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Inheritance of Reaction of a Diallel Set of Diploid Alfalfa Clones to Two Pathogens of the Blackstem Complex

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INHERITANCE OF REACTION OF A DIALLEL SET OF DIPLOID ALFALFA CLONES TO TWO PATHOGENS OF THE BLACKSTEM COMPLEX

BY

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INHERITANCE OF REACTION OF A DIALLEL SET OF DIPLOID ALFALFA CLONES TO TWO PATHOGENS OF THE BLACKSTEM COMPLEX

Abstract

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Under the supervision of Associate Professor M. D. Rumbaugh

In the North Central Region, Phoma herbarum var. medicaginis Rab. and Cercospora zebrina Pass. cause more damage to alfalfa than other components of the blackstem complex. The objectives of this investigation were: (1) to determine the ecological relationship between the two pathogens, and (2) to obtain some information regarding the genetic systems in alfalfa which condition the reaction to the two fungi.

A diallel set was established from eight diploid, heterozygous clones of alfalfa. The parental (P_1), the selfed (P_2) and the F_1 families were space-planted in two greenhouse benches, each bench constituting one replication. A family was represented by 10 plants per replication when possible.

One P. herbarum and two C. zebrina isolates were used. The five types of inocula, designated as treatments, were as follows: (a) P. herbarum alone (Phoma), (b) C. zebrina alone (Cercospora), (c) both pathogens mixed (mixture), (d) P. herbarum followed after 48 hours by C. zebrina (Phoma-Cercospora), and (e) C. zebrina followed after 48 hours by P. herbarum (Cercospora-Phoma). Three leaves from each plant were excised, placed in a Syracuse watch-glass, sprayed with the inoculum, floated on a 2 per cent sugar solution and incubated at 68°-72° F. in the dark for six days. Visual estimation of per cent infected leaf-

surface was based on a 0 (no infection) to 10 (severe infection) scale.

Since it was difficult to distinguish between the type of lesions produced by each pathogen parasitizing the same tissue, the gross infection was used as a basis to determine the relationship between the two fungi. The magnitude in level of infection produced by the various treatments suggested that the relationship was not neutral, and a certain type of interaction between the two pathogens did exist. Based on the mode and speed of penetration of the two pathogens, there was evidence that C. zebrina exhibited an antagonistic effect on P. herbarum.

Analysis of diallel crosses indicated that dominant and recessive genes in the host plants were involved in controlling resistance to both pathogens. Dominant genes, however, were more frequent than recessive. There was evidence also that dominance was not unidirectional. Lack of unidirectional dominance resulted in underestimation of number of loci showing dominance. It was evident, however, that at least two loci were involved.

In addition to dominance, epistatic gene action seemed to play a major role in controlling the reaction of the host plants to both pathogens. This non-allelic interaction appeared as h/d ratio in the range of overdominance and as heterosis of the F_1 families for resistance and susceptibility. In all analyses h/d ratio was more than 1.0, and over 42 per cent of the F_1 families were heterotic segregants in each of the five treatments. The extent to which this epistatic gene action was operational in relation to dominance was not estimated. The fact that at least three of the eight points on each graph fell below and to the right side of the regression line suggested that epistatic gene action

contributed considerably to the genetic variations in the reaction of the host plants to both pathogens.

Genetic and rank correlations between the reactions to Phoma and to Cercospora treatments indicated that the genetic factors which controlled the reaction to both pathogens were similar. The possibility that genes with pleiotropic effects were operational in the host population cannot be excluded. This observation suggested that concurrent selection for resistance to both fungi is feasible.

INHERITANCE OF REACTION OF A DIALLEL SET OF DIPLOID ALFALFA CLONES TO TWO PATHOGENS OF THE BLACKSTEM COMPLEX

This thesis is approved as a creditable, independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser	Head of the Major Department
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INTRODUCTION

Alfalfa in many regions of the world is adversely affected by a disease known as the blackstem complex. The disease, which is caused by several pathogens, inflicts heavy losses in seed and forage yields of alfalfa and other leguminous forages in the North Central States. In this region, Phoma herbarum var. medicaginis Rab. (Ascochyta imperfecta Pk.) and Cercospora zebrina Pass. cause more damage than any other component of the blackstem complex. The former pathogen spreads rapidly in the cool, humid weather of the spring and the fall, while the latter thrives in the warm summer months. Overlapping infection by these organisms does occur, but the ecological relationship between them is not known.

At present, alfalfa varieties available for commercial use do not possess sufficient resistance to infection by these two pathogens. However, variations in resistance are known to occur within these varieties. This resistance can be incorporated into new varieties through appropriate measures of breeding. Information relative to the genetic system of resistance in the host species is essential to the efficiency of the breeding programs. Also, diploid alfalfa is the best available plant material for such genetic studies, and the method of diallel crosses provides an effective statistical tool for understanding better the genetic mechanism of the host population.

The objectives of this experiment were: (1) to determine whether the ecological relationship between the two pathogens was neutral, antagonistic, metabiotic or synergistic; and (2) to obtain information relative

to the genetic mechanism or mechanisms in the host plants which condition reaction to these two pathogens.

LITERATURE REVIEW

I. Blackstem Disease of Alfalfa

Blackstem disease of alfalfa has a wide geographic distribution. It has been reported prevalent in many alfalfa-growing regions of the United States and Canada (45,25,9,44,32,17,20,28) and was encountered in several European countries (38) and in Argentina (1).

The disease is caused by several species of plant pathogens (26, 32,15,40). In the North Central Region of the United States, the two most important ones are Ascochyta imperfecta and Cercospora zebrina (7, 15,4,40), the causative agents of spring and summer blackstem, respectively. Schenck and Gerdemann (43) investigated spore-septation and host species of A. imperfecta and suggested the use of the name Phoma herbarum var. medicaginis in place of A. imperfecta. Edmund and Hanson (12) carried out a similar investigation and concluded that the name A. imperfecta was the correct name for this fungus.

Estimates of the damage to alfalfa caused by the disease were reported. Richards (41) estimated 40-50 per cent reduction in forage yield as a result of an outbreak in Utah. In several North Central States defoliation up to 75 per cent was observed in some alfalfa fields.[1] In greenhouse tests, severely infected plants with P. herbarum failed to produce seeds; mildly infected plants, on the other hand, produced shrivelled seeds a few of which germinated (30). The disease was also

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[1]Renfro, B. L., Ma. de la Isla, R. D. Wilcoxson and J. J. Christensen. Minnesota regional survey. Annual Report of Cooperative Regional Projects Supported by Allotments of Regional Research Funds Hatch Act as Amended Aug. 11, 1955, p. 3, 1957.

found to play a secondary role in winter-killing of alfalfa (45) and to reduce quality of hay (44) and total nitrogen in infected leaves (6).

Several reports on the progress of the disease have been published. Schenck and Gerdemann (43) found that germination tubes of P. herbarum spores penetrated alfalfa leaves directly and through the stomata, and the mycelia grew intercellularly at the beginning of the infection, then intracellularly in dead and dying tissue. In the stem, the mycelia grew inter- and intracellularly in living tissue. Infected leaves showed dark brown necrotic spots which enlarged rapidly, causing defoliation, while lesions on the stems were dark and elongated (37,7,14). Horsfall (21) described lesions on alfalfa leaves infected with C. zebrina as sunken, round, ashy-gray in color and on stems as elongated sunken reddish-brown to dark brown. Size and color of lesions were affected by environmental factors. Baxter (4) found that the long multiseptated conidia of C. zebrina germinated on alfalfa leaves after six hours of incubation, but penetration of the germ tubes was observed only after 48 hours of incubation. Penetration was through the stomata only.

The dissemination of the pathogens has also been investigated. Peterson and Melchers (38) reported that P. herbarum overwintered as mycelia and pycnidia in crop residue, and that the primary inoculum was disseminated as conidia by rain the following spring. Cormack (8) found the fungus to be seed and soil borne, capable of infecting roots as well as above-ground parts of the plants. Kernkamp and Hemerick (29,30) also demonstrated the seed-borne nature of the organism and they showed that the fungus affected seed yield and germination. In contrast, C. zebrina overwinters as mycelia in crop residue and conidia arise from these

mycelia in the following spring and infect young alfalfa leaves. Baxter (4) showed that this pathogen was not seed borne.

The morphological characteristics of P. herbarum were studied. Schenck and Gerdemann (43) described the growth of this fungus on agar as being grayish olive-green with abundant pycnidia. Optimum growth occurred at 21° C. Ellingboe (13) recorded measurements of pycnidia and conidia as 150-250 microns and 4-7 microns, respectively. Baxter (4) conducted cultural studies on C. zebrina. He reported the fungus grew best at 25° C. Spores failed to develop on acidified media. Best sporulation occurred on carrot agar, sterilized stems of alfalfa, red clover and sweet clover. Conidial measurements were 21.6-180.0 microns x 1.8-6.2 microns (21).

Single-spore isolates of P. herbarum were found to exhibit wide variations in virulence (39,7,15,42). Ellingboe (13) detected differences in rate of growth between single-spore isolates and related them to differences in carbohydrate metabolism. He (14) also found that some isolates were equally virulent on alfalfa and red clover, while others were more virulent on the host from which they were isolated. Reitz et al. (39) compared eight single-spore isolates of P. herbarum and found one to be more virulent than all others. A mixture of the eight isolates produced less infection than the highly virulent one. Using the detached-leaf technique, Ward (46) failed to observe any difference in virulence of four single-spore isolates of this pathogen.

