COMBINING ABILITY OF RED CLOVER CLONES

AND A BREEDING METHOD

(Suggested short title)

A STUDY OF THE COMBINING ABILITY OF

RED CLOVER CLONES AND THEIR USE IN A BREEDING PROGRAM

by

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A THESIS

Presented to the Faculty of Graduate Studies
and Research of McGill University in partial
fulfilment of the requirements for the degree
of -

DOCTOR OF PHILOSOPHY

May, 1955

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INTRODUCTION

Red clover, *Trifolium pratense* L., belongs to the family Leguminosae. It is one of that group of plants called the legumes which are so important in agriculture especially when the feeding of animals is the major enterprise. Here the legumes excel, not only because of the superior forage which they are capable of producing, but also because of their ameliorating effects on the soil. Red clover is a very worthy member of this group of plants and according to Clark and Malte (1913) "no other forage plant has been so important to agriculture as has red clover."

That statement is certainly true with reference to Quebec. An examination of the agricultural statistics for the province of Quebec reveals that over sixty per cent of the total acreage is devoted to hay and pasture crops. Of the hay crop acreage the greater proportion - possibly upwards of eighty per cent - has red clover as the principal seeded legume. Much the same is true with pasture seedings where again red clover may be the principal seeded legume.

Red clover is a comparatively new introduction to agriculture. It is known to have been grown in the Netherlands in the sixteenth century, was recorded in English agriculture in 1645 and is reported as having been grown in Rhode Island in 1663. According to Darlington and Ammal (1945) red clover has its centre of origin in Europe, West Africa and Algeria. From the New England States it no doubt spread into Canada.

However, unlike other agricultural crops, attempts at improvement of red clover are comparatively recent. Pieters and Hollowell(1937) record that in 1906 the Tennessee Agricultural Experiment Station selected a strain of red clover resistant to Colletotrichum trifolii S.M.Bain. They also report that a very intensive program was initiated by the U.S. Department of Agriculture in 1928. In Canada, and particularly, at Macdonald College a red clover breeding project was begun in 1911. Another program was initiated at the Central Experimental Farm, Ottawa, early in the century, while work has also been carried on at the Ontario Agricultural College. However in spite of these programs practically all of the seed used in Canada is of the commercial grade and not the product of deliberate breeding.

There seem to be two main reasons for this situation:

(1) A very real problem exists when attempting the large scale production of pedigreed seed of a red clover variety or for that matter of any herbage species which is cross-pollinated, red clover is entomophilous. This difficulty is being overcome gradually in Canada by the Canadian Forage Seeds Project and in the United States by the National Foundation Seeds Project.

(2) The improved strains of red clover resulting from the breeding programs have on the whole been rather disappointing and in most instances are but slightly - if at all - better than commercial seed stocks. Red clover is not only entomophilous but is also almost completely self-sterile and hence is extremely heterogenous. This extreme heterozygosity makes it almost impossible to select a plant which will breed true - a plant is not an entity as such and its progeny may bear no phenotypic relation to the parent selection.

It is this second feature which provides the background for this study, namely, to investigate certain aspects of the breeding behaviour of red clover and then to set down a method of breeding red clover which may be capable of producing a variety of red clover sufficiently superior to existing commercial stocks to warrant its release and introduction for large scale production.

REVIEW OF LITERATURE

Growth habit of red clover

According to Wilsie (1951) in the United States, red clover is a biennial or short lived perennial. There is in fact a good deal of conflicting evidence on the longevity of red clover. Gray's New Manual of Botany seventh edition (1908) lists *T.pratense* as a perennial, while the eighth edition (1950) lists it as a biennial or short lived perennial. Marie-Victorin (1935) in his Laurentian Flora gives red clover as "plante vivace". Again Flint (1887) an early agricultural worker in the New England States wrote that red clover could be made to last for three or four years. Recently Hollowell (1952) stated that in one instance when red clover was introduced for the first time into an area in the western United States and grown for seed production, the stand survived for three or four years. However, after it had been grown for a number of years it gradually assumed the characteristics of a biennial.

Crop classes of red clover

Williams (1927) at the Welsh plant breeding station made one of the first comprehensive studies of red clover. He grouped it into three classes;

(1) wild red clover

(2) early flowering red clover

(3) late flowering red clover.

These three types differed markedly not only in botanical characteristics but also with respect to their cropping capabilities, persistency and other agronomic characters.

Pieters (1928), Pieters and Hollowell (1937) and Hollowell (1940) recognized, in North America, the two classes (2) and (3) of Williams' classification. They also referred to these as "early or double cut" and "late or single cut". These classes are also referred to as "medium red clover" and "Mammoth red clover" respectively - which naturally leads to nomenclature confusion. When the terms double cut and single cut are used they have reference to the fact that the one class is capable of producing two cuts of herbage in a normal growing season, while the other will produce only one cut of herbage.

Bird (1948) stated that "the early or double cut type of red clover is the most commonly grown in Eastern Canada". He recognized five fairly discrete growth types of red clover as occurring in the early or double cut class of red clover in Quebec. These he called Types O, I, II, III and IV. They ranged from the Type O which produces a rosette and no flowers in the year of establishment to the Type IV which does not produce a rosette but has flowers and produces mature seed in the year of establishment. The Type 0 being typical of late or single cut while the Type IV is considered typical of an early or double cut red clover.

Bird (1948) associated differing degrees of winter hardiness with the different growth types. His data showed that the late type was superior to the early type for winter survival. For the period 1931-1946 he recorded the percentage survival on all nursery plantings, the pertinent data were:

late or single cut type ...... 62 per cent

early or double cut type ..... 43 per cent.

Fertility relationships of red clover

The earliest record of fertility relationships in red clover is that of Darwin (1876) who found that one hundred flowers when covered did not produce a single seed. De Vries (1877) tried artificial self-pollination and agreed with Darwin although he apparently obtained one fertile plant. Westgate et al (1915) reviewed early work on this subject and concluded that red clover was practically self-sterile. Malte (1921) agreed with Westgate et al.

Fergus (1922) while showing that red clover was nearly self-sterile did succeed in maintaining one inbred line for six generations before it was lost.

Kirk (1925) reporting on his study of red clover fertility carried on in Saskatchewan concluded that red clover was self-sterile but that self-fertile lines could be found. Williams (1925) reported on his extensive red clover studies in Wales in which he used various schemes for artificial self-pollination including hand-pollination, bees and rolling heads between fingers. He concluded that "a limited number of plants occur which are slightly self-fertile" but that "red clover is in the main practically self-sterile." Williams also demonstrated that the stage of maturity of the flowers was related to the apparent self-fertility of the plant. He obtained the data in Table 1.

Table 1. Amount of self-fertility in red clover at three stages of flower maturity (extract from Table IV in Williams)

	Bud stage	Early bloom	Late bloom
Seeds per 100 florets	2.87	0.82	0.09

This difference he attributed to the fact that the slow growing pollen tube had a greater chance to reach the ovule and fertilize it before disintegration when bud-pollinated rather than later in maturity. Mid-season or end of season self-fertility, which had been demonstrated in certain species of Nicotiana, was not found in red clover.

In a later paper, Williams (1931) outlined the mechanism for self-and cross-sterility in red clover. He concluded that the sterility of red clover followed the same genetic system as that postulated for Nicotiana by East and Mangelsdorf (1925). According to this theory self- and cross-sterility is controlled by a series of multiple alleles - called the "S" series - which regulate the pollen tube growth in the stylar tissue. Thus a plant with a constitution S_1S_2 is self-sterile and cross-sterile with any plant having the same S alleles but cross-fertile with any other plant having at least one different S allele, e.g., S_1S_3, S_1S_4, S_2S_3, or S_3S_4. With respect to red clover, out of 416 inter-plant crosses all were cross-fertile except 11 matings which Williams states were likely due to an unhealthy condition of these plants. In addition one plant showed a high degree of self-fertility. He concluded that this plant must be carrying a factor for self-fertility which he called S_f. This particular plant had a mean of 48.9 seeds per 100 florets based on three years data.

Williams and Silow (1933) reported that a very low percentage - 0.10% - of self-fertilization was effected by pseudo-fertility, i.e., pollen tubes carrying the same S alleles as the stylar tissue were able to penetrate and achieve fertilization of the ovule. Williams W. (1948a) demonstrated that homozygous genotypes of the constitution S_1S_1, S_2S_2, S_3S_3, etc., were obtained as a result of pseudo-fertility. He also showed that the self-fertility allele S_f belonged to the allelic series S_1, S_2, S_3 ....S_n. Williams W. (1948b) further reported a study of the frequency of the S alleles. Out of twenty-five unrelated plants of English late red clover, he isolated forty-one different S alleles,while out of twenty English broad red plants, thirty-seven of the possible forty S alleles were shown to be different. Thus there appears to be a reasonably good chance of cross compatibility in unrelated plants. Rinke and Johnson (1941) also isolated the self-fertility allele S_f during the course of their investigations on red clover in Minnesota.

Methods of breeding

Not all the possible methods of plant breeding will be discussed here but only those applicable to cross-pollinated crops. Methods which have been proposed by various workers and employed in breeding programs - not necessarily with red clover - include the following six main techniques.

Mass selection

(1) Under natural conditions

This means literally the survival of the fittest. It implies that naturally occurring phenomena (this could be considered to include a management regime peculiar to an area) acts upon the population to effect a selection of those plants which are best able to survive the conditions. Practically all of the locally adapted strains of red clover have been produced in this way. Thus if a farmer produces his own seed for a number of years from a continuing stock he will eventually produce a strain which will have certain characteristics as a result of the environment. Steppler and Lachance (1955) investigated twenty-three local farmer produced stocks of red clover from Quebec and found that they could be grouped according to the region in which they had been produced. Nilsson-Leissner and Nilsson (1940) list sixteen different strains of red clover which are adapted to the various regions in Sweden and which have arisen through natural selection. Pieters and Hollowell (1937) indicate that this is one of the more common methods of improvement for red clover. Varieties which have arisen in this manner in the United States include Emerson and Scott.

(2) <u>Under artificial conditions</u>

As soon as a crop is grown under unnatural conditions, e.g., spaced plants as opposed to solid seedings - then it might be considered as being under artificial conditions. Generally in addition to being grown under these unusual conditions the population is also subjected to an artificially induced selection pressure. This may take the form of an induced disease epidemic or insect pest infection in order to speed up the elimination of the susceptible plants. Thus a chance infection under natural conditions is replaced by an induced one under artificial conditions. For example the Swedish variety Karaby has some resistance to stem nematode and is the product of natural selection - more recently Bingefors (1952) has developed a technique for creating an artificial infestation of the stem nematode to be used in a mass selection program under controlled conditions. A technique similar to this but for the selection for resistance to Sclerotinia is being used in the breeding program for red clover at the University of Maryland - Ronnigen (1952) - and in the program at the U.S. Department of Agriculture at Beltsville, Maryland - Kreitlow (1952). It is significant that out of thirty-five improved varieties of red clover listed by Hall (1948) twenty-six of these were local strains developed by natural selection, four were by mass selection, while five did not have any breeding method clearly indicated. Either method of improvement will effect a change in the characteristics of the material. However, this is very slow and provides the breeder with little opportunity of evaluating and hence removing from the population those plants with an undesirable genotype.

