

L U C E R N E .BREEDING METHODS.

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A breeding project directed towards the type improvement of lucerne was commenced at the Plant Research Station in 1931. Such a proposition demanded a preliminary trial of numerous regional strains. Before proceeding, therefore, to discuss actual breeding methods I should like to give a brief outline of the origin, distribution and peculiarities of these various forms,

Lucerne is supposedly a "native of the temperate regions of Western Asia, the countries south east of the Caucasian range and the north western part of modern Persia." It was apparently confined to this area for a considerable period since, according to chronological records, its migration did not commence until about 500 B.C. Subsequent to this period, its distribution was influenced by the spread of civilization. It penetrated as far east as China, along the old caravan routes; it followed in the wake of the victorious Persian, Greek and Roman armies; it was transported to Mexico and South America by the Spaniards; and it was introduced into the more recently colonised countries by the early pioneers. To-day its dispersal is practically world-wide and is even yet being extended.

This universal distribution has been effected almost entirely by human agency, a fact which must constitute concrete evidence as to its extreme value as a forage crop,

Despite this acknowledged worth, though, the cultivation of lucerne could not have become so widely adopted had it not been for its great adaptability. Transported to regions where conditions differed considerably from those of its original source, it was able to survive but in many instances this survival was accompanied by a change in type. Forms were evolved which were better able to thrive in these foreign environments than those originally introduced. This presupposes that the original ancestor must have possessed a genetic complex with a wide range of potential characters and, when environmental forces of different intensities were imposed upon it, ^{together later with hybridization} the required variations more in harmony with the different conditions, were produced,

There exists, therefore, to-day a number of different types of lucerne, These have recently been classified in some detail but may be grouped roughly into the following classes:-

1. Prostrate forms: the se are winter dormant and are able to survive cold continental winters or rigorous mountain conditions. The Ladak variety and some Turkestan strain; are included in this group.

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2. Semi-erect forms: These are less hardy and are grown more extensively in temperate zones. They are probably the most widely-cultivated types, examples of which are Marlborough, South African, Hunter River, Provence, various strains of Grimm Baltic Cossack, some Canadian and American variegated strains and many others.

3. Erect forms: are non-hardy, are rapid growers, and have preference for high temperatures, Arabian and Peruvian varieties are typical representatives.

According to Bordakov, a Russian scientist who has been responsible for a recent classification of Lucerne, *Medicago falcata*, or the yellow flowered species may be grouped similarly,

Such information is directly relevant whenever it is proposed to effect an improvement in type by breeding or selection; it enables the investigator to choose that material which is likely to be the most suitable for his project.

MATERIAL. Through the courtesy of the Principal of Marlborough College, New Zealand, the Plant Research Station was fortunate in being able to obtain a collection of types introduced from America by the late Wm. Purdie. This series included such varieties as Grimm, Canadian and American variegated forms, Ladak, Baltic, Cossack, Turkestan, Arabian, Peruvian and others. Together with a number, of Marlborough strains secured direct from Blenheim these formed the bulk of the original breeding material and represented fairly typically the different growth habits of the lucerne species.

This collection was propagated as single plants and during establishment and at full development observations were made in an attempt not only to determine the most suitable varieties but also to select from within these varieties those plants showing superior qualities.

The behaviour of the different types was of interest in that each maintained its own distinct characteristics. The prostrate and semi-erect forms, developed in zones which experience severe winter conditions, were inclined to be spreading in habit and gave good summer yields but, being winter dormant, had a limited growing season; the erect types, originating from warmer climates, were upright in growth, quick in recovery and had an extended season of productivity but their permanency was limited and the yields of the individual cuts were not exceptionally high; while the Marlborough variety a regional strain of the semi erect type, developed naturally-by long sojourn in the province from which it takes its name, was semi-erect to erect in habit, was fairly rapid

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in recovery, had a prolonged growing season, gave good yields and was in fact superior to all other forms under local conditions. Recent trials indicate that the Hunter River and South African types are somewhat similar to the Marlborough form in their behaviour and may serve equally as well as sources for breeding material,

The accompanying graph indicates the relative seasonal productivity of a number of varieties during the period April 1932 to March 1933.

