This dissertation has been 64-10,658 microfilmed exactly as received

MINION, Gerald Douglas, 1926-

HERITABILITY OF RESISTANCE IN ALFALFA SELECTIONS TO <u>CERCOSPORA MEDICAGINIS</u> ELLIS AND EVERHART.

Iowa State University of Science and Technology Ph.D., 1964

Agriculture, plant culture

University Microfilms, Inc., Ann Arbor, Michigan

.

HERITABILITY OF RESISTANCE IN ALFALFA SELECTIONS TO <u>CERCOSPORA</u> <u>MEDICAGINIS</u> ELLIS AND EVERHART

by

Gerald Douglas Minion

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of The Requirements for the Degree of DOCTOR OF PHILOSOPHY

Major Subject: Crop Breeding

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean/pf' Graduat College

Iowa State University Of Science and Technology Ames, Iowa

TABLE OF CONTENTS

	Page
INTRODUCTION	1
REVIEW OF PERTINENT LITERATURE	3
The Causal Organism Breeding for Resistance Selection for Combining Ability Heritability in Forages	3 6 12 15
MATERIALS AND METHODS	18
Field Experiment No. 1 Greenhouse Experiment No. 1 Field Experiment No. 2 Greenhouse Experiment No. 2	20 21 23 25
EXPERIMENTAL RESULTS	26
Field Experiment No. 1 Greenhouse Experiment No. 1 Field Experiment No. 2 Greenhouse Experiment No. 2	26 49 63 74
DISCUSSION	86
SUMMARY AND CONCLUSIONS	91
ACKNOWLEDGEMENTS	95
LITERATURE CITED	96

.

.

.

INTRODUCTION

The selection and breeding of alfalfa for resistance to <u>Cercospora medicaginis</u> Ellis and Everhart, has been slow because of a lack of knowledge concerning the heritability of resistance to the organism.

<u>Cercospora</u> disease of alfalfa, often referred to as "summer blackstem", is an important component of the blackstem complex. It is prevalent in the central and eastern United States. The first symptoms are leaf spots followed by blackening of stems and petioles. Defoliation usually occurs, thus reducing the forage yield and quality. The occurrence of the disease in the North Central States region is sporadic but the economic losses are considered significant.

This dissertation is concerned primarily with the evaluation of nine selected parent clones of alfalfa and the ability of these clones to transmit <u>Cercospora</u> resistance to their offspring. Concurrently with the disease evaluations, it was desired to determine the relative merit of each clone for forage yield. To accomplish these objectives, the parent clones were selfed and crossed in a diallel manner and reciprocals were bulked.

Both field and greenhouse experiments were conducted with these crosses and self progenies. Field studies were required to obtain an estimate of the forage yielding poten-

tial of the parent material and to attempt <u>Cercospora</u> ratings under conditions of natural infestation. Greenhouse studies were conducted to obtain actual <u>Cercospora</u> reactions under more controlled conditions by assuring the presence of adequate inoculum and avoiding the masking of <u>Cercospora</u> reaction by other leaf spotting organisms.

Eleven single crosses were selected on the basis of parent clone reactions in diallel combination. Plants from these single crosses were then selfed to obtain the F_2 generation and backcrossed to each respective parent. The resultant progenies, and several check varieties, were then tested under field conditions for yield and under field and greenhouse conditions for comparative resistance or susceptibility to Cercospora.

REVIEW OF PERTINENT LITERATURE

The Causal Organism

On the basis of morphological study of <u>Cercospora</u> <u>medicaginis</u> on <u>Medicago</u> sp., <u>C. davissii</u> Jones on <u>Melilotus</u> sp., and <u>C. zebrina</u> Pass. on <u>Trifolium</u> sp., Horsfal (1929) placed the former two in synonymy with <u>C. zebrina</u>. Nagel (1932) and Jones (1944) from cross inoculation experiments indicated the three species are specialized at the host level. Chupp (1954) in a monograph of the fungus genus Cercospora considered them as three separate species on the basis of condiophore differences.

In greenhouse studies Baxter (1956) made inoculations with conidia of <u>C</u>. <u>medicaginis</u> and found the fungus to be pathogenic on <u>Medicago</u> but not on <u>Melilotus</u> and <u>Trifolium</u>. However, Berger and Hanson (1963) obtained cross-infection of <u>Trifolium</u>, <u>Medicago</u>, and <u>Melilotus</u> with isolates of <u>Cercospora</u> from these hosts. The <u>T</u>. <u>pratense</u> isolates were different in pathogenecity from isolates of <u>T</u>. <u>repens</u> and isolates from <u>M</u>. <u>sativa</u> were different from isolates of <u>M</u>. <u>lupulina</u>, which seemed to indicate the existence of distinct pathogenic races. Berger (1962) in studies of 19 leguminous genera obtained results that indicated pathological races. Generally, Berger and Hanson (1963) found isolates more pathogenic on species of the genus from which they were isolated. Similar

morphology of conidia was noted from leaves of <u>T</u>. <u>repens</u>, <u>T</u>. <u>pratense</u>, and <u>M</u>. <u>sativa</u>. It was also found that the different isolates were more variant in pathogenecity than morphology with <u>C</u>. <u>medicaginis</u> (isolates from <u>Medicago</u> spp.) and <u>C</u>. <u>zebrina</u> (isolates from <u>Trifolium</u> spp.) able to infect either or both <u>Trifolium</u> or <u>Medicago</u> hosts.

It may be pointed out that Chupp (1954) stated that Horsfal (1929) may eventually be proved right in reducing the three species <u>C. medicaginis</u>, <u>C. davissii</u> and <u>C. zebrina</u> to one species. Berger and Hanson (1963) stated more comprehensive comparisons need to be made to finally resolve the synonymy of <u>C. medicaginis</u> and <u>C. zebrina</u>, although their studies indicated they were identical.

The common names for the disease caused by <u>C</u>. <u>medicag</u>-<u>inis</u> are "Cercospora disease of alfalfa" "Cercospora blackstem" and "Summer blackstem". Baxter (1956) stated that "under Iowa conditions the disease caused by <u>C</u>. <u>medicaginis</u> first appears in mid June as small brown spots on the leaves. These spots enlarge to form circular lesions, reddish brown or smoky brown in color and from 2-6 mm in diameter. "When environmental conditions favor sporulation, the lesions become ashy gray in color because of the abundant production of conidia. In heavy infections, entire leaflets are killed and severe defoliation occurs. The leaf spot phase is followed by the appearance of dark brown, or elliptical, or linear lesions on petioles and stems. As the season pro-

gresses these lesions enlarge and coalesce. Under favorable conditions for disease development, entire stems become discolored. Smaller stems, petioles, and peduncles may be killed, resulting in further defoliation and seed loss."

The <u>Cercospora</u> organism causing blackstem of alfalfa is only one of a complex. Geise <u>et al</u>. (1957) in a study of 40 clones of alfalfa, their selfed and open pollinated progenies, isolated eight genera of microorganisms as members of the blackstem complex. Included were: <u>Ascochyta, Colletotrichum, Pleospora, Phoma, Pusarium, Rhizoctonia, Alternaria</u> and one bacterium, <u>Pseudomonas medicaginis</u>.

The significance of <u>Cercospora</u> blackstem is notably variable in its occurence from year to year and from season to season. Jones and Smith (1953) stated that summer blackstem or <u>G. zebrina</u> is less important than spring blackstem caused by <u>Ascochyta imperfecta</u> and very sporadic in occurrence. Carnahan and Graham (1956) indicated blackstem losses were generally greatest for first and last cuttings. Hanson (1956) referred to the variability of <u>G. medicaginis</u> and other alfalfa pathogens in their annual and seasonal occurrence. For the North Central States region Tamini and Rumbaugh (1963) state that among the pathogens of the blackstem complex <u>Phoma herbarum</u> West var. <u>medicaginis</u> Rab. (<u>Ascochyta</u> <u>imperfecta</u> Pk.) and <u>G. zebrina</u> Pass. are the most serious.

Breeding for Resistance

No reports were found that dealt directly with breeding for resistance to <u>Cercospora medicaginis</u>. Reports dealing with breeding for resistance to <u>C. zebrina</u> on alfalfa were found, and because of the possible synonymy these reports should be most pertinent. Studies of breeding for resistance in alfalfa to other disease organisms may also add information relative to our understanding of breeding for resistance to <u>C. medicaginis</u>.

Observations among inbred lines of alfalfa by Tysdal <u>et</u> <u>al</u>. (1942) indicated differences existed in susceptibility to leaf spot and blackstem. A significant and positive correlation was shown between the behavior of inbreds and their outcrossed progeny for both these diseases.

Geise <u>et al</u>. (1956 and 1957) reported studies on inheritance of resistance to <u>C. zebrina</u> and <u>Ascochyta imperfecta</u> in diploid <u>Medicago sativa</u> and <u>M. falcata</u>, and in tetraploid <u>M. sativa</u>. The diploid clones were significantly more resistant than the tetraploid clones. Although they found no difference in pathogenecity among three isolates tested there was a significant difference in host reaction at both the diploid and tetraploid level. A highly significant regression value of 0.68 was found for S₁ progeny reaction on diploid parent clone reaction. The regression value for open pollinated progeny reaction on the diploid parent clone reac-

tion, though only 0.37 was also highly significant. Correlation values for the above two regression values were 0.68 and 0.59 respectively. Renfro and Sprague (1959) in a study of reaction in alfalfa to eight pathogens also found diploids to have the highest degree of resistance.

Johnson (1958) obtained results from eleven alfalfa clones, their single crosses and reciprocals, open pollination progeny of ten clones, and nine commercial varieties for their resistance to <u>C. zebrina</u>. Differences in reaction were highly significant among the clones and among the single crosses. Also, high estimates of heritability were obtained. The mean of the progeny of certain crosses between resistant and susceptible parents was nearer the mean of the resistant parent suggesting at least partial dominance of resistance.

According to Tamimi and Rumbaugh (1963) there is a lack of genetic information relative to the inheritance of reaction to the pathogens <u>P. herbarum</u> var. <u>medicaginis</u> and <u>C.</u> <u>zebrina</u> and the complexity of the blackstem disease itself. For these reasons, they indicate, there is a delay in developing resistant varieties. In an effort to get at these factors in diploid alfalfa a comparison was made between the reaction of each F_1 family, within a diallel of eight clones, and the reaction of the two asexually propagated parental families involved in that cross. On the basis of phenotypic resemblance their results indicated about twice as many F_1 families resembled their more resistant parents as resembled

their more susceptible parents. These results indicated that dominant genes for resistance were more frequent than recessives in the host population tested. A further point of interest in this study was the close genetic correlation of 0.8839 found between the reaction of these plants to <u>P</u>. <u>herbarum</u> and to <u>C</u>. <u>zebrina</u>. Genes with pleiotropic effects were suggested as controlling the observed reaction to both pathogens. Linkage was not considered a factor because it was not thought that the consistently similar degrees of mean dominance, they found, could come from two sets of genes.

Information as to host reaction and breeding methods with other leaf and foliar diseases of alfalfa may apply to the general problem of obtaining resistance to Cercospora blackstem of alfalfa. Tysdal <u>et al</u>. (1942) noted less variability for hybrids and inbreds than was found for outcrossed progenies and original varieties, for leaf spot and blackstem. There was also a decrease in the variability of disease reaction, which indicated selection tends towards greater uniformity for disease reaction and suggests the possibility of selection within inbred lines for disease resistance.

Reitz et al. (1948) studied the reaction of alfalfa varieties, selections and hybrids to <u>Ascochyta imperfecta</u> and found the F_1 's to be intermediate between the parents, although some instances of dominance for resistance were noted. They determined also, that inbreeding followed by

selection and hybridization and a subsequent selection in the F_2 was valuable in raising resistance levels. The factors which effected resistance were not determined, although a glossy hairless leaf surface found in a Ladak selection was not easily wetted and this may have been related to the plant's resistance.

Davis (1951) in a study of common leafspot on alfalfa found highly significant correlations of 0.809 and 0.811 between the means of the selected F_1 progenies and the respective F_2 means from plants rated three and four on a one to five rating. This suggests that the reaction of the F_2 's was determined by the genotypes of the individuals involved in the crosses. The prepotency of one clone classed as being more homozygous resistant was manifested in the F_2 just as it was in the F_1 .

Jones and Smith (1953) suggested the utilization of crosses between <u>Medicago falcata</u> and <u>M. sativa</u> to develop resistance to many alfalfa diseases. Forty tetraploid and diploid clones, their selfed, and open pollinated progeny from crosses between <u>M. falcata</u> and <u>M. sativa</u> were tested by Geise <u>et al</u>. (1957) to determine to what extent certain plants differed in their reaction to pathogens of the blackstem complex and whether the reactions were heritable. The range of reaction was from highly resistant to susceptible, and from parent progeny regression analyses was found to be moderately to highly heritable. Such differential reactions

were found among plants of several sources indicating hereditary resistance is characteristic of all the material and not singular to an individual plant or a single gene of a particular introduction or strain.

In a diallel study of nine tetraploid clones including clonal, self, and diallel cross progeny for leaf spot resistance, Adams and Semeniuk (1958) obtained family heritability estimates that ranged from 79.26 to 89.62 per cent suggesting gene action was largely additive in the material studied. This level of additive genetic control over the phenotype reaction to leafspot disease indicated immediate progress in breeding for resistance to the disease could be expected if sufficient genetic reaction existed in the breeders material. This high additive gene action further suggested the selection of superior genotypes was possible on the basis of family means or by mass selection within replicated clonal nurseries. If, however, a low number of genes were associated with the high heritability of leafspot resistance, efficient selection could quickly reduce the available genetic variance associated with the trait. Synthetic performance and the average performance of clonal crosses for bacterial wilt and common leaf spot were found in close agreement by Pearson and Elling (1960). They suggested resistance to each of these diseases of alfalfa was inherited in a relatively simple and additive manner, and that performance of proposed synthetics could be accurately predicted from clonal cross

data.

Rumbaugh et al. (1962) in a study of the inheritance of reaction of diploid alfalfa clones to two isolates of Phoma herbarum var. medicaginis noted that the genes inducing resistance were recessive and at a low frequency in the population studied. Tamini and Rumbaugh (1963) in an analysis of diallel crosses suggests that dominance and recessive genes controlled resistance of alfalfa to Phoma herbarum var. medicaginis and C. zebrina with the dominance genes more frequent than the recessive. Evidence indicated dominance was not unidirectional and because of this there was an underestimation of the number of loci showing dominance. At least two loci appeared to be involved. The genetic and rank correlations between the reactions of the plants to the two organisms indicated that the genetic factors which controlled the reaction to both were similar. The possibility that genes with pleiotropic effects were involved was not discounted.

Dudley <u>et al</u>. (1963) found that rust resistance and leafhopper yellowing tolerance increased significantly with seven cycles of recurrent phenotypic selection in two pools of alfalfa germ plasm. The genetic variance for leafhopper reaction increased during the study, but, the genetic variance for rust was materially reduced. During this entire study estimates of heritability for rust reaction were higher for rust resistance than for leafhopper yellowing. This suggests the expression for rust reaction was influenced less by the

environment than was leafhopper yellowing.

Selection for Combining Ability

The application of corn breeding methods to the improvement of forage crops was suggested by Kirk (1933), Tysdal <u>et</u> <u>al</u>. (1942), Tysdal and Kiesselbach (1944), Johnson (1952) and Kalton <u>et al</u>. (1955).

Jenkin (1931) recommended the diallel cross to discover further the breeding characteristics of selected perennial ryegrass plants because of their loss of vigor from selfing. The diallel system was recommended also by Williams (1931) to determine the better combinations of selected lines in red clover, white clover and alfalfa.