Ellingboe (14) attempted to elucidate the source of variation in virulence of single-spore isolates of P. herbarum. He found single-cell and two-cell spores possessed one nucleus per cell. The germination tube also contained one nucleus per cell, except the terminal cell which was

multinucleated regardless of the source of the isolate. He suggested that variations in virulence were perhaps due to microenvironmental factors. He suspected that fusion between conidia and mycelia (hyphal anastomosis) may have contributed to these variations.

The studies of Horsfall (21) indicate that single-spore isolates of C. zebrina do not exhibit variations in virulence. This observation was confirmed by others (15).

Damage from the blackstem disease has been reduced by burning and sweeping the crop residue from the field (37) and by grazing (25). However, a major disease of forage crops, such as blackstem of alfalfa, can be controlled best through development of resistant varieties. Several workers (25,26,38,15,46,40,36) have indicated that variations between and within varieties of alfalfa in reaction to the blackstem pathogens were highly significant and selection for resistance is worth the effort. Several factors, however, have contributed to a delay in the development of such resistant varieties. One of these was the inability to distinguish between symptoms of the various pathogens in the field. Pearson and Elling (36) stated that "unless a satisfactory method of distinguishing among these organisms in the field has been developed, or until a reliable method of producing artificial epidemics of each individual disease has been devised, little progress can be expected in breeding blackstem-resistant varieties." One method of producing epiphytotics in the field was suggested by Ward (46). He sprayed rows of alfalfa plants with pure cultures of P. herbarum and obtained satisfactory infection.

Another major problem in developing resistant varieties of alfalfa to the blackstem pathogens has been the variations in virulence within some species of the causative agents. The variations may have been due to physiological forms of the pathogens. Carnahan and Graham (7) mentioned that the lack of highly resistant (immune) selections of alfalfa hindered progress in locating differentials for demonstrating whether physiological races were common.

Hanson (17) suggested each component of the disease complex should be handled separately in order to increase the efficiency of breeding alfalfa varieties resistant to the blackstem pathogens. Accordingly, most of the testing for resistance is made in greenhouse experiments with pure cultures of the pathogens as inoculum. Bean and Wilcoxson (5) conducted an extensive study with P. herbarum to determine the environmental factors which affect disease development on alfalfa and red clover. They found that spore concentration, temperature during and after the incubation period, moisture, stage of plant growth and mechanical injury to the leaves were important factors in disease incidence and severity. Satisfactory infection was obtained in the greenhouse with mixture of isolates (39,40,12) and with single-spore isolates of P. herbarum (39,15,42,46).

Ward (46) and Mead and Downey (35) inoculated detached leaves to evaluate alfalfa selection for resistance to P. herbarum and found the method quick and economical. Ward (46) obtained maximum infection in 4-5 days at 18°-23° C. in complete darkness. He floated the leaves on a 2 per cent sugar solution. The ratings obtained with this method were in agreement with field and greenhouse ratings on whole plants. On the other hand, Mead and Downey (35) incubated inoculated leaves at 65° F.

for three days, then at 70°-72° F. for three more days under strong light. The leaves were placed on a filter paper soaked in 3 per cent sugar solution. The results were consistent, but there was little if any agreement between ratings obtained with this technique and field and greenhouse ratings.

Reitz et al. (39) attempted to associate resistance in alfalfa to P. herbarum with morphological characteristics of the plants. They found that resistance was associated with glossy leaves which were more difficult to wet with the inoculum than pubescent leaves of the susceptible plants. Thus, resistance was a product of an escape mechanism. In the same study juices from resistant and susceptible plants were used in media to detect physiological differences between plants, but the fungal growth on both media was equally vigorous.

Studies aimed at investigating the genetic factors which condition the reaction of alfalfa plants to the blackstem pathogens were limited. Reitz et al. (39) studied selections of tetraploid alfalfa, their selfed and F_1 progenies in regard to reaction to P. herbarum. They concluded that the genetic system of the host species was not a simple one. Only in one case did they find resistance was conditioned by one dominant gene. They suspected that recessive and dominant genes were involved in controlling resistance to this pathogen. Rumbaugh et al. (42) studied the reaction of eight diploid alfalfa plants in a diallel set to two P. herbarum isolates and found that susceptibility was associated with genes operating at the complete dominance level. They also reported that resistance was conditioned by recessive genes which were of low frequency in the plant population tested. Epistatic gene action and lack of

unidirectional dominance were encountered in their investigation.

The reactions of selfed progenies of alfalfa plants to some of the blackstem pathogens were described (39,31). Reitz et al. (39) reported that S_1 progenies were more suitable for evaluating selections than the parental clones. Also, S_2 progenies from selected S_1 plants exhibited a much higher level of resistance than those from unselected S_1 plants. Koffman and Wilsie (31) observed that the inbred progenies were damaged more severely by the blackstem pathogens than non-inbred plants in the field. In greenhouse studies, Geise et al. (15) found a highly significant regression for the S_1 progenies' reaction on the diploid parents' reaction to both P. herbarum and C. zebrina.

II. Analysis of Diallel Crosses

Hayman (19,18) and Jinks (24) presented a detailed description of the method of analysis of diallel crosses. Hayman (19) discussed the theory underlying the method and listed the assumption required for the validity of the analysis as diploid segregation, no multiple allelism, homozygous parents, genes independently distributed between parents, no difference between reciprocals, and independent action of non-allelic genes. The method of analysis has been extended to apply to tetraploid segregation (10), heterozygous parents (11), arbitrary gene action (27), arbitrary number of genes per locus (16,27) and arbitrary inbreeding (34). Jinks (22) extended the method to apply to F_2 and backcross generations.

The analysis of diallel crosses was shown to be effective for studying genetics of quantitative characters. Jinks (24) showed that

the method gave estimates of over-all level of dominance, relative dominance of parents and distribution of dominant and recessive genes in these parents. The method was also used to estimate genetic environmental interaction (2,34).

Jinks (23) collected and analyzed diallel data from several crops and found that non-allelic interaction was always associated with overdominance. Several workers have removed parents and their arrays from diallel tables and reanalyzed the data to elucidate non-allelic interaction (23,3,33). In all cases such action resulted in a drop of the overdominance. Kempthorne (27) objected to removal of parents and their arrays from the data on the basis that the information obtained from the reanalysis would not apply to the original population of parents or the population from which these parents were selected.

MATERIALS AND METHODS

Plant Material

Eight heterozygous, diploid alfalfa clones were selected and crossed in every possible way to establish a diallel set. The parental (P_1), the selfed (P_2) and the F_1 families, including reciprocals were space-planted in two greenhouse benches. Spacing was 2.5 inches between and within rows. Each bench constituted a replication. A family was represented by a maximum of 10 plants in each replication. The designation of the clones, their origin[2] and species are listed below:

Parental designation	Origin	Species
1B	Iran	*Medicago sativa*
2	Turkey	*M. sativa*
3	Caucasus, U.S.S.R.	*M. falcata*
7 (S2128-9)	Armenian, S.S.R.	*M. sativa*
11 (Don S-1)	Don Province	*M. falcata*
14 (S33-1-7)	(unknown)	*M. falcata*
20 (Alaskan)	Tomsk Province, Siberia	*M. falcata*
40 (HF-9)	Hohenfels, Bavaria, Southeast Germany	*M. falcata*

Pathogens

The isolates of the pathogens used in this study were:

[2]Further information on the origin of these plants was given in the South Dakota Agr. Expt. St. (Agronomy) Pamphlet #69, p. 3, 1962.

a. A single-spore isolate of P. herbarum, isolate number 42.

b. A mass-conidial isolate of C. zebrina[3] obtained from infected alfalfa leaves in Missouri. This isolate was used in the first of two trials, then was discarded because of contamination.

c. A mass-conidial isolate of C. zebrina obtained from the Department of Plant Pathology at South Dakota State College and was used in the second trial.

Cultures of P. herbarum were grown on potato dextrose agar and used when 2-3 weeks old. Cultures of C. zebrina were grown on carrot agar and used when 2-4 weeks old.

Preparation of the Inoculum

Five types of inocula, henceforth designated as treatments, were used. The pathogen and preparation and the designation of these treatments are listed below:

Pathogen and preparation	Designation
P. herbarum, alone	Phoma
C. zebrina, alone	Cercospora
P. herbarum and C. zebrina, mixed	Mixture
P. herbarum followed after 48 hours by C. zebrina	Phoma-Cercospora
C. zebrina followed after 48 hours by P. herbarum	Cercospora-Phoma

In all preparations involving P. herbarum, one petri dish culture

[3]This isolate and that of P. herbarum were obtained through the courtesy of the Department of Plant Pathology and Botany, University of Minnesota.

was mixed in a liter of distilled water. The first isolate of C. zebrina had been mixed at a rate of three petri dish cultures per liter of water. The second isolate was used at the same rate in preliminary tests and produced satisfactory infection; after a few transfers, however, this isolate showed a marked reduction in virulence and the amount of culture per liter of water was doubled.

All cultures were mixed in a Waring-blender for approximately two minutes. Ten drops of Tween 20[4] were added as a wetting agent to each liter of inoculum. All inoculations were made with freshly mixed inoculum.