The performances of the various types may be summarised as follows:-

1. A lucerne strain evolved to meet the demands of a particular environment will, if transferred to a region differing from its former habitat, strive to retain those special characteristics through the development of which it had been able to adapt itself to its original surroundings. The period necessary for any change to occur would depend principally upon the degree of contrast between the exchanged environments and consequently between the type of plant essential to each and upon the variability potentiality of the species.

The Marlborough variety originating from a type somewhat similar to itself has taken only from 40-50 years to express upon itself characteristic features. It is obvious that, under similar conditions, such a form as Ladak would take a much longer period to become transformed into a Marlborough type.

2. A lucerne strain introduced from a region where external influences are somewhat similar to its own would probably be of more value in the new environment than any which may have been imported from localities subject to more extreme conditions. A winter dormant form removed to a more temperate zone will during the winter remain dormant; thus its season of productivity is rather limited. A non-hardy type, on the other hand, may under similar conditions, continue to develop even after experiencing a few degrees of frost but is disposed not to be permanent and does not yield particularly well. Regarded generally, varieties such as Hunter River, South African and Provence more nearly approach the superiority shown by the Marlborough type for New Zealand conditions than do the winter dormant or non-hardy forms,

3. A lucerne strain developed naturally is not absolutely homogeneous. Among the numerous lines planted out there were varying degrees of uniformity. Although each variety was characterised by some particular features the individuals within a variety varied considerably. Some plants possessed broad leaves, some narrow; some had open foliage others dense; the stems of some were coarse, those of

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others fine; although adhering to a definite type the plants differed extensively among themselves.,

From these preliminary investigations then it was evident not only that it might be possible to effect an improvement in type but also that the Marlborough strains were more suitably adapted to New Zealand conditions than any imported variety,

As a consequence the largest proportion of plants selected for their individual performances were chosen from this variety. Prior to their selection, though, the question arose as to what constituted an ideal type. It was decided finally that whether for hay or for grazing the chief aim should be a high yield to which should be coupled if possible leaf density, rapid recovery, disease resistance and permanency; a rather ambitious standard perhaps but one to which it seemed possible to adhere. Only those plants approximating this ideal were retained for breeding purposes; the rest were discarded.

But the apparent performance of a plant is no measure of its breeding potentialities. Considered as an individual it may be eminent but, until bred from, the probable nature of its offspring cannot be predicted. It is conceivable that if these untested selected plants were bulked together and a seed crop taken from them, the first generation may be an improvement on commercial strains but it would be very unlikely that in future generations this superiority would be maintained. Before any selected plant is utilised for further breeding work some indication has to be ascertained as to its genetic constitution. This is effected by inbreeding.

INBREEDING. In a self fertilised plant generations of inbreeding have brought about a state of complete homozygosity and except where mutations arise the form of the progeny is uniform and similar to that of its parent. This is not the case in a cross fertilized plant. Here the formation of the zygote is dependent upon the union of two gametes whose genetic constitution will invariably be unlike in some respect. This induces heterozygosity, a condition which affords opportunity for the accumulation of vigour factors and for the masking of undesirable characters, usually recessives, by the action of dominance. Thus the true constitution of a cross fertilized plant cannot be ascertained from its appearance..

Self fertilization or inbreeding of plants, normally cross fertilized, however, has the effect of exposing to some extent the make-up and is used extensively in the breeding of such individuals. The process itself tends to increase the proportion of homozygous factor pairs and to decrease heterozygosity.... These effects are reflected in the inbred progeny in the following ways:-

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1. Undesirable characters are exposed; inbreeding results in their segregation in a homozygous condition and allows them to be expressed.
2. Uniformity of type amongst the separate segregation is secured. Continued inbreeding increases the proportion of homozygotes which remain as such and tend therefore to establish uniformity.
3. Vigour is reduced among the inbred lines, This is due probably to the loss each may sustain, through the segregation of some of the factors on which maximum vigour depends.
4. The degree of self sterility is generally increased with each succeeding inbred generation.