Sprague and Tatum (1942) presented a method for estimating general and specific combining ability from single crosses in corn. The average performance of a line in hybrid combinations was used to designate general combining ability, whereas, specific combining ability referred to the deviation of certain combinations from their expected average performance. Mendelian segregation and recombination, incorrect genotype classification, and factor interactions were listed as possible causes for specific combining ability. This, as they indicate, would involve genes with dominance or epistatic effects. Their data suggested that genes with additive effects (general combining ability) were more important than those with epistatic or dominance effects in contributing to yield of single crosses of unselected lines. For previously selected material, however, genes conditioning specific combining ability were most effective in determining yield differences. The lines remaining from previous elimination trials probably would have a much higher degree of similarity in performance than the original population and hence genes with dominance and epistatic effects would be more important than those having additive effects.

Bolton, (1948) used the diallel cross to study combining ability in alfalfa in a group of 13 inbred alfalfa clones and in another group of 13 non-inbred clones, as the most refined technique for evaluating combining ability of the parents. Knowles (1950) also used this technique for measuringcombining ability in smooth bromegrass and two groups of crested wheatgrass. The relative importance of general and specific combining ability was shown by the method of Sprague and Tatum (1942). Specific combining ability effects for forage yield were considerably more important in the bromegrass material used than were general combining ability effects. The degree of crossing was uncertain, therefore, specific. effects were thought to be a result of this factor. In noninbred Fairway strains of crested wheatgrass, general and specific combining ability effects were similar, while in inbred Fairway strains, not previously selected for combining ability, general effects were decidedly greater.

Wilsie and Skory (1948) made crosses in all combinations among seven low-crown type alfalfa clones to determine their relative value in forage yield. General combining ability, as determined by yields of open pollination progenies, was positive, but not significantly correlated with specific combining ability as determined by yields of single crosses.

Kalton <u>et al</u>. (1955) related polycross, topcross and clonal studies to singlecross performance on the assumption that the latter gave the best estimate of combining ability of a clone. Pearson and Elling (1958) showed that synthetic varietal performance can be predicted on the basis of single cross performance for characters whose inheritance is conditioned by additive factors. Although the best synthetics yielded less than the average of the single crosses, results indicated the clones were properly rated by this method. These results were essentially substantiated by Downey (1960) who found single cross progenies of 16 unrelated clones to be the most accurate in predicting synthetics from these clones.

Kehr and Graumann (1958) found that general combining ability for forage yield was quite similar for six parental clones as measured by their average performance in two-clone synthetics. Specific combining ability for yield also was noted.

Frakes <u>et al</u>. (1961) analyzed a diallel of four alfalfa clones for general and specific combining ability effects with respect to natural height, long stem length, dry matter

yield, natural width, and stem number. Results indicated general combining ability effects were significant at the .01 level for the characters natural height and long stem length, whereas, for natural width and numbers of stems per plant combining ability effects were not significant. No significant effects were noted for specific combining ability. Wilcox (1962) however, found significant effects (.01 level) for both general and specific combining ability for fall growth habit, and spring vigor in a study of nine elite clones in single cross combination. For forage yield, general combining effects were significant at the .01 level and specific combining ability effects at the .05 level.

Heritability in Forages

The heritability of combining ability for yield of bromegrass was determined by Hawk and Wilsie (1952). They found values of 0.48 and 0.79 by regressing S_1 open pollination progeny on the S_0 open pollination progeny and the S_2 open pollination progeny on the S_1 open pollination progeny, respectively. Replicated parent progeny correlations in orchard grass studies by Kalton <u>et al.</u> (1952) showed values significant at the .01 level ranging from 0.52 for second cutting yields to 0.79 for panicle number.

Thomas and Kernkamp (1954) found a wide variation in heritability for the same character from test to test in

bromegrass with the same genotype. Heritabilities of 15, 19, and 25 per cent were determined for first cutting protein yield from three separate locations in a polycross study. Heritabilities for forage yields in the same studies ranged from 0-31 per cent. Grissom and Kalton (1956) obtained heritability values in bromegrass of 16, 19, 46 and 48 per cent for leaf disease score, leafiness percentage, spring vigor score, and forage yield respectively, as measured by the parent progeny regression.

Seedling vigor heritabilities in alfalfa, which included both additive and non-additive gene effects, were determined by Carnahan <u>et al</u>. (1959) for three locations from 14 clones in a diallel series. Heritability values were 66, 87, and 83 per cent for Indiana, Nebraska, and Pennsylvania respectively. Heritabilities for fall growth habit in Indiana, Minnesota, Nebraska, and North Carolina were 81, 83, 93 and 74 per cent respectively.

Pergament and Davis (1961) obtained heritability estimates in alfalfa using two widely differing alfalfa selections, their reciprocal F_1 crosses and their respective F_2 progenies. Differences included growth habit, height and size of leaves and stems. Heritabilities for mature height and yield were estimated from regression and variance components assuming both disomic and tetrasomic inheritance. Variances based on tetrasomic inheritance and those based on regression and analysis of variance components were in close

agreement. Estimates of total heritable variance by these methods ranged from 31.5 to 61.8 per cent for mature height and from 12.1 to 26.2 per cent for yield.

Six alfalfa clones in diallel crosses studies for combining ability by Kehr (1961) had heritabilities of 71, 58, 85 and 58 per cent respectively for spring and autumn growth rate, rate of recovery, and forage yield. Based on individual variance components, Wilcox (1962) obtained heritabilities of 0.91 for autumn growth habit, 0.76 for yield, 0.86 for autumn growth recovery, and 0.75 for spring vigor.

MATERIALS AND METHODS

Nine clones of alfalfa, seven with some resistance to <u>Cercospora medicaginis</u> and two susceptible, were crossed in a diallel series. The parental designation sources are as follows:

- M247 a Falcata type plant from a Minnesota selection of Siberian X Ladak.
- C609 Minnesota 277, a wilt resistant selection of Ladak origin.
- C610 Minnesota 281, a wilt resistant selection of Ladak origin, resistant to <u>Pseudopoziza medicaginis</u>.
- C605 Iowa 177-7, a 3-way cross from C610 X (C602 X C625), rated as having some resistance to <u>C</u>. <u>medicaginis</u>.
- C221 Nebraska 1563, a wilt resistant survivor from the F_1 of a cross Medicago falcata X (Turkestan FPI 107298 X Ladak selection).
- C618 South Dakota 1108, a cold-resistant, wilt resistant, and leafspot resistant clone from the cross Semipalatinsk X Turkestan SPI 20711).
- 414-10 Iowa selection from an F_1 of (Iowa 33 X Turkey 170446).
- C607 Iowa 186-11, a wilt resistant selection from a 3way cross C10 X (Iowa 35 X C2).

C628 Iowa 157-12, from a 3-way cross C10 X (C63 X Iowa 56) resistant to wilt and leafhopper.

Five propagules of each clone were established in the greenhouse in the fall of 1960. During the winter of 1960-1961 each clone was self pollinated by tripping each flower with a toothpick. The nine clones were also crossed in a diallel manner during this period. To aid in making the crosses the standard petal was clipped and each flower tripped onto a small piece of construction paper, formed into a V shape, to collect the pollen. Flowers were then emasculated by suction from a small vacuum pump and the pollen collected from the selected male was transferred to the stigma of the appropriate female parent.

When selfed and crossed seeds were mature, pods were harvested and threshed and reciprocals were bulked. Maturity normally occurred in four to five weeks after pollination.

Since greenhouse results are not necessarily indicative of the reaction of biological material under field conditions, both field and greenhouse studies for <u>Cercospora</u> reaction were conducted on the progeny. Field studies were also necessary to determine the forage yield potential of the progenies.

Field Experiment No. 1

On April 18, 1961 seeds were scarified and planted in three-quarter inch square paper bands filled with sterilized soil. Also seeded were two commercial varieties, Ranger and DuPuits, to be used as checks. These two varieties had previously been rated for reaction to <u>C. medicaginis</u>. Coincidental with this procedure, cuttings made from the parent clones were rooted in vermiculite.

During the period May 25-26, 1961, the 36 F_1 seedling progenies, nine self progenies, nine clonal progenies, and two check varieties were transplanted into the field. The experimental design used was a 7 X 8 rectangular lattice with three replicates repeated once. An individual plot consisted of eight single plants spaced two feet apart in plot rows 40 inches apart. Each entry was replicated six times, therefore each progeny included 48 plants in the experiment.

Two forage harvests were taken in the summer of 1962, the first cutting on June 6, and the second on July 7. Yields were recorded in pounds per plot and an analysis of variance was computed on the data from each harvest.

The complex of leaf spotting organisms attacking the plants during the latter part of the summer of 1962, made it impossible to obtain any reliable scores for <u>Cercospora</u> reaction. Individual entries were, however, rated September 3, 1963, with a score of from 1 to 9, 1 indicating no leaf-

spotting and 9 indicating extreme leaf-spotting. This same method of ranking for <u>Cercospora</u> reaction was used in other experiments referred to in this dissertation.

Data for forage yield and <u>Cercospora</u> reaction were taken on a plot basis and analyzed as a randomized block design. Means of pertinent entries were analyzed according to Method 4, Model 1, as proposed by Griffing (1956) to obtain estimates of general and specific combining ability of the clones.

Heritability estimates for forage yield and <u>Cercospora</u> reaction were computed by the analysis of variance technique and by the parent progeny regression technique.

Greenhouse Experiment No. 1

On March 30, 1961, other seedling populations of these F_1 progenies were established in the greenhouse. These were established in five four-inch clay pots with four plants per pot for each entry, therefore a total of 20 plants represented each entry. Varieties Banger and DuPuits were included as checks and the material arranged in a randomized block design with five replications. Check entries were duplicated making a total of 40 entries per replication.

Plants were inoculated June 13, with mycelial suspensions of the <u>C</u>. <u>medicaginis</u>, incubated in a humidity chamber for three days at $70-80^{\circ}$ F, and then allowed to grow an ad-

ditional 11 days in a warm greenhouse. Progenies and checks were then scored for reaction to the disease organism. The culture of <u>Cercospora</u> used had previously been isolated and tested for virulence by Dr. Don C. Norton.¹

Transfers of the culture were made to sterile potato dextrose agar, plated in 100 mm petri dishes, under asceptic conditions. The culture was then allowed to grow for three weeks at room temperature at which time it was ready for use. Preparation of the culture for inoculation of the plants was accomplished by blending the mycelial growth from one petri dish in 100 ml of distilled water. A Waring blendor was used for this purpose with about one minute blending time considered adequate. Following the blending procedure the solution was strained through folded cheesecloth to eliminate any material too large for the jets of the one liter "Sure-Shot" sprayer used to apply the inoculum. Distilled water was added to bring the total solution to 225 ml and then two or three drops of tween 20 emulsifier were included to assure satisfactory dispersion of the suspensions on the leaf surfaces. One petri dish of culture prepared in this manner was considered adequate inoculum for each 40 pots of planted material.

Material to be inoculated had been cut back previously

¹Associate Professor, Iowa State University, Department of Botany and Plant Pathology.

to a uniform height so that two weeks of new growth had accrued by treatment time. This was done to reduce the amount of foliage to be treated and to facilitate rating the plants for <u>Cercospora</u> reaction.

These same progenies were tested in two subsequent trials in the summer of 1961, the first on July 29, and the second on August 30. The material was arranged in a 7 X 7 partially balanced lattice with five replications for both trials. The nine parental clones, however, were now added and this made up the total of 49 entries. Data on disease reaction were analyzed as a partially balanced lattice, but since this design showed no increase in efficiency over a randomized block design, the error term from the randomized block design was used in the analysis of variance presented. Parent progeny relationships and heritability estimates were computed as described for Field Experiment No. 1.

Field Experiment No. 2

From the Greenhouse No. 1 screening data, 11 F_1 crosses were selected on the basis of parental performance in diallel combination and clonal progeny performance. The F_1 representatives in this group were from parents rated low X low, low X intermediate (two), low X high (two), intermediate X low, intermediate X intermediate, high X intermediate (two), and high X high (two). During the winter of 1961-62, these plants were selfed and backcrossed to their respective parents, and the parent clones also were selfed. Seed progeny from these crosses and selfs and seven checks were planted in the greenhouse in the early spring of 1962. Checks included the varieties Ranger, DuPuits, Culver, S.C. 118 and F.D. 100. Varieties Ranger and DuPuits were entered twice in each replication.

A field experiment designed as a 7 X 7 simple lattice, repeated once, was used for the evaluation of these seedlings. Entries consisted of 11 F_2 progenies (from selfing the 11 selected F_1 crosses) 22 backcross progenies, nine self progenies (one entry from selfing each of the original parent clones), and seven checks.

On May 16-17, 1962, six seedlings of each entry in each of four replicates were transplanted into the field. Individual plants were spaced two feet apart in rows 40 inches apart. Two forage harvests were made in the summer of 1963, the first on June 7, and the second on July 16. Leafhoppers were controlled with Malathion insecticide applied at weekly intervals from the last forage harvest until a scoring for <u>Cercospora</u> reaction was made August 30.

An analysis of variance was computed on the data obtained.

Greenhouse Experiment No. 2

Other population progenies of the same entries described in Field Experiment No. 2 were established in the greenhouse during the first week of April 1962. Sixteen plants in four four-inch clay pots with four plants per pot represented each entry. The same simple lattice design described in Field Experiment No. 2 was used also in this experiment. Inoculation procedures were similar to those indicated in Greenhouse Experiment No. 1. Three separate trials were conducted during the summer of 1962 in which the entries were scored for <u>Cercospora</u> reaction. After scoring the plants on July 3, August 8, and September 8, an analysis of variance was computed for the data obtained.

EXPERIMENTAL RESULTS

Field Experiment No. 1

Nine selected parent clones were selfed and crossed in a diallel manner. The 36 F1 progenies, eight self progenies, propagules of each parent clone, and two check varieties were established to obtain an evaluation of the ability of the parent clones to transmit resistance to their offspring. Further, it was considered essential to have an evaluation of the yielding potential of the material tested for Cercospora The analysis of variance for forage yield, with reaction. appropriate mean squares, is presented in Table 1. Data obtained are from three of the six replicates. Severe winter killing occurred during the winter of 1961 in many plots representing one-half of the lattice design; therefore, data were not obtained from this portion of the experiment. Mean squares for treatments, which includes genetically different types of entries, were significant at the .01 level of probability for the two cuttings June 6, and July 12, 1962. Orthogonal comparisons of the treatments showed mean squares significant at the .01 level for all components except among checks for all cuttings, and among selfs and among clones for the second cuttings. Drouth conditions during part of the period were undoubtedly responsible for these results in the second cutting. These results also showed that general com-

Table 1. Analysis of variance for forage yield of nine parent clones, eight self and all single cross progenies, and check varieties, Field Experiment No. 1, 1962

	Degrees	Mean s	Mean squares							
Source of variation	of freedom		Second cutting July 12	yield						
Replications	2	41.24**	3.23*	34.11**						
Treatments	2 55	41.29**	3.54**	60.77**						
Checks vs. others	1	169.21**	8.44**	245.50**						
Crosses vs. selfs and clones	1	538.97**	62.56**	968.80**						
Clones vs. selfs	1	140.15**	24.20**	280.84**						
Among checks	1	1.64	2.04	6.94						
Among clones	1	25.69**	1.47	26.75**						
Among selfs	1	17.01**	1.93	25.50**						
Among crosses	35	31.27**	2.00**	41.16**						
General combining ability	3		4.69**	151.55**						
Specific combining ability	27	° 4.35	1.20	8.45						
Error		3.22	0.94	5.46						
Standard error		1.036	• 560	1.349						

*Mean square significant at the .05 level.

**Mean square significant at the .01 level.

bining ability for forage yield was highly significant with no significance indicated for specific combining ability. This points up the fact that additive effects were of relatively greater significance than non-additive effects in the material tested.