Inoculation

The detached-leaf technique of inoculation was used for all treatments. From each plant three young leaves, uniform in size and free of blemishes, were excised with a forceps and placed in a Syracuse watch-glass. The watch-glasses for all plants were moved to the inoculation chamber where the leaves in each watch-glass received four dashes of a fine mist of inoculum from a hand atomizer. The procedure produced a uniform inoculum distribution. Thereupon, each Syracuse watch-glass received approximately 7 cc. of a 2 per cent sugar solution and the watch-glasses were then placed at 68°-72° F. in complete darkness for six days.

Rating of Infection

Ratings were based on visual estimation of per cent leaf surface showing necrosis. A scale of 0 (no infection) to 10 (severe infection)

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[4]Tween 20 is the commercial name for Polyoxyethylene sorbitan monolaurate, produced and distributed by Atlas Powder Company.

was used. Ratings were averaged over leaves per plant and plants per family. Analyses were based on the mean family score.

Analysis of the Data

The following methods of analysis were used:

1. Analysis of variance

Analysis of variance was conducted on the data from each of the two trials and each of the five treatments (10 analyses). Also, analysis of variance was conducted on the combined data of the two trials for each of the five treatments (five analyses).

2. Scaling

The mean score of each F_1 family was compared with the mean score of each of the two parental families. The scores were averaged over replications and trials. On this basis, the F_1 families were classified into the following classes:

Class I = F_1 more resistant than more resistant parent,

Class II = F_1 resembled the resistant parent,

Class III = F_1 resembled the mid-parent,

Class IV = F_1 resembled the susceptible parent, and

Class V = F_1 more susceptible than the more susceptible parent.

3. Analysis of diallel crosses

Diallel tables were obtained from ratings on each replication following inoculation with each of the five treatments in two trials (20 tables). The following genetic variances and covariances were computed from each of these tables:

Vp_1 = variance of parental families.

Vp_2 = variance of selfed families.

$\bar{V}r_1$ = mean variance of arrays with parental scores on diagonal.

$\bar{V}r_2$ = mean variance of arrays with selfed families' scores on diagonal.

$V\bar{r}_1$ = variance of array means with parental scores in arrays.

$V\bar{r}_2$ = variance of arrays with selfed families' scores in arrays.

$\bar{W}p_1/r_1$ = mean covariance of arrays and parental scores.

$\bar{W}p_2/r_2$ = mean covariance of arrays and selfed families' scores.

Wp_1/p_2 = covariance of parental and selfed families' scores.

From these statistics the following genetic parameters were computed:

$D_I = 2\ Vp_1+8\ Vp_2-8\ Wp_1/p_2$

$H_I = 4\ Vp_1+16\ Vp_2+16\ \bar{V}r_1+16\ \bar{W}p_1/r_1-32\ \bar{W}p_2/r_2-16\ Wp_1/p_2$

$H_{II} = 16\ \bar{V}r_1-16\ V\bar{r}_1$

$H_{III} = 16\ Vp_1+16\ Vp_2-32\ Wp_1/p_2$

$H_{IV} = 8\ Vp_1-16\ Wp_1/r_1+16\ Wp_2/r_2-8\ Wp_1/p_2$

$F_I = 8\ Vp_1+32\ Vp_2+16\ Wp_1/r_1-32\ Wp_2/r_2-32\ Wp_1/p_2$

$F_{II} = 16\ Vp_1+32\ Vp_2-48\ Wp_1/p_2$

$h/d = \sqrt{0.5\ (H_I+H_{IV})\ /\ D_I}$

Number of loci showing dominance = $16\ (\bar{F}_1-\bar{P}_2)\ /\ H_{II}$, where $\bar{F}_1$ and $\bar{P}_2$ are the mean scores of the F_1 and the selfed progenies' scores, respectively. The $\bar{V}r_2$ and $V\bar{r}_2$ were substituted for $\bar{V}r_1$ and $V\bar{r}_1$, respectively, which gave two estimates of H_I, H_{II}, h/d and number of loci showing dominance.

Before computing the genetic parameters, the variances and covariances were corrected with E_2 and E_3 for variations due to non-heritable components. E_2 was estimated from the analysis of variance of ratings

of the parental families. The form of analysis was as follows:

Source of variations	D. F.	Mean square expectation
Total	79	
Between parental families	7	$Ve+n_o\ Vp$
Within parental families	72	Ve

$E_2 = Ve / n_o$, where Ve = the variance within parental families, and n_o = number of clonal cuttings representing each parental clone. E_3 was estimated as $E_3 = E_2 / N$, where N = number of parental families. E_2 was used to correct Vp_1, Vp_2, Vr_1 and Vr_2, while $\bar{Vr}_1$, $\bar{Vr}_2$, Wp_1/r_1 and Wp_2/r_2 were corrected with E_3.

The genetic parameters used for interpretation were computed in two ways: (a) from means of variances and covariances of the four diallel tables of each treatment, and (b) by averaging the genetic parameters of the four diallel tables per treatment. The averages of the variances and covariances were also used to establish two graphs per treatment, henceforth designated as the Vr_1 and Vr_2 graphs.

4. Correlation and regression

Rank-correlation coefficients for the order of dominance on the Vr_1 and Vr_2 graphs were calculated between graphs of all treatments. Also, rank-correlation coefficients were calculated between the various combinations of the following scores: (1) parental families, (2) selfed families, (3) array means. The formula used in these calculations was $r_s = 1 - \frac{6\ (\Sigma di^2)}{N\ (N^2-1)}$, where (Σdi^2) is the sum of squared differences between paired ranks and N is the number of paired ranks.

A genetic correlation coefficient between the response to Phoma and the response to Cercospora treatments was estimated according to the following formula:

$$r_g = \frac{C_{f_p f_c}}{\sqrt{V_{f_p} \quad V_{f_c}}}$$

where $C_{f_p f_c}$ is the between families mean product component from analysis of covariance and V_{f_p} and V_{f_c} are the between families mean square components from analysis of variance of the Phoma and the Cercospora data, respectively.

Multiple correlation and standardized multiple regression coefficients for three variables were calculated. The data of the Phoma and the Cercospora treatments were considered as the independent variables (X_1 and X_2, respectively) and each of the mixture, Phoma-Cercospora and Cercospora-Phoma as the dependent variable (Y).

With the exception of the rank-correlation coefficients for order of dominance, all correlation and regression calculations were performed on the data averaged over replications and trials.

5. Estimates of heritability

Heritability was estimated as $h^2 = b_{\overline{po}}$ or the regression of the offspring on the mid-parent. Reciprocals were averaged to obtain the offspring scores. The h^2 estimates were obtained for each trial and for the combined data of the two trials.

RESULTS

The eight parents used to establish the diallel set showed wide variations in self- and cross-fertility and in ease of vegetative propagation. Therefore, it was difficult to obtain and maintain a complete set of plants throughout the period of investigation. Crossing and selfing were performed continuously to provide new plants to replace dead or weak ones.

Table 1 contains the mean ratings of the P_1, P_2 and F_1 families. Inoculation with P. herbarum resulted in similar disease ratings in three of the four inoculations. The C. zebrina isolate used in trial A produced 3-4 times more infection than the isolate used in trial B. The infection obtained with the mixture treatment was more severe than that obtained with either pathogen alone in trial A, but was intermediate in trial B. In general, the Phoma-Cercospora treatment produced twice as much infection as any other treatment. The Cercospora-Phoma treatment exhibited considerable variations in level of infection between and within trials. The difference between trials for the mixture, Phoma-Cercospora and Cercospora-Phoma treatments, was parallel to the difference in virulence of the two C. zebrina isolates.

The combined data of the two trials indicate that P_2 families were more susceptible than the P_1 families to four of the five treatments. The Cercospora treatment was the exception. Also, the F_1 families showed less infection than either the P_1 or the P_2 families.

Table 2 contains the mean scores of the eight parental families, their selfed and F_1 progenies. The mean scores of the parental families

Table 1. Mean Ratings on Parental (P_1), Selfed (P_2) and F_1 Families of an 8x8 Diallel Set of Diploid Alfalfa Clones Inoculated with Indicated Treatments. Ratings Were Based on a 0 (No Infection) to 10 (Severe Infection) Scale

Trial	Family	Treatment and replication									
		Phoma		Cercospora*		Mixture Phoma-Cercospora				Cercospora-Phoma	
		I	II	I	II	I	II	I	II	I	II
Trial A	P_1	2.25	2.32	3.64	3.91	4.73	3.76	5.83	5.74	4.06	2.10
	P_2	3.21	2.11	3.36	3.04	4.53	4.07	6.68	5.32	4.64	2.65
	F_1	1.95	1.86	2.82	3.10	3.30	3.19	5.28	4.77	3.43	2.11
	Grand Mean	2.12	1.94	2.97	3.19	3.59	3.35	5.49	4.94	3.64	2.17
Trial B	P_1	2.39	2.67	0.88	1.02	1.29	1.64	3.96	2.06	1.71	1.20
	P_2	2.49	3.25	1.25	1.22	1.54	1.85	4.08	2.00	1.94	1.14
	F_1	2.02	2.98	0.96	0.99	1.06	1.22	3.59	1.79	1.81	1.12
	Grand Mean	2.11	2.98	0.99	1.02	1.14	1.33	3.68	1.84	1.81	1.13
Combined data of two trials	P_1	2.41		2.36		2.68		4.40		2.27	
	P_2	2.76		2.22		3.00		4.52		2.59	
	F_1	2.20		1.97		2.19		3.68		2.12	
	Grand Mean	2.04		2.35		2.35		3.99		2.19	

*Two C. zebrina isolates were used in trials A and B, respectively.