Use of inbred lines

One of the outstanding advances in plant breeding was the utilization of inbreeding in the improvement of corn (Zea mays L.). As a result of the success obtained with corn it was natural that the same technique should be tried with other genera including red clover. It has been pointed out that red clover is largely self-incompatible and hence virtually impossible to inbreed on a large scale. However, Kirk (1927) felt that "controlled pollination with selection in self-fertilized lines provides a logical mode of attack in the systematic breeding". Williams (1925) on the other hand stated that inbreeding could not be employed "with any measure of success". Later he said (Williams, 1937) that "from the standpoint of crop improvement self-fertility in red clover is an undesirable feature as inbreeding invariably results in a very marked and progressive loss in vigour". Wexelson (1945) stated that in the course of his investigations of Norwegian red clover "no line resistant to inbreeding depression has been found".

Rinke and Johnson (1941) outlined a method of improvement for red clover which involved inbreeding. They proposed the introduction of the S_f allele into the selected plants, inbreeding to obtain the desired degree of homozygosity and then finally selection of progeny to eliminate the S_f allele. The resultant lines to be used in the synthetic variety. There are, however, factors other than loss of vigour and self-incompatibility which would make it very difficult to utilize the inbred line - heterosis technique in red clover breeding. Among these factors are (1) red clover is insect pollinated, and (2) red clover is a very short lived perennial which must be maintained either vegetatively or by seed - a much more difficult task in an insect pollinated plant than in a wind pollinated species. A search of the literature does not reveal any improved variety of red clover which has been produced by utilizing inbreeding. In support of this Wexelson (1952) said with reference to the S_f allele "it may be possible to transfer this fertility to larger material but as far as I know this has not been done in breeding experiments."

More recently Torrie et al (1952) have reported from Wisconsin on the effects of sibbing - a modified form of inbreeding - in red clover. The variety F.C. 13274 produced at Wisconsin by Torrie is the result of combining several sib-pollinated lines arising from controlled crosses between mildew resistant plants. While this variety is superior to other improved varieties of red clover regarding mildew resistance, nevertheless, at Macdonald College it is inferior with respect to forage yield to the varieties Ottawa Red, Dollard, Kenland and Emerson - Steppler (1953). Torrie noted that the yield of the synthetic was superior to that of the lines which had been sibbed for three generations. In addition he demonstrated by the polycross progeny test that there were differences in combining ability between sibbed lines.

Strain building

Stevenson (1939) defined the method of breeding called

Strain Building - a term which had been introduced earlier by Jenkins (1931) but not clearly defined. It involves the composite crossing of a number of parent plants which had been selected on the basis of type and breeding behaviour. This meant the use of a progeny test to evaluate breeding behaviour, generally following one generation of inbreeding. A large range of genotypes was used in order to maintain vigour. This system of breeding was used for Agropyron cristatum (L.) Guertn. and Medicago media Perss. - both long lived perennials - and for Melilotus alba Desr. which did not suffer from inbreeding. Stevenson said "an essential feature of strain building is the preservation for all time of the parent plants which enter into the strain". Thus this method was only applicable to (1) long lived perennials, (2) a species which does not suffer from inbreeding, or (3) a species which can be readily propagated by vegetative means. It has been shown that red clover does not fulfil either conditions (1) or (2) and earlier work of Scholz and Smidrkal (1939) indicated that it would not readily fit into (3).

Modified maternal line selection

Another method of breeding was that employed for the production of the variety Dollard at Macdonald College. This method was developed and described by Bird (1946) formerly in charge of forage investigations at Macdonald College. Briefly the method is as follows: an isolated breeding block is set out each year and consists of some 75 to 100 lines with thirty plants representing each line. These plants are each individually evaluated and scored according to the classification set up by Bird and described by Steppler and Raymond (1954). Inferior lines are discarded and new lines may be added. In the fall, one plant considered typical for the line is selected from each progeny, its seed harvested and used for establishing that line in the new block in the following year. Bulk seed is harvested from the remaining plants and used to produce breeder seed of Dollard. This breeding technique affords a degree of maternal genotype selection based on the progeny evaluation each year. However, the superior genotype is not retained as such and hence the rate of improvement is rather slow. Some of the lines show considerable uniformity compared to others which are still quite variable. While it has been possible to produce by this means a variety which is superior to commercial stock in local tests, nevertheless, the variety leaves much to be desired and is not so outstandingly superior in tests further afield.

Single plant selection

Frandsen (1940) indicated that two varieties of red clover Otofte early and Tystofte No.40 had been produced in Denmark and released for commercial use. Both these varieties were superior to imported commercial seed and each resulted from single original mother plants. Nordenskiold (1949) has since suggested that these varieties are not as good as had been expected and attributed that to the narrow gene base on which they were established. As far as can be determined this method has not been used for red clover breeding in North America.

Hybrid variety method

Sometime ago Tysdal et al (1942) and more recently Bolton (1948) have outlined a method of breeding for alfalfa which presented a somewhat different approach. The technique involved the following:

(a) Selection of highly desirable single plants, the basis for selection dependent on the objective of the program.

(b) Test of the combining ability of the selected plants by means of the polycross progeny test.

(c) Maintenance of the original selection by vegetative propagation until either rejected or accepted for the program.

(d) Combining the best lines, first as single crosses, then to form a double cross to produce the new hybrid alfalfa. The fields for the production of the single cross would be established by vegetative propagation. Tysdal et al (1942) maintained that the original selections should be highly self-sterile, while Bolton (1948) said that this was not important, that self-fertile but preferentially cross-fertilized plants could be used and he modified the increase procedure accordingly. However, the program retains the same essential features.

This technique has not been tried with red clover (at least a search of the literature does not reveal any pertinent reports) and it was the purpose of this study to investigate the possibilities of applying it to red clover and at the same time to study related problems of combining ability. It was also planned to outline a scheme of production for an improved variety if red clover proved amenable to the technique.

EXPERIMENTAL METHODS

Definition of Terms

In the discussions which follow several terms which are relatively new in plant breeding will be used. For the sake of clarity they will be defined at this time. If an official definition exists, i.e., has been published, then that definition will be given.

Polycross nursery

An isolated block consisting of selections, lines or clones planted in such a way as to facilitate maximum cross-pollination between the various plants in the nursery. A system of replication and randomization is used in order to ensure this (author).

Polycross progeny

Progeny from a selection, line or clone naturally outcrossed to other selections etc., growing in the same isolated nursery. (Agronomy Jour. 46: 599).

Synthetic variety

Advanced generations of open pollinated seed mixtures of a number of clones or inbred lines or of hybrids among them (Agronomy Jour. 46: 599).

Clone

All the individuals derived from a single original selection by vegetative propagation (Hayes and Immer 1942).

Combining ability

The relative ability of a biotype to transmit desirable performance to its crosses (Hayes and Immer 1942). Obviously "desirable performance" must be stated before combining ability can be evaluated.

Diallele crossing

The crossing of a group of lines or clones by pairs and in all possible combinations (author). This means hand-pollinations with red clover.

The material used in this study was selected from various space-planted blocks of red clover used in the regular Dollard variety breeding nursery at Macdonald College. On the average a new nursery is established each year and may contain upwards of seventy-five lines, each represented by twenty to thirty plants. These nurseries are isolated from all other red clover material.

The first selections, aimed at eventual use in this program, were made in 1950 - five clones were selected at that time but none were retained for testing owing to inability to maintain them. In 1951 two lots were selected viz. lot (a) from a two-year old stand, twenty-four clones were selected, examined during the winter of 1951-52 and eventually six were retained for use in the study; lot (b) from a one-year old stand seven clones were selected, studied in the greenhouse and three retained for use. In 1952 six clones were selected and only one was retained. In all a total of ten clones was used in the study. It should be pointed out here that in the early stages of the work clones were discarded if they could not be easily propagated vegetatively - hence the large number of rejects. A description of each of the clones finally retained follows.

Before giving the descriptions it is in order to indicate the scheme used in identifying these clones. Thus, in using the designation 14(51-41/14), the number outside the bracket refers to the line number used in the regular breeding program previously mentioned. This number provides a means of tracing the line through its maternal parent to the original source. The numbers inside the bracket refer to the specific plant within the line which was selected to establish the clone. The number preceding the dash viz. 51 indicates the year of establishment of the nursery (identifies the field plan) - the numbers following the dash indicate row number and plant number, in that order, of the selected plant. For the sake of brevity the clone will be referred to by its line number only in subsequent discussion.

Further, each plant in the breeding nursery and hence each individual clone was classified in the year of establishment into one of the five growth types recognized by Bird (1948) and described by Steppler and Raymond (1954) as follows:

Type 0 - produces rosette only in year of establishment, no flower formation.

Type I - produces strong rosette in the year of establishment with one or a very few prostrate flower stems.

Type II - produces fairly prominent rosette in the year of establishment with a ring of flower stems, generally prostrate.

Type III - produces indistinct rosette in year of establishment with many flower stems, generally upright.

Type IV - produces no rosette in year of establishment, many sparsely leaved upright flower stems.

<u>Clone 14(51-41/14)</u>

The line 14 originated in 1930 from an individual plant selection out of Silesian. The Silesian bulk had been introduced in 1911 and mass selected during the years up to 1930.

This clone was selected in the fall of 1951. The clone had not gone through one winter when selected. It was classified as a type III plant in the field in 1951 with no disease evident. Seed was harvested from the selected plant as shown below:

20 heads	2.2 grams
Remainder of plant	10.4 grams
Total	12.4 grams

The total progeny of the line was classified in the field in 1951 and gave:

Type 0	0	
Type I	1	average type 2.9
Type II	1	
Type III	16	modal type III
Type IV	1	

Hence this clone comes from a medium early line with considerable uniformity.

<u>Clone 38(50-88/7)</u>

The line 38 originated from a single plant selection out of 3 Orel bulk and was made in 1930. Three Orel bulk came from surviving plants out of Orel strain, first planted at Macdonald College in 1911.

This clone was selected in October 1952. It was classed as a type III in 1950 in the field and recorded as "good" healthy plant in 1951. Seed was not harvested from this plant in 1950 or 1951. In 1952 the seed performance was recorded as:

20 heads .... 0.5 grams

The total progeny of the line was classed in the field in 1950 as follows:

Type 0 2		
Type I 7	average type	1.7
Type II 7	modal type	I and II equal
Type III 3		
Type IV 1		

The plants surviving the winter - twelve in all - were again evaluated in 1951 with four classed as fair and six as good.

Thus this clone originates from a mid-late to late line showing considerable variation but possessing some winter hardiness.

Clone 188(50-32/14)

The line 188 originated from a single plant selection out of a space planted nursery of Dollard red clover and was made in 1932.

