nor so high seeds per pod, or seeds per 100 florets emasculated and pollinated. The percentages however, are still reasonably high and the percentage that can be ascribed to cross-

(94%) is higher than in any other method. The lower number of seeds obtained by this method may be due to either a poor rupture of the stigma surface than where the natural means are employed, or to the lack of proper moisture conditions for germination when the standard is removed, and the stigma surface left exposed to the atmosphere. Further experimentation will prove which factor is primarily responsible, and whether or not greater care in pollination to ensure rupture of the stigma surface, will result in as good fertilisation as in methods (1) and (2).

(c). Method (4) gives low results in percentage of florets setting pods (39%) seeds per pod (3.74) and seeds per 100 florets emasculated and pollinated (145.4). The percentage of seed set due to crossing is about 89%, which is slightly better than methods (1) and (2), but actually below that obtained when method (3) is used. The poor seed setting by the use of this method may have been due to a combination of three factors -

(1). The stigma may have been still damp when pollinated, resulting in the bursting of many pollen grains due to excessive intake of water.

(2). The water may have damaged the stigma surface making it unreceptive.

(3). The stigma cells may not have been sufficiently ruptured during pollination.

Comparing this method with method (3) it seems likely that it is the excess water and probable damage to stigma that are the main reasons for low fertility. Armstrong & White say that lucerne pollen will not germinate where 100% saturation is reached in the atmosphere, but germinate freely where saturation is 90%. Method (4) besides taking longer time, has a rather adverse effect upon pollination and fertilisation, and consequently cannot be recommended for general field work. It is indeed doubtful if under New Zealand conditions it has any place at all in lucerne crossing work.

E. CONCLUSIONS FROM RESULTS:

If some method of depollination of lucerne flowers is necessary before cross-pollination, and Messrs. Hadfield and Calder (1936) have demonstrated this necessity, then it is indeed likely that method (3) will prove most successful. In the first place this method is easily the quickest, gives a greater degree of emasculation, and the highest percentage of seeds due to crossing. It also gives quite a fair return of seed per 100 florets crossed. Where the maximum number of seeds is an aim in crossing, methods (1) and (2) have an advantage over method (3), if no allowance is made for the extra time taken in these two methods. Also we know that 86% of the seed is due to crossing. Taking the relative times to cross by method (3) compared to methods (1) and (2), as 2 : 3, which is about the ratio in practice and allowing for the difference in efficiency, all the methods take as long to produce 500 seeds. As we know there is less admixture of seed from self-pollination where method (3) is used, this method can be strongly advocated under New Zealand conditions. It has been tried out at Palmerston North during 1935-6, but owing to the very poor seeding season, and the fact that the crossing this year was between plants of the second inbred generation, no comparisons could be drawn with method (1), which was used during 1934-5.

## SECTION III

### A MEASURE OF LOSS OF VIGOUR ON SELFING LUCERNE PLANTS FOR TWO GENERATIONS.

i. The Purpose of the Experiment.

Self fertilisation of lucerne plants results in progeny which exhibit reduced vegetative activity, when compared with the parent plant. (Hadfield & Calder 1936 and others). The extent of this reduction in vigour had been investigated overseas, but information suggested reductions from very little to 40 - 50% in one generation of selfing. The following experiment was designed to gain information as to the relative importance of this loss in vigour when inbreeding. Due to the variation that exists in lucerne types and their offspring, any figures produced could not be statistically applicable to all inbreeding with lucerne, but would have a general application of the extent of the loss in vigour one might expect. As some doubt was felt as to the chance of getting good results from this experiment, the experiment on "Regain in Vigour on Crossing", was designed to include material which of itself would answer the questions asked in this experiment.

ii. The Material of the Experiment.

The work was carried out on material which came originally from the Plant Research Station, Palmerston North. The following is a list of plants taken over from this Station during August 1934. These comprise Parents and 1st. inbred generation plants of the following 5 families - 39/9, 53/18, 91/10, 99/3, 111/21.

FIG. I SHOWING DIAGRAM OF PLANTS RECEIVED IN ORDER OF PLANTING

| | | | | | |
|---|---|---|---|---|---|
| 39/9/22 | 39/9/23 | 53/18/2 | 53/18/3 | 111/21/20 | 111/21/21 |
| 39/9/21 | 91/10/P | 53/18/1 | 53/18/4 | 111/21/18 | 111/21/22 |
| 39/9/19 | 91/10/P | 99/3/17 | 53/18/5 | 111/21/17 | |
| 39/9/18 | 91/10/P | 99/3/16 | 53/18/6 | 111/21/16 | |
| 39/9/17 | 91/10/P | 99/3/15 | 53/18/7 | 111/21/15 | |
| 39/9/16 | 91/10/P | 99/3/14 | 53/18/8 | 111/21/13 | |
| 39/9/15 | 91/10/1 | 99/3/13 | 53/18/9 | 111/21/12 | |

| | | | | |
|---|---|---|---|---|
| 39/9/13 | 91/10/2 | 99/3/11 | 53/18/10 | 111/21/11 |
| 39/9/12 | 91/10/3 | 99/3/9 | 53/18/11 | 111/21/10 |
| 39/9/10 | 91/10/4 | 99/3/8 | 53/18/12 | 111/21/9 |
| 39/9/9 | 91/10/5 | 99/3/7 | 53/18/13 | 111/21/8 |
| 39/9/8 | 91/10/8 | 99/3/6 | 53/18/14 | 111/21/7 |
| 39/9/7 | 91/10/9 | 99/3/4 | 53/18/15 | 111/21/6 |
| 39/9/6 | 91/10/11 | 99/3/3 | 53/18/16 | 111/21/5 |
| 39/9/5 | 91/10/12 | 99/3/2 | 53/18/17 | 111/21/3 |
| 39/9/4 | 91/10/13 | 99/3/1 | 53/18/18 | 111/21/2 |
| 39/9/3 | 91/10/16 | 99/3/12 | 53/18/19 | 111/21/1 |
| † 39/9/1 | 91/10/17 | 99/3/P | 53/18/20 | 111/21/P |
| * 39/9/P | 91/10/18 | 91/10/22 | 111/21/P | 111/21/P |
| 39/9/P | 91/10/19 | 91/10/21 | 111/21/P | 111/21/P |

* P means parent plant, e.g., 39/9/P a parent plant in family 39/9.

† similarly 1, 2, 3 etc., are the L.1 plants of the family.

The above plants were planted on the 22nd. of August 1934, spaced 2'6" apart each way. Weather rather wet, but soil in good condition.

During November, plants of the following L.2 families were planted alongside the L.1 and parent plants. Weather fine, dry. Soil Moist underneath, surface dry.