Table 2. Mean Scores on Eight Diploid Alfalfa Selections, Their Selfed and F_1 Progenies Inoculated in a Diallel Set with Indicated Treatments. Ratings were Based on a 0 (No Infection) to 10 (Severe Infection) Scale, and were Averaged Over Two Replications and Two Trials

Families and assigned no.		Treatment					
		Phoma	Cercospora	Mixture	Phoma-Cercospora	Cercospora-Phoma	Mean
Parental families	1B	1.53	1.84	1.45	4.48	1.60	2.18
	2	1.39	1.32	1.89	2.98	1.69	1.85
	3	3.50	2.93	3.58	6.16	2.86	3.81
	7	1.90	2.41	2.40	3.49	2.59	2.56
	11	3.83	3.41	4.05	5.24	1.86	3.68
	14	2.60	1.92	2.72	5.34	2.19	2.95
	20	1.77	1.64	2.02	2.56	1.62	1.92
	40	2.73	3.43	4.73	4.93	3.71	3.91
Selfed families	1B	2.22	2.55	2.11	4.29	1.83	2.60
	2	2.41	2.18	2.08	4.27	2.42	2.67
	3	3.06	3.07	3.15	5.23	3.37	3.58
	7	2.84	2.28	3.40	4.26	3.31	3.22
	11	2.92	2.23	4.10	4.62	2.85	3.34
	14	1.99	1.32	3.10	4.82	2.17	2.68
	20	2.38	1.74	1.90	3.57	1.87	2.29
	40	4.29	2.38	4.13	5.11	2.93	3.77
F_1 families	1B	2.27	2.16	1.96	3.88	1.99	2.45
	2	1.96	1.78	2.17	3.66	2.07	2.33
	3	2.40	1.98	2.25	3.98	2.13	2.55
	7	2.29	1.67	2.53	4.13	2.35	2.59
	11	2.17	2.01	2.17	3.64	2.07	2.41
	14	2.49	1.87	2.37	4.38	2.21	2.66
	20	1.99	1.66	1.98	3.71	2.09	2.29
	40	2.15	2.62	2.09	3.45	2.04	2.47

show parents 1B, 2 and 20 were the most resistant to all treatments. Parents 7 and 14 were intermediate in reaction, while parents 3, 11 and 40 were the most susceptible. The Duncan multiple range test indicated that the difference in reaction of the three most resistant parents and that of the most susceptible ones was significant. The differences between the most resistant and intermediate parents on the one hand, and the susceptible and intermediate on the other, were non-significant.

Mean scores of the selfed families also indicated that parents 1B, 2 and 20 were the most resistant, 7 and 14 were intermediate and 3, 11 and 40 were the most susceptible. Parent 14 gave the most resistant P_2 progenies to both Phoma and Cercospora treatments. The difference was significant only between the reactions of the most resistant parent (parent 20) and the most susceptible one (parent 40).

Parents 2 and 20 produced the most resistant F_1 families. Generally, however, differences between the reactions of the F_1 families from the eight parents were small.

The mean square values obtained by the analysis of variance on ratings from individual trials are presented in Table 3. In general, differences between replications, genotypes and diallel families were significant, while differences between parental families and between arrays were non-significant.

The mean square values obtained by the analysis of variance of ratings from the combined data of the two trials are presented in Table 4. Differences between trials were highly significant in all treatments. With the exception of the Cercospora treatment, differences between genotypes and diallel families were significant. Differences between

Table 3. Mean Square Values Obtained from Analysis of Variance of Ratings Recorded on an 8x8 Diallel Set of Diploid Alfalfa Clones Following Inoculation with Indicated Treatments in Two Replications

Source of variations	D. F.	Treatments and trials					
		Phoma		Cercospora		Mixture	
		A	B	A	B	A	B
Replications (R)	1	1.2395	26.9620**	1.6320	0.0427	2.0640	1.3669**
Genotypes (G)	71	1.5186**	1.0321**	2.6885**	0.2036	2.4553**	0.4625**
Parental families (Pa)	7	1.9132	1.8562*	4.5879	0.1368	6.2190*	0.9929*
Progenies (Pr)	63	1.4808**	0.9568**	2.3832**	0.2132	1.9070*	0.3943**
Between arrays (BA)	7	1.8802	2.6324*	5.8907	0.2939	2.0453	0.6465
Within arrays (WA)	56	1.4309**	0.7474**	1.9448**	0.2031	1.8897*	0.3628**
Pa vs Pr	1	1.1350	0.0036	8.6268	0.0489	10.6511	1.0462
R x G	71	0.6867	0.3941	1.1119	0.1967	1.0669	0.1505
R x Pa	7	0.6766	0.3310	1.7421	0.1808	0.9394	0.2624
R x Pr	63	0.6943	0.3825	1.0541	0.2008	1.0603	0.1403
R x BA	7	0.6890	0.6929	2.6755	0.5602	1.4248	0.2768
R x WA	56	0.6950	0.3437	0.8515	0.1558	1.0147	0.1242
R x Pa vs Pr	1	0.2769	1.5620	0.0174	0.0539	2.4099	0.0115

Table 3. (Continued)

Source of variations	D. F.	Treatment and trials			
		Phoma-Cercospora		Cercospora-Phoma	
		A	B	A	B
Replications (R)	1	11.0390**	122.3974**	77.3522**	16.7418**
Genotypes (G)	71	3.0300**	1.1620*	1.8291**	0.4994
Parental families (Pa)	7	8.6223**	2.1620	3.0734	0.9498
Progenies (Pr)	63	2.3657*.	0.9205	1.7110**	0.4572*
Between arrays (BA)	7	5.0566	2.1290	4.0136	2.1168
Within arrays (WA)	56	2.0294*	0.7694	1.4232**	0.2498
Pa vs Pr	1	5.7333	0.8759	0.5615	0.0074
R x G	71	1.2411	0.6742	0.8212	0.3373
R x Pa	7	0.7588	0.8309	0.8678	0.7667
R x Pr	63	1.2992	0.6673	0.8110	0.2928
R x BA	7	1.6312	1.6112	1.3749	0.7705
R x WA	56	1.2576	0.5493	0.7405	0.2331
R x Pa vs Pr	1	0.9574	0.0141	1.1379	0.1311
Total	143				

*Significant at the 0.01 level.
**Significant at the 0.05 level.

Table 4. Mean Square Values Obtained from Analysis of Variance of Ratings on an 8x8 Diallel Set of Diploid Alfalfa Inoculated with Indicated Treatments in Two Replications and Two Trials

Source of variations	D. F.	Treatment				
		Phoma	Cercospora	Mixture	Phoma-Cercospora	Cercospora-Phoma
Trials (T)	1	18.7834**	310.3578**	360.2599**	433.5286**	147.6335**
Replications (R) in T	2	14.1008**	0.8374	1.7154	66.7182**	47.0470**
Genotypes (G)	71	1.6940**	1.6248	1.9761**	2.4917*	1.4464*
Parental families (Pa)	7	3.3430**	2.6513	5.3093	6.3839	2.2279
Progenies (Pr)	63	1.5297*	1.4780	1.4944**	2.0053*	1.3791**
Between arrays (BA)	7	3.6249*	3.6428	1.9889	5.1612	4.9648**
Within arrays (WA)	56	1.2678	1.2074	1.4326*	1.6108	0.9309
Pa vs Pr	1	0.5059	3.6880	8.9949	5.8876	0.2201
T x G	71	0.8567*	1.2672**	0.9416*	1.5834**	0.8821*
T x Pa	7	0.4264	2.0752	1.9154*	4.4000**	1.7954
T x Pr	63	0.9080**	1.1060**	0.8069	1.2809	0.6975
T x BA	7	0.8877	2.4302	0.7029	2.0245	0.3411
T x WA	56	0.9106**	0.9405**	0.8199	1.1879	0.7421*
T x Pa vs Pr	1	0.6325	5.7690*	2.6114	0.9233	6.1203
R in T x G	142	0.5404	0.6543	0.6087	0.9591	0.5792
R in T x Pa	14	0.5038	0.9847	0.5986	0.7948	0.8173
R in T x Pr	126	0.5384	0.6274	0.6003	0.9832	0.5519
R in T x BA	14	0.6909	1.6179	0.8508	1.6212	1.0727
R in T x WA	112	0.5194	0.5036	0.5690	0.9035	0.4868
R in T x Pa vs Pr	2	0.9195	0.0356	1.2107	0.5856	0.6345
Total	287					

were non-significant except for the Phoma treatment. The interaction of trials x genotypes was significant in all treatments.

The frequency distribution of the F_1 families as classified according to the mean score of each cross in relation to the mean score of its parents is shown in Table 5. On the average, about 30 per cent of the F_1 families showed heterosis for resistance and 13 per cent for susceptibility. Also twice as many F_1 families resembled their more resistant parent than those which resembled their more susceptible parent. In all treatments approximately 30 per cent of the F_1 families resembled the mid-parent.

The frequency distributions of the F_1 families in the five classes followed a similar trend in the Phoma, Cercospora, mixture and Phoma-Cercospora treatments, but deviated considerably in the Cercospora-Phoma treatment.