The clone was selected in October 1951 and had lived through one winter in the field. It was classed as a type II plant in 1950, with a trace of mildew and recorded as "good" no disease in 1951. Seed was not harvested from this plant in 1950 but in 1951 it produced 2.3 grams.

The total progeny of this line was classed in the field in 1950 as follows:

Type 0 0		
Type I 1		average type 2.8
Type II 7		
Type III 7		modal type II and III equal
Type IV 3		

The plants surviving the winter - sixteen in all - were evaluated in 1951 and of these five were classed as fair and five as good.

This clone comes from a medium early line showing considerable variation but possessing a fair degree of winter hardiness.

Clone 284(50-40/19)

The line 284 traces through two other lines A 218 (originating in 1941) and C 150 (originating in 1938) to a lot known as Accession Number 118. This number designates a strain obtained by combining material out of Silesian and Orel which did not have leaf marks. The lot 118 was selected and numbered in 1932.

The clone was selected in October 1951 having survived one winter. It was classed as type I with slight mildew in 1950 and recorded as "good" no disease in 1951. Seed was harvested from the plant in 1950 and 1951 with the following results:

1950 produced 0.6 grams

1951 produced 4.2 grams

The total progeny of the line was classed in the field in 1950 as shown below:

Type 0	 0		
Type I	 5	average type	2.2
Type II	 7		
Type III	 6	modal type	II
Type IV	 1		

Twelve plants survived the winter - only three of these were recorded as good and five as fair.

Therefore, this clone comes from a line which is medium late in maturity and fairly variable. It also shows a slight degree of winter hardiness.

<u>Clone 339(50-62/4)</u>

Line 339 traces through line A 280 (originating in 1942) to Accession No. 118 which has been described in detail under clone 284.

The clone was selected in 1951 after having survived one winter. It was classed as a type III plant in the field in 1950 with no disease evident and recorded as "good", no disease in 1951. Seed was harvested from this plant in both 1950 and 1951 with the results shown below:

1950 produced 12.1 grams

1951 produced 8.2 grams

The total progeny of the line was classed in the field in 1950 as follows:

Type 0	 0	average type	3
Type I	 1	modal type	III
Type II	 2		
Type III	 13		
Type IV	 4		

Fifteen plants survived the winter and of these eleven were classed good and three fair.

Thus the parent line of this clone could be classed as medium early is relatively uniform and has shown fairly high degree of winter hardiness.

Clone 366(50-68/5)

The line 366 traces through two lines A 224 (originating in 1941) and C 152 (originating in 1938) to the material designated as Accession No.118 previously described.

The clone named above was selected in October 1951. It had been classed as a type IV plant in the field in 1950 and recorded as "good" in 1951. Seed was harvested from this plant in both 1950 and 1951 and produced the following:

1950 produced	5.4 grams
1951 produced	3.6 grams

The total progeny from the line was classed in 1950 as follows:

Type 0	0	
Type I	4	average type 2.2
Type II	8	
Type III	4	modal type II
Type IV	1	

Twelve of the plants survived the winter and of these four were recorded as good and four as fair.

Hence this clone originated from a line which could be classed as medium late which exhibited considerable variation and which showed about average winter hardiness.

<u>Clone 376(50-73/9)</u>

The line 376 traces through two lines A 287 (originating in 1943) and C 149 (originating in 1938) to the seed lot Accession No. 118.

The clone was selected in October 1951 after having survived one winter. This plant had been classed in the field in 1950 as a type II and in 1951 recorded as "good" no disease evident. Seed was harvested from the plant in both 1950 and 1951 with the results shown below:

1950 produced 1.4 grams

1951 produced 3.1 grams

The total progeny of the line was classified in the field in 1950 and gave the following:

Type 0 2		
Type I 3		average type 2.3
Type II 4		
Type III 7		modal type III
Type IV 3		

Ten of the plants survived the winter and of these four were classed as good and two as fair. The evidence is, therefore, that this clone comes from a line which shows considerable variation which could be classed as medium early to medium late in maturity and with average winter hardiness.

Clone 392(50-58/14)

The line 392 originated through lines A 333 (originating in 1943) and A 280 (originating in 1942) from the previously described lot Accession No. 118.

The clone was selected in October 1951 after having gone through one winter. The plant was classed as a type II in the field in 1950 and recorded as "good" no disease in 1951. Seed was harvested in 1950 and 1951 with the following results:

1950 produced 1.6 grams
1951 produced 8.1 grams

The total progeny was classed in the field in 1950 as follows:

Type 0	....	1	
Type I	....	3	average type 2.4
Type II	....	4	
Type III	....	10	modal type III
Type IV	....	1	

Thirteen of the plants survived the winter and of these two were classed as fair and four as good in 1951. Thus the line from which this clone originates could be classed as medium late to medium early, shows some variation and possesses an average degree of winter hardiness.

<u>Clone 425(51-11/21)</u>

The line 425 originating in 1947 traces through lines A 277 (originating in 1942), A 209 (originating in 1940), A 136 (originating in 1937) to A 45 which originated in 1934 from a lot known as Accession No. 2605. This was a selection out of Ottawa Red made in 1926.

The clone was selected in 1951 without having gone through one winter. It was classed in the field as a type III, disease free. Seed was not harvested from this plant, however a sib produced the following seed in the same year:

20 heads .... 2.6 grams
Remainder of plant ..... 11.0 grams
Total 13.6

The total progeny of this line was classed in the field in 1951 as follows:

Type 0	 0		
Type I	 0	average type	2.9
Type II	 4		
Type III	 13	modal type	III
Type IV	 2		

Hence this clone comes from a line which may be classed as medium early and which is relatively uniform.

Clone 463(51-22/23)

The line 463 originated in 1948 from line A 403 (originating in 1945) which traces through line A 345 (originating in 1945) to line A 284. This line has been described under clone 284 and comes initially from Accession No. 118.

The clone was selected in October 1951 without having gone through one winter. It was classed in the field in 1951 as type III with no disease. Seed was harvested from the plant in that year and was as shown below:

20 heads	1.0 grams
Remainder of plant	10.7 grams
Total	11.7

The total progeny of this line was classed in the field in 1951 as follows:

Type 0	0	
Type I	1	average type 2.9
Type II	1	modal type III
Type III	16	
Type IV	1	

Hence the clone comes from a line that can be classed as medium early and quite uniform.

The pertinent data on these clones are summarized in Table 2. It will be noted that with respect to type there is one clone of type I, three clones of type II, five clones of type III, and one clone of type IV. With reference to seed yielding ability, there are five clones in the high class, one in the medium and four in the low group.

Following the selection of the clones various plantings were made which provided the material for this study. In the case of the polycross nurseries, it was necessary to propagate the clones vegetatively in order to provide sufficient material to establish the planting. These various plantings are now described in chronological order.

<u>1952</u>

An isolated polycross nursery -PN1- of the following clones was established in the field in the spring of 1952.

Clones	14	376
	188	392
	339	425
	366	

Seed was harvested from these clones in the fall of 1952 and retained for a polycross progeny test in 1953. The above clones were further propagated vegetatively and transferred to the greenhouse for the winter of 1952-53. Two new clones were added to the above list viz 38 and 463, and diallele crosses

TABLE 2. Summary of Pertinent Data on Red Clover Clones.

Clone No.	Clone Type	Line Type	Seed Yield Group*	Original Material of Line
14	III	III	High	Silesian
38	III	I-II	Low	3 Orel
188	II	II-III	Low	Single Plant Dollard
284	I	II	Low	Acc. No. 118
339	III	III	High	Acc. No. 118
366	IV	II	Medium	Acc. No. 118
376	II	II-III	Low	Acc. No. 118
392	II	II-III	High	Acc. No. 118
425	III	III	High	Acc. No. 2605
463	III	III	High	Acc. No. 118

* High - over six grams of seed.

Medium - five to six grams of seed.

Low - below five grams of seed.

were made in the greenhouse among the nine clones. Seed was not obtained in the case of every cross, but it did not seem to be due to the presence of cross-incompatibility in the selected clones. A complete list of the successful crosses is given in Appendix Table 1.

1953

A second isolated polycross nursery - PN2 - was established in the field in the spring of 1953. It consisted of the following clones:

Clones	14	366	463
	188	376	
	284	392	
	339	425	

Clone 38 was not carried through to this nursery due to the inability to propagate it satisfactorily.

Seed was harvested from the nursery to be used for the planting of a polycross progeny test in 1954.

A polycross progeny test - PT1 - of the clones used in PN1 was planted in the field in 1953. This was not replicated due to the limitations of seed. In addition a planting was made of all the crosses obtained in the diallele crossing program carried in the greenhouse during the winter of 1952-53.

All the plants in these two tests - a total of 585 - were classified as to growth type, while forage was harvested from one-half of the material in the polycross progeny test - these data are given in Appendix Tables 1a, 2 and 3.

Seed was harvested from each plant in the remaining half of the polycross progeny test and all of the diallele cross-planting, these results are given in Appendix Table 4.

Winter survival was noted for all plants in 1954 and the results given in Appendix Table 5.

<u>1954</u>

A second polycross progeny test - PT2 - representing the material produced from PN2 was established in the spring of 1954. This was a much more extensive test than that conducted in 1953. It was planted as a randomized block experiment with four replications. The plants - 868 in all - were classified as to type (see Appendix Table 6).

Twenty heads were collected for seed production on one-half of the plants in each progeny - the results are given in Appendix Table 7. Forage yields were taken on October 20th, 1954, on each progeny and are reported in Appendix Table 8.

RESULTS AND DISCUSSION

As has been pointed out previously, the ultimate objective of this study was to set down a new breeding procedure for red clover. However, before this can be done, it will be necessary to examine and evaluate certain other problems which are inherent in the breeding procedure. Thus it will be necessary to determine whether or not clones can be selected which possess differing combining abilities and then to decide how this can best be utilized in the program.

Distribution of growth types in the clonal progeny

All plants grown in the diallele cross test, planted in 1953, were classified according to type as mentioned previously; in addition all plants in the two polycross tests PT1 and PT2 were similarly classified. An examination of these data (Appendix Tables 1a, 2 and 6) shows that there is considerable variation in the progeny produced by clones of different growth type and also between clones of the same growth type - for example the percentage of type III varies in PT2 from 28 in clone 284 to 73 in clone 339.

This gives rise to the question - are the results obtained from the polycross progeny test similar to those from the examination of diallele crosses? Since diallele crosses are made by hand, it is obvious that if the polycross gives the same results then the latter is to be preferred, as hand crossing is eliminated and much larger progenies can be obtained.

A study has been made of the correlation between numbers of plants in each growth type group as obtained for the diallele cross and for the polycross. This was done for each clone, correlating the diallele cross for 1953 with the polycross for 1953 and the diallele cross for 1953 with the polycross for 1954. In the diallele cross, only those crosses containing clones found in the polycross were used for the calculations. The growth type distributions and the correlation coefficients are given for each clone in the following appendix tables:

clone 14 -	Appendix	Table	9
clone 188 -	"	"	10
clone 339 -	"	"	11
clone 366 -	"	"	12
clone 376 -	"	"	13
clone 392 -	"	"	14
clone 425 -	"	"	15

Since clones 284 and 463 did not appear in the diallele crosses, correlation coefficients could not be calculated for them.