The analyses of this experiment, and other experiments in this dissertation, showed no increase in efficiency for the lattice designs used so the results presented are those obtained using randomized complete block designs.

Mean yield of the first cutting in pounds per plot for the clones, eight self progenies, and 36 single crosses are presented in Table 2, second cutting in Table 3, and total yield in Table 4. Self progenies of C607 failed to establish and a check variety, Du Puits, was substituted. Also presented in these tables are estimates of the general combining ability effects of the clones and the predicted single cross yields based on combining ability effects. Crosses involving either M247 or C618 show noticeably higher means. This is to be expected on the basis of the good general combining ability effects for yield of these two clones. Clone 414-10 also showed good general combining ability effects for yield but clones C605, C607 and C221 showed poor general combining ability effects for yield. The remainder of the clones showed slightly below average general combining ability effects. Generally the mean yields of the selfs were lower than those indicated by the mean of the

Parent clone ^a	M247	C609	C610	C605	C221	C618	414- 10	C607	C628	gi	x of line	x of clone	x of self
$\begin{array}{c} \text{M247}^{b}\\ \text{M247}^{c}\\ \text{C609}^{b}\\ \text{C609}^{c}\\ \text{C610}^{b}\\ \text{C610}^{c}\\ \text{C605}^{b}\\ \text{C605}^{c}\\ \text{C221}^{c}\\ \text{C618}^{c}\\ \text{C618}^{c}\\ \text{414-10}^{b}\\ \text{414-10}^{c}\\ \text{C607}^{b}\\ \text{C607}^{c}\\ \text{C628}^{b}\\ \text{C628}^{c}\\ \end{array}$		19.25 18.63	19.38 17.16 14.95 14.57	10.48 16.50 12.05 12.40 12.16 12.73	17.92 19.00 13.49 13.43 13.62 14.53 10.72 9.60	22.45 20.13 18.02 19.16 18.15 17.73 15.25 16.93 16.69 16.40	20.48 20.23 16.05 15.23 16.18 17.06 13.28 14.06 14.72 15.70 19.25 19.00	16.81 18.63 12.38 12.77 12.51 12.93 9.61 9.23 11.05 9.40 15.58 15.63 13.61 12.97	18.70 21.00 14.28 14.30 14.41 14.70 11.51 9.60 12.95 13.10 17.48 17.60 15.51 14.53 11.84 11.83	+4.20 -0.21 -0.08 -2.98 -1.54 +2.98 +1.02 -2.65 -0.75	18.93 15.05 15.18 12.63 13.90 17.82 10.10 12.92 14.59	15.7 13.76 16.40 8.23 12.56 16.50 11.16 11.10 10.33	9.63 6.40 11.26 8.30 7.03 13.53 8.96 - 11.20

٩

Table 2. Mean yield in pounds per plot of nine parent clones, eight self and all single closs progenies, and estimates of their general combining ability, and predicted single cross yields, first cutting, Field Experiment No. 1, June 6, 1962

^aReciprocals bulked.

^bPredicted yield $\cdot \cdot = \pm 0.518$.

^cObserved yield $\cdot \cdot = \pm 1.035$.

Table 3. Mean yield in pounds per plot of nine parent clones, eight self and all single cross progenies, and estimates of their general combining ability, and predicted single cross yields, second cutting, Field Experiment No. 1, July 12, 1962

Parent clone ^a M247	C 609	C610	C605	C221	C618	414 10	C607	C62 8	Ei	T of line	x of clone	x of self
M247 ^b M247 ^c C609 ^b C609 ^c C610 ^b C610 ^c C605 ^b C605 ^c C221 ^b C221 ^c C618 ^c 414-10 ^b 414-10 ^c C607 ^b C607 ^c C607 ^c C628 ^b C628 ^c	4.26 4.37	4.53 3.53 4.62 5.00	3.83 3.80 3.92 4.33 4.19 3.87	4.10 4.77 4.19 4.33 4.46 4.17 3.76 3.63	5.22 4.47 5.33 5.58 4.88 5.67 4.88 5.15 5.15 4.57	5.08 5.17 5.23 5.40 5.40 5.40 5.40 5.40 5.40 5.43 6.00	4.979 4.929 4.9290 4.40 3.8130 5.4.90 5.4.90 5.4.90	4 5 4 5 5 4 4 5 6 5 5 4 5 5 4 4 5 6 5 5 4 5 6 6 8 7 9 3 1 3 7 3 9 3 5 6 6 8 7 9 3 1 3 7 3 9 3 5 6 6 8 7 9 3 1 5 6 5 5 4 5 6 5 5 5 4 5 6 5 5 5 4 5 6 5 5 5 4 5 6 5 5 5 4 5 6 5 5 5 4 5 6 5 5 5 4 5 6 5 5 5 6 5 5 5 4 5 6 5 5 5 6 5 5 5 6 5 5 5 6 5 5 5 6 5 5 5 6 5 5 5 6 5 5 5 6 5 5 5 6 5 5 5 6 5 5 5 6 5 5 6 5 5 5 6 5 5 6 5 5 6 5 5 6 5 5 5 6 5 5 6 5 5 6 5 5 6 5 5 5 6 5 5 5 6 5 5 6 5 5 6 5 5 5 6 5 5 6 5 5 5 6 5 6 5 5 5 5 6 5	-0.29 -0.20 +0.07 -0.63 -0.36 +0.76 +0.62 -0.26 +0.30	4.51 4.59 4.81 4.20 4.44 5.42 5.30 4.51 5.02	2.60 4.60 5.00 3.60 4.27 3.70 4.37 4.40 4.00	1.90 1.70 4.67 2.27 2.37 2.90 3.30 -

^aReciprocals bulked.

^bPredicted yield $\cdot = \pm 0.288$.

^cObserved yield . . = ± 0.559 .

ς Θ

Table 4. Mean yield in pounds per plot, total of two cuttings of nine parent clones, eight self and all single cross progenies, and estimates of their general combining ability, and predicted single cross yields for two cuttings, Field Experiment No. 1, June 6 and July 12, 1962

Parent clone ^a	M247	C609	C610	0605	C221	C618	41 <i>4</i> 1 10	C607	C628	g _i	x of line	र्र of clone	x of self
$\begin{array}{c} \text{M247}^{b} \\ \text{M247}^{c} \\ \text{C609}^{b} \\ \text{C609}^{c} \\ \text{C610}^{b} \\ \text{C610}^{c} \\ \text{C610}^{c} \\ \text{C605}^{b} \\ \text{C605}^{c} \\ \text{C221}^{b} \\ \text{C221}^{c} \\ \text{C618}^{b} \\ \text{C618}^{c} \\ \text{414-10}^{b} \\ \text{414-10}^{c} \\ \text{C607}^{b} \\ \text{C607}^{c} \\ \text{C628}^{b} \\ \text{C628}^{c} \\ \text{C628}^{c} \end{array}$		23.52 23.00	23.91 20.70 19.59 19.56	20.30 20.30 15.98 16.73 16.37 16.60	22.02 23.76 17.70 17.77 18.09 18.70 14.48 13.23	27.67 24.60 23.35 24.50 23.74 24.40 20.13 22.56 21.85 20.96	25.56 24.80 21.24 19.46 21.63 23.60 18.02 19.46 19.74 21.13 25.39 25.27	20.98 23.60 16.66 16.67 17.05 17.33 13.44 13.10 15.16 13.60 20.81 20.30 18.70 17.86	23.48 26.63 19.16 19.50 19.55 19.56 15.94 12.67 17.66 17.53 23.31 23.63 21.20 20.36 16.62 16.96	+3.92 -0.40 -0.01 -3.62 -1.90 +3.75 +1.64 -0.44	19.65 20.05 16.83 18.34 23.28 21.49 19.61	18.30 18.36 21.40 11.83 16.83 20.30 15.33 14.33 14.33	11.53 8.10 15.43 10.57 9.40 16.43 12.27 14.03 14.03

^aReciprocals bulked.

^bPredicted yield $\cdot \cdot = \pm 0.674$.

^cObserved yield . . = \pm 1.349.

single crosses or the clonal mean. This occurred for both first and second cuttings. Combining ability effects for clone M247 in the second cutting were below average, possibly reflecting the dry conditions that existed during the growth period after the first cutting.

Duncan's multiple range tests were made on the ranked mean yields of these treatments to determine the least significant ranges for plot means. The results are shown in Table 5 for the first cutting, Table 6 for the second cutting, and Table 7 for the means representing the total of the two cuttings.

The analysis of variance for <u>Cercospora</u> reaction, September 3, 1963, with appropriate mean squares is presented in Table 8. Readings were not made in 1962, as previously indicated, because of the complex of leaf spotting organisms attacking the alfalfa plants the latter part of the summer. There were other leaf spotting organisms present in the late summer of 1963, however, quite uniform <u>Cercospora</u> reaction was indicated by comparison with the previous year's observations.

Significant differences were obtained for treatments, but this mean square when compared with the error mean square does not appear of sufficient magnitude to indicate a large selection differential among the alfalfa plants. This is further borne out by an observation of the ranked means in Table 9 where the Duncan's multiple range test is used as a

Table 5. Ranked means for first cutting forage yield of nine parent clones, eight self and all single cross progenies, and check varieties, Field Experiment No. 1, June 6, 1962

Treatments	Mean plot yield			Le	ast lev	si el	gni (Du	fica	ant n's	ra Mu	nge lti	s at ple	t ti Rai	ne nge	1 pe Tea	er (st)	ent	
M247 x C628 M247 x 414-10 M247 x C618 C618 x 414-10 C609 x C618 M247 x C221 M247 x C607 M247 x C609 C610 x C618 C618 x C628 M247 x C610 C610 x 414-10 C605 x C618 M247 x C605 C618 M247 x C605 C618 M247 x C605 C618 C221 x 414-10 C618 x C607 C609 x C618 C609 x C610 C610 x C221 414-10 x C221 414-10 x C221 C609 x C628 C605 x 414-10 C609 C618 selfed	21.06 20.23 20.13 19.26 19.16 19.00 18.63 17.07 17.07 16.94 16.50 16.40 15.70 15.64 14.54 1	888888888888888888	ע ע ע ע ע ע ע ע ע ע ע ע ע ע ע ע ע ע ע	000000000000000000000000000000000000000			111111111111111111111111111111111111111	සි ස	hhhhhhhhhhhhhhhhh		うりりりりりりりりり	*****	1111	n n	n	0		

 $\frac{\omega}{\omega}$

ì

Table 5 (Continued)

•

Treatments	Mean plot yield	Least significant ranges at the 1 per cent level (Duncan's Multiple Range Test) ^a
C609 x C221 C221 x C628 414-10 x C607 C610 x C607 C609 x C607 C609 x C605 C221 C609 x C605 C607 x C628 C610 selfed C628 selfed 414-10 C607 C628 Ranger Du Puits M 247 selfed C605 x C628 C605 x C628 C605 x C628 C605 x C607 414-10 selfed Du Puits C605 selfed C605 C221 selfed C609 selfed	$13.44 \\ 13.10 \\ 12.97 \\ 12.94 \\ 12.77 \\ 12.74 \\ 12.57 \\ 12.40 \\ 11.84 \\ 11.27 \\ 11.20 \\ 11.84 \\ 11.27 \\ 11.20 \\ 11.17 \\ 11.11 \\ 10.34 \\ 10.14 \\ 9.74 \\ 9.60 \\ 9.6$	f gg h i j k l m n n o o o pp p q q q q q q q q q q q q q q

^aMeans belonging to the same subgroup (same letter) are not significantly different.

Table 5 (Continued)

Treatments	Mean plot yield	Least significant ranges at the 1 per cent level (Duncan's Multiple Range Test) ^a
Mean	13.74	

•

-

monto 1													
Treatments	Mean plot yield		Leas le	t si evel	gnif (Dur	'ican Ican'	t re s Mu	inges iltir	: at ble F	the lange	1 pe Tes	r ce t) ^a	nt
C610 x C618 C618 x C628 C618 x 414-10 C610 x 414-10 414-10 x C628 C605 x C618 M247 x C628 C221 x 414-10 C605 x 414-10 C609 x C618 C609 x C628 C607 x C628 C609 x C610	6.67 6.03 6.00 5.83 5.63 5.63 5.57 5.43 5.40 5.33 5.20 5.13 5.00	8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4		d d d d d d d d d	e e e e e e e e	f f f f f f	5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	h	<u> </u>			
C610 M247 x C607 414-10 x C607 C610 x C628 M247 x C221 C610 selfed C618 x C607 C609 C221 x C618 M247 x 414-10 M247 x C618 C221 x C628 C610 x C607 C607 414-10	5.00 5.00 4.97 4.90 4.87 4.67 4.67 4.67 4.67 4.67 4.67 4.67 4.57 4.57 4.43 4.40 4.40 4.37	a a a a a a a a a a a a a a a a a a a	ע ע ע ע ע ע ע ע ע ע ע ע ע ע					ත හ හ හ හ හ හ හ හ හ හ හ හ හ හ හ	"hhhhhhhhhhhhhhhhh		りりりりりりりりりりりりり	******	1 1 1

.

Table 6. Ranked means for second cutting forage yield of nine parent clones, eight self and all single cross progenies, and check varieties, Field Experiment No. 1, July 12, 1962

~

· .

Treatments	Mean plot yield		Leas le	st si evel	lgni: (Du	ficar ncan	nt ra 's Mu	inges iltij	s at ple l	the Range	1 pe e Tes	er ce st) ^a	ent	
M247 x C609 C609 x C605 C609 x C221 C221 C609 x 414-10 C221 x C607 C610 x C221 C628 C609 x C607 C605 x C607 C610 x C605 Banger M247 x C605 C618 C605 x C221 C605	y1eld 4.37 4.33 4.33 4.27 4.23 4.20 4.17 4.00 3.90 3.87 3.80 3.63 3.60	8 8 8 8 8 8 8 8 8 8	10 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	c c c c c c c c c c c c c c c c c c c		e e e e e e e e e e e e e e e e e e e	s M ffffffffffffffffffffffffffffffffffff	ngti) Beeseeseeseeseeseeseesee	hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhh	the Bange 1 1 1 1 1 1 1 1 1 1 1 1 1	1 pe Tes j j j j j j j j j j j j j	t) kkkkkkkkkkkkkkkkkkkkkk		
M247 x C610 414-10 selfed C605 x C628 Du Puits C618 selfed C628 selfed Du Puits M247 C221 selfed C605 selfed	3.53 3.30 3.07 3.00 2.90 2.83 2.73 2.60 2.37 2.27		Ъ	C C	đ đ	e e e	ſ ſ ſ	80 80 80 80 80	h h h h	1 1 1 1 1 1	りむりむりりりり	K K K K K K K K K	111111111111111111111111111111111111111	

Table 6 (Continued)

÷

^aMeans belonging to the same subgroup (same letter) are not significantly different.

Table 6 (Continued)

.

•

. .

.

.

Treatments	Mean plot yield	Least significant ranges at the 1 per cent level (Duncan's Multiple Range Test) ^a
M247 selfed C609 selfed	1.90 1.70	l m m
Mean	4.27	
		

.

86

.