Figure 1. Diagram of L.2 plant families received during November 1934 in order of planting.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 111/21/8 | 111/21/8 | 111/21/8 | 91/10/10 | 91/10/10 | 91/10/8 | 91/10/8 | | |
| | 111/21/8 | 111/21/8 | 111/21/8 | 91/10/10 | 91/10/10 | 91/10/8 | 91/10/8 | | |
| x111/21/8 | 111/21/8 | 111/21/8 | 111/21/8 | 91/10/10 (4) | 91/10/10 | 91/10/8 | 91/10/8 | | |
| 111/21/8 | 111/21/8 | 99/3/12 (4) | 99/3/12 | 91/10/10 | 91/10/10 | 91/10/8 (4) | 91/10/8 | | |
| 111/21/8 | 111/21/8 | 99/3/12 | 99/3/12 | 91/10/10 | 91/10/10 | 91/10/8 | 91/10/8 (3) | | |
| 111/21/8 | 111/21/8 | 99/3/12 (3) | 99/3/12 | 91/10/10 (2) | 91/10/10 (3) | 91/10/8 | 91/10/8 | | |
| 111/21/8 | 111/21/8 | 99/3/12 | 99/3/12 (2) | 91/10/10 | 91/10/10 | 91/10/8 (1) | 91/10/8 (2) | | |
| 111/21/8 | 111/21/8 | 99/3/12 | 99/3/12 (1) | 91/10/10 | 91/10/10 (1) | 91/10/20 (4) | 91/10/20 | 53/18/21 | 53/18/21 |
| 111/21/8 | 111/21/8 | 99/3/10 | 99/3/10 | 91/10/7 | 91/10/7 (4) | 91/10/20 | 91/10/20 | 53/18/21 | 53/18/21 |
| 99/3/15 | 99/3/15 (4) | 99/3/10 | 99/3/10 (4) | 91/10/7 | 91/10/7 | 91/10/20 (3) | 91/10/20 | 53/18/21 | 53/18/21 |
| 99/3/15 | 99/3/15 | 99/3/10 (3) | 99/3/10 | 91/10/7 | 91/10/7 (3) | 91/10/20 (2) | 91/10/20 | 53/18/21 | 53/18/21 |
| 99/3/15 | 99/3/15 | 99/3/10 | 99/3/10 | 91/10/7 | 91/10/7 (2) | 91/10/20 | 91/10/20 | 53/18/21 | 53/18/21 |
| 99/3/15 | 99/3/15 (3) | 99/3/10 | 99/3/10 | 91/10/7 (1) | 91/10/7 | 91/10/20 (1) | 91/10/20 | 53/18/21 | 53/18/21 |
| 99/3/15 | 99/3/15 | 99/3/10 | 99/3/10 | 99/3/8 (4) | 99/3/8 | 91/10/20 | 91/10/20 | 53/18/21 | 53/18/21 |
| 99/3/15 | 99/3/15 | 99/3/10 | 99/3/10 | 99/3/8 | 99/3/8 | 39/9/5 | 39/9/5 | 53/18/2 | 53/18/2 |
| 99/3/15 (1) | 99/3/15 (2) | 99/3/10 (2) | 99/3/10 | 99/3/8 | 99/3/8 (3) | 39/9/5 | 39/9/5 | 53/18/2 | 53/18/2 |
| 99/3/15 | 99/3/15 | 99/3/10 | 99/3/10 | 99/3/8 | 99/3/8 | 39/9/5 | 39/9/5 | 53/18/2 | 53/18/2 |
| 99/3/15 | 99/3/15 | 99/3/10 | 99/3/10 | 99/3/8 (1) | 99/3/8 (2) | 39/9/5 | 39/9/5 | 53/18/2 | 53/18/2 |
| 99/3/5 | 99/3/15 | 99/3/10 | 99/3/10 | 99/3/8 | 99/3/8 | 39/9/5 | 39/9/5 | 39/9/20 | 39/9/20 |

x 111/21/8 here refers to seedling L.2 plants from the L.1 family 111/21/8, and so with the other families.

The plants with numbers 1-4 in circles are plants picked for cuttings.

The plants received, did not include all plants necessary for the first years work, but use was made of plants growing on the P.R. Station area to complete the series for cuttings necessary. It was finally decided to restrict the experiment to the two plant families 91/10 and 99/8. From these two families were chosen the following plants on which to work.

| | | |
|---|---|---|
| Parents | 91/10/P | 99/3/P |
| 1st. inbred generation. | 91/10/7 | 99/3/8 |
| | 91/10/8 | 99/3/10 |
| | 91/10/10 | 99/3/12 |
| | 91/10/20 | 99/3/15 |
| 2nd. inbred generation | 91/10/7 (1)-(4) | 99/3/8 (1)-(4) |
| | 91/10/8 (1)-(4) | 99/3/10(1)-(4) |
| | 91/10/10(1)-(4) | 99/3/12(1)-(4) |
| | 91/10/20(1)-(4) | 99/3/15(1)-(4) |

(1)-(4) refers to plants in the previous figures. All the inbred plants were chosen as representatives of their generation.

iii. The Method of the Experiment.

Cuttings were taken from each of the plants above (P. R. Station material has to be utilised for several lots). It was hoped by proper interspacing of the plants from these cuttings to be able to compare yields from rows of each. The cuttings were planted on August 14th., to 20th., 1935, in boxes of sandy soil innoculated with nodule bacteria, and placed in the green house at Massey Agricultural College. The size of the boxes was 21" x 14½" and 6" deep, and cuttings were planted in each box.

The total plantings were as follows:-

| Plant Title | Genera tion | No. Planted. | No. Recd. | Plant Title | Genera tion | No. Planted. | No. Recd. |
|---|---|---|---|---|---|---|---|
| 91/10/P | P. | 96 | 40 | 99/3/P | P. | 96 | 40 |
| 91/10/7 | I.1 | 48 | 20 | 99/3/8 | I.1 | 48 | 20 |
| 91/10/8 | I.1 | 48 | 20 | 99/3/10 | I.1 | 48 | 20 |

| Plant Title | Generation | No. Planted | No. Required | Plant Title | Generation | No. planted | No. Reqrd: |
|---|---|---|---|---|---|---|---|
| 91/10/10 | L.1 | 48 | 20 | 99/3/12 | L.1 | 48 | 20 |
| 91/10/20 | L.1 | 49 | 20 | 99/3/15 | L.1 | 48 | 20 |
| 91/10/7 (1) | L.2 | 28 | 10 | 99/3/8 (1) | L.2 | 28 | 10 |
| " (2) | L.2 | 28 | 10 | " (2) | L.2 | 28 | 10 |
| " (3) | L.2 | 28 | 10 | " (3) | L.2 | 28 | 10 |
| " (4) | L.2 | 28 | 10 | " (4) | L.2 | 28 | 10 |
| 91/10/8 (1) | L.2 | 28 | 10 | 99/3/10 (1) | L.2 | 28 | 10 |
| " (2) | L.2 | 28 | 10 | " (2) | L.2 | 28 | 10 |
| " (3) | L.2 | 28 | 10 | " (3) | L.2 | 28 | 10 |
| " (4) | L.2 | 28 | 10 | " (4) | L.2 | 28 | 10 |
| 91/10/10 (1) | L.2 | 30 | 10 | 99/3/12 (1) | L.2 | 28 | 10 |
| " (2) | L.2 | 30 | 10 | " (2) | L.2 | 28 | 10 |
| " (3) | L.2 | 30 | 10 | " (3) | L.2 | 28 | 10 |
| " (4) | L.2 | 30 | 10 | " (4) | L.2 | 28 | 10 |
| 91/10/20 (1) | L.2 | 30 | 10 | 99/3/15 (1) | L.2 | 28 | 10 |
| " (2) | L.2 | 30 | 10 | " (2) | L.2 | 28 | 10 |
| " (3) | L.2 | 30 | 10 | " (3) | L.2 | 28 | 10 |
| " (4) | L.2 | 30 | 10 | " (4) | L.2 | 28 | 10 |

Previous experience at the P. R. Station (unpublished) indicated that rather more than 50% of the cuttings grew. So far as the parent plants were concerned, slightly more than 50% grew, but with the inbred progeny the results were very variable.

Every possible care was taken to select cuttings which were similar, and also representative of the plants and generation concerned, and which also compared in stage of development with plants of different generations. It was impossible however, to select perfectly even lines, firstly, because the plants from which the cuttings were taken were of different ages, and secondly, because some of the 2nd. inbred generation (L.2) plants were not growing sufficiently vigorously when cuttings were taken, limiting the choice of cuttings.

Mortality among cuttings was so high in some cases that the original design of the experiment had to be modified in several ways.

This mortality may be due to -

(1). the season of the year at which cuttings were taken.

(2). the selfed plants lacked the vigour to establish well from cuttings.

(3). other accidental causes not known, such as wrong soil, wrong water content of soil, too much or too little heat in greenhouse etc., If the experiment is repeated it is considered advisable to plant cuttings in the Autumn, rather than early spring, allowing cuttings better chances of establishment, and also giving one the opportunity to replace dying plants before the spring. Also, too, a greater number of L.1 and L.2 plants could be planted.