The correlation coefficients obtained for the various clones are summarized in Table 3.

TABLE 3. Summary of Correlation Coefficients Calculated for the Diallele 1953 with Polycross 1953 and Diallele 1953 and Polycross 1954

Clone Number	Diallele 1953 with Polycross 1953	Diallele 1953 with Polycross 1954
14	.831[1]	.994
188	.927	.936
339	.934	.988
366	.859	.966
376	.840	.887
392	.856	.936
425	.948	.835

(1) Correlation coefficients required for significance at various probabilities are:

p at .10 r =.805; p at .05 r =.878;

p at .01 r =.959.

Studying the coefficients obtained for the diallele 1953 with polycross 1953, indicates that three of the seven coefficients show a significant relationship at the five per cent level. The remaining four did not reach the five per cent level but have a probability of occurrence of about six to seven per cent. Now examining the coefficients for the diallele 1953 and polycross 1954, shows that three of the coefficients have a probability of occurrence of less than one per cent, three have a probability of occurrence of less than five but greater than one per cent, while only one did not reach the five per cent level but is less than ten per cent. Averaging these correlation coefficients by transforming to Z, calculating the mean and retransforming to r gives a correlation coefficient of .934 which has a probability of occurrence of much less than five per cent. It therefore seems safe to conclude that the polycross test gives results similar to those of the diallele crosses insofar as classifying the genotype of the clone for its growth type potential.

Since the polycross progeny test - PT2 - was randomized and replicated and had a much greater population than PT1 (due to a shortage of seed) the following discussions will be based on PT2 only.

Looking at the percentages of the different growth types in the various clones (Appendix Table 6) for the polycross test 1954 (PT2) reveals that there is considerable variation between clones. This variation in progeny was studied by means of the Chi square test of independence, assuming that if all clones produced progeny showing similar numbers of the different growth types, this would be indicated by a Chi square value with a probability of occurrence greater than five per cent, i.e. independence. The pertinent date were summarized and a Chi square value calculated as shown in Table 4.

Since this Chi square has a probability of much less than .001, it is apparent that the clones do not produce similar progeny and hence have different genotypes as far as growth type is concerned. Further study of Table 4 reveals that five of the clones, viz. 14, 188, 339, 366, 425, appear similar. These were tested using the same assumption as before with the results given in Table 5.

In this case the Chi square value for all clones has a probability of occurrence of between ten and twenty per cent.

A closer examination of these five clones indicates that they can be further divided into two groups of 188, 339 and 366, and 14 and 425, as indicated by the Chi square values reported in Table 5. The test shows similarity between the clones within the two groups.

Looking at the remaining clones from Table 4, and testing for similarity using the Chi square as before, the results

TABLE 4. Test of the Similarity of the Distribution of the Growth Types in the Various Clonal Progenies as Measured by the Polycross Test 1954 (PT2)

Clone	Growth Type		
	O & I (1)	II	III & IV
14	2	23	65
188	6	13	70
284	37	27	28
339	2	13	71
366	6	18	66
376	22	25	44
392	27	32	33
425	7	26	57
463	18	24	48

(1) Growth type classes were grouped in order to meet requirements for Chi square test.

Chi square = 146.13 p is less than .001

TABLE 5. Test of the Similarity of the Distribution of the Growth Types in the Conal Progenies of 14, 188, 339, 366, 425 as Measured by the Polycross 1954 (PT2)

Clone No.	Growth Type		
	0 & I	II	III & IV
14	2	23	65
188	6	13	70
339	2	13	71
366	6	18	66
425	7	26	57

Chi square value for clones 14, 188, 339, 366, 425

= 13.10 .20 p .10

Chi square value for clones 188, 339, 336

= 2.803 .70 p .50

Chi square value for clones 14, 425

= 3.088 .30 p .20

reported in Table 6 were obtained. Here the Chi square value for all clones indicates a lack of similarity between them. A closer study reveals that clones 376, 392 and 463 appear similar, while 284 seems to be different. Testing the group of three, the Chi square value has a probability of occurrence of between twenty and thirty per cent which indicates a similarity in growth type distribution.

Collecting the data from tables 4, 5 and 6 and assembling it into table 7 on the basis of similarity, makes it apparent that the clones can be divided into three main progeny classes, namely, 1, 2 and 3. There is a definite shift in composition of these three classes relative to their growth type distributions. Thus from class 1 to class 3 there is a reduction in the percentage of types III and IV - the earlier types - and an increase in the percentage of types 0, I and II - the later types. Also there is a decided change in the degree of variation within a group - class 1 being quite uniform, while class 3 is the most variable. Further, class 1 may be subdivided into 1 (a) and 1 (b) with 1 (b) showing a shift towards a slightly later maturity.

The three progeny classes may be described as follows:

Class 1 (a)

Composed of clones 188, 339 and 366. This class shows a fairly high degree of uniformity in growth type with a medium early to early maturity.

TABLE 6. Test of the Similarity of the Distribution of the Growth Types in the Clonal Progenies of 284, 376, 392 and 463 as Measured by the Polycross 1954 (PT2)

Clone No.	Growth Type		
	0 & I	II	III & IV
284	37	27	28
376	22	25	44
392	27	32	33
463	18	24	48

Chi square value for clones 284, 376, 392, 463

= 13.99 .05 p .02

Chi square value for clones 376, 392 , 463

= 8.006 .30 p .20

TABLE 7. Composition in Percent of the Three Progeny Classes with References to Growth Type Distribution in PT2

Progeny Class	Clones in Class	Growth Type 0	I	II	III	IV
1 (a)	188,339,366	0.8	4.5	16.5	67.9	10.1
(b)	14,425	0.0	5.0	27.2	61.1	6.6
2	376,392,463	8.7	15.7	29.6	43.5	2.1
3	284	13.0	27.1	29.3	28.2	2.1

Class 1 (b)

Composed of clones 14 and 425. This class exhibits a fairly high degree of uniformity in growth type with a medium early maturity.

Class 2

Composed of clones 376, 392 and 463. The class shows a wider distribution of growth type with less tendency to concentrate in one growth type i.e. no one growth type accounts for more than 45 per cent.

Class 3

Composed of clone 284 only. This class shows the greatest diversity with nearly equal numbers in the three central growth types I, II and III. Thus this class exhibits the maximum shift found - within the clones studied-to a late maturity red clover.

Hence it would seem to be possible to select clones such that their progeny would be either relatively uniform, or possessing differing amounts of variation as measured by the distribution of growth types.

Forage yields of polycross progeny tests

The forage yields of the two polycross tests are given in Appendix Tables 3 and 8. Since the polycross test for 1953 was not replicated (owing to limitation of seed) this discussion will be limited to the polycross progeny test for 1954. The analysis of variance of these data (Appendix Table 8) indicates no significant difference in yielding ability of the nine clones. The mean yields of the clones in per cent of the general mean are given in Table 8.

The forage yielding ability varies from 72.7 per cent of the general mean to 116.7 per cent. In the right hand column of Table 8 has been placed the progeny class into which the clone was placed on the basis of growth type distribution. It is now apparent that the clones with more uniform progeny are poorer as far as forage yield,while the less uniform are better. Testing this statistically, i.e. progeny classes 1 (a) and (b) versus classes 2 and 3, or uniform versus variable, gives an F = 6.38 (see Appendix Table 8) which is significant. This suggests superior performance with the less uniform group which, in turn, possesses higher proportions of the later growth types O and I.

This conclusion must, however, be qualified and carefully studied. Firstly, the forage yield was taken on the 20th of October and the plots had not been previously cut. This was done in order to allow the heads to ripen seed - in fact, forage yield was measured after seed yield had been obtained. Again, this was not by design but rather dictated by scarcity of seed. The procedure outlined above is not the regular

TABLE 8. Forage Yield of Clones in Polycross Test PT2 1954 in Per Cent of General Mean

Clone No.	Yield in Per Cent Mean	Progeny Class
284	116.7	3
463	114.2	2
392	110.6	2
188	109.3	1 (a)
376	102.3	2
425	100.6	1 (b)
339	91.0	1 (a)
14	82.0	1 (b)
366	72.7	1 (a)

practice in forage yield tests. Secondly, the average behaviour of the later growth types O and I is such that they do not produce an aftermath and hence if they had been cut previously in the season, e.g., mid summer, it is unlikely that any further growth would have taken place. On the other hand, the earlier types III and IV will produce an aftermath and hence would have produced a second growth. For example, tests of the forage yielding ability of red clover, conducted at Macdonald College, have consistently shown that at the first cut the late types of red clover will generally outyield the early types. However when the total season's production is taken into account - two forage cuts - the early types will outyield the later. Thus, under a normal test procedure involving two harvests - one mid season and one end of season - the situation could well have reversed with the more uniform progenies - in this instance those with a predominant percentage of the medium early type III - in the superior position.

Seed yields of polycross progeny tests

The seed yields of the polycross progeny test PT1 are given in Appendix Table 4, along with the analysis of variance, while seed yields and analysis of variance for the polycross progeny test PT2 are given in Appendix Table 7. The pertinent date from these tables are summarized and given in Table 9.

TABLE 9. Seed Yields of Clonal Progeny in Polycross Progeny Tests PT1 and PT2 (Grams per 20 heads)

Clone No.	PT1 - 1953 Yield	PT1 - 1953 Rank	PT2 - 1954 Yield	PT2 - 1954 Rank	Progeny Class	Clone Evaluation at time of selection (see Table 2)
14	1.05	(4.5)	1.63	(1)	1 (b)	High
188	1.23	(2)	1.23	(5)	1 (a)	Low
284	-	-	1.12	(8)	3	Low
339	1.36	(1)	1.54	(2)	1 (a)	High
366	.97	(6)	1.30	(4)	1 (a)	Medium
376	1.05	(4.5)	1.14	(7)	2	Low
392	1.12	(3)	1.20	(6)	2	High
425	.75	(7)	.97	(9)	1 (b)	High
463	-	-	1.39	(3)	2	High

L.S.D. ..29 .32

It will be noted that significant differences in seed yield were found in both years the appropriate least significant differences are reported in Table 9. Looking at the rank of the clones in the two tests one sees that only the best and poorest were reasonably consistent, i.e. 339 was first in 1953 and second in 1954, while 425 was poorest in both years. In the column at the right is given the evaluation of the clone at time of selection, while in the second column from the right is the progeny class into which the clone was placed on the basis of growth type. Examining firstly the clonal evaluation at selection it will be seen that there is reasonably good agreement in that in PT2 the clones ranking first, second and third had been evaluated as "high" at the time of selection. Also, those clones ranking seventh and eighth had been ranked as low. Only clone 425 which ranked ninth in the test but which had been evaluated as high, appears as a contradiction. However, even this may be explained since the specific clone 425 was not evaluated as high on the basis of its own performance, but rather on that of a sib plant (see description of clones). Thus, it appears that a plant breeder could make a fairly satisfactory evaluation of the seed yielding ability of the progeny of a clone by examining that of the clone itself.