The means of the variances and covariances from the four diallel tables of each treatment are shown in Table 6. The Vp_1 exceeded the Vp_2 in four of the five treatments. A comparison between the Phoma and the Cercospora treatments for these statistics revealed that the $\bar{V}r_2$, $\bar{W}p_1/r_1$ and Wp_1/p_2 were approximately of the same magnitude.

Since the two methods of calculating the genetic parameters yielded approximately the same values for seven of the nine parameters, only one set of these estimates is shown in Table 7. In general, the variations in magnitude of these parameters between treatments were considerable, especially for the H_I, H_{III}, F_I and F_{II}. The parameter H_{II} was comparatively consistent between treatments. In all analyses the F_I and F_{II} were positive. In general, there was a certain degree of resemblance

Table 5. Frequency Distributions (%) of F_1 Family Means in Relation to the Performance of the Parents in Each Cross of an 8x8 Diallel Set of Diploid Alfalfa Inoculated with Indicated Treatments. Ratings were Averaged over Two Replications and Two Trials

Treatment	Phenotypic class*					Heterotic segregants
	I	II	III	IV	V	%
Phoma	25.0	19.6	30.4	7.1	17.8	42.8
Cercospora	32.1	17.8	33.9	5.4	10.7	42.8
Mixture	35.7	23.2	23.2	10.7	7.1	42.8
Phoma-Cercospora	37.5	12.5	37.7	8.9	5.4	42.9
Cercospora-Phoma	23.2	16.1	23.2	12.5	25.0	48.2
Mean	30.7	17.8	29.7	8.9	13.2	

*The phenotypic classes were:

Class I F_1 more resistant than the most resistant parent,

Class II F_1 as resistant as the most resistant parent,

Class III F_1 as mid-parent in resistance,

Class IV F_1 as susceptible as the most susceptible parent, and

Class V F_1 more susceptible than the most susceptible parent.

Table 6. Mean Variances and Covariances of Disease Ratings on an 8x8 Diallel Set of Diploid Alfalfa Clones Inoculated with Indicated Treatments. Two Inoculations were Made on Each of Two Replications, and the Data from Each Inoculation were Analyzed Separately

Variances and covariances	Treatment				
	Phoma	Cercospora	Mixture	Phoma-Cercospora	Cercospora-Phoma
Vp_1	0.9417	1.4289	1.8338	2.8064	1.1941
Vp_2	1.2572	0.5555	1.0769	0.8427	0.7244
$\bar{V}r_1$	0.4888	0.6937	0.6553	1.0617	0.5390
$\bar{V}r_2$	0.6065	0.6065	0.5643	0.8851	0.5372
$V\bar{r}_1$	0.1207	0.2496	0.1116	0.3174	0.0964
$V\bar{r}_2$	0.1018	0.2020	0.1186	0.2699	0.0810
$\bar{W}p_1/r_1$	0.2782	0.2909	0.2537	0.5350	0.2037
$\bar{W}p_2/r_2$	0.1397	0.3288	0.2780	0.1521	0.3478
Wp_1/p_2	0.5188	0.4900	1.1276	0.7680	0.3287

Table 7. Genetic Parameters Computed from the Statistics in Table 6

Genetic parameters	Treatment				
	Phoma	Cercospora	Mixture	Phoma-Cercospora	Cercospora-Phoma
	Vr_1				
D_I	7.7906	3.3818	3.2620	6.2104	5.5538
H_I	23.3828	11.9956	12.1720	33.1008	11.8612
H_{II}	5.8896	7.1056	8.6992	11.9088	7.0816
H_{III}	18.5808	16.0704	10.4880	33.8096	20.1776
H_{IV}	1.1672	8.1176	6.0384	10.1808	9.2288
F_I	31.1432	7.6600	8.2112	28.5344	14.3448
F_{II}	30.3952	17.1184	9.6768	35.0048	26.5088
	Vr_2				
H_I	25.2660	10.6004	10.7160	30.2752	11.8324
H_{II}	8.0752	6.4720	7.1312	9.8432	7.2992

in magnitude of these genetic parameters between the Phoma and the Phoma-Cercospora on the one hand, and between the Cercospora and Cercospora-Phoma treatments on the other.

The two methods of calculating the genetic parameters gave estimates of h/d ratios and number of loci showing dominance which were different in magnitude. Estimates by the two methods are presented in Tables 8 and 9. In both tables h/d ratios exceeded 1.0 in all treatments. Also, estimates of both parameters were somewhat larger when averaged over the four inoculations (Table 9) than from the genetic parameters derived from means of the statistics (Table 8). In general, estimates of number of loci were very small, especially for the Cercospora treatments. At least two loci were involved in the mixture treatment and one locus in each of the other three treatments.

The Vr_1 and Vr_2 graphs for each of the five treatments are shown in Figures I to V. The following observations were made from these graphs:

1. The regression coefficient of the variances on the covariances was less than 1.0 in nine of the 10 graphs. In the mixture treatment the regression coefficient was 1.27 in the Vr_1 graph.
2. The unit slope intersected the lower curvature of the parabola below the Vr axis in all graphs.
3. In general, points representing the eight parents fell closer to the regression line in the Vr_2 than in the Vr_1 graphs. This was more pronounced in the mixture and the Phoma-Cercospora treatments.
4. The distribution of points which indicated order of dominance in the parents was fairly consistent between the first four treatments.

Table 8. Number of Loci Showing Dominance Calculated from Ratings of Disease Infection on an 8x8 Diallel Set of Diploid Alfalfa Clones Inoculated with Indicated Treatments, and h/d Ratios Computed from the Genetic Parameters Shown in Table 7

Parameters	Treatment				
	Phoma	Cercospora	Mixture	Phoma-Cercospora	Cercospora-Phoma
Vr_1					
Loci	0.9139	0.1407	1.2067	0.5852	0.4991
h/d	1.2553	1.7244	1.6707	1.8668	1.3780
Vr_2					
Loci	0.6665	0.1545	1.4720	0.7081	0.4842
h/d	1.3025	1.6636	1.6025	1.8048	1.3770

Table 9. Means of Four Estimates of Number of Loci and h/d Ratios Obtained from Analysis of Disease Ratings on an 8x8 Diallel Set of Diploid Alfalfa Inoculated with Indicated Treatments in Two Replications and Two Trials

Parameters	Treatment				
	Phoma	Cercospora	Mixture	Phoma-Cercospora	Cercospora-Phoma
Vr_1					
Loci	1.1500	0.3526	2.0402	0.6358	0.7840
h/d	1.4849±0.2221	1.8419±0.6453	1.6476±0.7491*	2.2292±0.4137	3.1243±1.1468
Vr_2					
Loci	0.6796	0.3011	2.0124	0.7855	0.6404
h/d	1.5148±0.2051	1.7785±0.5343	1.6448±0.5169*	2.0531±0.4007	2.9174±1.0619

*One of the four estimates of h/d ratio was omitted due to negative D_I value.