Sufficient plants were obtained however, to allow an experiment to be conducted. These plants were planted on November 20th 1935 as follows:-

FIG. 3. Showing order of planting of Cuttings.

| | |
|---|---|
| Control | Control |
| 99/3/10 (1) L.2 | 99/3/I5 (I) |
| 99/3/10 | 99/3/I5 |
| 99/3/10 (2) & (3) | 99/3/I5 (2) |
| 99/3/P | 99/3/P |
| 99/3/10 (4) | 99/3/I5 (3) |
| 99/3/8 (1) | 99/3/I5 (4) |
| 99/3/8 | 99/3/I2 (I) |
| 99/3/8 92) | 99/3/I2 |
| 99/3/P | 99/3/I2 (2) |
| 99/3/8 (3) | 99/3/P |
| 99/3/8 (4) | 99/3/I2 (3) |
| 9I/IO/8 (2) | 99/3/I2 |
| 9I/IO/8 | 99/3/I2 (4) |
| 9I/IO/8 (3) | 9I/IO/P |
| 9I/IO/8 | 91/10/10 (1) |
| 91/10/8 (4) | 91/10/10 (2) |
| 91/10/7 | 91/10/P |
| 91/10/7 | 91/10/10 (3) |

| | |
|---|---|
| 91/10/P | 91/10/10 |
| 91/10/7 | 91/10/10 (4) |
| Control | Control |

There were 10 plants in each row, 21" apart. Rows were 2'6" apart and blocks 3ft. apart. The "control" row was really an outside row, not being used for comparison. Soil was moist and establishment good.

A preliminary trimming was given to the plants about one month after planting. The stand was cut and weighed in rows on February 10th., 1936, March 25th, 1936, and June 4th, 1936, three cuts in all.

iv. The Results of the Experiment.

The weights of the rows from the three cuts are recorded in the following table under the column headings, 1, 2, and 3. The weight for the season is totalled and compared for convenience with the average of the two parent rows on either side of the row concerned as the base of 100. Although this base is condemned by some statisticians, it has the advantage of practical convenience. Here too, differences are expected to be large, and consequently the refinement of technique is not so important as where small differences are to be observed. The figures in parenthesis are the average of the parent row, about which they occur, and the next parent row below.

TABLE V: LOSS OF VIGOUR ON SEIFING

| No. | Pl. | 1 (lbs) | 2 (lbs) | 3 (lbs) | Total | P = 100 |
|---|---|---|---|---|---|---|
| Block 2. | | | | | | |
| 1. | L.1 X | 2.4 | 5.2 | 4.8 | 12.4 | - |
| 2. | L.2 | .7 | 1.6 | 0.7 | 3.0 (8.7) | 33.7 |
| 3. | P. | .8 | 3.8 | 4.3 | 8.9 | 100 |
| 4. | L.2 | .9 | 2.0 | 1.5 | 4.4 | 50.6 |
| 5. | L.1 | .9 | 2.4 | 1.7 | 5.0 | 57.5 |
| 6. | L.2 | .9 | 3.3 | 4.5 | 8.7 (9.7) | 100 |
| 7. | P. | 1.1 | 3.7 | 3.7 | 8.5 | 100 |
| 8. | L.2 | 1.2 | 2.8 | 3.9 | 7.9 | 81.4 |
| 9. | L.1 | .9 | 2.1 | 1.8 | 4.8 | 49.5 |
| 10. | L.2 | .75 | 2.9 | 4.5 | 8.15 | 84.0 |
| 11. | L.2 | 1.3 | 3.2 | 1.7 | 6.2 | 63.9 |
| 12. | L.2 | 1.2 | 2.5 | 2.7 | 6.4 (11.7) | 66.0 |
| 13. | P | 1.2 | 3.6 | 6.0 | 10.8 | 100 |
| 14. | L.2 | 1.3 | 2.8 | 2.9 | 7.0 | 59.8 |
| 15. | L.1 | .75 | - | 2.5 | 3.2 | - |
| 16. | L.2 | 2.0 | 4.0 | 3.8 | 9.8 | 83.7 |
| 17. | L.2 | 1.8 | 3.7 | 4.3 | 9.8 | 83.7 |
| 18. | P. | 1.5 | 4.4 | 6.6 | 12.5 | 100 |
| 19. | L.2 | 1.3 | 2.8 | 3.6 | 7.7 | 61.6 |
| 20. | L.1 | .4 | 1.7 | 2.5 | 4.6 | 36.8 |
| 21. | L.2 | .8 | 1.3 | 1.7 | 3.8 | 30.4 |
| 22. | L.1 X | 3.0 | 7.5 | 8.3 | 18.8 | - |
| Block 3. | | | | | | |
| 1. | L.1 X | 2.8 | 5.7 | 4.7 | 13.2 | - |
| 2. | L.2 | .9 | 2.7 | 2.0 | 5.6 | 60.2 |
| 3. | L.1 | .4 | 1.3 | 0.8 | 2.5 | 26.9 |
| 4. | L.2 | .75 | 1.5 | 0.8 | 3.0 (8.9) | 32.2 |
| 5. | P | 1.2 | 3.9 | 4.2 | 9.3 | 100 |
| 6. | L.2 | 1.4 | 3.1 | 2.3 | 6.8 | 76.4 |
| 7. | L.2 | 1.1 | 2.0 | 2.5 | 5.6 (9.1) | 62.9 |
| 8. | P | 1.4 | 4.1 | 3.1 | 8.6 | 100 |
| 9. | L.2 | 1.3 | 2.3 | 2.4 | 6.0 | 65.9 |

| No. | Pl. | 1 (lbs) | 2 (lbs) | 3 (lbs) | Total | P = 100 |
|---|---|---|---|---|---|---|
| 10. | L.1 | 1.7 | 3.9 | 4.6 | 10.2 | 112.1 |
| 11. | L.2 | 1.3 | 2.5 | 2.3 | 6.1 | 67.0 |
| | | | | | (9.8) | |
| 12. | P | 1.4 | 3.6 | 4.6 | 9.6 | 100 |
| 13. | L.2 | 2.1 | 4.0 | 2.8 | 8.9 | 90.8 |
| 14. | L.1 | 1.7 | 4.3 | 4.5 | 10.5 | 107.2 |
| 15. | L.2 | 1.4 | 2.8 | 1.5 | 5.7 | 58.2 |
| 16. | L.2 | 1.6 | 3.1 | 3.0 | 7.7 | 78.6 |
| 17. | L.2 | 1.1 | 1.7 | 2.2 | 5.0 | 51.0 |
| 18. | P | 1.4 | 3.2 | 5.3 | 9.9 | 100 |
| 19. | L.2 | 1.6 | 2.4 | 2.0 | 6.0 | 60.6 |
| 20. | L.1 | 1.0 | 2.1 | 3.5 | 6.6 | 66.7 |
| 21. | L.2 | 1.2 | 2.7 | 4.2 | 8.1 | 81.8 |
| 22. | L.1 X | 2.4 | 7.2 | 8.4 | 18.0 | - |

Summary of Table I.

| | Parent | L.1 | L.2. |
|---|---|---|---|
| Average | 100 | 65.2 | 66.0 |
| Standard deviation | - | 33.1 | 18.1 |
| Standard error | - | 12.5 | 3.7 |
| Results become | 100 | 62.5 ± 12.5. | 66.0 ±3.7 |

V. Discussion on Results.

The results obtained in this experiment are not altogether what one would desire. It was expected and other experiments confirm the expectation, that there would be a drop in yield from parent to 1st. selfed generation, and a further though smaller drop from 1st., to 2nd., selfed generation. Results were however, very variable particularly in the L.1 series, as the standard error shows. This can be mainly attributed to -

(1). The impossibility of accurately duplicating the yielding capacity of a plant by the use of clones, particularly in a single season.

(2). In the case of the 1st., inbred generation (L.1 series) a greater number of rows would have been an advantage in giving a more reliable result.

(3). A small number of rows from L.1 plants occur in the experiment because for some reason not discovered, such plants were exceedingly difficult to grow from cuttings, only a few surviving.