Looking now at the second column from the right in Table 9, it appears that the clones placed in progeny class 1 are superior to those in the other classes on the basis of the test PT2. Evaluating this statistically - as was done for forage yield - one obtains an F value of 5.06 for the comparison of uniform versus not so uniform or predominantly earlier types (clones 14, 188, 339, 366 and 425) versus others (clones 284, 376, 392 and 463). In this the earlier type clones are superior in seed yield which is in agreement with expectation on the basis of flowering habit of the various growth types, since the earlier types would possibly bloom under more favourable pollinating conditions than that prevailing when the later types come into bloom. Once more, however, a word of caution is needed on a too dogmatic statement regarding seed yield. Povilaitis (1955) who has been studying seed setting in red clover at Macdonald College has found little correlation between seed production and number of mature embryo sacs in data collected in 1954, while a positive correlation was found in 1953. In addition, 1953 was considered as a year of good seed setting while 1954 was not considered satisfactory. Thus it would seem on the basis of this work that evaluation for seed setting ability should not be done in a year when poor seed setting conditions prevail. However, in spite of this the results of Table 9 are presented and do show reasonably good agreement with clone genotype evaluation at the time of selection.

Winter hardiness of progeny of clones

One polycross progeny test - PT1-went through the winter

1953-54 in the field. Winter survival was recorded on the planting in mid-summer 1954 and the results are presented in Appendix Table 5. These are briefly summarized in Table 10. It was not possible to analyze these data statistically owing to the limitation of the original field planting imposed by seed supply. However, one interesting point is apparent when one looks at the progeny classes of the clones and winter killing. The lowest percentages of winter killing occur in the two clones in class 2, that is, the "not so uniform" class with the higher proportion of the medium late to late growth types.

Summary of pertinent data

Some of the relevant data presented on the various clones and their progeny have been brought together in Table 11. Two points are evident on study of this table, namely -

i. Variation in the sibs of the line from which a clone has been selected gives no indication of the type of progeny which it will produce. Thus clone 188 came from a mother line which was rated as having considerable variation yet on the basis of its progeny clone 188 was placed in progeny class 1 (a) - most uniform.

TABLE 10. Winter Killing in the Polycross Progeny Test PT1 as Recorded in 1954

Clone No.	Per Cent Winter Killed	Progeny Class
14	37.2	1 (b)
188	37.2	1 (a)
339	40.0	1 (a)
366	54.3	1 (a)
376	28.3	2
392	21.7	2
425	40.9	1 (b)

TABLE 11. Summary of Pertinent Data on Clones from Original Selection Evaluation and Polycross Progeny Tests

Clone No.	Clone Growth Type	Degree of Variation in Mother Line	Progeny Class	Forage Yield in Per Cent (PT2)	Seed Yield Rank (PT2)	Per Cent Winter Killed (PT1)
14	III	slight	1 (b)	82.0	1	37.2
188	II	considerable	1 (a)	109.2	5	37.2
284	I	medium	3	116.7	8	-
339	III	slight	1 (a)	91.0	2	40.0
366	IV	considerable	1 (a)	72.7	4	54.3
376	II	considerable	2	102.3	7	28.3
392	II	medium	2	110.6	6	21.7
425	III	slight	1 (b)	100.6	9	40.9
463	III	slight	2	114.2	3	-

ii. The growth type assigned to the clone does indicate to some extent the maturity range of its progeny. Clone 284 was classed as growth type I and produced progeny which had a large percentage of late types. Similarly clones 376 and 392 were rated as growth type II and likewise produced progeny which resulted in placing them in progeny class 2 - showing a tendency towards the medium late types.

A BREEDING PROCEDURE

It is now proposed to set down, phase by phase, a breeding procedure for the production of a hybrid variety of red clover and then to examine each step in more detail. However before doing this it is proposed to state the terms which will be used to refer to the various classes of pedigreed seed. The nomenclature followed is that of the International Crop Improvement Association. The terms with a brief description of each are as follows:

Breeder seed - the seed produced under the direct supervision of the plant breeder who originated the variety. This seed provides the basic stock for the production program.

Foundation seed - the seed produced from breeder seed with the appropriate isolation requirements etc. set down by the certifying agency.

Registered seed - the seed produced from foundation seed with the appropriate regulations observed.

Certified seed - the seed coming from registered seed with the required regulations observed. This class of seed is the one normally offered for sale to farmers, etc.

The equivalent terms used by the Canadian Seed Growers' Association are, in order of production, foundation, elite or approved, registered and certified.

The program can be briefly tabulated as follows:

Phase 1. The selection of clones from a space planted nursery of red clover.

Phase 2. The determination of the combining ability of these clones by means of the polycross test.

Phase 3. The combination of a small number of the best clones to produce breeder seed of the hybrid variety.

Phase 4. The increase of the breeder seed to the level at which it will be used by the seed consumers, i.e. farmers, likely to the certified seed level.

Phase 5. The testing of the certified seed for suitability over the area for which it is intended, and particularly to determine if it is sufficiently superior to existing stocks to warrant release.

Phase 6. Large scale production of seed and release to the farmers of the improved variety.

It is immediately apparent that from Phase 1 to Phase 3 the selected clones must be maintained as such, and further if a clone is selected for the production of an improved variety, it must be maintained for as long as required in order to provide its share of the germplasm in the breeder seed. Since, as previously pointed out, red clover acts as a biennial or short lived perennial, one could only expect the original plant to live for two or possibly three years. Further, since red clover is self-incompatible a clone cannot be maintained by seed. Hence this is clearly a case of maintenance by vegetative propagation.

This problem has been investigated for the past few years by B.G. Cumming, a graduate student in the Department of Agronomy, Macdonald College. Cumming (1955) reports* that it is feasible to propagate red clover vegetatively but that lines differ markedly in their ability to produce rooted propagules (a propagule being defined as a portion of a plant and used for vegetative propagation, i.e. a stem cutting, crown cutting, stipular shoot etc.). Under three different management regimes he gives the following possibilities for propagation.

(1) Maintained only in the greenhouse and cold frame for one year (March to March), one plant can produce 150 rooted propagules.

(2) Maintained in the field, from May until the end of the growing season, one plant can produce 300 rooted propagules,

*A complete and detailed discussion of this question will be given by Cumming in his thesis which will be presented to the Graduate Faculty of McGill University.

the rooted propagules being stored during the winter.

(3) Maintained in the field, from August over winter until the end of the next growing season, one plant can produce 600 rooted propagules, the rooted propagules being stored during the winter.

Since the problem of vegetative propagation does not present a barrier, it is now in order to examine in more detail each phase of the breeding program.

Phase 1. In the initiation of the breeding program it is absolutely essential to state the objective of the program. This may be increased seed production, increased forage production, resistance to a disease, longevity of stands, growth habit, etc. With certain of these objectives some measure of success may be achieved in the initial selection of the clone, e.g., it has been shown that a clone of high seed setting ability tends to produce progeny with high seed production (see Table 8).

On the question of disease Hollowell (1952) felt that it was possibly an important factor limiting the longevity of red clover. Thus disease resistance could well be an objective in the program. Observations in the breeding blocks for red clover at Macdonald College have indicated that under these conditions there are three main diseases of red clover of which only one - Sclerotinia root and crown rot - has been known to cause death. These diseases are:

Sclerotinia trifoliorum Eriks. - Sclerotinia root and crown rot.

Kabatiella caulivora (Kirch.) Karak - northern anthracnose.

Erysiphe polygoni D.C. - powdery mildew.

In addition, an insect pest, the red clover root borer *Hylastenus obscurus* Morchon, is becoming increasingly important in red clover stands in Quebec. Thus, resistance to any one of the above diseases or pests in addition to some other agronomic characteristics might form the objectives of the program and hence dictate criteria for selection. With disease, obviously an artificial epidemic would be created if possible in order to speed up selection.

Finally any red clover material could be used as a source of germplasm and it may be an advantage to use as widely differing sources as possible. It has been shown with corn that the wider the diversity of the germplasm used in establishing the inbred lines the greater is the possibility of obtaining increased heterosis in the resultant double cross (the method of combining the inbred lines is important in the realization of optimum heterosis). Thus it is reasonable to assume that the more divergent the source of the selected clones the greater is the chance of obtaining heterosis in the hybrid variety. The selection of the clones in this phase will be on the basis of phenotype only and hence the criteria of selection will take that into account.

Phase 2. As stated under Phase 1 the selection was phenotypic - at this stage the evaluation will be for combining ability and hence will be a measure of the suitability of the genotype of these selected clones. This will be done by an evaluation of the progeny on the basis of all of the phenotypic characteristics given in Phase 1 along with other pertinent characteristics especially growth type distribution. That problem will be discussed later in this section.

As has been shown (Table 3), the results of the polycross test agreed with the results of the more laborious diallele cross method and hence the latter need not be used except to assess cross-compatibility. The selected clones from Phase 1 would be propagated vegetatively and an isolated polycross nursery established similar to that used in this study. Randomization and replication of the clones would be used in order to ensure a maximum opportunity for cross-pollination between all clones.

Seed would be harvested from each clone and used to establish the polycross progeny test. The ideal situation would be to have two tests as follows: (a) a randomized and replicated test of single plants, space planted, where growth type composition of the progeny may be evaluated as well as other agronomic characteristics, and (b) a randomized and replicated small plot test in order to give better evaluation of the progeny yield performance under more normal conditions. Since seed will frequently be a limiting factor this may not be possible and hence preference would be given to a test of the first type.

The evaluation of the progeny on the basis of distribution of growth types would be done at this time. This is considered of major importance since the growth type is so closely related to the type of red clover ultimately produced, that is, early double cut, medium early double cut, late single cut, etc. However, the question of the "ideal" progeny immediately presents a fundamental problem in plant breeding. In most cases plant breeders strive for "uniformity" in the bred strain, whether it be for disease resistance, height, strength, quality of product, etc. In self-fertilized crops e.g. wheat, oats or barley, this uniformity means not only homogeneity but also homozygosity. With cross-fertilized crops uniformity will generally mean homogeneity and in some instances e.g. double cross corn, it will imply a closely regulated degree of heterozygosity. Thus uniformity can have a different connotation depending on the genus under discussion. However, in nearly every case this striving towards uniformity has resulted in reducing the range of adaptation of the improved variety, with hybrid corn as an extreme case of restriction in adaptation. The problem is therefore - should one select for a uniform progeny (based on growth types) and possibly limit the range of adaptation of the variety, or, should one deliberately select clones which produce progeny that exhibit a fair degree of variation, in the expectation that such a variety would be more widely adapted.

