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

L. M. Driver.

DURING THE PERIOD - AUGUST 1934 TO SEPTEMBER 1936.

TABLE OF CONTENTS:

		PAGE.
SECTION I.		1.
i.	Introduction	1.
ii.	A General Outline of the main experiment.	5.
iii.	The Source of Material	6.
SECTION II.		
	EXPERIMENTS AND OBSERVATIONS ON THE TECHNIQUE OF LUCERNE CROSSING.	8.
i.	How pollination is effected	8.
ii.	The Reason for the Experiment	9.
iii.	A Description of the Methods of Emasculation	10.
iv.	The Source of Material.	11.
v.	A Comparison of the Efficiency of several Methods of Emasculating Lucerne Flowers.	12.
	A The method of the Experiment	12.
	B The results of the Experiment	12.
	C Discussion on the results	12.
	D Conclusions from experiment	14.
vi.	The Effect of the Method of Emasculation upon the subsequent pollination.	15.
	A The purpose of the Experiment	15.
	B The method of the Experiment	15.
	C The results of the Experiment	15.
	D Discussion on the Results.	16.
	E Conclusions from results.	18.
SECTION III.		19.
	A MEASURE OF LOSS OF VIGOUR ON SELFING LUCERNE PLANTS FOR TWO GENERATIONS.	

SECTION III (continued) PAGE.

i. The purpose of the Experiment 19.

ii. The material of the Experiment 19.

iii. The method of the Experiment 22.

iv The results of the Experiment 25.

v. Discussion of Results. 27.

SECTION IV 29.

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

i. The purpose of the Experiment 29.

ii. The material of the experiment 29.

iii. The method of the Experiment 31.

iv. The results of the Experiment 33.

v. Discussion on results 35.

SECTION IV.A. 39.

A GENERAL COMPARISON BETWEEN YIELDS FROM CROSSBRED AND INBRED LUCERNE PLANTS.

i. The purpose of the Experiment 39.

ii. The material of the Experiment 39.

iii. The method of the Experiment 40.

iv. The results of the Experiment 41.

v. Discussion on Results. 43.

SECTION V.

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

i. The purpose of the Experiment 45.

ii. The material of the Experiment 45.

iii. The method of the Experiment 45.

iv. Discussion on the Experiment 45.

PAGE

SECTION VI 47

PRELIMINARY OBSERVATIONS ON STERILITY IN LUCERNE (MEDICAGO SATIVA)

i. Conditions necessary for seed production 47

ii. A general outline of sterility 47

iii. Pollen sterility 48

iv. Sterility of the ovary 49

SUMMARY 51

LIST OF REFERENCES: 52

SECTION 1.

1. 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	
	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 heterzygosity 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

SECTION III (continued) PAGE.

i. The purpose of the Experiment 19.

ii. The material of the Experiment 19.

iii. The method of the Experiment 22.

iv The results of the Experiment 25.

v. Discussion of Results. 27.

SECTION IV 29.

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

i. The purpose of the Experiment 29.

ii. The material of the experiment 29.

iii. The method of the Experiment 31.

iv. The results of the Experiment 33.

v. Discussion on results 35.

SECTION IV.A. 39.

A GENERAL COMPARISON BETWEEN YIELDS FROM CROSSBRED AND INBRED LUCERNE PLANTS.

i. The purpose of the Experiment 39.

ii. The material of the Experiment 39.

iii. The method of the Experiment 40.

iv. The results of the Experiment 41.

v. Discussion on Results. 43.

SECTION V.

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

i. The purpose of the Experiment 45.

ii. The material of the Experiment 45.

iii. The method of the Experiment 45.

iv. Discussion on the Experiment 45.

PAGE

SECTION VI 47

PRELIMINARY OBSERVATIONS ON STERILITY IN LUCERNE (MEDICAGO SATIVA)

i. Conditions necessary for seed production 47

ii. A general outline of sterility 47

iii. Pollen sterility 48

iv. Sterility of the ovary 49

SUMMARY 51

LIST OF REFERENCES: 52

SECTION 1.

1. 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	
	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 heterzygosity 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

not this vigour which had been apparently lost was still present in the plants, and could be recovered by combination or two or more inbred lines. Crossing had been resorted to between some inbred lines to save them from extinction, but no comparisons had been made between these crosses and the original parents.

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 F 1 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. SOURCE OF MATERIAL:

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.