A better method to use in this experiment would be to grow from seed obtained by selfing parent and 1st., inbred generation, 1st., and 2nd., inbred generation plants. The difficulty is to get plants from seed representative of the parent plant. Probably seed from the parent when open pollinated would give the best control here. Seedlings from a definite parent cross can be compared to seedlings of the first and second inbred generations in the next experiment in "Regain in Vigour on Crossing", the inbred plants being deliberately included here to allow this comparison.

If we leave out the results from the 1st., inbred generation, and compare only parent and 2nd., selfed generation we find that the results differ by at least three times the probable error, and can therefore, be considered to be significant.

## SECTION IV.

### A MEASURE OF THE REGAIN IN VIGOUR ON CROSSING INBRED PLANTS.

i. The Purpose of the Experiment.

As inbreeding results in a loss in vigour (as measured by a reduction in yield), it becomes imperative to know whether or not this vigour can be recovered on crossing these inbred plants. If the vigour is not recovered, then it is exceedingly difficult to make use of the more uniform plants resulting from inbreeding. If however, vigour can be shown to be recovered on subsequent crossing of inbred plants, then we can concentrate on producing uniform types of lucerne by inbreeding, neglecting to some extent, the reduction in vigour occasioned by such methods, knowing that a later crossing of inbred lines will result in a recombination of vigour factors to give plants as vigorous as, or more vigorous than the parents. This experiment was designed to measure the yields of green matter from rows of a parent cross and rows of crosses between the 1st., inbred generation of each parent, and rows of crosses between the 2nd., inbred generation of each parent. The aim was to decide whether or not the vigour lost on selfing was recovered on crossing. Included in the experiment were rows of 1st., and 2nd., inbred generations to act as a check on the previous experiment.

ii. The Material for the Experiment.

The same original plants were received in this experiment as in the previous experiment. For the original plantings see Figs. 1 and 2.

The two families 91/10 and 99/3, were again chosen for this experiment, the following plants being chosen on which to work.

| | | |
|---|---|---|
| Parents | 91/10/P | 99/3/P |
| 1st. inbred generation | 91/10/7 | 99/3/12 |
| | 91/10/8 | 99/3/8 |
| | 91/10/10 | 99/3/10 |
| | 91/10/20 | 99/3/15 |
| 2nd. inbred generation | 91/10/7 (1)-(4) | 99/3/12 (1)-(4) |

91/10/8 (1)-(4) 99/3/8 (1)-(4)
91/10/10 (1)-(4) 99/3/10(1)-(4)
91/10/20 (1)-(4) 99/3/15(1)-(4)

See Fig. 2 - for explanation of number (1)-(4).

As all these plants were not available on my own area, recourse had to be taken to material of the P. R. Station, which was kindly made available.

On January 4th, 1935, the following cross was made - 91/10/Parent ♀ X 99/3/Parent ♂

On January 5th

| | | | |
|---|---|---|---|
| 99/3/P ♀ | X | 91/10/P ♂ | |
| 99/3/10 ♀ | X | 91/10/10 ♂ | L.1 cross. |
| 99/3/12 ♀ | X | 91/10/7 ♂ | L.1 cross. |
| 99/3/15 ♀ | X | 91/10/20 ♂ | L.1 cross. |
| 99/3/8 ♀ | X | 91/10/8 ♂ | L.1 cross. |

On January 7th, 1935, repeated the L.1 crosses - (reciprocals)

On January 9th 1935, made the following crosses between plants of the 2nd., inbred generation (L.2)

| ♀ | | ♂ | ♀ | | ♂ |
|---|---|---|---|---|---|
| 99/3/10 (1) | X | 91/10/10 (1) | 99/3/12 (1) | X | 91/10/7 (1) |
| 99/3/10 (2) | X | 91/10/10 (2) | 99/3/12 (2) | X | 91/10/7 (2) |
| 99/3/10 (3) | X | 91/10/10 (3) | 99/3/12 (3) | X | 91/10/7 (3) |
| 99/3/10 (4) | X | 91/10/10 (4) | 99/3/12 (4) | X | 91/10/7 (4) |

On January 10th 1935, the following L.2 crosses were made.

| | | | | | |
|---|---|---|---|---|---|
| 99/3/15 (1) | X | 91/10/20 | 91/10/8 (1) | X | 99/3/8 (1) |
| 99/3/15 (2) | X | 91/10/20 | 91/10/8 (2) | X | 99/3/8 (2) |
| 99/3/15 (3) | X | 91/10/20 | 91/10/8 (3) | X | 99/3/8 (3) |
| 99/3/15 (4) | X | 91/10/20 | 91/10/8 (4) | X | 99/3/8 (4) |

The reciprocals of the crosses were made in each case, and the resultant seed - from cross and reciprocal - mixed. In all the crosses the pin and brush method (See experiments on technique of crossing), the other method of cutting the standard not being developed at this time.

iii. The Method of the Experiment.

The inbred plant 91/10/8 has proved to be a very poor parent, and in this experiment had to be left out owing to the very few seed produced by the cross, in which it occurred. Seed was planted on July 6th., and 7th., as follows:-

TABLE VI  Showing planting of Lucerne seed.

| Plant Description. | No. sown. | No. Reqd. | Plant Description | No. sown | No. Reqd. |
|---|---|---|---|---|---|
| 99/3/P x 91/10/P P.x | 101 | 80 | 99/3/12 x 91/10/7(1) | 24 | 10 |
| 99/3/10 x 91/10/10 (L.1 x) | 160 | 40 | " (2)x " (2) | 25 | 10 |
| 99/3/12 x 91/10/7 (L.1 x) | 72 | 40 | " (3)x " (3) | 33 | 10 |
| 99/3/15 x 91/10/20 (L.1 x) | 49 | 40 | " (4)x " (4) | 25 | 10 |
| 99/3/10 (1) x 91/10/20 (1) (L.2 x) | 12 | 10 | 99/3/15 x 91/10/20(1) | 12 | 10 |
| " (2) x " (2) (L.2 x) | 12 | 10 | "(2) x " (2) | 13 | 10 |
| " (3) x " (3) (L.2 x) | 20 | 10 | "(3) x " (3) | 13 | 10 |

Besides the above crosses the following seed from selfing was also sown.

| Plant Description | | No. sown. |
|---|---|---|
| 91/10 | L.1 | 45 |
| 99/3 | L.1 | 48 |
| 99/3/12 | L.2 | 46 |
| 99/3/8 | L.2 | 55 |
| 99/3/12 | L.2 | 84 |
| 91/10/7 | L.2 | 55 |
| 91/10/20 | L.2 | 55 |

Germination was good from seed which resulted from crosses, but not good for seeds from inbreeding. The seedlings were grown in the green-house, and then hardened off outside before planting on November 19th. 1935. To make the fullest use of the material that grew, the plants were arranged in the following order of planting:

FIG 4. Showing the arrangement of plants to allow a measure of regain in vigour on crossing.

10 plants per row.

| | | | | |
|---|---|---|---|---|
| 42 | 91/10/P | x | 99/3/P | P. X |
| 41 | 91/10/10 | | self | L.2 |
| 40 | 91/10/10 | x | 99/3/10 | L.1 X |
| 39 | 91/10 | | self | L.1 |
| 38 | 91/10/10 | x | 99/3/10 (1) | L.2 X |
| 37 | 91/10/10 | | self | L.2 |
| 36 | 91/10/P | x | 99/3/P | P. X |
| 35 | 91/10/10 | | self | L.2 |
| 34 | 91/10/10 | x | 99/3/10 (2) | L.2 X |
| 33 | 91/10 | | self | L.1 |
| 32 | 91/10/10 | x | 99/3/10 | L.1 X |
| 31 | 91/10/10 | | self | L.2 |
| 30 | 91/10/P | x | 99/3/P | P. X |
| 29 | 99/3/12 | | self | L.2 |
| 28 | 91/10/7 | x | 99/3/12 (1) | L.2 X |
| 27 | 91/10/7 | | self | L.2 |
| 26 | 91/10/7 | x | 99/3/12 | L.1 X |
| 25 | 99/3/12 | | self | L.2 |
| 24 | 91/10/7 | x | 99/3/12 (2) | L.2 X |
| 23 | 91/10/7 | x | 99/3/12 | L.1 X |
| 22 | 91/10/7 | | self | L.2 |
| 21 | 91/10/P | x | 99/3/P | P. X |
| 20 | 91/10/7 | | self | L.2 |
| 19 | 91/10/7 | x | 99/3/12 (3) | L.2 X |
| 18 | 91/10/7 | x | (self [illegible] | L.2 [illegible] |
| 17 | 91/10/7 | x | 99/3/12 | L.1 X |
| 16 | 91/10/7 | | self | L.2 |
| 15 | 91/10/7 | x | 99/3/12 (4) | L.2 X |
| 14 | 91/10/7 | x | 99/3/12 | L.1 X |
| 13 | 99/3/12 | | self | L.2 |
| 12 | 91/10/P | x | 99/3/P | P. X |
| 11 | 91/10/20 | | self | L.2 |
| 10 | 91/10/20 | x | 99/3/15 (1) | L.2 X |