Unfortunately with red clover, the dilemma does not end with adaptation. As was pointed out in the introduction to this study, "a definite problem exists in the large-scale increase of pedigreed seed of red clover or for that matter of any herbage species which is cross-pollinated". As indicated this is being overcome through the Canadian Forage Seeds Project which contracts for production of the higher classes of pedigreed seed of forage crops, these to be grown in a seed producing rather than seed consuming area. Steppler and Raymond (1954) demonstrated that just one season's production of a bred variety in an area possessing a different climate and/or different management regime was sufficient to so materially change the composition of the variety that it was not recognizable as such. Steppler (1954) further demonstrated this using the variety Lasalle with its components Dollard and Ottawa and with the American variety Kenland. This problem may develop when a management regime - generally made possible by climate - favours the seed production of one growth type in excess of others. Obviously, then, if a variety were produced which was very uniform with respect to growth type, it would "resist" the forces of change of a different management regime and would be returned to the area for which it was bred relatively unharmed.

Thus it seems that the plant breeder must weigh the possible limitation of the range of adaptation on the one hand against the possibility of change in composition by management on the other hand in order to establish his criteria for evaluation of progeny. The tests reported herein indicate that both types of clones (those which produce variable and those which produce uniform progeny) can be selected and it would seem that final choice would fall to the uniform progeny. What would be the advantage of producing a variety of wide adaptation and then have it changed by the management regime practised for the production of its seed? However, even this statement must be qualified since it has been made having in mind present management practices. A more rigidly defined seed production procedure may completely nullify that conclusion.

There is one other factor which should be determined in Phase 2. Since red clover is self- and may be cross-incompatible, the clones selected for superior combining ability in the polycross progeny test should be tested to ensure that none are cross-incompatible. This cannot be done in the polycross but may be done in the greenhouse during the winter by diallele crossing of the selected clones. While the probabilities are in favour of cross-compatibility, nevertheless, this should be checked. No cross-incompatibility was encountered in the nine clones used in this study.

Phase 3. Following the selection in Phase 2 the best clones will be combined to produce an improved variety. This presents another problem, namely, what is the optimum number of clones to use? Tysdal et al (1942) and Bolton (1948) envisaged the use of four clones in their hybrid alfalfa production. Fransden (1952) stated that in Denmark ten clones were considered as satisfactory, although he intimated that up to twenty may be used. No experiments have been reported to date to determine the best number of clones to be used in a hybrid program of this nature.

With hybrid corn it has been conclusively shown that a maximum number of four inbred lines can be used and still realize an optimum expression of heterosis. A certain degree of similarity exists between the situation with hybrid corn and that for a hybrid red clover variety. Thus a selected clone of red clover will be similar to a single plant of a single cross corn in that both represent the combining of two genotypes. However with reference to corn these two genotypes come from two inbred lines and are, therefore, homozygous within themselves, while in red clover the two original genotypes are almost certainly extremely heterozygous. The plant breeder has some prior knowledge of the behaviour of the single cross, with red clover the selected clone is an unknown factor and will remain such until tested for combining ability.

Leaving aside these points of difference one could argue - using hybrid corn as the example - that two clones should be used in the production of the hybrid variety. While there is no experimental evidence on this matter, nevertheless knowledge of the breeding behaviour of red clover would lead one to reject this proposal. There are three reasons for this rejection. Firstly, the narrow gene base could only provide in the case of the S alleles a maximum of four alleles. This could interfer with seed production. Secondly, in hybrid corn the double cross seed is the class sold to the consumer (advanced generations show a very marked reduction in heterosis in comparison to the double cross), while in a red clover hybrid the seed is likely to go through at least two additional increases and there could be considerable drop in the hybrid effect. Moreover, it is quite possible that a farmer may decide to continue producing his own seed beyond the certified level - a practice which would contribute to even more decline and obviously an argument against a narrow gene base. Thirdly, the narrow gene base may seriously circumscribe the range of adaptation.

Hence it would seem better to at least double the number of basic clones and use four. This would be somewhat comparable to the crossing of two double crosses in corn and would infer the mixing of eight genotypes. Naturally, the fewer the number of clones entering the hybrid the greater would be the probability of selecting clones which produce progeny that will be truly superior to commercial stocks.

Coincident with the question of number of clones is the problem of how to combine these clones. Again there is no experimental evidence to indicate the best method of combination. Assuming four clones, the minimum number of S alleles - and still have cross-compatibility - is five and the maximum is eight. The five alleles will give rise to ten different S genotypes which are cross-compatible and these will be realized irrespective of the method of combination. It would seem that the most convenient method would be to establish an isolated block in which the four clones will appear in equal numbers. In addition, the planting would preferably be of single spaced plants arranged so that a clone appears in a row and the rows are randomized as shown in Fig. 1, where A, B, C, etc., represent single plants of the clones A, B, C, etc., with the rows spaced three feet apart

D	A	C	B	C	D
D	A	C	B	C	D
D	A	C	B	C	D
D	A	C	B	C	D

Fig. 1. Suggested planting plan for the production of breeder seed.

and plants spaced one and one-half feet apart in the row. By maintaining the clones in rows, the relative stands of the various clones can be determined and the block discarded as soon as one clone begins to kill out, or that clone could be replanted with new propagules. The actual management of the block for seed production will be consistent with the objectives of the program.

This seed production block will have been established from propagules. The seed harvested from the block will be termed breeder seed for the new hybrid variety.

Phases 4 and 5. It is now important to test the new hybrid variety for adaptation. While it is desirable that these tests be conducted with seed similar in status to that which the farmer-grower will purchase, it is nevertheless also desirable to initiate testing as soon as possible. Thus, tests should be established with the breeder seed and with seed arising from each successive generation of increase which are in sequence as previously mentioned Breeder seed, Foundation seed, Registered seed and Certified seed. This will not only allow for immediate testing, but will also - and possibly this is even more important - permit the plant breeder to determine whether any material changes are occurring in the variety as a result of the advancing generations of seed production, and what effect these changes, if any, have on the usefulness of the variety.

Phase 6. Once the superiority of the hybrid variety has been clearly demonstrated and the area to which it is adapted determined, it would become necessary to establish a program for the increase of seed. This would follow the sequence in Phases 4 and 5. Diagrammatically the program may be shown as in Fig. 2.

As indicated, the one acre field for the production of breeder seed is established from propagules, approximately 2,500 propagules per clone. Using the system (1) outlined by Cumming (1955) one would be required to maintain approximately seventeen plants per clone or sixty-eight plants in all to provide these propagules. These would be carried in the greenhouse and would only require about ten to twelve square feet of bench space. In order to have insurance against loss of a clone, it would be desirable to carry a parallel set under system (2) or (3). With system (2) this would require about eight plants per clone, maintained in the field with propagules taken in the late fall, rooted and stored over winter in a cool place e.g. a root cellar. Thirty-two plants in all would be required and, as indicated, these would not be carried in the greenhouse.

The remaining steps in the actual seed production will entail routine seed production practices with fields being established from seed. The expected seed production has been placed at a conservative figure and does not envisage the

One acre field, four clones vegetatively propagated, planted in rows, randomized and replicated with equal numbers of plants of each clone. Approximately 2500 plants per clone.

Will produce →

BREEDER SEED
expected production
100 pounds

Seed 20 acres at 5 pounds per acre

Will produce →

FOUNDATION SEED
expected production
5000 pounds
(250 pounds per acre)

Seed 1000 acres at 5 pounds per acre

Will produce →

REGISTERED SEED
expected production
250,000 pounds
(250 pounds per acre)

Seed 50,000 acres at 5 pounds per acre

Will produce →

CERTIFIED SEED
expected production
10,000,000 pounds
(200 pounds per acre)

WILL SEED 1,250,000 ACRES
ANNUALLY FOR FORAGE PRODUCTION
(seeded at rate of 8 pounds per acre)

Fig. 2. SEED PRODUCTION SCHEME FOR HYBRID RED CLOVER

phenomenal yields which have been obtained in some areas, e.g. upwards of 500 lbs. per acre under irrigation in the western United States. As will be seen, the potential of this program would be the seeding of approximately 1,250,000 acres in red clover annually. This is equal to the estimated acreage seeded to red clover each year in Quebec, namely, about one million acres.

It would not be difficult to double or even triple the area established vegetatively for breeder seed production and hence effect a material increase in seed production at the certified seed level. Also if tests show that it was unwise to use more than three advanced generations - i.e. not proceed beyond registered seed level - then an increase in the area devoted to breeder seed production would be required in order to provide a satisfactory amount of seed, e.g. a ten-fold increase in breeder seed production would provide registered seed for about 500,000 acres annually, seeded at the rate of five pounds per acre.

It is envisaged that there would be continual selection and evaluation of new clones using all possible sources of germplasm. This should, therefore, mean that the hybrid variety will be constantly improved as new and better clones are selected. As a final consideration of this program some of the advantages and disadvantages will now be discussed.

Advantages of the hybrid breeding method

There seem to be two major advantages to this type of breeding program, namely:

1. When a clone or clones of superior genotype is found, for example, outstanding in winter hardiness or resistance to Sclerotinia, it can be very quickly and easily utilized in the production of the hybrid variety and will exert a profound effect upon that variety. Thus, one superior clone for Sclerotinia could immediately contribute 25 per cent of the germplasm of the breeder seed.

2. Since one is dealing with a very small number of basic clones, it may be possible to introduce a genetic marker which will identify the variety. Williams, W. (1950) said with reference to the breeding of herbage legumes "Breeding for authentication is one of the most immediate and tangible contributions that can be made by plant breeding in this field". Thus, the variety of red clover Dollard - mentioned previously - is almost free of leaf mark. It might be possible to produce a variety which was entirely free of leaf-mark, at least at the breeder seed level. In addition, during the course of these studies, it was noted that some clones produced seed which was very light in colour, particularly clones 425 and 463. Thus it may also be possible to produce a variety which has mainly yellow seed. These two markers

could aid immeasurably in maintaining the genuineness of the seed and thus the authenticity of the variety.

Disadvantages of the hybrid breeding method

There seem to be three major disadvantages to this scheme of breeding.

1. Since the variety is based on four clones, it will have a relatively narrow gene base in comparison to a mass selected variety which may possess several hundred different genotypes. This could limit the range of adaptation. However, it is possibly better to have a variety superior in a relatively small area than one which can be grown over a wide region but is not significantly better in any portion of that region. To hope to produce a variety significantly superior over a wide area may be a mere will-o-the-wisp, a goal which forever eludes one's grasp.

2. It is apparent that the cost of producing breeder seed may be high in comparison to normal seed production. However, since the seed will normally pass through three increases, the final cost of certified seed to the farmer-consumer may be little more than commercial and, it is hoped, he would be more than repaid by the improved performance of the variety.

3. The basic clones used in the production of the hybrid variety must be retained as such for as long as they are required in the hybrid. If one of these is lost the variety can

no longer be produced. Hence extreme care must be exercised in the maintenance of the basic clones.