• -		age yield, total of two suttings of nine parent and single cross progenies, and check varieties, Field 062
Treatments	Mean plot yield	Least significant ranges at the 1 per cent level (Duncan's Multiple Range Test) ^a
M247 x C62	28 26.63 8	

Table 7.	Ranked means for forage yield, total of two cuttings of nine parent	
	clones, eight self and single cross progenies, and check varieties, Field	
	Experiment No. 1, 1962	

Treatmentes	yield		1	eve	1 (Dun	can	's	Mul	tip	le	Ran	ge T	est) ^a	
M247 x C628 C618 x 414-10 M247 x 414-10 M247 x C618 C609 x C618 C609 x C618 C609 x C618 M247 x C221 C618 x C628 M247 x C607 C610 x 414-10 M247 x C609 C605 x C618 C610 C221 x C618 C221 x C618 M247 x C607 C610 x C610 414-10 x C607 M247 x C605 C618 C618 x C607 C609 x C610 C610 x C628 C609 x C628 C609 x 414-10 C605 x 414-10 C610 x C221 C609	21.40 e 21.13 e 20.96 e	1 b 1 b 1 b 1 b 1 b 1 b 1 b 1 b			111111111111111111111111111111111111111	සි ස	hhhhhhhhhhhhhhhhh			**************************************		m	n		
						9			~						

Table 7 (Continued)

Treatments	Mean plot yield	Lea	ast Leve	sig l (nif Dun	ica can	nt 's	ran Mul	ges tip	at le	th Ran	e 1 ge	pe Tes	r c t) ^a	ent		
M247 C618 x 414-10 C609 x C221 C221 x C628 C610 x C607 C607 x C628 C605 C609 x C605 C609 x C605 C610 x C605 C610 x C605 C610 selfed Hanger C628 C628 selfed C221 x C607 C605 x C221 C605 x C607 C605 x C628 Du Puits 414-10 selfed C605 Du Puits M247 selfed	18.30 17.86 17.77 17.53 17.33 16.96 16.83 16.73 16.67 16.60 16.43 15.53 15.53 15.53 15.53 14.63 14.63 14.03 13.23 13.60 13.23 13.10 12.67 12.47 12.87 11.83 11.70 11.53	đ	e e e	f f f f	50 50 50 50 50 50 50 50 50 50 50 50 50 5	h hhhhhhhhhh	11111111111	もっとうようようようようようようようようよう	*************	11111111111111		nnnnnnnnnnnnnnnnnn	000000000000000000000000000000000000000	υυυυυυυυυυυυυυυυ υ υυ	ט ט ט ט ט ט ט ט ט ט ט ט ט ט ט ט ט ט ט		ちちちちちちちちちちちちちちちちちちちちちちちちちちちちちちちちちちちちちちち

^aMeans belonging to the same subgroup (same letter) are not significantly different.

Table 7 (Continued)

Treatments	Mean plot yield	Least significant ranges of the 1 per cent level (Duncan's Multiple Range Test) ^a	st significant ranges of the 1 per cent evel (Duncan's Multiple Range Test) ^a r s t s t t	
C605 selfed C221 selfed C609 selfed	10.57 9.40 8.10	r		もちち
Mean	18.01			

Table 8. Analysis of variance for <u>Cercospora</u> reaction of nine parent clones, eight self and all single cross progenies, and check varieties, Field Experiment No. 1, September 3, 1963

Source of variation	Degrees of freedom	Mean squares
Replication Treatments Checks vs. others Crosses vs. clones and self Clones vs. selfs Among checks Among clones Among selfs Among crosses General combining ability Specific combining Error	$ \begin{array}{c} 1 \\ 1 \\ 2 \\ 8 \\ 7 \\ 35 \\ 8 \\ 27 \\ \end{array} $	10.02** 3.04** 28.67** 8.26** 0.00 0.12 3.87** 2.52** 2.16** 4.60** 1.44* 0.84
Standard error		• 529

**Mean square significant at the .01 level.

test of the mean Cercospora scores.

Table 10 presents the mean <u>Cercospora</u> scores for the nine parent clones, their self and single cross progenies and estimates of their general combining ability effects. The negative general combining ability effects of C618, C609 and M247 indicate that these clones were better than average in contributing resistance to the crosses in which they were involved. Clone C618, itself, and cross progenies had means lower than representatives of the other clones and their progenies. The mean square for general combining ability (Table 8) is significant at the .01 level. This indicates

Ireatments	Mean plot score	Leas le	t signi: vel (Du	ficant ncan's	ranges a Multipla	at the e Rang e	5 per c Test) ^a	ent
414-10	7.00	a.	·····					
Du Puits	7.00	a						
Du Puits	6.67	a	Ъ					
Ranger	6.67	a	ъ					
C221 selfed	6.33	a	ъ	С				
$C609 \times 414 - 10$	6.33	a	Ъ	С				
414-10 selfed	6.00	a	ъ	С	đ			
414-10 x C628	6.00	a	ъ	C	đ			
414-10 x C6-7	6.00	a	ъ	С	đ			
C221 x 414-10	6.00	a	ъ	С	đ			
C607	6.00	a	ъ	С	đ			
C62 8	6.00	a	ъ	С	đ			
C221	6.00	a	Ъ	C	đ			
C605 selfed	6.00	a	ъ	С	đ			
C605 x C607	5.67	a	ъ	C	đ	е		
$M247 \times C221$	5.67	a	ъ	C	đ	e		
C605 x C628	5.67	a	ъ	С	đ	е		
C605 x C221	5.67	a	ъ	С	đ	е		
C628 selfed	5.67	a	ъ	C	đ	е		
$C610 \times C605$	5.67	a	ъ	G	đ	е		
C610 x C607	5.67	a	ъ	C	đ	е		
C610 selfed	5.33	a	ъ	C	đ	е	f.	
C605	5.33	a	ъ	C	đ	e	f	
C221 x C628	5•33	a	ъ	C	đ	е	f f f	
M247	5•33	a	ъ	C	đ	е	f	
$C610 \times 414 - 10$	5•33	a	ъ	C	đ	е	f	
$M247 \times C607$	5.33	a	ъ	С	đ	е	f f	
M247 x C628	5.33	a	ъ	С	đ	е	f	

Table 9. Ranked means for <u>Cercospora</u> reaction of nine parent clones, eight self and all single cross progenies, and check varieties, Field Experiment No. 1, September 3, 1963

Treatments	Mean plot score	Least sign level (D	ificant uncan's	; ranges ; Multip	at th e le Rang	5 per je Test)	cent a
C609 x C610 M247 x 414-10	5.00	þ	С	đ	e	f f	g
C609 selfed	5.00 5.00	b b	C	d d	e	ı f	8
C609 Serred	4.67	5	с с	đ	e	f	e E
C618 x C607	4.67		c	đ	e	f	ზ თ
C610 x C628	4.67		c	đ	e	f	5 0*
C609 x C607	4.67		c	đ	ē	ŕ	8 07
M247 x C610	4.67		c	ã	e	f	5
C609 x C221	4.67		č	ã	ē	f f	0 &
M247 x C605	4.67		c	đ	e	f	g
C607 x C628	4.67		Ċ	đ	e	f	ğ
C221 x C618	4.33			đ	е	f	g
C609 x C605	4.33			đ	e	f	g
C610 x C221	4.33			đ	е	f	ğ
C605 x C618	4.33			đ	е	f	ğ
M247 selfed	4.33			đ	е	f	ĝ
C618 x 414-10	4.33			đ	е	f	g
C610	4.00				е	f	g
C221 x C607	4.00				e	f	g
$C605 \times 414 - 10$	4.00				е	f	g
C618 selfed	3.67					f	g
C609 x C628	3.67					f	_ g
C609 x C618	3.67					f f	ġ
C618 x C628	3.67					I	g
C610 x C618	3.33						ති කි
C618	3•33						ß

•

•

Table 9 (Continued)

^aMeans belonging to the same subgroup (same letter) are not significantly different.

Table 9 (Continued)

Treatments	Mean plot score	Least significant ranges at the 5 per cent level (Duncan's Multiple Range Test) ^a
M247 x C618 M247 x C609	3•33 3•33	e e
Mean	5.06	

.

Table 10. Mean <u>Cercospora</u> scores of nine parent clones, eight self and their single cross progenies, and estimates of their general combining ability and predicted single cross scores, Field Experiment No. 1, September 3, 1963

Parent clone ^a M247	C609	C610	C605	C221	C 618	414- 10	C 607	C62 8	gi	₹ of line	₹ of clone	x of self
M247 ^b M247 ^c C609 ^b C609 ^c C610 ^c C610 ^c C605 ^b C605 ^c C221 ^c C618 ^b C618 ^c 414-10 ^b 414-10 ^c C607 ^b C607 ^c C628 ^b C628 ^c	4.25 3.33	4.68 4.67 4.44 5.00	4.87 4.67 4.63 4.33 5.06 5.67	4.87 5.67 4.67 5.06 5.25 5.67	3.68 3.47 3.67 3.33 4.33 4.33 4.33 4.33 4.33	5.30 5.06 5.06 5.39 5.38 5.38 5.68 5.00 5.64 5.09 4.33	4 5 • 3 7 7 5 5 5 5 4 4 • 5 • 6 7 5 7 5 5 5 5 4 4 • 6 • 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	4 5 4 3 4 4 5 5 5 5 5 3 3 5 6 5 4 6 9 2 7 1 7 1 3 2 7 4 0 1 7 5 5 5 5 3 3 5 6 5 4 0 1 7 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	-0.16 -0.40 +0.03 +0.22 +0.22 -0.97 +0.65 +0.32 +0.08	4.67 4.46 4.83 5.00 5.00 3.96 5.37 5.09 4.88	5.33 4.67 4.00 5.33 6.00 3.33 7.00 6.00 6.00	4.33 5.00 5.33 6.00 6.33 3.67 6.00 - 5.67

^aReciprocals bulked.

^bPredicted score . . \pm \pm 0.265. ^cObserved score . . = \pm 0.529. 5

.

that additive genetic variance is a major factor for transmission of resistance to <u>Cercospora</u>. Some non additive gene action is indicated by the significant mean square (.05 level) for specific combining ability. This may be due to dominance, or non-allelic interaction of resistance.

Heritabilities for forage yield and <u>Cercospora</u> reaction were computed by components of variance and regression. The components of variance method is as follows:

Heritability =
$$\frac{2 \sigma_g^2 + \sigma_s^2}{2 \sigma_g^2 + \sigma_s^2 + \sigma_s^2 + \sigma_e^2}$$

where d^2g = additive and additive x additive gene action, d^2s = the specific combining ability or that portion of the genetic variance attributed to dominance epistasis, and other factor interactions, and d^2e = error variance.

The determination of $\sigma^2 g$ is by subtracting the components of mean square for specific combining ability, $\sigma^2 e + k_1 \sigma^2 s$, from the components of mean square for general combining ability, $\sigma^2 e + k_1 \sigma^2 s + k_2 \sigma^2 g$, and dividing the remainder by the coefficient of $\sigma^2 g$, or k_2 , which is equivalent to n-2, where n = the number of parent clones.

The second method used for computing heritabilities was the regression of progeny means (determined as general combining ability effects) on the means of the parents.

Estimates of heritability for the two characters varied considerably as determined by the two techniques as shown in Table 11. Heritability estimates were sufficiently high, how-

Method	First cutting 1962	Forage_yield Second cutting 1962	Total of two cuttings 1962	Cercospora reaction September 3, 1963
Variance components	•918	•916	.889	.641
Regression	.602*	•115	• 543	•355**

Ł

Table 11. Estimates of heritability based on variance components^a and regression of progeny means on means of parents for forage yield and <u>Cercospora</u> reaction, Field Experiment No. 1

*Significant at the .05 level of probability.

****Significant** at the .01 level of probability.

^aHeritability =
$$\frac{2 \sigma_g^2 + \sigma_g^2}{2 \sigma_g^2 + \sigma_g^2 + \sigma_g^2 + \sigma_e^2}$$

ever, that it appears that good progress could be made by selecting for higher forage yield and for <u>Cercospora</u> resistance within the material in this study.

Greenhouse Experiment No. 1

This experiment consisted of three separate trials. Diallel cross progenies of the nine parent clones and two check varieties made up the first trial, whereas the subsequent two trials involved these same progenies with clonal progenies in addition. <u>Ceroospora</u> reaction scores were made 14 days after inoculation, as previously described.

Greenhouse screening was an essential part of the <u>Cer-</u> <u>cospora</u> studies. In the field, natural inoculum often is absent or, if present, symptoms of infection of alfalfa often are obscured by the presence of other leafspotting organisms. Greenhouse conditions provided a minimum of interference from other sources. Typical <u>Cercospora</u> leaf spots under greenhouse conditions are shown on the check variety Du Puits (Figure 1) and the single cross M247 x C607 (Figure 2).

The analysis of variance for the first trial is presented in Table 12. The large error mean squares, by comparison with the standard error of the mean in the first trial, is a result of the sampling procedure used on this occasion which increased n from 5 to 20. Each of the four plants per replication was scored separately and not as a single score per

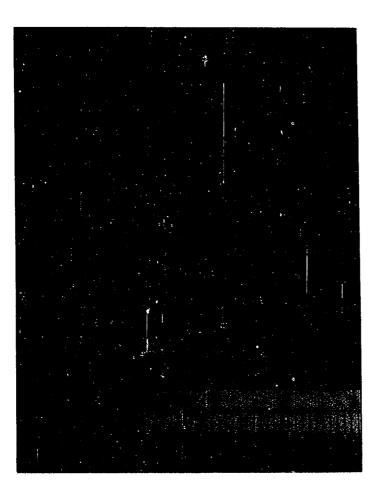


Figure 1. <u>Cercospora</u> leaf spots on the variety Du Puits under greenhouse conditions

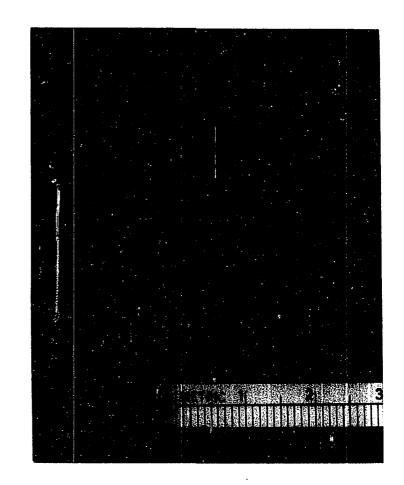


Figure 2. <u>Cercospora</u> leaf spots on the single cross M247 X C607 under greenhouse conditions

Table 12. Analysis of variance for <u>Cercospora</u> reaction of single cross progenies of nine parent clones, and check varieties, Greenhouse Experiment No. 1, June 27, 1961

Source of variation	Degrees of freedom	Mean squares
Replications Treatments Checks vs. crosses Ranger vs. Du Puits Within Ranger Within Du Puits Among crosses General combining abilit Specific combining abilit Error		98.91** 18.60** 129.04** 0.00 1.10 11.70 16.67** 55.79** 5.08 3.93
Standard error of the	mean	•443

**Mean square significant at the .01 level.

replication, in which case n would be 5. The very large mean square for checks vs. crosses in the orthoganol comparisons of the treatment components is an indication of the greater susceptibility to <u>Cercospora</u> by the checks. Means of the varieties and of the crosses are compared in Table 13 with the use of Duncan's multiple range test.

Table 14 shows the means of the single cross progenies and the estimated general combining ability effects. It is noted that clones M247, 414-10, C610 and C628 all show above average general combining ability effects. Most significant of these is the effect for M247 which is highest for <u>Cercos</u>-<u>pora</u> reaction. Clones C618, C221 and C605 appear most

2(, 19	01													
Treatments	ž	<u> </u>	·····	Leas	st s: evel	ignii (Dur	ficar ncan'	nt ra 's Mu	inges iltig	s at ble H	the Bange	5 pe e Tes	r ce t) ^a	nt
Du Puits Ranger M247 x C607 M247 x 414-10 M247 x C628 M247 x C628 M247 x C618 M247 x C609 M247 x C609 M247 x C610 Ranger 414-10 x C628 M247 x C605 Du Puits 414-10 x C607 C609 x C607 C609 x C607 C609 x C628 C607 x C628 C609 x C628 C610 x C618 C610 x C628 C610 x C628 C	6.30 5.90 5.82 5.75 5.65 5.65 5.65 5.60 5.30 5.21 5.15 5.65 5.30 5.21 5.15 5.25 5.25 5.25 5.25 5.25 5.25	2222222222222222222	ע ס ס ס ס ס ס ס ס ס ס ס ס ס ס ס ס ס ס ס				111111111111111111111111111111111111111	8 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	hhhhhhhhhhhhhh		1;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;	********		m m

.