| | | |
|---|---|---|
| 9 | 91/10/20 x 99/3/15 | L.1 X |
| 8 | 91/10/20 x 99/3/15 (2) | L.2 X |
| 7 | 91/10/20 self | L.2 |
| 6 | 91/10/P x 99/3/P | P. X |
| 5 | 91/10/20 x 99/3/15 (3) | L.2 X |
| 4 | 91/10/20 self | L.2 |
| 3 | 91/10/20 x 99/3/15 | L.1 X |
| 2 | 91/10/20 self | L.2 |
| 1 | 91/10/P x 99/3/P | P. X |

P. X signifies the cross between the two parents, L.1 X the cross between plants of the 1st., inbred generation, and L.2 X, the cross between plants of the 2nd., inbred generation.

A preliminary cutting was given to the plants about 6 weeks after planting to reduce all plants to the same level. Weighed cuts were taken from the area on February 10th., 1936, March 25th., 1936, and June 4th., 1936.

iv. The Results of the Experiment.

The row weights from these three cuts are given in the following table, under 1, 2, and 3, and the season total in the next column. The season total for each row is then compared to the parent total, using the average of the two parents on either side of the row as the base of 100.

The figures in parenthesis refer to the average between the parent row concerned and the parent row next below.

TABLE VII: Regain in Vigour on Crossing

| No. | Cross. | 1 (lbs) | 2 (lbs) | 3 (lbs) | Total | P. X = 100 |
|---|---|---|---|---|---|---|
| 1 | P X | 1.4 | 5.2 | 4.8 | (12.8) 11.4 | 100 |
| 2 | L.2 | 1.2 | 3.9 | 3.3 | 8.4 | 65.6 |
| 3 | L.1 X | 2.2 | 6.4 | 5.5 | 14.1 | 110.2 |
| 4 | L.2 | 1.8 | 5.0 | 3.2 | 10.0 | 78.1 |
| 5 | L.2 X | 2.3 | 6.0 | 5.3 | 13.6 | 106.3 |
| 6 | P X | 2.0 | 6.2 | 6.1 | (12.6) 14.3 | 100 |
| 7 | L.2 | 1.8 | 4.7 | 2.9 | 9.4 | 74.9 |
| 8 | L.2 X | 2.2 | 6.0 | 5.5 | 13.7 | 108.7 |
| 9 | L.1 X | 2.3 | 6.1 | 4.9 | 13.3 | 105.5 |
| 10 | L.2 X | 2.1 | 6.1 | 5.5 | 13.7 | 108.7 |
| 11 | L.2 | .5 | 2.6 | 1.8 | 4.9 | 38.9 |
| 12 | P X | 1.3 | 4.7 | 5.0 | (11.4) 11.0 | 100 |
| 13 | L.2 | 1.3 | 3.9 | 2.6 | 7.8 | 68.4 |
| 14 | L.1 X | 2.3 | 6.2 | 5.6 | 14.1 | 123.7 |
| 15 | L.2 X | 2.3 | 6.7 | 5.1 | 14.1 | 123.7 |
| 16 | L.2 | .9 | 2.2 | 1.5 | 4.6 | 40.3 |
| 17 | L.1 X | 1.7 | 6.1 | 5.9 | 13.7 | 120.1 |
| 18 | L.2 | 1.1 | 3.4 | 3.0 | 7.5 | 65.8 |
| 19 | L.2 X | 1.8 | 5.5 | 5.5 | 12.8 | 112.3 |
| 20 | L.2 | .6 | 1.9 | 1.1 | 3.6 | 31.6 |
| 21 | P X | 1.2 | 5.0 | 5.7 | (12.5) 11.9 | 100 |
| 22 | L.2 | .55 | 2.4 | 1.4 | 4.3 | 34.4 |
| 23 | L.1 X | 1.3 | 4.0 | 4.4 | 9.7 | 77.6 |
| 24 | L.2 X | 1.4 | 4.6 | 4.2 | 10.2 | 81.6 |
| 25 | L.2 | 1.1 | 2.7 | 2.5 | 6.3 | 50.8 |
| 26 | L.1 X | 1.5 | 5.4 | 5.6 | 12.5 | 100 |
| 27 | L.2 | 1.0 | 1.4 | 2.0 | 4.4 | 35.1 |
| 28 | L.2 X | 2.4 | 5.4 | 4.9 | 12.7 | 101.6 |
| 29 | L.2 | 2.0 | 4.1 | 3.2 | 9.3 | 75.0 |
| 30 | P X | 1.9 | 5.2 | 6.1 | (14.4) 13.2 | 100 |
| 31 | L.2 | 1.0 | 2.7 | 2.3 | 6.0 | 41.7 |
| 32 | L.1 X | 2.3 | 5.7 | 6.6 | 14.6 | 101.4 |
| 33 | L.1 | 2.0 | 4.6 | 3.2 | 9.8 | 68.1 |

| No. | Cross | 1 (lbs) | 2 (lbs) | 3 (lbs) | Total | P X = 100 |
|---|---|---|---|---|---|---|
| 34 | L.2 X | 2.3 | 5.6 | 4.8 | 12.7 | 88.2 |
| 35 | L.2 | 1.8 | 3.5 | 2.6 | 7.9 (13.4) | 54.9 |
| 36 | P X | 2.6 | 6.5 | 6.3 | 15.4 | 100 |
| 37 | L.2 | 1.8 | 3.9 | 2.7 | 8.4 | 62.7 |
| 38 | L.2 X | 2.1 | 5.3 | 5.1 | 12.5 | 93.3 |
| 39 | L.1 | 2.2 | 5.5 | 4.2 | 11.9 | 88.8 |
| 40 | L.1 X | 1.4 | 4.6 | 4.8 | 10.8 | 80.6 |
| 41 | L.2 | 1.3 | 3.1 | 2.0 | 6.4 | 47.8 |
| 42 | P X | 1.5 | 4.4 | 5.5 | 11.4 | 100 |

SUMMARY

| | P X | L.1 | L.1 X | L.2 | L.2 X |
|---|---|---|---|---|---|
| Average | 100 | 78.4 | 102.3 | 54.1 | 102.7 |
| Standard deviation | | 14.56 | 16.6 | 16.0 | 13.0 |
| Standard Error | | 10.3 | 5.9 | 4.0 | 4.4 |
| Results become | 100 | 78.4 ± 103 | 102.3 ±5.9 | 54.1 ± 4.0 | 102.7 ± 4.4 |

v. The Discussion on Results.

The results of the crosses are significantly different from the selfings, and show a complete recovery in vigour when compared with the parent cross. The yield from L.2 progeny is significantly lower than the yield from L.1 progeny. It is thus possible to group the results as follows:-

| | | |
|---|---|---|
| Crosses between Parents | 100 | ) |
| " " 1st. selfed generations | 102.3 ± 5.9 | ) = |
| " " 2nd. selfed generations | 102.7 ± 4.4 | ) |
| 1st. Generation of selfed plants | 78.4 ± 10.3 | |
| 2nd. " " " " | 54.1 ± 4.0 | |

It will be noticed that the L.1 and L.2 plants are significantly different in yield, differences being as expected.