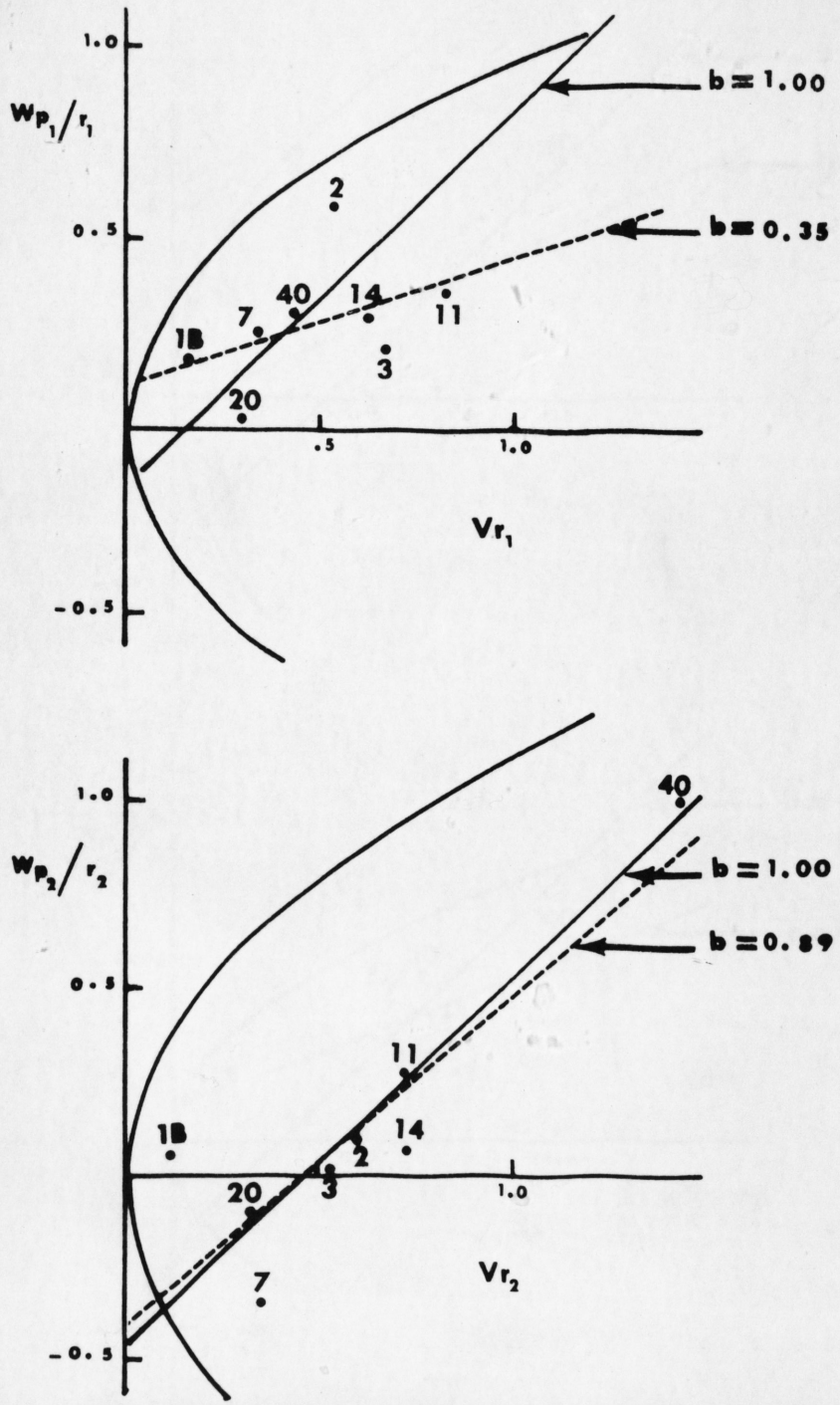

Figure I. The distributions of mean variances and covariances of the reaction of a diploid alfalfa diallel to the Phoma treatment, when the parent clones (Vr_1) and the selfed families (Vr_2) are included in the arrays

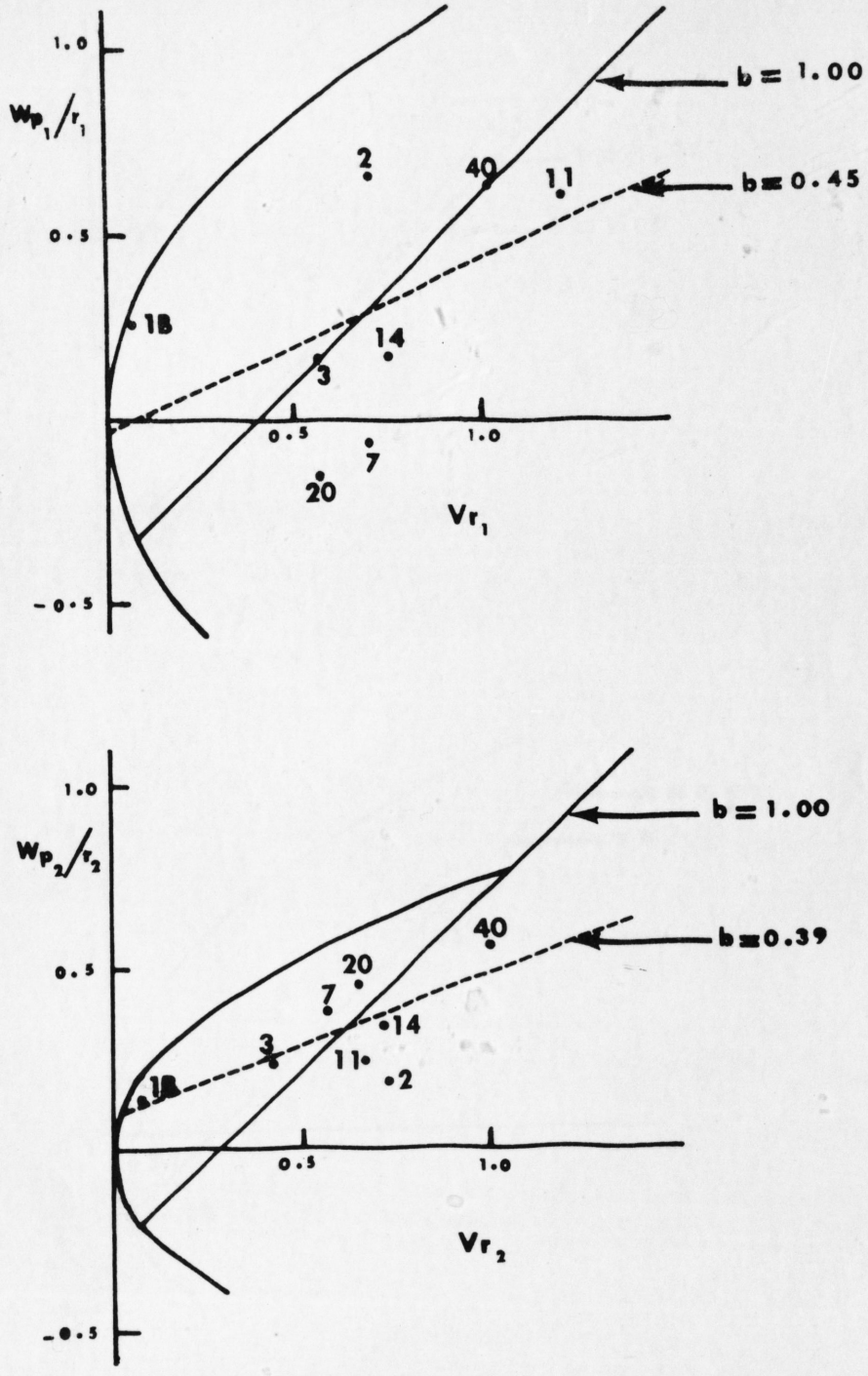

Figure II. The distributions of mean variances and covariances of the reaction of a diploid alfalfa diallel to the Cercospora treatment, when the parent clones (Vr_1) and the selfed families (Vr_2) are included in the arrays

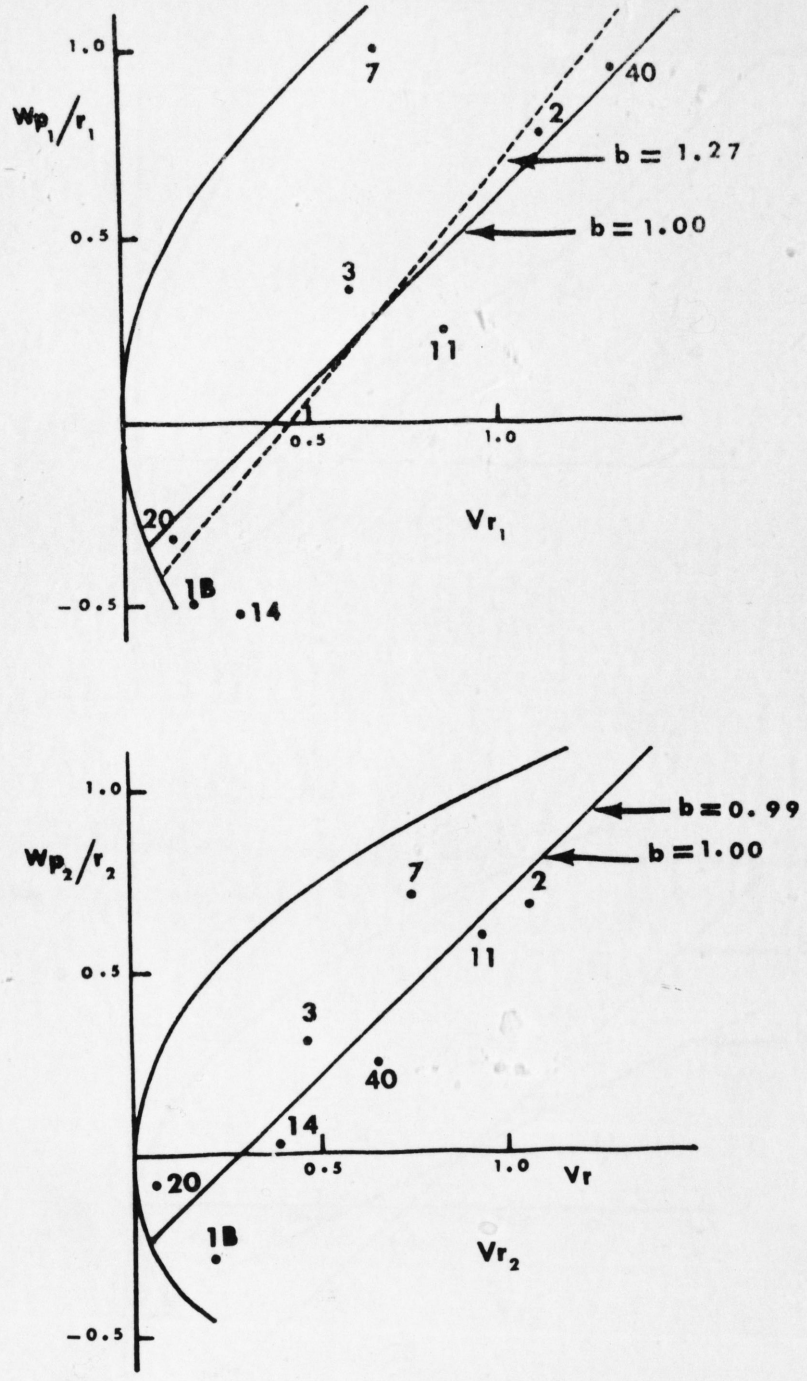

Figure III. The distributions of mean variances and covariances of the reaction of a diploid alfalfa diallel to the mixture treatment, when the parent clones (Vr_1) and the selfed families (Vr_2) are included in the arrays