.

Table 13. Ranked means for Cercospora reaction of single cross progenies of nine parent clones, and check varieties, Greenhouse Experiment No. 1, June 27, 1961

Table 13 (Continued)

Treatments	ž	Least significant ranges at the 5 per cen level (Duncan's Multiple Range Test) ^a
C605 x C607 C610 x C605 C618 x 414-10 C609 x C618 C609 x 414-10 C609 x C610 C221 x C628 C609 x C221 C618 x C607 C221 x C618 C605 x C221 C618 x C605	3.80 3.80 3.78 3.70 3.51 3.50 3.50 3.46 3.35 3.35 2.98 2.50	g h i j k l g h i j k l h i j k l j k l j k l j k l j k l j k l k l k l k l
Mean	4.55	

^aMeans belonging to the same subgroups (same letter) are not significantly different.

Parent clone ^a	M247	C 609	C610	C605	C221	C618	414- 10	C607	C 628	gi	x of line
M247 ^b M247 ^c C609 ^b C609 ^c C610 ^c C605 ^b C605 ^c C221 ^c C618 ^c C221 ^c C618 ^c C618 ^c C618 ^c C618 ^c C618 ^c C618 ^c C618 ^c C618 ^c C618 ^c C607 ^c C607 ^c C607 ^c C628 ^b C628 ^c C628 ^c		5.73 5.65	5.50 5.64 4.03 3.50	5.41 5.30 3.85 4.85 3.72 3.80	5.31 5.65 3.75 3.47 3.62 3.80 3.43 2.99	5.24 5.75 5.16 3.70 3.54 4.30 3.36 2.50 2.82 3.35	6.14 5.82 4.57 3.52 4.45 4.05 4.26 4.85 4.16 4.80 4.08 3.79	5.95 5.85 4.39 4.26 4.25 4.25 4.25 4.25 3.97 3.97 3.97 3.997 3.997 3.997 3.997 5.15	$\begin{array}{c} 6.01\\ 5.75\\ 4.80\\ 4.32\\ 4.20\\ 4.13\\ 4.00\\ 3.50\\ 3.96\\ 4.85\\ 4.85\\ 4.55\\ 4.55\\ 4.55\end{array}$	+1.44 -0.13 -0.26 -0.44 -0.54 -0.62 +0.29 +0.10 +0.16	5.68 4.28 4.19 4.03 3.94 3.88 4.67 4.48 4.5

Table 14. Mean <u>Cercospora</u> scores of single cross progenies of nine parent clones, estimates of their general combining ability effects, and predicted single cross scores, Greenhouse Experiment No. 1, June 27, 1961

a Reciprocals bulked.

^bPredicted score . $= \pm 0.222$. ^cObserved score . $= \pm 0.443$.

favorable for contributing <u>Cercospora</u> resistance to their offspring.

Non additive gene action is indicated by the significant (.05 level) mean square for crosses vs. clones (Table 15). This is possible due to dominance or partial dominance of resistance to Cercospora. However, since the mean square for specific combining ability is not significant (also an indication of non additive effects) and the mean square for general combining ability is significant at the .01 level, it would appear that inheritance of resistance to Cercospora is due primarily to an additive gene action. There is no indication of dominance or partial dominance of resistance in the June 27 (Table 12), or the August 30 trials (Table 15). Mean squares for general combining ability were significant at the .01 probability level in the three greenhouse trials, which indicates the greater importance of additive over non additive gene action for resistance to Cercospora.

Checks were observed to be significantly different from the clones and single crosses as indicated by the mean squares for checks vs. others. The significance of this may be further observed in Tables 16 and 17 where a Duncan's multiple range test of the means is presented. These tables also show clone C618 to have the lowest means for <u>Cercospora</u> reaction which is an indication of resistance to the organism. General combining ability effects as shown in Tables 18 and 19 are similar to those observed in the first trial (Table 14)

Source of variation	Degrees of freedom	July 29	Mean squares August 30
Replications Treatments Checks vs. others Crosses vs. clones Among checks Among clones Among crosses General combining ability Specific combining ability	$ \begin{array}{c} 4 \\ 48 \\ 1 \\ 1 \\ 3 \\ 8 \\ 35 \\ 8 \\ 27 \\ \end{array} $	8.34** 5.17** 35.77** 6.14* 0.26 7.52** 4.24** 14.15** 1.31 1.48	6.55* 5.59** 46.30** 0.40 2.98 7.67** 4.34** 14.30** 1.38
Error Standard error of the mean	192	1.48 .544	1.99 .631

Table 15. Analysis of variance for <u>Cercospora</u> reaction of nine parent clones, their single cross progenies, and check varieties, Greenhouse Experiment No. 1, 1961

*Mean square significant at the .05 level.

**Mean square significant at the .01 level.

reatment	Mean plot score		Least s: level	ignific (Dunce	cant an's	ranges a Multiple	t the Range	5 per Test)	cent
Du Puits	6.4	a				<u></u>			
1247 x C610	6.2	a	Ъ						
1247 x C628	6.2	a	b						
lone M247	6.2	a	Ъ						
1247 x C609	6.2	a	Ъ						
1247 x 414-10	6.2	a	Ъ						
langer	6.0	a	Ъ	C					
Du Puits	6.0	a	Ъ	C	-				
628	5.8	a	Ъ	C	đ				
1247 x C607	5.6	a	þ	C	đ	e			
14-10 x C607	5.6	a	Ъ	С	đ	e			
langer	5.6	a	b	С	đ	e			
lone C605	5.6	a	Ъ	С	đ	е			
607 x C628	5.4	a	Ъ	C	đ	е			
lone C607	5.4	a	Ъ	С	đ	e	•		
1247 x C605	5.2	a	Ъ	C	đ	e	f		
lone 414-10	5.2	a	Ъ	C	đ	е	f		
C610 x C628	5.2	a	b	C	đ	е	f		
2618 x C628	5.0	a	b	C	đ	e	f	g	
1247 x C618	5.0	a	Ъ	С	đ	е	f	g	
C609 x C610	4.8	a	р	C	đ	е	f	g	h
C610 x C607	4.8	8.	Ъ	C	đ	е	f	g	h
C610 x 414-10	4.8	a	р	C	đ	е	f	g g	h
C610 x C605	4.8	a	Ъ	С	d	е	f	g	h
M247 x C221	4.6	a	Ъ	С	đ	е	f	g	h
C605 x C628	4.6	a	Ъ	C	đ	e	f f	g	h
C609 x C607	4.6	a	ъ	C	đ	е	f	g	h
C609 x C628	4.6	a	Ъ	С	đ	е	f	g	h
C609	4.6	ล	ъ	C	đ	е	f	g	h

Table 16. Ranked means for <u>Cercospora</u> reaction of nine parent clones, their single cross progenies, and check varieties, Greenhouse Experiment No. 1, July 29, 1961

,

Treatment	Mean plot score	Least leve	signif: l (Dun	icant : can's	ranges Multipl	at the Le Rang	e 5 per ge Test	c cent t) ^a	
414-10 x C628	4.4	b	C	đ	e	f	g	h	<u></u>
C609 x C618	4.4	þ	С	đ đ	е	f	සා හා හා භා භා භා භා භා භා භා	h	
C605 x C221	4.4	b	С	a	е	f	g	h	
Clone C610	4.4	b	C	đ	е	f	g	h	
$C609 \times 414 - 10$	4.4	ď	С	đ	е	f	g	h	
C605 x 414-10	4.2		С	d d	e	f f	g	h	
C609 x C605	4.2		С	đ	е	f	g	h	
C221 x C607	4.0			đ	е	f	g	h	1
C610 x C618	4.0			đ	e	f f	g	h	ļ
C610 x C221	4.0			đ	е	f	g	h	i
C605 x C607	3.8				е	f	g	h	1
C605 x C618	3.8 3.8 3.4				e	f f	g	h	1
C221 x C628	3.8				е		g	h	1
Clone C221	3.4					f	g	h	1
C609 x C221	3.4					f f	g	h	1
C221 x 414-10	3.4					f	g	h	1
C618 x C607	3.2						g	h	i
C221 x C618	3.0							h	i
C618 x 414-10	2.4								1
Clone C618	2.4								1
Mean	4.7								

Table 16 (Continued)

^aMeans belonging to the same subgroup (same letters) are not significantly different.

Treatments	Mean plot score		Least s level	ignific (Dunca	ant ran n's Mul	ges at tiple R	the 5 p ange Te	er cent st) ^a	
Ranger	7.2	a							
M247 x C605	6. 8	a	ъ						
Du Puits	6.8	a	Ъ						
M247 x 414-10	6.6	a	Ъ	С					
M247 x C609	6.6	a	Ъ	С					
M247 x C628	6.6	a	Ъ	С					
Ranger	6.4	a	ъ	C	đ				
M247 x C610	6.4	a	Ъ	С	đ				
M247 x C607	6.2	a	ъ	С	đ	е			
Clone C628	6.2	a	ъ	С	đ	е			
$M247 \times C618$	6.0	a	Ъ	С	d	е	f		
Clone M247	5.8	a	ъ	С	đ	е	f		
C618 x C628	5.6	a	ъ	С	đ	e	f	g	
Clone C605	5.6	a	ъ	С	đ	е	f	g	
C609 x C618	5.4	a	Ъ	С	đ	е	f	g	
414-10 x C607	5.4	a	Ъ	C	đ	e	f	g	
Clone 414-10	5.4	a	ъ	C	đ	е	f	g	
C607 x C628	5.4	a	ъ	С	đ	е	f	g	
Du Puits	5.4	a	Ъ	С	đ	е	f	g	
Clone C607	5.2	a	ъ	C	đ.	е	f	g	
C609 x C628	5.2	a	Ъ	С	đ	е	f	g	
C610 x C607	4.8		ъ	С	đ	е	f	g	
C605 x C618	4.8		ъ	С	đ	e	f	g	
C605 x C628	4.8		Ъ	С	đ.	е	f	g	
$M247 \times C221$	4.8		ď	С	đ	e	f	ත ත ත ත ත ත ත ත ත ත	
C605 x C607	4.8		Ъ	С	đ	е	f	g	
C610 x C605	4.8		Ъ	С	d	е	f	g	
C221 x C628	4.6		Ъ	С	đ	е	f	g g	
Clone C609	4.6		ъ	С	đ	е	f	g	

.

Table 17.	Ranked means for Cercospora reaction of nine clones, their single cross
	progenies, and check varieties, Greenhouse Experiment No. 1, August 30,
	1961

Treatments	Mean plot score		signific L (Dunca					
C221 x C607 414-10 x C628 C609 x C610 C605 x 414-10 C221 x 414-10 Clone C221 C609 x C607 C610 x 414-10 C610 x C618 C610 x C628 C609 x 414-10 C609 x C605 C221 x C618 C609 x C221 C610 x C221 C610 x C221 C610 x C221 C615 x C221 Clone C610 C618 x 414-10 Clone C618	4.6 4.4 4.4 4.4 4.4 4.2 4.2 4.2 4.2 4.2 4.2	b	С С С С	d d d d d d d d d d d	e e e e e e e e e e e	1111111111111111111	භ සා භ භ භ භ භ භ භ භ භ භ භ භ භ භ භ භ	h h h h h h h h h h h h h h h h h h h
Mean	5.0							

Table 17 (Continued)

^aMeans belonging to the same subgroup (same letter) are not significantly different.

.

Table 18. Mean <u>Cercospora</u> scores of nine parent clones, their single cross progenies, and estimates of their general combining ability effects, and predicted single cross scores, Greenhouse Experiment No. 1, July 29, 1961

Parent clones ^a	M247	C 609	C610	C605	C221	C618	414- 10	C607	C 628	gi	x of line	x of clone
M247 ^b M247 ^c C609 ^b C609 ^c C610 ^b C610 ^c C605 ^b C221 ^b C221 ^c C618 ^b C618 ^c 414-10 ^b 414-10 ^c C607 ^b C607 ^c C628 ^b C628 ^c		7.45 6.2	7.75 6.2 5.10 4.8	6.23 5.2 3.98 4.2 4.89 4.8	5.00 4.6 2.56 3.4 3.46 4.0 3.34 4.4	5.43 5.0 3.59 4.4 3.49 4.0 2.77 3.8 1.35 3.0	7.29 6.2 4.24 4.4 5.4 3.83 4.2 3.4 3.4 3.4 3.4 3.4 3.4 3.4 3.4	6.92 5.6 4.68 4.6 5.18 5.18 3.66 3.8 3.66 3.2 3.2 3.2 5.6	7.83 6.2 4.99 4.6 5.2 4.6 5.2 4.6 3.8 5.4 5.6 3 4.5 5.4 4.5 5.4 5.4 5.4 5.4	+1.24 +0.01 +0.30 -0.22 -0.84 -0.81 -0.16 +0.07 +0.39	5.7 4.6 4.8 4.4 3.8 3.9 4.4 4.6 4.9	6.2 4.6 4.4 5.6 3.4 2.4 5.2 5.4 5.8

^aReciprocals bulked.

^bPredicted scores $\cdot = \pm 0.272$. ^cObserved scores $\cdot = \pm 0.544$.

Table 19. Mean <u>Cercospora</u> scores of nine parent clones, their single cross progenies, and estimates of their general combining ability effects and predicted single cross scores, Greenhouse Experiment No. 1, August 30, 1961

Parent clones ^a	M247	C 609	C 610	C605	C221	C618	414- 10	C607	C62 8	gi	ī of line	x of clone
M247 ^b M247 ^c C609 ^c C610 ^c C610 ^c C610 ^c C605 ^b C605 ^c C221 ^c C618 ^c C618 ^c C618 ^c 414-10 ^c C618 ^c C618 ^c 414-10 ^c C607 ^c C607 ^c C628 ^b C628 ^c		6.29	6.14 6.4 4.43 4.4	6.32 6.8 4.61 4.0 4.46 4.8	5.74 4.8 4.03 4.0 3.88 4.0 4.06 3.8	6.17 6.2 4.46 5.4 4.31 4.2 4.49 4.8 3.91 4.0	6.14 6.63 4.28 4.28 4.28 4.28 4.28 4.28 4.28 4.28	6.25 4.25 4.272 4.4.4 4.4 4.4 4.6 3 4.6 5.4	6.69 6.69 54.20 54.45 54.45 54.45 54.45 55 5 5 5 5 5 5	+1.56 -0.15 -0.30 -0.12 -0.70 -0.27 -0.30 +0.02 +0.25	6.3 4.8 4.6 4.8 4.3 4.7 4.6 4.9 5.1	5.8 4.6 3.8 5.6 4.2 2.2 5.4 5.2 6.2

^aReciprocals bulked.

^bPredicted scores . . = \pm 0.316. ^cObserved scores . . = \pm 0.631.

except for 414-10 which in this trial apparently contributed <u>Cercospora</u> resistance to its crosses. Continued above average effect of M247 and below average effect of C618 and C221 are noted.

Heritability estimates were computed by the components of variance technique (Table 20) for the three trials and by the regression method for the last two trials, which included clonal progeny. Both methods indicate good progress could be made by breeding for <u>Cercospora</u> resistance within the material studied. The one exception to this is the third trial which shows a low heritability estimate by the regression method.