(See discussion on Loss of Vigor on Selfing)
Here the L.1 plants are significantly lower than the parent x, on an average 21.6% - and the L.2 plants significantly lower than the L.1 plants - on an average 24.3%. These differences may be taken as a general indication of the reduction in yield with selfings, being a better indication than the actual experiment on loss of vigor on selfing in which cuttings were used. It is, of course, impossible to suggest a percentage loss of vigor for all plant families, as this has recently been shown to vary considerably. Some plants have been demonstrated which show very little loss of vigor on selfing, and many of these are being used in future selection and breeding at the Plant Research Bureau, Christchurch.

The most important part of the above experiment is the fact that, plants which have been selfed to obtain eveness of line will fully recover vigor, as measured by yield, on subsequent crossing. Not only do plants of the 1st., inbred generation, when crossed, fully recover in vigour, but plants of the 2nd. inbred generation also recover as fully. There is a slight actual, though not significant, difference in favour of the crossed selfings, as compared to the parent cross. In maize breeding, the more homozygous the plants are made by inbreeding, and the greater the yield is from crosses between such inbred material. Whether such phenomena will arise in lucerne is a matter for conjecture. At the present time it is difficult to inbreed beyond two generations with the material available.

However, it is of great importance to know when breeding lucerne, that the loss of vigour occasioned on selfing is recovered on crossing. The knowledge enables one to concentrate on purifying families by inbreeding, Knowing that judicious crosses between selected selfings will recover in vigour, and yet be reasonably homozygous compared with the parent material. One method of breeding adopted by the Plant Research Bureau, is to test the selected parents by progeny tests, then to use the best parents in either mass crosses, diallel crosses or individual crosses, to produce improved races. It can easily be seen that the use of inbred material in crosses, being more homozygous will result in greater improvement in uniformity of lines. In this experiment the inbred plants selected for crossing were not the best of the line, but representative of the generation they came from. Yet the plant grown from crosses between such inbred material was as high yielding as the parent cross, which can be considered as the most vigorous parent. May it not be logical to suggest that selection of the better progeny - such being no more heterozygous than the poorer progeny - for crossing, should results in offspring which surpass the parent in yield. Whether this is actually so, or whether the better plants are actually more heterozygous than the poorer plants and on crossing not necessarily resulting in increased vigour, needs further experimentation to find out.

If it were possible to combine vigour factors from the best lucerne plants, with the factor for autogamy, the production of a vigorous lucerne plant would be simplified. Autogamous lucerne has been propagated by Kirk in Canada, but as yet nothing seems to be known of what is being done with such lucerne. At the present time, even when a good crossbred lucerne is produced, successive generations tend to revert to the original average in yield, due to selfing and segregation taking place. The elimination of undersirable factors by inbreeding may result in plants which do not deteriorate so rapidly. Progeny tests are used in the selection of parents to overcome this deterioration due to selfing. It might also be suggested that if plants are produced which are high yielders, and will stay down more or less indefinitely in the field, seed not being produced from an area of this type, but continually being produced at some central station, then the problems of deterioration would not concern the farmer, who would have a high grade plant which, more or less, lasted his lifetime. Much work still needs to be done on these ...

of inbreeding, and also on such problems as the relation between yield of green material, and seeding capacity, fertility and sterility.

## SECTION IV.A.

### A GENERAL COMPARISON BETWEEN YIELD FROM CROSSBRED AND INBRED LUCERNE PLANTS.

i. The Purpose of the Experiment.

This experiment was arranged as a general amplification of the previous two experiments, and illustrates the application of some of the results to general practice. A comparison was made between the yields of green material, from the first inbred generation of several parents and the F.1 of the cross between each parent and several other parents. In this way it was hoped to show the importance of crossing in maintaining yield under average conditions.

ii. The Material of the Experiment.

Some surplus seed was given to the writer by the Officers of the Plant Research Station, and comprised seed from 8 different parent plants when self pollinated (1st., inbred generation), and seed from each of the parents. when crossed with from one to five other parents. The seed was planted on July 16th, 1935, in boxes in the greenhouse.

A list of Plantings:

| Plant Title. | No. Sown. | Plant Title | No. Sown |
|---|---|---|---|
| 7/25 Selfed | 13. | 75/24 Selfed | 26 |
| 7/25 x 23/2 | 26. | 75/24 x 114/14/14 | 26. |
| " x 26/23 | 20. | 39/9 Selfed | 26. |
| " x 53/18 | 26. | 39/9 x 108/15 | 26. |
| " x 68/13 | 21. | 111/21 Selfed | 24. |
| " x 74/11 | 26. | 111/21 x 125/11 | 24. |
| 73/12 Selfed | 26. | " x 132/16 | 24. |
| 73/12 x 75/24 | 23. | " x 120/14 | 24. |
| " x 114/14/14 | 26. | 8/4 Selfed | 36. |
| 26/24 Selfed | 40. | 8/4 x 26/24 | 24. |
| 26/24 x 70/13 | 26. | " x 70/13 | 24. |
| " x 129/9 | 24. | " x 129/9 | 24. |
| " x 87/25 | 24. | | |
| 26/23 Selfed | 24. | | |
| 26/23 x 74/11 | 24. | | |

| Plant Title. | No. Sown | Plant Title | No. Sown. |
|---|---|---|---|
| 26/23 x 68/13 | 12 | | |
| 26/23 x 53/18 | 23. | | |

The seed from selfings did not grow very well in most cases, but crossbred seed germinated freely. After being hardened outside for several weeks, the seedlings were planted out on November 21st., 1935, as follows:-

iii. The Method of the Experiment.

FIG. 5 Showing arrangement of material in this experiment.

| Block 2. | | Row Number. | Block 3. | |
|---|---|---|---|---|
| Outside row | | 41. | | |
| 26/24 | x 70/3 | 40. | Outside row | |
| | x 8/4 | 39. | 8/4 | x 129/9 |
| | x 87/25 | 38. | | x 70/13 |
| | Selfed | 37. | | Selfed |
| | x 70/13 | 36. | | x 26/24 |
| | x 129/9 | 35. | 111/21 | x 120/14 |
| 39/9 | x 108/5 | 34. | | x 132/16 |
| | Selfed | 33. | | Selfed |
| 75/24 | x 114/14/14 | 32. | | x 120/14 |
| | Selfed | 31. | | x 125/11 |
| | x 114/14/14 | 30. | 26/23 | x 74/11 |
| 7/25 | x 53/18 | 29. | | x 53/18 |
| | x 74/11 | 28. | | Selfed |
| | x 68/3 | 27. | | x 74/11 |
| | x 53/18 | 26. | 73/12 | x Selfed |
| | x 26/23 | 25. | | x 114/14/14 |
| | Selfed | 24. | | Selfed |
| | x 23/2 | 23. | | x 75/24 |
| Outside row | | 22. | Outside row. | |

Ten plants were planted per row in each block, plants 1'9" apart, rows 2'6" apart, blocks 3 ft. apart.

A preliminary trimming was given to the plants about one month after planting. Three cuts were taken and weighed on the following dates - February 10th, March 25th., and June 4th., 1936.

iv. The Results of the Experiment.

The row weights will be found in the following table, the three cuttings under 1, 2 and 3, and these columns totalled and compared to the parent selfed as the base of 100. No attempt has been made to work out probable errors or statistical significance, but the magnitude of the results and their consistency should indicate a practical significance.