SUMMARY AND CONCLUSIONS

The study reported here was conducted on nine clones of red clover which had been selected from the variety Dollard at Macdonald College. These clones were combined as diallele crosses and as polycrosses. The seed resulting from these crosses was studied for combining ability using as the main selection criteria, the distribution of the growth types within the clonal progenies. In addition seed yield and forage yield were measured and studied statistically, however, because of the circumstances surrounding these tests they have not been interpreted too dogmatically. Winter hardiness was recorded in the case of the one polycross which had gone through one winter.

These data were studied with two objectives in mind. Namely to examine certain aspects of the breeding behaviour of red clover and to set down a breeding procedure for red clover which could be used to produce a hybrid variety. These two aspects of the study will be treated separately.

Conclusions which may be drawn from these studies with reference to breeding are:

1. The diallele cross and the polycross give essentially the same results when compared on the basis of distribution of

the growth types in their clonal progenies. This was determined by calculating correlation coefficients. Thus the polycross can be used in place of the diallele cross to determine combining ability.

2. The nine clones can be divided into three main classes - one with two sub classes - on the basis of the variation in growth types shown by their clonal progenies. These are:

Class 1.(a) Mainly of one growth type, medium early in maturity.

(b) Similar to (a) in uniformity but slightly later in maturity.

Class 2. More variation in growth type composition, no one type accounts for more than 45 per cent of the progeny.

Class 3. Maximum variation of the progeny studied, no one type accounts for more than 30 per cent of the progeny.

3. On the strength of the preceding statement, it appears possible to select clones that will produce progenies which are either uniform or with a constant degree of variation among the various growth types.

4. Clones differ in their ability to produce seed. Clones which were placed in class 1 were superior to those in class 3 when evaluated for seed yielding ability. There was reasonably good agreement between the evaluation of the clone at selection and the evaluation of its progeny in the polycross with respect to seed yield.

5. Significant differences were not found between the clones with respect to their forage yielding ability. Because of the abnormal conditions of the forage tests no conclusions could be made.

6. Because of the fact that the one test which has gone through the winter was not replicated, the data on winter hardiness were not analyzed. They did indicate, however, that clones in class 2 were more winter hardy than those of class 1.

The breeding program proposed may be briefly tabulated as follows:

Phase 1. The selection of clones from a space planted nursery of red clover.

Phase 2. The determination of the combining ability of these clones by means of the polycross test.

Phase 3. The combination of a small number (4) of the best clones to produce breeder seed of the hybrid variety.

Phase 4. The increase of the breeder seed to the level at which it will be used by the seed consumers, i.e farmers, likely to the certified seed level.

Phase 5. The testing of the certified seed for the suitability over the area for which the variety was intended. Particularly to determine if it is sufficiently superior to existing stocks to warrant release.

Phase 6. Large scale production of seed and the release to the farmers of the hybrid variety.

Clones must be maintained vegetatively for as long as they are used in the variety. It is envisaged that there will be continual selection of new material and that new clones would be added and old ones withdrawn from the variety.

A seed production scheme is outlined whereby it would be possible to seed 1,250,000 acres to red clover annually - all this seed to be of pedigreed status.

Throughout the discussion of the breeding procedure there was constant reference to problems related to the program for which an answer based on experimental data does not exist. Those problems are now listed but not necessarily in the order of importance.

1. To study the effect on the range of adaptation of a variety by restricting variation within that variety with

reference to its growth type composition.

2. To determine the effect of a seed management regime and /or climate on the maintenance of genuineness of a variety using (a) a variety with considerable homogeneity of growth type, and (b) a variety with a constant degree of variation in growth type.

3. To determine the optimum number of clones to be used in the production of a hybrid variety of red clover.

4. To determine the best manner of combining the selected clones in the production of a hybrid variety.

5. To determine the maximum number of advanced generations of seed production which are consistent with the optimum expression of heterosis.

6. To determine whether or not genetic markers can be used which will aid in maintaining the genuineness of the variety.

Finally, it is in order to restate the original problem of this study, namely, to outline a breeding procedure which may overcome some of the characteristics of red clover that make it so difficult to breed. To date the breeding programs have yielded very little in return for the energy expended on them and particularly in comparison to breeding with crops like wheat or corn. This is mainly due to the virtually complete self-incompatibility which exists in red clover. As a result it is practically impossible to establish pure lines or to use the methods of breeding which have proved so successful with other crops. It is felt that the breeding procedure outlined herein - while it has many drawbacks - may nevertheless achieve a measure of success where others have failed.

ACKNOWLEDGEMENTS

The writer wishes to acknowledge at this time the friendly criticisms and helpful suggestions of Professor L.C. Raymond.

He would also like to acknowledge the privilege accorded to him by Professor Raymond in allowing him to conduct these studies within the confines of the Agronomy Department of Macdonald College.

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APPENDIX TABLES - Nos. 1 to 15 inclusive

APPENDIX TABLE 1. Diallele Crosses made in the Greenhouse in the Winter of 1952-53 and planted in the field in 1953.

14 x 188	38 x 14	366 x 339	366 x 463
188 x 14	14 x 463	339 x 392	376 x 425
14 x 339	188 x 339	392 x 339	425 x 376
339 x 14	366 x 188	38 x 339	376 x 38
14 x 366	188 x 376	366 x 376	376 x 463
366 x 14	376 x 188	366 x 392	392 x 425
14 x 376	188 x 392	392 x 366	392 x 38
376 x 14	392 x 188	366 x 425	38 x 392
392 x 14	425 x 188	425 x 366	463 x 425
14 x 425	188 x 38	366 x 38	
425 x 14	188 x 463	38 x 366	
14 x 38			

APPENDIX TABLE 1a. Field Notes on Diallele Crosses - Distribution of Growth Types

Cross	Growth Type 0	I	II	III	IV
14 x 188			2	5	3
188 x 14			2	8	1
14 x 339				2	
339 x 14				4	
14 x 366	1	1	1	4	1
366 x 14				6	3
14 x 376		1	4	6	
376 x 14		1	2		
392 x 14	2	1	10	3	
14 x 425			1	4	
425 x 14	1		1	3	1
14 x 38	1		2	1	
38 x 14			1	5	
14 x 463	1				
188 x 339					2
366 x 188	1		4	4	1
188 x 376		1	1	1	
376 x 188				2	
188 x 392		1	3	4	
392 x 188			3		1
425 x 188				1	

APPENDIX TABLE 1a. (continued)

Cross	Growth Type 0	I	II	III	IV
188 x 38			1	3	
188 x 463		1			1
366 x 339				2	
339 x 392		1	4	10	
392 x 339	1		5	1	
38 x 339		2	1	1	
366 x 376				1	
366 x 392			2	2	1
392 x 366				4	
366 x 425				3	2
425 x 366				5	1
366 x 38	1	2		2	
38 x 366	1	1		3	2
366 x 463	2	2		1	2
376 x 425		3		2	
425 x 376	1	2			1
376 x 38				1	
376 x 463	2	1	4		
392 x 425	1	1	1	1	
392 x 38		1	2		
38 x 392	6			1	
463 x 425	1				

APPENDIX TABLE 2. Field Notes on Polycross Test (PT1) - 1953 Growth Types

Clone No.	Growth Type 0		I		II		III		IV	
	No.Plts	%	No.Plts	%	No.Plts	%	No.Plts	%	No.Plts	%
14	8	18.6	4	9.3	14	32.6	17	39.5	0	0
188	6	13.9	6	13.9	9	20.9	17	39.5	5	11.6
339	1	2.2	5	11.1	15	33.3	24	53.3	0	0
366	4	8.7	4	8.7	13	28.3	22	47.8	3	6.5
376	6	13.0	11	23.9	15	32.6	14	30.4	0	0
392	6	13.0	5	10.9	24	52.2	10	21.7	1	2.2
425	1	2.3	0	0	6	13.6	37	84.1	0	0

APPENDIX TABLE 3. Dry Matter Yields of Clones in Polycross (PT1) 1953

Clone No.	Dry Matter	Yield in Per Cent of Mean	Progeny Class
14	1499	81.8	1b
188	1760	96.1	1a
339	1808	98.7	1a
366	1834	100.1	1a
376	2004	109.4	2
392	1825	99.6	2
425	2092	114.2	1b

APPENDIX TABLE 4. Seed Yield (grams) of Clones in Polycross (PT1) 1953 Based on 20 heads per plant and analysis of variance

Clone Number						
14	188	339	366	376	392	425
1.1	.7	1.4	1.3	1.0	.8	.6
1.0	.5	.9	.4	1.4	1.5	.9
1.2	.4	1.7	.9	.5	1.6	1.0
.7	1.6	1.0	1.6	.9	.7	1.0
.9	.7	2.1	1.2	1.2	1.6	.3
.6	1.4	.9	.8	.6	1.0	.7
2.1	1.5	1.0	.4	1.3	1.9	1.0
.6	.6	1.3	1.0	1.5	1.1	.5
1.9	2.2	1.5	.8	1.3	.5	.5
.8	1.4	.6	1.0	1.6	1.9	.6
.4	1.2	1.1	1.0	1.3	.8	.6
.3	1.8	2.2	.7	.6	1.5	.5
1.2	1.4	.5	1.0	.6	.4	1.3
1.1	1.6	1.9	.8	1.3	.4	.8
.7	.6	1.6	1.7	1.0	1.1	.9
2.5	2.2	2.2	1.3	.9	1.0	.7
1.3	1.6	1.7	.4	.8	1.0	.9
.9	1.2	1.7	.8	1.1	.7	.2
1.0	.9	.9	.9		1.6	.5
.7			.7		1.5	1.3
			.9		1.0	
			.6			
			2.0			
			1.2			
Mean Yield						
1.05	1.23	1.36	.97	1.05	1.12	.75

Analysis of Variance

Source	d.f.	S.S.	M.S.	F.	5%	1%
Clones	6	4.66	.77	3.85	2.17	2.95
Error	134	27.76	.20			

L.S.D. = .29 for mean of 18.