Field Experiment No. 2

Eleven single cross progenies were selected for further study from the 36 diallel crosses of the nine parent clones. Plants from these selected F_1 progenies were selfed and backcrossed to each of their respective parents in order to obtain more information on the inheritance of resistance to <u>Cercospora</u> reaction. It was also desirable to test the material for forage yield in comparison with standard varieties to detect if any of the progenies showing improved resistance to <u>Cercospora</u> also possessed good yield potential. Results were measured in pounds of green forage per plot.

An analysis of variance for forage yield obtained from this experiment for two cuttings in 1963 is presented in

Table 20. Estimates of heritability based on variance components^a and regression of progeny means on means of parents for <u>Cercospora</u> reaction, Greenhouse Experiment No. 1, 1961

Method	First trial June 27, 1961	Second trial July 29, 1961	Third trial August 30, 1961			
Variance components	•799	.710	•639			
Regression		•393**	•252			

**Significant at the .01 level.

^aHeritability =
$$\frac{2 \mathcal{J}_g^2 + \mathcal{J}_s^2}{2 \mathcal{J}_g^2 + \mathcal{J}_s^2 + \mathcal{J}_e^2}$$

Table 21. Treatment mean squares were significant at the .01 level as was each orthogonal comparison presented in the table. The large mean square value for checks vs. others, when contrasted with other mean squares, is a reflection of the low mean yields for the check varieties. This was to be expected because four of the check varieties are relatively non-hardy in spaced plantings under Iowa conditions. The significant mean square for within F_1 (between backcross parents) is an indication of the difference in effect on yield of each clonal parent to which each single cross was backcrossed.

Duncan's multiple range test was used to evaluate the difference among the means. Table 22 presents the ranked means for the first cutting, Table 23 the second cutting, and Table 24 the total for the two cuttings combined. Yields of the progeny of M247 x C618 backcrossed to either of the clonal parents suggest a high yield prepotency of these parents. This is further observed in that most crosses which involved either M247 or C618 are among the higher yielding progenies. An averall evaluation of these means shows a wide range of variability for the forage yield.

The analysis of variance of the scores for <u>Cercospora</u> reaction obtained August 30, 1963, is presented in Table 25. The orthogonal comparisons show a non significant difference among selfs, F_2 and backcross progenies. This would be expected if there were equal representation of genes for

Table 21.	Analysis of variance for forage yield of F ₂ and backcross progenies from
	selected single crosses, self progenies from the nine parent clones, and
	seven check varieties, Field Experiment No. 2, 1963

	Degrees	Mean squares	Total
Source of variation	of freedom	First cutting Second cu June 7, 1963 July 16,	itting yield
Replications Treatments Checks vs. others Among checks Among selfs, F_2 and BC Among selfs Among F_2 Among F_2 Among F_1 Within F_1 (between parents) Error	3 48 1 6 2 8 10 21 21 10 11 144		37.91** 281.95** 17.85** 203.61** 15.55** 16.80** 34.80** 52.52**
Standard error of	the mean	.714 .224	•883

**Mean square significant at the .01 level.

Treatment	Treatment mean			_]	Lea	ast Le	t ve	si; 1	gn: (D	ifi und	ica cai	ant a's	ran Mul	ge ti	s pl	at e	t Ra	he nge	1 • 1	pe: Fes	r c t) ^a	ent
(C221 x C618) C618 (M247 x C618) C618 (M247 x C618) M247 (C618 x 414-10) C618 (M247 x C628) M247 (M247 x C628) M247 (M247 x C607) M247 (M247 x C607) M247 (M247 x C607) C607 (C221 x C618) C221 (C221 x C618) Selfed M247 selfed (M247 x C221) C221 (C618 x 414-10) 414-10 (M247 x C221) Selfed (C610 x 414-10) M247 (M247 x C618) Selfed Culver (C610 x 414-10) M247 (M247 x C618) Selfed Culver (C610 x 414-10) Selfed (C618 x 414-10) Selfed (C618 x 414-10) Selfed (M247 x C628) C628 (C221 x C628) C221 (M247 x C628) Selfed C610 selfed (C605 x C221) C221 (M247 x C607) Selfed	41.4 40.4 40.3 39.0 37.6 37.3 36.9 36.1 36.1 36.1 36.0 35.9 34.4 34.3 33.1	aaa	b b		đ	ee	ff	E E	h h	1	ملک هنگ هنگ هنگ ملک ملک ملک ملک	k k k	1111111	n n n n n	000000	p p	đ	r	s				

Table 22. Ranked means for first cutting forage yields of F₂ and backcross progenies, self progenies from the nine parent clones, and seven check varieties, Field Experiment No. 2, June 7, 1963

Treatment	Treatment mean	Least significant ranges at the 1 per cent level (Duncan's Multiple Range Test) ^a
(C610 x 414-10) selfed C609 selfed (C607 x C628) C628 414-10 selfed (C605 x C221) C605 Hanger (C221 x C628) C628 C605 selfed Hanger C607 selfed (C221 x C628) selfed C221 selfed (C607 x C628) C607 (C605 x C221) selfed Du Puits C628 selfed FD 100 Du Puits (C607 x C628) selfed SC 118	29.4 29.3 28.9 28.6 28.2 27.1 24.7 24.6 24.3 23.4 22.9 22.3 21.5 20.6 18.2 18.1 18.1 18.1 16.9 16.6 16.0	pqrs pqrs qrs qrs st tuv tuv tuv uvw uvw uvw uvw vw vw xy xy xy yy
Mean Standard error	32.86 .714	

Table 22 (Continued)

^aMeans belonging to the same subgroup (same letter) are not significantly different.

• •

		· · · · · · · · · · · · · · · · · · ·
Treatments	Mean plot yield	Least significant ranges at the 1 per cent level (Duncan's Multiple Range Test) ^a
(C221 x C618) C618	14.2	a
(C618 x 414-10) C618	12.7	b
$(M247 \times 414-10) 414-10$	12.5	b
(M247 x C618) C618	11.9	bc
(C221 x C618) C221	11.2	c d
(C221 x C618) selfed	11.1	c d
(C605 x C221) C221	10.9	c d e
$(c610 \times 414-10) 414-10$	10.5	def
C618 selfed	10.4	defg
$(C618 \times 414-10) 414-10$	10.3	defg
(C221 x C628) C221	10.3	defg
Culver	10.3	defg
(M247 x C618) M247	10.0	efgh efgh
(M247 x C628) M247	10.0	e f g h
414-10 selfed	9.8	fgh
(M247 x C221) C221	9•5 9•4	fghi
(C607 x C628) C628	9.4	ghij
(M247 x C221) M247	9.2	hijk
(C610 x 414-10) C610	9.1	h 1 j k l
(M247 x C607	9.1	hijkl
Ranger	9.1	hijkl
Ranger	8.9	1 j k 1
(M247 x C221) selfed	8.7	i j k l m
C610 selfed	8.5 8.5	1 j k l m n
(C610 x 414-10) selfed	ؕ5	jklmn
(C221 x C628) C628	8.4	jklmno
$(C605 \times C221) C605$	8.4	klmno
(M247 x C607) M247	8.3	klmno
(M247 x 414-10) selfed	8.2	klmnop

Table 23. Ranked means for second cutting forage yields of F_2 and backcross progenies, self progenies from the nine parent clones, and seven check varieties, Field Experiment No. 2, July 16, 1963

`

.

Treatments	Mean plot yield	Least significant ranges at the 1 per cent level (Duncan's Multiple Range Test) ²
C607 selfed (M247 x C628) C628 (M247 x C618) selfed (M247 x 414-10) M247 C605 selfed M247 x C607 selfed Du Puits C221 selfed C605 x C221 selfed C605 x C221 selfed C608 x 414-10) selfed C609 selfed (C618 x 414-10) selfed C609 selfed (C607 x C628) C607 (C221 x C628) selfed Du Puits FD 100 SC 118 M247 selfed (C607 x C628) selfed (M247 x C628) selfed	8.1 8.1 7.7 7.6 7.4 7.4 7.2 7.4 7.2 7.2 7.1 7.0 7.0 6.9 6.3 6.2 5.5 5.4 5.5	l m n o p q l m n o p q l m n o p q r n n o p q r o p q r o p q r p q r s p q r s p r s r s t r s t r s t t u t u u u
Mean	8.73	

Table 23 (Continued)

.

^aMeans belonging to the same subgroup (same letter) are not significantly different.

reatments	Mean plot yield	 Le														1 per c Test) ^a	
C221 x C618) C618 M247 x C618) C618 M247 x C618) M247 C618 x 414-10) C618 M247 x C628) M247 M247 x C628) M247 M247 x C221) M247 M247 x 414-10) 414-10 M247 x C607) M247 C618 selfed C221 x C618) selfed M247 x C607) C607 C618 x 414-10) 414-10 M247 x C221) C221 (C610 x 414-10) C610 C610 x 414-10) C610 C610 x 414-10) 414-10 M247 x C221) selfed Julver M247 x C618) selfed M247 x C618) selfed M247 x C628) C221 (C605 x C221) C221 (C605 x C221) C221 (M247 x 414-10) M247 M247 x C618) selfed (M247 x 414-10) selfed (M247 x C628) C628 (C618 x 414-10) selfed (C610 selfed 414-10 selfed (C607 x C628) C628	71.6 68.6 65.8 65.5 59.9 57.6 51.5 51.5 51.5 59.8 57.6 51.5 51.5 44.5 44.8 44.8 44.3 43.0 41.3 41.3 40.2	c c c	d d d	 e e e e	ff	s s h h h h h h h h h	1 1 1 1 1 1 1	****	1111111	nnnnnn	0000000	4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	q	rrrr	S S S S		

Table 24. Ranked means for total forage yield from two cuttings of F₂ and backcross progenies, self progenies from the nine parent clones, and seven check varieties, Field Experiment No. 2, 1963

.

Table 24 (Continued)

Treatments	Mean plot yield	Least significant ranges at the 1 per cent level (Duncan's Multiple Range Test) ^a
(C610 x 414-10) selfe (M247 x C607) selfed (M247 x C628) selfed (C605 x C221) C605 C609 selfed Ranger Ranger C605 selfed (C221 x C628) C628 C607 selfed (C221 x C628) selfed (C607 x C628) c607 (C605 x C221) selfed Du Puits C628 selfed FD 100 Du Puits (C607 x C628) selfed SC 118	d 37.9 37.5 37.1 36.6 36.3 36.2 33.2 33.2 33.1 31.5 30.5 29.6 28.4 27.8 25.6 25.2 24.3 23.2 24.3 23.2 24.3 23.2 21.9	pqrs qrs rst rst st tu tu tu tu vw vw vw vw vw vw vw vw vw vw vw vw vw
Mean Standard error	41.60 .883	

^aMeans belonging to the same subgroup (same letter) are not significantly different.

Table 25. Analysis of variance for <u>Cercospora</u> reaction of F₂ and backcross progenies from selected single crosses, self progenies from the nine parent clones, and seven check varieties, Field Experiment No. 2, August 30, 1963

Source of variation	Degrees of freedom	Mean squares
Replications	3	24.95**
Treatments	48	27.30**
Checks vs. others	1	230.26**
Among checks	6	10.38
Among selfs, F2 and backcross	2	6.39
Among selfs	2 8	52.65**
Among F2	10	21.88 **
Among backcrosses	21	18 .57**
Among F ₁	10	24.13**
Within \overline{F}_1 (between backcross parents)	11	13.51**
Error		3.89
Standard error		.402

**Mean square significant at the .01 level.

resistance and susceptibility within the selected F_1 progenies. However, when comparisons were made separately for among selfs, among F_2 and among backcross, means squares were significant at the .01 level indicating good variability existed for resistance within the material tested. These results also indicated significant effects of the different parent clones to which the selected single crosses were backcrossed.

The ranked means for <u>Cercospora</u> reaction and the test of significance of these means by use of the Duncan's multiple range test are presented in Table 26. Clones C618 and M247 would appear to be desirable parents for crosses evaluated under field conditions because their self progenies showed relatively low mean infection per plot. The average mean scores of the check varieties for <u>Cercospora</u> reaction were somewhat higher than those of the F_2 and backcross progenies, indicating progress toward resistance in the experimental material.

Greenhouse Experiment No. 2

This study included the same treatments found in Field Experiment No. 2. As previously indicated, greenhouse studies are necessary in order to get an evaluation of the plant material following more positive inoculation with the pathogen. Though results when compared with field experiments may

Treatment	Treatment mean	Leas le	t s vel	ign (D	ifi unc	can an'	t ra s Mi	ang ult	es a iplo	at th e Ran	e 5 p .ge Te)er ;st
Du Pults	6.6	a										
FD 100	6.6	a										
414-10 selfed	6.3	a	ъ									
C605 selfed	6.0	a	ъ									
Du Puits	6.0	a	ъ									
(C221 x C628) selfed	6.0	a	ъ									
(C607 x C628) C628	5•9	a	ъ	С								
SC118	5.8	a	ъ	С	đ							
Ranger	5.8	a	Ъ	С	đ							
$(C610 \times 414-10) 414-10$	5.8	a	ъ	С	đ							
(M247 x 414-10) 414-10	5.8	a	ъ	С	đ							
C221 selfed	5•7	a	ъ	С	đ							
$(M247 \times C628) C628$	5•7	a	ъ	С	đ							
$(C607 \times C628) C607$	5.6	a	ъ	С	đ							
(C605 x C221) C605	5•5	a	ъ	С	đ	е						
(C605 x C221) selfed	5.5	a	Ъ		đ	е	f					
C628 selfed	5.3	a	Ъ	0	đ	е	f	g				
$(C221 \times C628) C221$	5.3	a	ъ	С	đ	е	f) 2 0 28 28				
(C221 x C618) C221	5.3	a	ъ	C	đ	е	f	g				
C607 x C628 selfed	5.2	a	ъ	C	đ	е	f	g	h			
Ranger	5.1		ъ	С	đ	е	f f f f f f	g	h			
C607 selfed	5.0		Ъ	С	đ	е	f	g	h	1		
$(M247 \times C221) C221$	5.0		Ъ	C	đ	е	f f	g g	h	1		
$(C610 \times 414-10) C610$	5.0		Ъ	С	đ	е	f	g	h	1		
$(M247 \times C607) C607$	5.0		Ъ	С	đ	е	f f	g	h	1		
Culver	4.9		Ъ	C	đ	е	f	g	h	1		
(M247 x 414-10) M247	4.9		Ъ	C	đ	е	f	g	h	i		
C610 selfed	4.8			C	d	е	f	g	h	1		
(C610 x 414-10) selfed	4.8			С	d	e	f	g	h	i		

Table 26.	Ranked means for Cercospora reaction of F2 and backcross progenies, self
	progenies from the nine parent clones and seven check varieties, Field
	Experiment No. 2, August 30, 1963

•

Table 26 (Cont	inued)	
----------------	--------	--

.

Treatment	Treatment mean	Least significant ranges at the 5 per cent level (Duncan's Multiple Range Test) ^a
(C221 x C618) selfed M247 x 414-10 selfed M247 x C607 selfed (C618 x 414-10) 414-10 (C221 x C628) C628 (M247 x C628) M247 C609 selfed (M247 x C621) M247 (M247 x C607) M247 (C605 x C221) C221 (C618 x 414-10) C618 (C221 x C618) M247 (M247 x C618) M247 (M247 x C618) selfed (C618 x 414-10) selfed (M247 x C628) selfed (M247 x C618) C618 M247 x C618) C618 M247 x C618) Selfed (M247 x C618) C618 M247 x C618) selfed (M247 x C221) selfed (M247 x C221) selfed C618 selfed	4.7 4.7 4.6 4.5 4.4 4.1 4.0 4.0 3.9 3.6 3.6 3.6 3.3 3.0 2.8 2.6 1.9	c d e f g h i j c d e f g h i j c d e f g h i j k d e f g h i j k d e f g h i j k l e f g h i j k l f g h i j k l f g h i j k l f g h i j k l j k l h i j k l h i j k l m h i j k l m n m n
Mean	4.8	

^aMeans belonging to the same subgroup (same letter) are not significantly different.

not be identical, greenhouse trials provide the assurance that each plant is given equal opportunity for infection. There is no assurance of this under field conditions. The method of inoculation and scoring for <u>Cercospora</u> reactions was the same as for Greenhouse Experiment No. 1, explained in the Materials and Methods section.