The character and degree of the variation of the inbred progeny is a representation of the constitution of the parent and serve to evaluate its breeding possibilities. Thus some cross fertilized plants on selfing may give rise to a comparatively uniform progeny; in such a case if the type be good the plant itself or some of the offspring may serve as useful parents for further breeding work. Conversely other plants may exhibit extreme variability; these should be eliminated.

In lucerne, fertilization is brought about as a result both of cross and of self pollination. For breeding purposes, then, selected individuals should be subjected to an inbred progeny test.

From our original material about 250 plants had been selected for some particular quality. Each of these were forced to undergo self pollination by being enclosed within a ~~carcase cloth~~ cover. The resulting families were grown together in groups for comparison. Some closely resembled their parents and showed little variability; others exhibited much inconsistency in the habit and form of the separate plants; while a few were composed of nothing but dwarfed or subnormal individuals. During this comparative study of the L1 material, outstanding individuals among the most satisfactory families were noted; these were selfed and although the increase in the degree of self sterility was very marked, sufficient seed was obtained from most plants. This provided the second inbred generation which is now under observation. In both generations there was in most families an obvious reduction in vigour relative to that of the parent plant and none of the families inbred to the second generation have this season outyielded their contiguous controls. An outstanding feature in this second inbred generation was the remarkable degree of uniformity among many of the families, some certainly varied to quite an extent but the larger proportion was extremely uniform,

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The utilization of this material for type improvement will now be discussed.

As breeding work with plants is necessarily rather a slow process it is perhaps advisable to attempt to produce partially improved strains during the progression towards the development of that product which should represent the consummation of the breeder's efforts. Such a system has been adopted at the Plant Research Station. The work is advancing by stages and it is proposed to introduce in succession a series of improved types of which the later ones should be superior to the earlier forms. It may be impossible to produce a strain where each individual plant is able to maintain an enhanced superiority over any appearing in commercial lines but it is more probable that a form may be raised which will contain a greater proportion of satisfactory plants than is exhibited in most varieties at present on the market.

This is being attempted by the following methods:-

1. Interpollination of those originally selected plants which produced satisfactory L1 progeny.

It has been mentioned above that about 250 selected plants of the original material were selfed and their families studied and compared. From the behaviour of these L1 progenies it was possible to eliminate all those selected plants whose constitution had proved to be unsatisfactory. Of the remainder about 30 of the best, regardless of type, were transplanted together in a block and allowed to inter-pollinate. The seed was harvested and later used to form a second seeding area. This second crop on establishment consisted of rather a mixed population of plants, a condition which had been anticipated, however, as the parents concerned were themselves so different in type and had undergone no test as to their breeding behaviour when crossed one with another. Despite the presence of a few poor plants the crop was quite satisfactory judging from the performance of a normal lucerne stand. Strict roguing has been and will still be carried out to eliminate undesirable plants. The remainder will be left to seed together and should provide the first slightly improved material.

2. Combination of the original parents and their offspring subsequent to testing by selfing and controlled crossing.

About 45 L1 families were retained after having been under observation for two seasons. During that period, as has been mentioned previously, the best plants, about 200, within these families had been noted and were ultimately selfed. The seed from these produced the second inbred generation. Again after a studied comparison it was possible to discard a number of families leaving only comparatively few with which to continue the breeding work.

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The methods of proceeding with these were somewhat limited for the reasons that:-

a. None of the families inbred to the second generation excelled in vigour that of the Marlborough controls. This can be seen from the following figures which indicate the average weight per plant within 10 Marlborough control plots compared with that within 20 inbred lines taken at random.

cuts	<u>Marlborough</u>	<u>Inbred lines</u>	<u>Best Inbred</u>
	<u>Control</u>		<u>Line</u>
	<u>Av. wt. of plant.</u>	<u>Av. wt. of plant.</u>	<u>Av. wt. of plant.</u>
1st cut.	1.12 lbs.	0078 lbs.	1.32
2nd cut.	1.16 lbs.	0.77 lbs.	1.05
3rd cut.	1.14 lbs.	0.82 lbs.	1.20

Loss of vigour has reduced the average weight of plants originating from selected material to below that of even an ordinary commercial line. It seemed futile, therefore, to attempt to develop an improved strain from merely an inbred line.

b. It has been suggested by other workers that, though synthesis of selected inbred plants will restore vigour to some extent, certain valuable characters may have been lost through being associated with the discarded segregates.