TABLE VIII

Increase in Vigour on Crossing (General)

| No. | Plant | Cut 1. 10.2.36 | 2. 25.3.36 | 3. 4.6.36. | Total | S = 100 |
|---|---|---|---|---|---|---|
| (2). | | | | | | |
| 23 | 7/25 cross. | 2.6 | 5.6 | 4.2 | 12.4 | 137.7 |
| 24 | 7/25 self. | 2.0 | 4.1 | 2.9 | 9.0 | 100 |
| 25 | 7/25 cross. | 2.8 | 5.8 | 3.9 | 12.5 | 138.8 |
| 26 | 7/25 cross. | 2.9 | 7.1 | 4.4 | 14.4 | 160.0 |
| 27 | 7/25 cross | 3.4 | 6.9 | 4.9 | 15.2 | 168.8 |
| 28 | 7/25 cross. | 3.3 | 7.2 | 4.9 | 15.4 | 171.1 |
| 29 | 7/25 cross. | 3.5 | 8.0 | 5.1 | 16.6 | 184.4 |
| 30 | 75/24 cross. | 4.6 | 9.0 | 6.3 | 19.9 | 140.1 |
| 31 | 75/24 self | 2.9 | 6.1 | 5.2 | 14.2 | 100 |
| 32 | 75/24 cross | 3.4 | 7.7 | 5.3 | 16.4 | 115.5 |
| 33 | 39/9 self | 2.4 | 5.4 | 3.8 | 11.6 | 100 |
| 34 | 39/9 cross | 3.5 | 7.2 | 5.1 | 15.8 | 136.2 |
| 35 | 26/24 cross | 4.0 | 6.8 | 3.9 | 14.7 | 154.7 |
| 36 | 26/24 cross | 4.1 | 7.5 | 3.3 | 14.9 | 156.8 |
| 37 | 26/24 self | 2.6 | 4.7 | 2.2 | 9.5 | 100 |
| 38 | 26/24 cross | 4.5 | 8.6 | 4.7 | 17.8 | 187.4 |
| 39 | 26/24 cross | 6.6 | 10.0 | 4.0 | 20.6 | 216.8 |
| 40 | 26/24 cross | 5.5 | 8.7 | 3.5 | 17.7 | 186.3 |

| No. | Plant | Cut 1. 10.2.36 | 2. 25.3.36 | 3. 4.6.36 | Total | S = 100 |
|---|---|---|---|---|---|---|
| (3). | | | | | | |
| 23 | 73/12 cross | 2.3 | 5.9 | 5.9 | 14.1 | 152.5 |
| 24 | 73/12 self | 1.9 | 4.0 | 3.3 | 9.2 | 100 |
| 25 | 73/12 cross | 3.0 | 5.3 | 3.9 | 12.2 | 125.8 |
| 26 | 73/12 cross | 2.3 | 4.4 | 3.5 | 10.2 | 100 |
| 27 | 26/23 cross | 3.0 | 5.6 | 4.5 | 13.1 | 140.9 |
| 28 | 26/23 self | 2.2 | 4.2 | 2.9 | 9.3 | 100 |
| 29 | 26/23 cross | 2.9 | 5.6 | 3.3 | 11.8 | 126.9 |
| 30 | 26/23 cross | 2.8 | 5.5 | 3.6 | 11.9 | 127.9 |
| 31 | 111/21 cross | 3.1 | 6.5 | 4.2 | 14.8 | 125.4 |
| 32 | 111/21 cross | 4.6 | 6.6 | 4.8 | 16.0 | 135.6 |
| 33 | 111/21 self | 2.1 | 6.0 | 3.7 | 11.8 | 100 |
| 34 | 111/21 cross | 3.2 | 5.9 | 4.2 | 13.3 | 112.7 |
| 35 | 111/21 cross | 3.8 | 7.3 | 5.2 | 16.3 | 138.1 |
| 36 | 8/4 cross | 4.1 | 11.4 | 3.2 | 18.7 | 125.5 |
| 37 | 8/4 self. | 3.3 | 9.3 | 2.3 | 14.9 | 100 |
| 38 | 8/4 cross | 5.0 | 13.4 | 3.7 | 22.1 | 148.4 |
| 39 | 8/4 cross | 4.7 | 12.9 | 3.2 | 20.8 | 139.6 |
| 40 | | | | | | |

SUMMARY OF RESULTS:

| Plant Family. | Self. | Cross Average. |
|---|---|---|
| 7/25 | 100 | 160.1 |
| 75/24 | 100 | 127.8 |
| 39/9 | 100 | 136.2 |
| 26/24 | 100 | 180.4 |
| 73/12 | 100 | 139.1 |
| 26/23 | 100 | 131.6 |
| 111/21 | 100 | 127.9 |
| 8/4 | 100 | 137.8 |
| General Average | | 142.6 |

v. Discussion on results.

It will be seen that in every case the parent cross is higher yielding than the parent selfed. The crossed plants establish better, have a higher germination rate, are not so much affected by adverse conditions and continue growing at a fair rate until late in the season. Lucerne may be either cross pollinated or self pollinated in the field. Literature suggests that natural areas exist where selfing is dominant or where crossing is dominant, depending on the agencies available for crossing. New Zealand experience (Hadfield & Calder, (1936)) would suggest that of the florets that set seed, about 44 per cent. results from crossing and 56 per cent. from self fertilisation. The effect on a subsequent crop grown from seed as a result of open pollination is apparent. In-bred plants resulting from self pollination are slower to establish, are easily crowded out by weed invasion, and never reach the yield of the cross-bred plants. Fortunately, the very fact that crossed plants are more vigorous in yield, and seed setting ability (See later) tends to ensure their succession rather than that of the poorer selfed plants. If we allow, say, 6 seeds per pod under cross fertilisation, and 2 seeds per pod under self fertilisation, using the figures for percentage crossing above, we find that 70 per cent. of the seed produced, would be the result of crossing. In bred plants would produce fewer seeds than crossbred plants, resulting in their gradual elimination from the seed crop. It may be possible to isolate lines which are continually self pollinated, and use these in breeding work. Autogamous lucerne has been isolated by Kirk at Ottawa, Canada, but as yet no information has appeared, as to its comparative yielding ability, compared with normal lucerne. As, however, self-pollination has always been associated with a decrease in vigour in the offspring, the general tendency in breeding work has been to retain hybrid vigour by crossing. Inbreeding has been used, mainly as a "progeny test" of the original parent plants. The parents giving the best offspring are combined to give a better average material. These parents have been selected from areas of lucerne grown from commercial seed. It is indeed likely, that many of these plants so selected are the plants which exhibit the most hybrid vigour in the field. These plants may thus be very heterozygous, and no better for breeding work than the more average plant, which may be more homozygous. However, in a variety in which natural crossing is very high, it may be inferred that the best plants could be the result of the combination of most of the vigour factors, and other desirable factors present in the population of lucerne plants from which the seed was taken. It may not be possible to further improve such material by crossing, within the population from which it came, but it would be a very useful type of plant for crossing with other varieties. If, of course, we could make the plant homozygous for these vigour factors, we would have progressed a fair way in producing a high yielding variety.

Generally speaking, even the best lucerne stands consist of a mixture of types from the high producing prostrate types to the rather lower producing tall types. Consequently, selection of the best individuals, and concentration of their best qualities within a new crossbred line should give a marked increase in yield.

It may also be mentioned, that any agency assisting cross fertilisation in the field would greatly assist the breeder in preventing deterioration, which occurs in the offspring when crossbred lines are self pollinated.

## SECTION V.

### A COMPARISON OF THE SEED SETTING ABILITY OF PARENT PLANTS, AND THEIR INBRED PROGENY.

i. The Purpose of the Experiment.

Overseas literature suggested that inbred plants were poor seeds compared with the parent plants. Hadfield & Calder (1936) report that self-fertilisation gives plants which produce fewer seeds. In order to confirm these results, and to obtain some measure of this reduced fertility in inbred plants, the following experiment was designed

ii. The material of the Experiment.

The Experiment was carried out on plants of the Plant Research Station, which were allowed to go to seed. Four families of plants were chosen. In each family 3 - 5 parent plants were available, 3 - 5 plants from 4 of the L.1 progenies, and 4 plants from as many of the L.2 progenies as were available.

iii. The method of the Experiment.

A seed crop was harvested from the above four plant families on April 28th., 1936, and the bundles of seed stalks allowed to dry to be threshed during the writer's spare time. Unfortunately, the material was mixed by some outside person, making it impossible to continue the experiment.

iv. Discussion.