The analysis of variance for three separate trials conducted during the summer of 1962 is found in Table 27. The contrasting magnitude of the mean squares for checks vs. others over the non significant mean squares for among checks, and among selfs, F_2 and backcrosses should be noted. As a general rule, the plot means for <u>Cercospora</u> reaction, among checks, are not randomly distributed over the total range of the treatment means for the trials. Their reaction is well within the upper one-half of the range as indicated on Tables 28, 29 and 30 where the significance of the mean differences is shown by the Duncan's multiple range test.

Mean square for among selfs, F_2 and backcrosses was not significant in the July 3, nor in the September 8 trials (Table 27), which would be expected if there were equal representation of genes for resistance and susceptibility within the selected F_1 progenies. A review of the F_1 selections in the Methods and Materials section would suggest equal representation of genotypes with resistance and susceptibility. Significance at the .05 level was found for the mean square among selfs, F_2 and backcrosses in the August 8 trial (Table

Table 27. Analysis of variance for <u>Cercospora</u> reaction of F_2 and backcross progenies from selected single crosses, self progenies from the nine parent clones, and seven check varieties, Greenhouse Experiment No. 2, 1962

Source of variation	Degrees of	1	lean squares	
	freedom	July 3	August 8	September 8
Replications Treatments Checks vs. others Among checks Among selfs, F_2 and backcrosses Among selfs Among F_2 Among backcrosses Among F_1 Within F_1 (between backcross	3 48 1 6 2 8 10 21 21 10 11		2.45 7.95** 38.22** 3.98 7.84* 6.19* 7.32** 8.62** 15.04** 2.78	3.15 8.12** 115.16** 1.12 2.73 6.00* 5.15* 7.59** 12.31** 3.31
parents) Error	144	2.25	2.43	2.67
Standard error		•750	•779	.816

*Mean square significant at the .05 level.

**Mean square significant at the .01 level.

Ireatment	Mean plot yield	I	least lev	; sig rel (mifi Dunc	cant an's	ran Mul	ges tipl	at t e Ra	he 5 nge '	p er Test	oent) ^a
Ranger	7.25	a										
(M247 x C607) M247	7.00	a	ъ									
(M247 x 414-10) M247	6.75	a	ъ	С								
FD 100	6.75	a	ъ	С								
M247 selfed	6.75	a	ъ	C								
(M247 x C628) M247	6.25	a	ъ	С	đ							
(M247 x C607) selfed	6.25	a	ъ	C	đ							
Ranger	6.25	a	ъ	С	đ							
(M247 x C628) selfed	6.00	a	b	C	đ	e						
Du Puits	6.00	a	ъ	С	đ	e						
(C628 x C607) selfed	6.00	a	Ъ	С	đ	e						
(C628 x C607) C607	6.00	a	ъ	С	đ	е						
(M247 x C618) selfed	6.00	a	ъ	С	đ	е						
414-10 selfed	6.00	a	ъ	C	đ	e						
Du Puits	5.75	а.	ъ	C	đ	e	f					
(M247 x C628) C628	5.50	a	ъ	С	đ	е	f	g				
SC 118	5.50	a	ъ	С	đ	е	f	g				
C628 selfed	5.50	a	Ъ	С	đ	е	f	g	_			
$(M247 \times 414-10) 414-10$	5.25	a	Ъ	C	đ	e	f	g	h			
(C628 x C607) C628	5.25	a	b	С	đ	e	f	න හ හ හ හ	h			
(M247 x 414-10) selfed	5.25	a	b	С	đ	e	f f f f f f f f f f f	g	h			
(M247 x C618) M247	5.00	a	Ъ	С	đ	е	f	g	h	i		
(C221 x C628) selfed	5.00	a	b	С	đ	е	f	g	h	i ,		
(M247 x C221) selfed	4.75	a	b	С	đ	e	f f f f	g	h	i		
(C610 x 414-10) selfed	4.75	a	ъ	्ट	đ	e	f	ğ	h	i	1	
C607 selfed	4.75	a	Ъ	`C	đ	e	ſ	ğ	h	1		
(M247 x C607) C607	4.75	a	b	С	đ	ę	f	g	h	1		
(M247 x C221) M247	4.75	a	Ъ	C	a	e	f	g	h	i		
C610 selfed	4.50		ъ	С	đ	е	f	g	h	i	j	

Table 28. Ranked means for Cercospora reaction of F_2 and backcross progenies, self progenies from the nine parent clones, and seven check varieties, Greenhouse Experiment No. 2, July 3, 1962

Treatment	Mean plot yield	Least lev	; si 7el	gnif: (Dun	icant can's	ran Mul	ng es Lt:1p:	at 1 le Ra	the game	5 pei Test	; cer	nt
C609 selfed (C610 x 414-10) C610 Culver (C618 x 414-10) 414-10 (C610 x 414-10) 414-10 (C211 x C628) C221 (M247 x C618) C618 (C605 x C221) C605 (M247 x C221) C221 C605 selfed (C618 x 414-10) selfed (C605 x C221) C221 (C221 x C618) selfed (C605 x C221) selfed (C605 x C221) selfed (C605 x C221) selfed (C221 x C618) C221 (C221 x C618) C628 (C618 x 414-10) C618 (C221 x C618) C618 (C221 x C618) C618 (C618 selfed Mean	4.50 4.25 4.25 4.00 3.50 3.50 3.25 3.25 3.00 3.00 2.75 2.75 2.75 2.50 2.50 2.50 2.50 2.50 2.50 2.50 2.5	Ъ	C C C	đ đ đ	e e e e	1 1 1 1 1 1 1	ති කි ක ක ක ක ක ක ක ක	h h h h h h h h h h h h		ううちりりりりりりりりりりりりりり	*************	111111111111111111111111111111111111111

Table 28 (Continued)

^aMeans belonging to the subgroup (same letter) are not significantly different.

Ireatment	Mean plot yield	Leas le	st s: evel	lgnii (Dur	ficar ican'	nt re 's Mu	inges iltig	at le F	the lange	5 per Test) ^a
(M247 x 414-10) M247	7.75	a									
SC 118	7.50	a	ъ								
Banger	7.25	a	ъ	C							
(C610 x 414-10) C610	7.00	a	ъ	С	đ						
(M247 x C618) selfed	7.00	a	ъ	С	đ						
(C628 x C607) C607	6.75	a	ъ	C	đ						
(M247 x C628) C628	6.50	a	ъ	С	đ	е					
Ranger	6.50	a	ъ	С	đ	е					
(M247 x 414-10) 414-10	6.50	a	ъ	С	đ	e					
(M247 x C221) selfed	6.25	a	ъ	С	đ	е	ſ ſ				
(M247 x C607) selfed	6.25	a	ъ	С	đ	е	f				
Culver	6.00	a	ъ	С	đ	е	f	g			
C607 selfed	6.00	a	ъ	С	đ	е	f	න න න න න න න			
Du Puits	6.00	a	ъ	С	đ	е	f	g			
414-10 selfed	6.00	a	ъ	С	đ	e	f f	g			
(C518 x 414-10) selfed	5.75	9	Ъ	С	d.	e	f	g			
$(M247 \times C607)$ M247	5•75	a	ъ	C	đ	е	f	g			
(M247 x 414-10) selfed	5•50	a	ъ	C	đ	е	f	g	h		
(M247 x C221) C221	5•50	a	ъ	C	đ	е	f	g	h		
(M247 x C618) M247	5.50	a	ъ	С	đ	е	f	g	h		
FD 100	5.25	a	Ъ	C	đ	е	f f f	g	h		
(C628 x C607) C628	5.25	a	Ъ	C	đ	е	f	g	h		
(M247 x C221) M247	5.25	a	ъ	С	đ	e	f	g	h		
(C628 x C607) selfed	5.25	a	Ъ	C	đ	е	f	ğ	h		
(M247 x C628) selfed	5.25	a	Ъ	С	đ	е	f	g	h		
M247 selfed	5.00	a	b	С	đ	е	f	g	h	i	
(M247 x C618) C618	5.00	a	ъ	С	đ	е	f	g	h	i	
(C610 x 414-10) selfed	5.00	a	ъ	С	đ	е	ſ	g	h	1	
$(C610 \times 414 - 10) 414 - 10$	5.00	a	ъ	С	đ	е	f	ğ	h	1	

Table 29. Ranked means for Cercospora reaction of F_2 and backcross progenies, self progenies from the nine parent clones, and seven check varieties, Greenhouse Experiment No. 2, August 8, 1962

•75 •75 •50 •50 •50	g Q	o c c	d d d	e e	f f	g	h h	i	j	
50 25 25 75 75 50 50 50 50 25 00 50 25 75		с с с	d d d d	e e e e e e	f f f f f f f f f f f f f f f f f f f	የ የ ም ም ም ም ም ም ም ም ም ም ም	"hhhhhhhhhhhhhhh	1111111111111	ھے ملے ھے ھے سے ملے ھے ملے سے ملے سے ملے ملے ھے ملے ملے ملے ک	*********
} } ? ?	•50 •25 •00 •50 •25	•50 •25 •00 •00 •50 •25 •75	• 50 • 25 • 00 • 00 • 50 • 25 • 75	• 50 • 25 • 00 • 00 • 50 • 25 • 75	• 50 • 25 • 00 • 00 • 50 • 25 • 75	.25 .00 .00 .50 .25 .75	.25 g .00 .00 .50 .25 .75	.25 g h .00 h .00 h .50 .25 .75	.25 g h i .00 h i .00 h i .00 i i .50 i i .25 .75 i	.25 g h i j .00 h i j .00 h i j .50 i j .25 j .75

Table 29 (Continued)

^aMeans belonging to the same subgroup (same letters) are not significantly different.

Standard error of the mean .779.

Treatment	Treatment mean		ast sig level (
Ranger	7.75	a							
SC 118	7.75	a							
Du Puits	7.00	a	ъ						
Ranger	7.00	a	ъ						
$(C6\bar{2}8 \times C607) C628$	7.00	a	Ъ						
(C628 x C607) C607	7.00	a	ъ						
(C610 x 414-10) C610	6.57	a	ъ	С					
(M247 x C607) selfed	6.75	a	Ъ	C					
Du Puits	6.75	a	ъ	C					
Culver	6.50	a	ъ	С	đ				
FD 100	6.50	a	ъ	C	đ				
M247 selfed	6.25	a	ъ	С	đ	f			
C607 selfed	6.25	a	ъ	С	đ	f			
(M247 x 414-10) selfed	6.00	a	ъ	С	đ	f	g		
$(C610 \times 414 - 10) 414 - 10$	6.00	a	ď	С	đ	f	g		
$(M247 \times 414-10) 414-10$	6.00	a	ъ	C	đ	f	g		
$(M247 \times C628) M247$	6.00	a	ъ	С	đ	f	g		
(M247 x C618) selfed	6.00	a	ъ	С	đ	f	g		
(C221 x C628) selfed	5•75	a	ď	С	đ	f	g		
$(M247 \times C618) M247$	5•75	a	ъ	C	đ	f	භ භ භ භ භ භ		
$(M247 \times C607) C607$	5.75	a	Ъ	C	đ	r	g		
(C221 x C618) selfed	5.50	a	Ъ	С	đ	f f f	g	h	
$(M247 \times 414-10)$ M247	5.50	a	Ъ	С	đ	f	g	h	
(M247 x C628) C628	5.50	a	Ъ	C	đ	f \	e E	h	
$(C618 \times 414 - 10) 414 - 10$	5.50	a	Ъ	C	đ	f	g	h	
(C221 x C628) C221	5.50	a	b	С	đ	f	g	h	
(M247 x C628) selfed	5.00	a	Ъ	С	đ	f	g	h	j
(M247 x C607) M247	5.00	a	Ъ	C	đ	f f	g	h	1
C609 selfed	5.00	a	ъ	C	đ	f	g	h	j

Table 30. Ranked means for <u>Cercospora</u> reaction of F_2 and backcross progenies, self progenies from the nine parent clones and seven check varieties, Green-house Experiment No. 2, September 8, 1962

Treatment	Treatment mean	Least significant ranges at the 5 per cent level (Duncan's Multiple Range Test) ^a							
C610 selfed (M247 x C221) selfed (C605 x C221) C221 414-10 selfed (C610 x 414-10) selfed C221 selfed (M247 x C618) C618 C605 selfed (C628 x C607) selfed (C221 x C618) C618 (M247 x C221) M247 (C221 x C628) C628 C628 selfed (C605 x C221) selfed (C618 x 414-10) selfed (C618 x 414-10) C618 (C605 x C221) C605 (C221 x C618) C221 (M247 x C221) C221 C618 selfed Mean	4.75 4.50 4.25 4.25 4.00 4.00 4.00 4.00 4.00 4.00 3.75 3.75 3.25 3.25 3.25 3.25 3.00 2.75 2.50 2.50 5.16	b b b b	с с с с с с с с с с с с с с с с с с с	d d d d d d d d d d d d d	ſ ſ ſ ſ ſ ſ ſ ſ ſ ſ ſ ſ ſ ſ ſ ſ ſ ſ ſ	ති හි	h h h h h h h h h h h h h h h h h h h	もうち ちょう ちょう ちょう ちょう ちょう ちょう ちょう ちょう	

Table 30 (Continued)

^aMean belonging to the same subgroup (same letter) are not significantly different.

Standard error of the mean .816.

27). This may be due to chance rather than a difference in gene frequency for resistance to <u>Cercospora</u> in the selected F_1 progenies. Non significant mean squares (Table 27) for within F_1 (between backcross parents) indicates that the difference in each backcross parent, for <u>Cercospora</u> resistance, was not detectable by the analysis of variance.

DISCUSSION

Self and single cross progenies of nine alfalfa clones were evaluated under field and greenhouse conditions, with check varieties, for forage yield and <u>Cercospora</u> reaction. Eleven single crosses were selected for further breeding on the basis of greenhouse performance of the parent clones in diallel crosses. F_1 plants from each selected single cross were selfed and backcrossed to their respective parents. The F_2 and backcross progenies were tested under field conditions for forage yield and under field and greenhouse conditions for reaction to <u>Cercospora</u>.

Data from Field Experiment No. 1 indicated a wide range of variability in forage yield. Clones M247, C618 and 414-10 showed the greatest general combining ability effects, an indication of their value in transmitting high yielding characteristics to their offspring. Most of the single crosses outyielded the check varieties, Ranger and Du Fuits, but it must be remembered that yield data were obtained from spaced plantings. Both clones M247 and C618 are prostrate in growth habit. This characteristic may tend to favor their comparative yield under spaced conditions because of their greater total leaf exposure to sunlight. Possibly the more vertical plants would be favored in terms of leaf area efficiency in a solid stand.

The poor clonal yield of C605 in contrast with the mean

of the single crosses in which the clone was represented, may have been due to poor root development of the vegetative propagules of this clone. In general, however, favorable yields of the clones compared with the means of their single crosses.

Cercospora reaction in the first field experiment should not be regarded as conclusive. Readings under field conditions are difficult because of masking by other leaf spotting organisms and the possible non-uniform distribution of The vertical distribution of the plant may also inoculum. have an influence on the degree of infection. M247 did not respond the same under field and greenhouse conditions. The general combining ability effects for reduced Cercospora reaction of this clone in the field were above average, but greenhouse results were the extreme opposite. This clone's prostrate growth habit may have influenced this reduced reaction, or possibly its apparent higher leaf to stem ratio compared to more erect growing plants. Whatever the factors involved, this clone and its offspring generally exhibited a clean appearance in the field.