To avoid these difficulties the original parents themselves which had produced favourable L1 and L2 progeny are at this stage being utilised in preference to the inbred material. They are to be combined, according to type, for the formation of composite strains, but before this is proceeded with the interaction of one upon the other has to be determined. This is being done by the process referred to as diallel crossing. The selected parents, depending on whether the type has been erect, semi-erect or prostrate have been divided into three groups each of which includes 6 individuals. Within each group the plants have been crossed one with the other; their progenies have yet to be studied and compared and representative individuals again selfed or intercrossed. From the behaviour of the families of both generations it should be possible to ascertain, in each particular group, which of the selected individuals or which of their progeny should form the nuclei for further improved composite strains. These should be superior to the first endeavour as the parents included will have been subjected to stricter examination.

3. The use of inbred material for strain building purposes.

By inbreeding, undesirable characters are unmasked during segregation and are able to be eliminated by selection. Thus, theoretically it could be assumed that in a few generations a uniform line of good plants suitable to form the foundation of an improved strain could be evolved; practically

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this is not so. If after the first one or two generations the degree of self sterility has not increased to such an extent as to prevent the production of further generations, it is almost certain that vigour reduction will already have been expressed,, There is little chance of raising an improved strain merely from an inbred line,

If we assume that vigour reduction has been due to the expression of deleterious recessive factors in a homozygous condition and to the segregation of Factors for vigour, then, by eliminating the poorest families, those that are retained should possess the more desirable characters. Due to an incomplete complement of vigour factors though, none of these families are as productive as the original parent despite the fact that they have been rid of some undesirable traits. The following figures indicate the average yield per plant of some L2 families from three cuts taken during the past season.'

39/9/2 = 1.09 lbs.	99/3/8 = 0.86
39/9/5 = 0.64 "	99/3/10 = 0.73
39/9/11 = 0.70 "	99/3/12 = 0.85
39/9/14 = 0.67 "	99/3/13 = 0.80
39/9/20 = 0.55 "	99/3/15 = 0.78
53/18/2 = 0.59 lbs.	111/21/1 = 1.14.
53/18/8 = 0.53 "	111/21/10 = 1.19
53/18/11 = 0.38 "	111/21/18 = 0.91
53/18/15 = 0.40 "	111/21/21 = 1.07
53/18/18 = 1.23 "	111/21/22 = 1.04
91/10/7 = 0.73	
91/10/8 = 0.84	
91/10/10 = 0.62	
91/10/14 = 0.63	
91/10/20 = 0.89	

The yields under similar conditions, of the respective parents of these families were not obtainable but that of the Marlborough commercial controls = 1.14 lbs. There has evidently been a segregation for vigour accompanied by a consequent reduction in yield. If the poorest of these families are discarded it may be possible by a synthetic building up of the remaining lines to recombine most of the characters necessary for full vigour but three possibilities militate against the chances of ever securing a maximum permanent improvement.

1. Some desirable factors may be linked with those responsible for the rejection of the discarded families; they are, therefore, for all practical purposes, unattainable.
2. When two unrelated inbred strains are crossed, any increased vigour which may be observed in the progeny is due to a greater accumulation of genes, influencing size and vigour favourably than was present in either of the parents.

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The probable linkage of such genes to others that influence vigour adversely, minimises the possibility of securing a maximum complement of desirable vigour factors. only.

3. Even if it were possible to establish a germplasm containing a large proportion of the most beneficial vigour factors they still might be in a heterozygous condition and the initial improvement would be merely a temporary one,

With due regard to these facts it is proposed, nevertheless, to combine members of the best of the inbred families first in pairs to test their mutual reaction and then, systematically, in order to recombine if possible a large number of favourable vigour factors in a homozygous condition.

These are the methods then which have been adopted in the endeavour to secure an improvement of type in lucerne. Should they be successful to the extent that forms are produced which in the trial plots exhibit a superiority over commercial lines strict comparisons under field conditions will still be necessary; not until they are proved definitely to be superior will they be released into commercial channels.

Graph
Seasonal Behaviour of Lucerne Varieties
 1932-33.