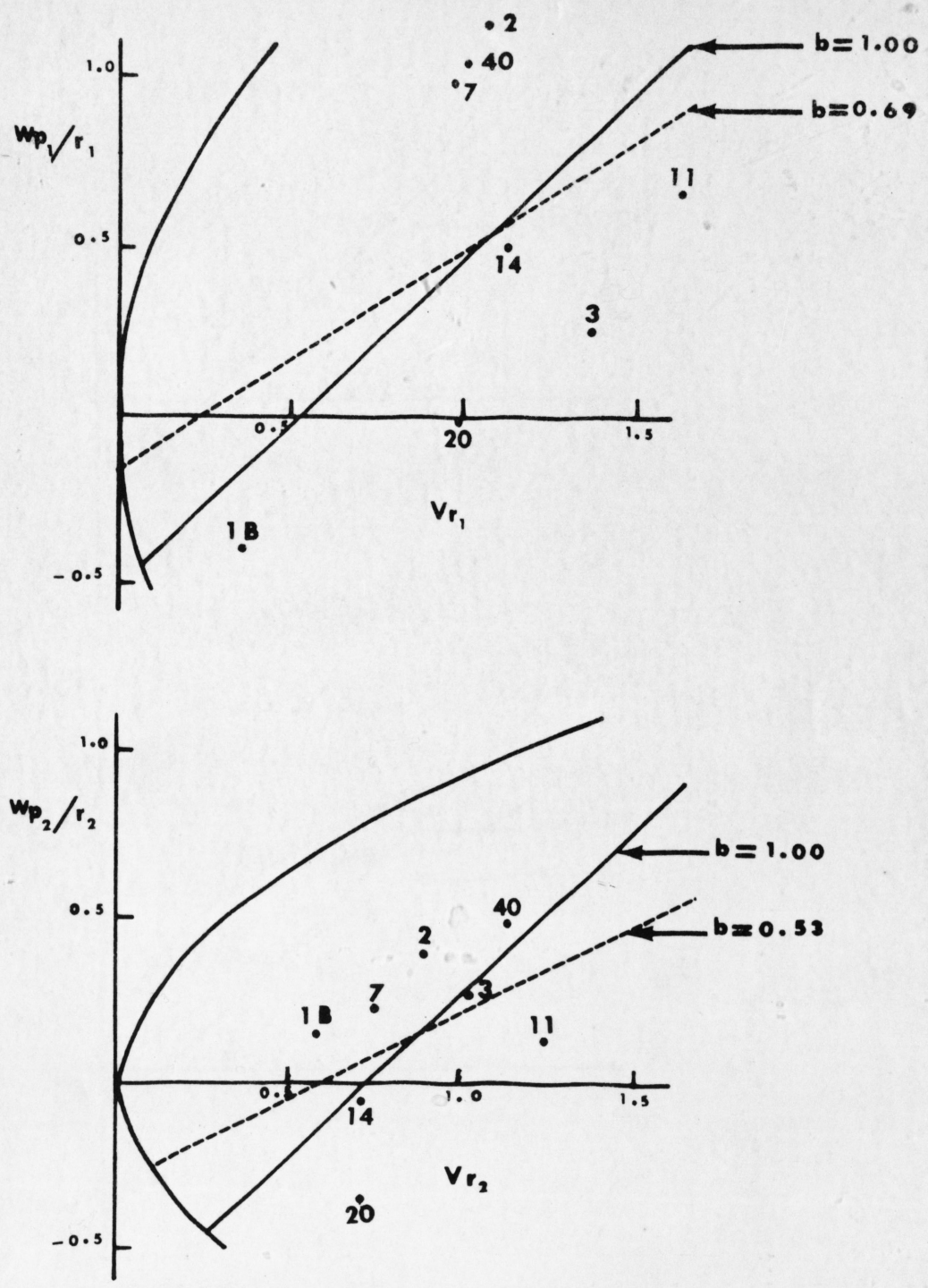

Figure IV. The distributions of mean variances and covariances of the reaction of a diploid alfalfa diallel to the Phoma-Cercospora treatment, when the parent clones (Vr_1) and the selfed families (Vr_2) are included in the arrays

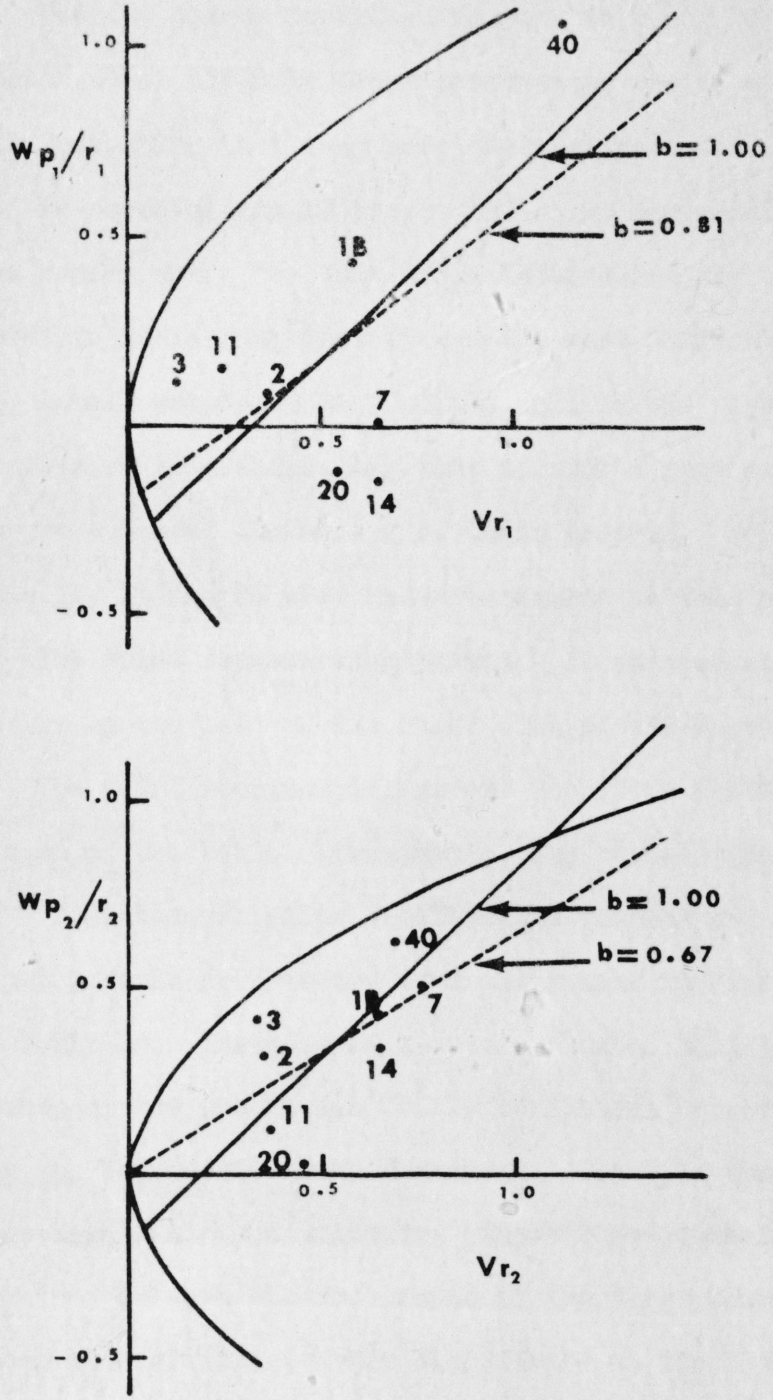

Figure V. The distributions of mean variances and covariances of the reaction of a diploid alfalfa diallel to the Cercospora-Phoma treatment, when the parent clones (Vr_1) and the selfed families (Vr_2) are included in the arrays

5. The two points representing parents 2 and 40 usually fell in the region where the unit slope intersected the upper curvature of the parabola indicating that they were the most recessive parents.

6. In seven of the 10 graphs the point representing parent 1B fell in the region where the unit slope intersected the lower curvature of the parabola indicating that it was the most dominant parent.

7. In all graphs, 3 to 5 points fell to the right side and below the regression line indicating that epistatic gene action was functional in the reaction of the arrays of these parents. Points representing parents 11, 14 and 20 were most consistent in this respect.

8. The point representing parent 3 fluctuated about the mean and fell either to the left of the right side of the regression line.

9. The point representing parent 7 shifted position consistently between graphs of the various treatments, and between graphs of the same treatment.

Rank-correlation coefficients for the order of dominance of the eight parents as detected from the graphs in Figures I to V are shown in Table 10. These coefficients indicated that the distribution of points on the graphs was fairly consistent between treatments, except for the Cercospora-Phoma treatment. Eight of the 16 coefficients for this treatment were negative. The highest correlation coefficients were obtained between the Vr_1 graphs of the first four treatments. Three of these 10 coefficients were significant at the 1 per cent level, and two at the 5 per cent level.