Clone C618 showed good general combining ability for reduced <u>Cercospora</u> reaction both under field and greenhouse conditions. This clone has a similar appearance to M247, prostrate growth, high leaf to stem ratio, and general clean appearance.

Clones C221 and C605 showed good general combining

ability effects for reduced <u>Cercospora</u> reaction in the greenhouse, but failed to respond similarly in the field. Combining ability effects for yield of these clones were below average.

Clones 414-10, C610 and C609 exhibited above to near average general combining ability effects for reduced <u>Cer-</u> <u>cospora</u> reaction in the greenhouse. Field results showed C609 as one of the better clones for transmitting factors for reduced <u>Cercospora</u> reaction, while 414-10 and C610 showed positive effects for <u>Cercospora</u> reaction.

Clones C607 and C628 rated as susceptible before the study began showed above average combining ability effects for increased <u>Cercospora</u> reaction in field and greenhouse trials and below average effects for yield. This combination of characteristics indicates they would be a poor choice for breeding purposes.

Environments are quite different in the field vs. greenhouse conditions which results in different physiological responses in plants. It is not known whether physiological responses or morphological characters condition the variant reactions that occur under the two situations.

Non additive gene action is indicated by the significant (.05 level) mean square for specific combining ability, and by significance at the .01 level for the mean square crosses vs. selfs and clones in Field Experiment No. 1. Mean square for crosses vs. others was significant at the .05 level in

Greenhouse Experiment No. 1, July 29, also an indication of non-additive gene action. This may be due to dominance or non-allelic interaction of resistance to <u>Cercospora</u>. However, inheritance of resistance to Cercospora appears to be mainly due to additive gene action since the mean squares for general combining ability were significant at the .01 level in the field experiment (Table 8) and in the three greenhouse trials (Tables 12 and 15).

Heritability values indicate good progress can be made by selection for either resistance to <u>Cercospora</u> or for higher forage yield. In the case of clone C618, there appears to be good phenotypic correlation for both factors, thus good genetic advance would be expected. Favorable results may be expected for M247, but some caution is indicated because of the radical difference in greenhouse vs. field conditions for <u>Cercospora</u> reaction.

Data from Field Experiment No. 1 and Greenhouse Experiment No. 2 indicate a high level of variability for both forage yield and <u>Cercospora</u> reaction. The highest forage yield was obtained from the backcross (c221 x C618) C618. The two clones represented by this cross had the highest mean <u>Cercospora</u> resistance from diallel studies in the greenhouse. Clone C221 did not show good general combining ability for yield in previous studies. This high yield for the above backcross may be a reflection of the good combining ability of C618. Entry (C221 x C618) C618 did not respond as favor-

ably for high <u>Cercospora</u> resistance in the field as in the greenhouse. However, under greenhouse conditions, when either C618 or C221 was: used as the recurrent backcross parent, reduced <u>Cercospora</u> scores normally were obtained.

Crosses involving M247 expressed undesirable susceptibility to <u>Cercospora</u> in the greenhouse but showed considerable resistance to <u>Cercospora</u> as well as high forage yield under field conditions. Self, F_2 , and backcross progenies of M247 showed similar responses. The high mean scores for <u>Cercospora</u> susceptibility in the greenhouse, and the low incidence of disease in the field suggest that environmental factors are of great importance in determining the response of certain alfalfa genotypes to this pathogen.

Heritability estimates, or combining ability effects of the parent clones, were not feasible from the backcross studies because of the unequal representation of parents in the selected single crosses. However, the continued highly significant (.01 level) mean squares for treatments indicates a high level of variability was maintained within the screened progenies and that progress could be made in breeding for resistance to <u>Cercospora</u> reaction. Clones such as C618, M247, and 414-10 are likely choices to include in a synthetic breeding program.

SUMMARY AND CONCLUSIONS

The general objective of this study was to evaluate selected clones of alfalfa for their ability to transmit resistance to <u>Cercospora medicaginis</u> Ellis and Everhart to their progeny. Nine parent clones, seven with some resistance to <u>Cercospora</u> and two susceptible, were selfed and crossed in a diallel manner. Single cross, self, and clonal progenies were established with check varieties in a field experiment to determine forage yield and <u>Cercospora</u> reaction. Field infection was dependent upon natural inoculum.

Single cross and clonal progenies were also established in the greenhouse and screened for resistance to <u>Cercospora</u>. Plants were inoculated with mycelial suspensions of the organism, incubated in a humidity chamber for three days at 70-80°F, and allowed to grow an additional 11 days in a warm greenhouse. Progenies and check varieties were scored on the basis of a 1 to 9 scale (1 being resistant) and ratings indicated the heritable nature of resistance.

An analysis of variance for general and specific combining ability showed mean squares significant at the .01 probability level for general combining ability, but nonsignificant for specific combining ability for both yield and <u>Cercospora</u> reaction in the field and for <u>Cercospora</u> reaction in the greenhouse.

Clones M247, C618 and 414-10 had the highest general

combining ability values for forage yield. This was an indication of the relative value of these clones in transmitting high yielding characteristics to their offspring. Both M247 and C618 are prostrate in growth habit, a character which may give them a comparative yield advantage under space planted conditions.

Clone M247 did not respond to <u>Cercospora</u> the same under field and greenhouse conditions. General combining ability effects for <u>Cercospora</u> reaction suggested resistance in the field, but greenhouse results showed marked susceptibility. The clone and its offspring generally had a clean appearance in the field.

Clone C618 showed good general combining ability for resistance to <u>Cercospora</u> under both field and greenhouse conditions. This clone has an appearance similar to M247, prostrate growth, high leaf to stem ration and general clean appearance.

Clones C221 and C605 showed good general combining ability effects for reduced <u>Cercospora</u> reaction in the field but failed to respond similarly in the greenhouse. Both were below average in general combining ability for yield.

Clones 414-10, C610, and C609 showed above to near average general combining ability effects for reduced <u>Cercos</u>pora reaction in the greenhouse. Field results showed C609 as one of the better clones for transmitting factors for reduced <u>Cercospora</u> reaction, while 414-10 showed positive

general combining ability effects for Cercospora reaction.

Clones C607 and C628, the two parent clones selected for their susceptibility, showed above average general combining ability effects for <u>Cercospora</u> reaction in the field and greenhouse and were below average for yield.

Inheritance of resistance to <u>Cercospora</u> was due primarily to additive gene action, although there is some indication of dominance or partial dominance (Tables 8, 12 and 15).

Eleven single crosses, selected on the basis of clonal reactions in the greenhouse diallel studies, were selfed and backcrossed to their parent clones. F_2 and backcross progenies were tested in the field for yield and in the field and greenhouse for <u>Cercospora</u> reaction.

Treatment mean squares were significant at the .01 level of probability. The entry with the highest mean forage yield was the backcross (C221 x C618) C618. The two clones involved showed the highest general combining ability for <u>Cercospora</u> resistance in greenhouse diallel trials. This progeny had a low mean score for <u>Cercospora</u> reaction in the greenhouse but did not respond as favorably in the field. In the greenhouse, clones C618 and C221, when used as recurrent backcross parents, appeared to contribute <u>Cercospora</u> resistance to their progenies.

Crosses in which M247 was a parent showed above average susceptibility to <u>Cercospora</u> in the greenhouse, but in the field appeared to contribute to <u>Cercospora</u> resistance and

high yield.

Clones C618, M247 and 414-10 are possible choices for a synthetic breeding program for producing a high yielding <u>Cercospora</u> resistant variety. Results suggest that clone 414-10 will not make a positive contribution to <u>Cercospora</u> resistance but it would be desirable for its good combining ability for yield.

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to Dr. C. P. Wilsie for suggesting the problem and for his consideration and guidance throughout the conduct of the experiment and during the preparation of the dissertation. Thanks are extended to Dr. Don C. Norton for assistance and advice during the greenhouse studies. Appreciation is also extended Dr. Dewey L. Harris for his helpful suggestions in the combining ability analysis. Special thanks go to the author's wife, Carolyn, for her continued encouragement and assistance throughout the period of study.

LITERATURE CITED

Adams, M. W. and G. Semeniuk 1958. The heritability of reaction in alfalfa to common leaf spot. Agronomy Journal 50: 677-679. Baxter, J. W. Cercospora blackstem of alfalfa. Phytopathology 1956. 46: 398-400. Berger, R. D. 1962. Factors affecting growth, sporulation, pathogenecity, and survival of Cercospora zebrina. Dissertation Abstracts 23: 1155. Berger, R. D. and E. W. Hanson Pathogenecity, host parasite relationships, and 1963. morphology of some forage legume Cercosporae. Phytopathology 53: 500-508. Bolton, J. L. 1948. A study of combining ability of alfalfa in relation to certain methods of selection. Scientific Agriculture 28: 97-126. Carnahan, H. L. and J. H. Graham 1956. Blackstem of alfalfa. Alfalfa Improvement Conference Report 15: 50-57. Carnahan, H. L., A. W. Hovin, H. O. Graumann, W. R. Kehr, R. L. Davis, L. J. Elling and C. H. Hanson General vs. specific combining ability in alfalfa 1959 for seedling vigor and fall growth in the year of establishment. Agronomy Journal 52: 511-516. Chupp, C. 1954. A monograph of the fungus genus Cercospora. Published privately by the author. Ithaca, New York. Davis, R. L. 1951. A study of the inheritance of resistance in alfalfa to common leaf spot. Agronomy Journal 43: 331-337. Downey, R. K. 1960. Formation of synthetic varieties of alfalfa. Alfalfa Improvement Conference Report 17: 8-11. Dudley, J. W., R. R. Hill, Jr. and C. H. Hanson 1963. Effects of seven cycles of recurrent phenotypic

selection on means and genetic variances of several characters in two pods of alfalfa germ plasm. Crop Science 3: 543-546.

Frakes, R. V., R. L. Davis and F. L. Patterson

- 1961. The breeding behavior of yield and related variables in alfalfa. III. General and specific combining ability. Crop Science 1: 210-212.
- Geise, H. A., M. W. Adams and G. Semeniuk 1956. The blackstem complex. Alfalfa Improvement Conference Report 15: 57-58.
- Geise, H. A., M. W. Adams and G. Semeniuk

1957. Reaction of certain diploid and tetraploid alfalfas to some phytopathogens inducing blackstem disease. South Dakota Academy of Science Proceedings xxxvi: 165.

- Griffing, B.
 - 1956. Concept of general and specific combining ability in relation to diallel crossing systems. Australia Journal of Biological Science 9: 463-493.
- Grissom, D. B. and R. R. Kalton
 - 1956. Inheritance of combining ability for forage traits in bromegrass, <u>Bromus inermis</u> Leyss. Agronomy Journal 48: 289-293.
- Hanson, E. W.
 - 1956. The blackstem complex. Alfalfa Improvement Conference Report 15: 47-49.
- Hawk, V. B. and C. P. Wilsie
- 1952. Parent-progeny yield relationships in bromegrass, Bromus inermis Leyss. Agronomy Journal 44: 112-118.

•

- Horsfal, J. G.
 - 1929. Species of Cercospora on Trifolium, Medicago and Melilotus. Mycologia 21: 304-312.
- Jenkin, T. J.
 - 1931. The method and technique of selection, breeding, and strain-building in grasses. Imperial Bureau Plant Genetics Herbage Plant Bulletin 3: 5-34.
- Johnson, E. C.
 - 1958. Inheritance studies, including reaction to certain foliage diseases, in alfalfa. Dissertation Abstracts 19: 12-13.

Johnson, I. J. 1952. Evaluating breeding materials for combining ability. Sixth International Grassland Congress Proceedings 1: 246-252.

- Jones, F. R. 1944. Life history of Cercospora on sweetclover. Mycologia 36: 518-525.
- Jones, F. R. and O. F. Smith 1953. Sources of healthier alfalfa. U.S.D.A. Yearbook of Agriculture 1953: 228-237.

Kalton, R. R., R. C. Leffel, C. E. Wassom and M. G. Weiss 1955. Evaluation of combining ability in <u>Dactylis</u> <u>glomerata</u> L. I. clonal and outcross progeny performance. Iowa State College Journal of Science 29: 631-658.

Kalton, R. R., A. G. Smit and R. C. Leffel

- 1952. Parent inbred progeny relationships of selected orchardgrass clones. Agronomy Journal 44: 481-486.
- Kehr, W. R.

1961. General and specific combining ability for four agronomic traits in a diallel series among six alfalfa clones. Crop Science 1: 53-55.

- Kehr, W. R. and H. O. Graumann
 - 1958. Specific combining ability in alfalfa. Alfalfa Improvement Conference Report 16: 9-16.
- Kirk, L. E.

1933. The progeny test and methods of breeding appropriate to certain species of crop plants. American Naturalist 67: 515-531.

- Knowles, R. P.
 - 1950. Studies of combining ability in bromegrass and crested wheatgrass. Scientific Agriculture 30: 275-302.
- Nagel, C. M.
 - 1932. The sporulation and host range of six species of Cercospora. Unpublished M. S. thesis. Library, Iowa State University of Science and Technology. Ames, Iowa.

Pearson, L. C. and L. J. Elling

1958. Predicting synthetic varietal performance from single cross information. Alfalfa Improvement

Conference Report 16: 17-21.

Pearson, L. C. and L. J. Elling

1960. Predicting disease resistance in synthetic varieties of alfalfa from clonal cross data. Agronomy Journal 52: 291-294.

Pergament, E. and R. L. Davis 1961. Quantitative inheritance of height and yield in alfalfa, <u>Medicago sativa</u> L. Crop Science 1: 221-224.

Reitz, L. P., C. O. Grandfield and E. D. Hansing 1948. Reaction of alfalfa varieties, selections and hybrids to <u>Ascochyta imperfecta</u>. Journal of Agricultural Research 76: 307-323.

Renfro, B. L. and E. W. Sprague 1959. Reaction of Medicago species to eight alfalfa pathogens. Agronomy Journal 51: 481-483.

Bumbaugh, M. D., G. Semeniuk and H. A. Geise 1962. Inheritance of reaction of diploid alfalfa clones to two isolates of <u>Phoma herbarum</u> var. <u>medicaginis</u>. Crop Science 2: 13-15.

Sprague, G. F. and L. A. Tatum 1942. General vs. specific combining ability in single crosses of corn. American Society of Agronomy Journal 34: 923-932.

Tamini, S. A. and M. D. Rumbaugh 1963. Resistance of diploid alfalfa to <u>Phoma herbarum</u> var. medicaginis and Cercospora zebrina. Crop Science 3: 227-230.

Thomas, H. L. and M. F. Kernkamp

1954. The use of heritability ratios on correlation coefficients for measuring combining ability with smooth bromegrass, <u>Bromus inermus</u> Leyss. Agronomy Journal 46: 553-556.

Tysdal, H. M. and T. A. Kiesselbach 1944. Hybrid alfalfa. American Society of Agronomy Journal 36: 649-667.

Tysdal, H. M., T. A. Kiesselbach and H. L. Westover 1942. Alfalfa breeding. Nebraska Agricultural Experiment Station Research Bulletin 124. Wilcor, J. R.

1962. Combining ability of nine clones and reciprocal differences among their hybrids. Unpublished Ph.D. thesis. Library, Iowa State University of Science and Technology. Ames, Iowa.

Williams, R. D.

1931. Methods and techniques of breeding red clover, white clover, and lucerne. Imperial Bureau Plant Genetics Herbage Plant Bulletin 3: 46-76.

Wilsie, C. P. and J. Skory

1948. Self-fertility of erect and pasture-type alfalfa clones as related to the vigor and fertility of their inbred and outcrossed progenies. American Society of Agronomy Journal 40: 786-794.