Although the actual experiment was a failure, some observations may be of interest. As a general rule the parents set most seed, the 1st., inbred generation (L.2) produced the least seed. There is however, very much variation in the seeding capacity of inbred plants, particularly in the "L.2" generation. Here plants were observed which set practically no seed, while others approached the parents in seeding capacity. The possibility of selection is thus apparent. In many cases the high seeders were poor yielders of green material and vice versa, though this cannot as yet, be considered general.

It is now realised that the experiment designed as above would not have given a very accurate indication of the seeding capacity of the plants. Given the opportunity in the future it is hoped to repeat the experiment, but rather on the following lines.

A number (as large as possible) of florets is selected on each parent plant considered, and also on a representative sample of L.1 and L.2 progeny. The percentage of florets setting seed, and the number of seeds per pod would give a better idea of seeding capacity under open pollination conditions than the measure of the total seed set on the individual plants or small groups of plants. Allowance would here have to be made for differences in number of flowers produced. This method has been used in New Zealand by Messrs. Hadfield & Calder, - see "Investigations relative to pollination and seed production in New Zealand". N.Z. Jour. Sc. & Techn. V. XVII. 4. 1936.

SECTION VI.

PRELIMINARY OBSERVATIONS ON STERILITY IN LUCERNE:

i. Conditions necessary for Seed Production.

Seed production in lucerne is dependent upon the following (Armstrong & White, 1935)

(1). Abundant viable pollen must be shed at the erect standard stage. (They distinguish four stages in the bud, prior to tripping, the straight bud stage, pointed bud stage, hooded bud stage, and erect standard stage. For further explanation see Article in Journal of Agric. Science, 1935)

(2). The stamenal column must be released from the keel (i.e., flowers must be tripped).

(3). In the act of tripping the column must strike the standard with sufficient force to rupture the surface cells of the stigma.

(4). After tripping occurs, the proper moisture relationship for pollen germination and pollen tube growth must be maintained to effect fertilisation

(5). After fertilisation the water metabolism of the plant must be such as to prevent ovule abortion.

ii. A General outline of Sterility.

Lucerne is normally (or perhaps preferably) cross pollinated, though much self pollination can occur (Piper, 1914, Hayes & Garber 1921, Yarashevsky, 1931, Jenkins, 1931, Torssel, 1931, etc.,). The progeny resulting from self-fertilisation usually show a marked reduction in self-fertility, and often in cross-fertility (Williams, 1931). Successive selfings cause a greater decrease in fertility until a stage is often reached in the 2nd., or 3rd., inbred generation, where the plant is completely self sterile, and often only slightly cross-fertile.

Sterility may be due to pollen sterility or sterility of the ovary, or some combination of both. It may be due to some heritable defect in the germ plasm (e.g., lethal factors), or to some structual defect in the pollinating mechanism, resulting in incomplete fertilisation.

iii. Pollen Sterility.

Armstrong & White, 1935, found that in three cases out of eight investigated, pollen sterility could be suspected as the cause of low pod setting. They considered that 70% or more of viable pollen was ample for complete fertilisation. They showed a definite correlation in the plants studied between the proportion of good pollen and pod bearing. Plants which had a high percentage of sterile pollen were shown to be deficient in amount of pollen as well, and had a high proportion of shrunken grains associated with faulty dehiscence of the anthers. Such defects, frequently result in the failure of the stigma to capture viable pollen grains between itself and the standard when tripped. They found in comparing parents with the 1st., and 2nd., inbred generation progeny, a definite correlation between pollen sterility and the number of seeds per pod, and also a definite segregation of a factor or factors for pollen sterility in the plants used.

Several lines of lucerne under observation by the P. R. Station, were tested by the writer for pollen sterility in parent and 1st., and 2nd., inbred generations. The method used was to introduce pollen grains into a drop of 10% sugar solution on a cover slip, and examine as a hanging drop (Bower & Gwynne-Vaughan "Practial Botany for Beginners). In all cases, germination occurred within 25-30 minutes, whatever the generation, and in no case was any degree of sterility noticeable. In one case (family 91/10/8)which is the 2nd inbred generation showed great sterility in crosses with other plants, the inbred pollen seemed slightly more vigorous than the parent plant. In all the families studied, from 95 to 100% of the pollen grains germinated and produced pollen tubesunder the conditions of the test. Sufficient pollen could generally be said to be present to effect fertilisation, though observation would tend to suggest a reduction in amount of pollen in inbred plants compared with the original parent.

Where the parent plant is a poor producer of pollen, the inbred plants are also poor producers. It is hoped to continue experiments on testing pollen, with observations on size and qualities of grains, and quantity of pollen produced during this coming season, 1936-7.

iv. Sterility in the female gamete.

Sterility caused by some lack in the female part of the flower or by some physiological disturbance in the flower has not been investigated as yet to the same extent as pollen sterility. Several cases may occur

(1) The ovule fails to develop, and the flower drops early in development.

(2) The ovule aborts subsequent to fertilisation. Here incompatibility or the presence of lethal factors concentrated during selfing cause non-fertilisation or abortion.

(3). Some physiological disturbance in the plant may

(a) prevent tripping.

(b) not cause the stigma to strike with sufficient force against the standard to rupture the stigma cells.

(c) not give the proper moisture requirement for germination of the pollen, and also the water metabolism of the plant after fertilisation may not be such as to allow normal development.

(4). It may also be supposed that a lack of vigour in both male and female plants may cause the failure of fertilisation, even where no incompatibility exists between the male and female germ cells.

During February and March last, an attempt was made to measure germination of pollen grains on the stigmas of parents and L.1 and L.2 generations in families exhibiting sterility. However, no germination was obtained on any stigma, either due to faulty technique or to the generally poor seeding season during 1935-6. Cotton blue stain was used to show up the pollen grains, and also the pollen tubes through the stigma. The value of the stain could not be determined due to lack of germination of the pollen tubes.

Armstrong & White, 1935, used a staining solution of lacto-phenol, containing an acid fuchsin light green stain. The stain was made up of 8 parts of 1% aqueous acid fuchsin and 2 parts of 1% light green in 95% alcohol. Sufficient stain added to lacto-phenol to give it a deep colour. When the pistil was flattened slightly the pollen tubes could be traced through the stigma. This method will be tried out next season, and if possible while the flowers are in bloom about January.

It can be suspected that it is either lethal factors, or else that the plant cannot provide adequate facilities for fertilisation which are mainly responsible for sterility in inbred lucerne at Palmerston North.

SUMMARY:

1. Experiments on the technique of lucerne cross pollination.

Several methods of emasculation of lucerne flowers, and their effect upon subsequent pollination were compared. Greatest efficiency was obtained by removing the standard and blowing away the surplus pollen. It is likely that this greater efficiency is due to the less chances of rupture of the stigma cells compared with florets in which the standard is left. Washing the pollen off was impracticable and adversly effected pollination.

2. A measure of loss of Vigour on Selfing.

The experiment using cuttings turned out very unsatisfactorily, and demonstrated to the writer the difficulty of securing representative and comparative samples of plants by this method. A loss of vigour occur from parent to 1st., selfed generation, but no further loss from 1st., to 2nd., selfed generation.

Using plants grown from seed in the next experiment, there was a successive loss of vigour from parent to 1st., and 2nd., selfed generation, of the order 21.6% from parent to 1st., selfed generation, and 24.3% from 1st., selfed to 2nd., selfed generation.

3. A measure of return in vigour and crossing.

Recovery of vigour (expressed by yield) on crossing selfed progeny was complete when compared with the parent cross. It may be suggested that crossings between specially selected plants of both 1st., and 2nd., selfed generations would give a higher yield than the parent cross.

4. A Comparison of Seed Setting Ability.

Preliminary observation would support the belief of a general reduction in seed yield on selfing. As yet no statistical results have been obtained.

5. Sterility in Inbred plants.

No cases of pollen sterility were recorded in families of lucerne tested at Palmerston North during the 1935-6 season. Investigations are proceeding on other aspects of sterility

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