APPENDIX TABLE 5. Field Notes on Winter Killing in Polycross Progeny Test PT1 - 1954

Clone No.	% Winter Killed in Growth Type 0	I	II	III	IV	% Total Progeny Winter Killed
14	26.7	25.0	42.8	35.3	-	37.2
188	50.0	33.3	44.4	29.4	40.0	37.2
339	0.0	40.0	33.3	45.8	-	40.0
366	25.0	50.0	38.5	63.6	100.0	54.3
376	16.7	9.1	40.0	35.7	-	28.3
392	33.3	20.0	12.5	30.0	100.0	21.7
425	100.0	-	33.3	40.5	-	40.9
% Survived averaged over clones	33.3	25.7	32.2	41.8	66.7	

APPENDIX TABLE 6. Field Notes on Polycross Test 1954 (PT2)
Distribution of Growth Types within Progeny of Clones

Clone No.	Growth Types 0		I		II		III		IV	
	No.Plts	%	No.Plts	%	No.Plts	%	No.Plts	%	No.Plts	%
14	0	0	2	2.2	23	25.5	60	66.6	5	5.5
188	2	2.2	4	4.4	13	14.6	63	70.7	7	7.8
284	12	13.0	25	27.1	27	29.3	26	28.2	2	2.1
339	0	0	2	2.3	13	15.1	63	73.2	8	9.3
366	0	0	6	6.6	18	20.0	54	60.0	12	13.4
376	9	9.8	13	14.2	25	27.4	43	47.2	1	1.0
392	11	11.9	16	17.3	32	34.7	32	34.7	1	1.1
425	0	0.0	7	7.7	26	28.8	50	55.5	7	7.7
463	4	4.4	14	15.5	24	26.6	44	48.8	4	4.4

APPENDIX TABLE 7. Seed Yield of 20 Heads per plant, 11 Plants per row. Polycross Test (PT2) 1954 and analysis of variance

Rep. No.	Clone Number									Replication Total
	366	339	376	463	284	14	425	392	188	
I	2.05	1.15	1.05	3.45	.95	2.55	1.15	1.70	1.25	
	2.00	1.45	.05	1.70	1.15	1.65	.90	1.05	.65	
	1.55	1.85	.50	1.35	1.25	2.45	.75	3.00	1.70	
	1.55	2.40	2.10	1.15	.70	1.25	1.20	.05	2.25	
	1.45	2.30	1.30	1.85	1.65	2.60	1.10	.90	1.35	
	1.50	1.25	.55	1.60	1.05	1.90	.25	1.55	1.30	
	2.40	1.70	2.45	1.35	1.50	2.60	1.65	.05	1.45	
	1.70	1.95	2.15	2.20	.55	.80	1.15	2.85	1.50	
	2.30	1.60	.05	2.10	1.25	1.75	.80	1.50	1.65	
	1.15	2.25	.45	1.40	2.30	1.20	.75	1.85	1.90	
	1.50	2.65	1.25	1.50	1.55	1.80	.50	2.20	1.25	
	19.45	20.55	11.90	19.65	13.90	20.55	10.20	16.70	16.25	149.15

APPENDIX TABLE 7 - continued

Rep. No.	366	339	376	Clone Number 463	284	14	425	392	188	Replication Total
II	1.40	1.70	.50	1.40	.55	1.65	.65	1.45	.85	
	1.45	1.60	.15	1.25	1.75	1.45	1.45	.95	1.10	
	2.20	2.40	1.05	1.35	.35	2.30	1.45	1.20	.60	
	.65	.95	.65	1.15	1.20	1.25	.80	.85	1.30	
	1.55	1.25	1.05	1.85	1.05	1.05	1.25	.75	.10	
	1.00	.25	1.00	.95	1.20	1.70	.55	1.40	1.05	
	1.10	1.00	.70	1.70	1.00	2.05	.60	1.10	1.25	
	.30	1.40	1.00	1.45	.60	1.00	1.15	.85	1.80	
	.40	1.10	1.35	1.50	1.85	2.00	.95	1.55	.20	
	1.15	1.05	.45	1.05	1.15	2.45	1.15	1.25	.60	
	.45	2.40	1.35	1.25	.10	1.10	1.25	2.10	2.30	
	11.65	15.10	9.25	14.90	10.80	18.00	11.25	13.45	11.15	115.55

APPENDIX TABLE 7 - continued

Rep. No.	Clone Number 366	339	376	463	284	14	425	392	188	Replication Total
III	1.25	2.15	1.85	.70	.50	1.15	1.35	2.30	1.50	
	2.25	.95	1.20	1.00	1.85	1.25	1.40	1.80	1.95	
	1.10	1.05	1.50	1.00	1.50	2.15	1.30	.90	2.05	
	.20	2.20	1.25	1.20	1.75	.80	.40	1.65	1.10	
	1.45	2.70	1.55	.50	1.35	1.45	1.20	1.00	1.05	
	1.80	1.70	.30	1.15	1.15	1.85	.80	1.75	1.55	
	.95	1.55	2.05	1.85	1.25	2.25	.75	.70	1.05	
	.80	2.00	1.30	1.05	1.25	1.00	1.20	1.10	1.40	
	1.10	2.00	1.70	.85	.50	1.10	1.10	1.40	1.75	
	1.35	1.25	1.40	.95	1.65	.60	.75	.95	1.90	
	1.35	1.90	.30	1.45	2.05	.95	.65	.85	1.05	
	13.60	19.45	14.40	11.70	14.80	14.55	10.90	14.40	16.35	130.15

APPENDIX TABLE 7 - continued

Rep. No.	Clone Number 366	339	376	463	284	14	425	392	188	Replication Total
IV	1.10	1.50	1.65	1.65	.70	.85	.15	.75	.95	
	1.55	.95	.90	1.20	1.05	2.20	1.55	.90	.80	
	1.20	.05	1.00	1.15	1.10	1.45	.35	.15	.30	
	1.40	2.20	.85	1.95	.10	1.35	1.45	1.25	.70	
	1.20	1.35	1.10	1.25	.55	1.90	.60	.40	.85	
	.80	1.25	.80	1.85	.85	1.00	.35	.40	1.50	
	1.35	2.30	1.80	.75	1.00	1.95	1.60	1.60	.35	
	1.50	.60	1.95	.60	.75	1.60	.80	.75	1.00	
	1.30	.35	2.15	1.20	1.10	2.35	1.30	.85	.80	
	.35	1.10	1.45	1.65	1.95	2.05	1.20	1.10	1.95	
	.75	1.20	.95	1.55	.75	2.10	1.00	.30	1.10	
	12.50	12.85	14.60	14.80	9.90	18.80	10.35	8.45	10.30	112.55
Mean Yield	1.30	1.54	1.14	1.39	1.12	1.63	.97	1.20	1.23	

APPENDIX TABLE 7 - continued

Analysis of Variance

Source	d.f.	S.Square	M.Square	F.	5%	1%
Clones	8	15.6532	1.9566	3.71	2.36	3.36
Replicates	3	8.4884	2.8295			
Error	24	12.6255	.5261			
Between Plants with plots	360	96.8746	.2690			

L.S.D. = .32 grams

Single degree of freedom comparison.

(clones 14, 188, 339, 366, 425) vs (clones 463, 376, 392) i.e. Progeny class 1 vs Progeny class 2. Calculated using linear orthogonal comparison gives the following:

S. Square	M. Square	F.	5%	1%
2.6662	2.6662	5.06	4.26	7.82

with superiority for Progeny class 1.

APPENDIX TABLE 8. Dry Matter Yield Polycross Test (PT2)
1954 - Harvested 20/10/54, and Analysis of Variance

Rep. No.	Clone Number 366	339	376	463	284	14	425	392	188	Total Rep.
I	2267	3068	2836	3798	4478	2422	3428	2388	3666	28351
II	1888	2732	3026	2801	3493	1428	3064	3149	2690	24271
III	2359	2581	3044	3680	4294	2660	3974	3458	3335	29385
IV	3063	3606	4563	4757	3095	4294	2783	5575	4704	36440
Total Clone	9577	11987	13469	15036	15360	10804	13249	14570	14395	118447
Mean Yield	2394	2997	3367	3759	3840	2701	3312	3642	3599	

Analysis of Variance

Source	d.f.	S. Square	M. Square	F.	5%
Clones	8	7,935,163	991,895	2.22	2.36
Replicates	3	8,532,168	2,844,056		
Error	24	10,689,241	445,385		

Single degree of freedom comparison
(Clones 14, 188, 339, 366, 425) vs (Clones 463, 376, 392) i.e. Class 1 vs Class 2. Calculated using linear orthogonal comparisons give the following:

S. Square	M. Square	F.	5%	1%
2,845,766	2,845,766	6.38	4.26	7.82

Indicates superior performance with Progeny class 2.

APPENDIX TABLE 9. Distribution of Growth Types in Progeny of Clone 14 Diallele Cross 1953 and Polycross 1953 and 1954 with Correlation Coefficients

Growth Type	Diallele Cross 1953	Polycross 1953	
0	5	8	
I	4	4	r = .831
II	23	14	
III	45	17	
IV	9	0	

Growth Type	Diallele Cross 1953	Polycross 1954	
0	5	0	
I	4	2	r = .994
II	23	23	
III	45	60	
IV	9	5	

APPENDIX TABLE 10. Distribution of Growth Types in Progeny of Clone 188 - Diallele Cross 1953 and Polycross 1953 and 1954 with Correlation Coefficients

Growth Type	Diallele Cross 1953	Polycross 1953	
0	1	6	
I	2	6	r = .927
II	13	9	
III	25	17	
IV	8	5	

Growth Type	Diallele Cross 1953	Polycross 1954	
0	1	2	
I	2	4	r = .936
II	13	13	
III	25	63	
IV	8	7	

APPENDIX TABLE 11. Distribution of Growth Types in Progeny of Clone 339 - Diallele Cross 1953 and Polycross 1953 and 1954 with Correlation Coefficients

Growth Type	Diallele Cross 1953	Polycross 1953	
0	1	1	
I	1	5	r = .934
II	9	15	
III	28	24	
IV	2	0	

Growth Type	Diallele Cross 1953	Polycross 1954	
0	1	0	
I	1	2	r = .988
II	9	13	
III	28	63	
IV	2	8	

APPENDIX TABLE 12. Distribution of Growth Types in Progeny of Clone 366 - Diallele Cross 1953 and Polycross 1953 and 1954 with Correlation Coefficients

Growth Type	Diallele Cross 1953	Polycross 1953	
0	3	4	
I	1	4	r = .859
II	6	13	
III	31	22	
IV	9	3	

Growth Type	Diallele Cross 1953	Polycross 1954	
0	3	0	
I	1	6	r = .966
II	6	18	
III	31	54	
IV	9	12	

APPENDIX TABLE 13. Distribution of Growth Types in Progeny of Clone 376 - Diallele Cross 1953 and Polycross 1953 and 1954 with Correlation Coefficients

Growth Type	Diallele Cross 1953	Polycross 1953	
0	1	6	
I	8	11	r = .841
II	7	15	
III	12	14	
IV	1	0	

Growth Type	Diallele Cross 1953	Polycross 1954	
0	1	9	
I	8	13	r = .887
II	7	25	
III	12	43	
IV	1	1	

APPENDIX TABLE 14. Distribution of Growth Types in Progeny of Clone 392 - Diallele Cross 1953 and Polycross 1953 and 1954 with Correlation Coefficients

Growth Type	Diallele Cross 1953	Polycross 1953	
0	4	6	
I	4	5	r = .856
II	28	24	
III	25	10	
IV	2	1	

Growth Type	Diallele Cross 1953	Polycross 1954	
0	4	11	
I	4	16	r = .936
II	28	32	
III	25	32	
IV	2	1	

APPENDIX TABLE 15. Distribution of Growth Types in Progeny of Clone 425 - Diallele Cross 1953 and Polycross 1953 and 1954 with Correlation Coefficients

Growth Type	Diallele Cross 1953	Polycross 1953	
0	3	1	
I	6	0	r = .948
II	3	6	
III	19	37	
IV	5	0	

Growth Type	Diallele Cross 1953	Polycross 1954	
0	3	0	
I	6	7	r = .835
II	3	26	
III	19	50	
IV	5	7	