Rank-correlation coefficients between the scores of the parental families, selfed progenies and array means are shown in Table 11. In general, the scores of the selfed families were better correlated with

Table 10. Rank-Correlation Coefficients (r_s) for Order of Dominance of Eight Diploid Alfalfa Selections Inoculated in a Diallel Set with Indicated Treatments. Ranking of Parents was Obtained from the Vr_1 and Vr_2 Graphs of Figures I to V

		Treatment							
		Phoma		Cercospora		Mixture		Phoma-Cercospora	
		Vr_1	Vr_2	Vr_1	Vr_2	Vr_1	Vr_2	Vr_1	Vr_2
Cercospora	Vr_1	0.8095*	0.8810**						
	Vr_2	0.0476	0.4524						
Mixture	Vr_1	0.5000	0.4524	0.6905*	0.3810				
	Vr_2	0.7381	0.3333	0.7143*	0.1190				
Phoma-Cercospora	Vr_1	0.8333**	0.4881	0.8333**	0.3333	0.8571**	0.9524**		
	Vr_2	0.5714	0.7143*	0.8571**	0.1429	0.7857*	0.3333		
Cercospora-Phoma	Vr_1	-0.3333	0.0714	0.0000	0.1905	0.2024	-0.1904	-0.0476	-0.0238
	Vr_2	-0.1950	0.0952	0.1548	-0.3095	0.3333	-0.0714	0.2619	-0.0476

Table 11. Rank-Correlation Coefficients (r_s) Between Scores on Parental Families (P_1), Selfed Progenies (P_2) and Array Means (A) Obtained from Disease Ratings on an 8x8 Diallel Set of Diploid Alfalfa Inoculated with Indicated Treatments. The Correlation Coefficients were Averaged Over Two Trials

Pathogenic treatment		Rank-correlation coefficient	
		P_2	A
Phoma	P_1	0.4881	0.2857
	P_2	-	0.4762
Cercospora	P_1	0.4881	0.2024
	P_2	-	0.6667*
Mixture	P_1	0.7143*	0.5000
	P_2	-	0.7619*
Phoma-Cercospora	P_1	0.3810	0.3689
	P_2	-	0.3810
Cercospora-Phoma	P_1	0.5238	0.3214
	P_2	-	0.6310

*P less than 0.10.

the array mean scores than those of the parental families. There was some resemblance between the Cercospora and the Cercospora-Phoma treatments in magnitude of these coefficients.

A genetic correlation coefficient (r_g) between the reaction of the 72 families to Phoma and their reaction to Cercospora treatments was estimated at 0.8839.

Multiple correlation coefficients (R_s) between the Phoma and the Cercospora as the independent variables and each of the mixture, Phoma-Cercospora and Cercospora-Phoma as the dependent variables were 0.5968, 0.7185 and 0.6549, respectively. All these coefficients were highly significant.

Standardized multiple regression coefficients (b') for three variables are shown in Table 12. These coefficients indicated that in all three treatments where the two pathogens parasitized the leaf-tissue at the same time, P. herbarum was more effective than C. zebrina.

Table 12. Standardized Multiple Regression Coefficients (b') for the Phoma (X_1) and the Cercospora (X_2) Data as the Independent Variables, and Each of the Mixture, Phoma-Cercospora and Cercospora-Phoma Data as the Dependent Variable (Y)

	Y		
	Mixture	Phoma-Cercospora	Cercospora-Phoma
b' Y 1.2	0.5601**	0.9446**	0.4584**
b' Y 2.1	0.0692	0.2004	0.1697

**Significant at the 1% level.

Estimates of heritability (h^2) are presented in Table 13. There was considerable difference in magnitude of these estimates between

treatments and between trials of each treatment. The estimates based on the combined data were less variable than those based on individual trials.

Table 13. Estimates of Heritability (h^2) for the Reaction of an 8x8 Diallel Set of Diploid Alfalfa Inoculated with Indicated Treatments in Two Trials

Treatment	h^2			
	Trial A	Trial B	Mean of the two trials	Combined data of the two trials
Phoma	0.0492	0.4985	0.2738	0.1685
Cercospora	0.4196	0.0389	0.2292	0.3965
Mixture	-0.0318	0.2157	0.0920	0.0388
Phoma-Cercospora	0.1105	0.2874	0.1990	0.1292
Cercospora-Phoma	-0.0760	0.5499	0.2370	0.1043

DISCUSSION

Several investigators (15,21) have reported no detectable difference in virulence between single-spore isolates of C. zebrina. In this study, two isolates of this fungus exhibited a marked difference in virulence. It should be pointed out, however, that both isolates were obtained by mass-conidial isolation, and were tested at different times. Also, the two isolates produced a satisfactory infection when first used, but after a few transfers, one isolate showed a considerable reduction in virulence, while the other was highly consistent.

One objective of this experiment was to determine the ecological relationship between P. herbarum and C. zebrina. For this purpose, the diallel set was inoculated with the two pathogens in various sequences with the intention of distinguishing between the types of lesions produced by each pathogen. Unfortunately, this distinction was not possible, and instead, the gross infection was used as a basis to determine the relationship between the two fungi.

The consistency in level of infection obtained by inoculation with the P. herbarum isolate indicated that the differences in level of infection produced by the various treatments were due to the treatments themselves, rather than to drastic fluctuation in environmental factors. A comparison between levels of infection presented in Table 1 indicated that Phoma-Cercospora produced on the average twice as much infection as any other treatment. Had the relationship between the two pathogens been neutral, the mixture treatment would have resulted in the highest level of infection, since the two pathogens were associated with the

leaf-tissue for a longer period of time than in any other treatment. The fact that the Phoma-Cercospora treatment produced approximately twice as much infection as the mixture treatment suggested that a type of interaction between the two fungi did take place. Accordingly, an antagonistic effect for C. zebrina on P. herbarum is proposed. Evidence for this hypothesis may be derived from the mixture treatment in trial B where the level of infection was less than that obtained with Phoma alone. The question which may arise here is why the level of infection obtained with the mixture treatment in trial A was higher than that obtained with either pathogen alone. Reports in the literature indicate that the spores of C. zebrina germinated after six hours, but penetration into the leaf-tissue occurred only after 48 hours of incubation, and the penetration was through the stomata only (4). In contrast, P. herbarum produced infection within two days, which indicated that germination and penetration took place within 48 hours, and such penetration was direct and through the stomata as well (43). According to these observations, the mixture treatment in trial A resulted in more infection than either pathogen alone because P. herbarum penetrated the leaves first, but before it produced its maximum effect it was interrupted by the slow penetrating C. zebrina. The difference between the two trials in level of infection was due to the difference in virulence of the two C. zebrina isolates.

Further evidence of the antagonistic effect of C. zebrina on P. herbarum was detected in the treatments Phoma-Cercospora and Cercospora-Phoma. The former treatment resulted in the highest level of infection because P. herbarum had a 96-hour period (48 hours between inoculations, and 48 hours for C. zebrina to penetrate the leaves) to produce its

effect before it was subjected to the influence of C. zebrina. The Cercospora-Phoma treatment on the other hand produced only half the level of infection produced by Phoma-Cercospora. This was due to the presence of C. zebrina in the leaf-tissue when P. herbarum penetrated the leaves. According to the hypothesis presented, this treatment should behave like the Cercospora treatment. In two of four inoculations this was true. In general, however, the Cercospora-Phoma treatment deviated from all other treatments in more than one respect.

Although evidence of an antagonistic effect of C. zebrina on P. herbarum was detected in this study, the latter pathogen seemed to be more effective than the former in production of infection. The multiple regression coefficients (Table 12) clearly demonstrated that this was true. This perhaps was due to the distinct difference between the two pathogens in mode and speed of penetration reported in the literature and discussed above. Also, the inoculum of P. herbarum contained abundant spores, while that of C. zebrina contained only mycelial fragments. Usually, spores are more effective than mycelia in producing infection.

The discussion presented above is relative to one P. herbarum and two C. zebrina isolates. Undoubtedly, a better evaluation of the relationship between the two pathogens would be achieved if more isolates are used, if symptoms produced by each are distinguishable and if other factors are considered.

The major objective of this investigation was to obtain some information regarding the genetic factor or factors in diploid alfalfa which condition its reaction to the two pathogens. Such information may aid considerably in designing more efficient breeding programs for

developing commercial varieties of alfalfa with substantial amount of resistance to the two parasites.

The mean scores of the parental families indicated that parents 1B and 2 were among the most resistant parents to all treatments. The graphs in Figures I to V indicated that the reactions of the arrays of parents 1B and 2 were conditioned by dominant and recessive genes, respectively. This suggested that dominant and recessive genes were involved in controlling resistance to both pathogens. Other workers suggested that dominant and recessive genes have conditioned the resistance in tetraploid alfalfa to P. herbarum (39). The fact that the genetic parameters which estimate the over-all dominance in the population were large in magnitude and the F_I and F_{II} parameters were positive in all treatments suggested that dominant genes were at higher frequency than recessive genes. Rumbaugh et al. (42) found that recessive genes which conditioned the resistance in diploid alfalfa clones to two isolates of P. herbarum were of low frequency.

From the comparison between the reaction of each F_1 family and the reaction of the two parents that produced it, 19.6 and 17.9 per cent of the F_1 families resembled their most resistant parents, while 7.1 and 5.4 per cent resembled their most susceptible parents to P. herbarum and C. zebrina, respectively. This indicated that dominance was not unidirectional. Rumbaugh et al. (42) found genes operating on the complete dominance level have conditioned susceptibility in diploid alfalfa to P. herbarum. Dickinson and Jinks (11) reported that lack of unidirectional dominance resulted in underestimation of number of loci showing dominance. In this investigation estimates of number of

loci of less than 0.5 were encountered. It was evident, however, that at least two loci were functional in producing the reaction observed.

In addition to dominance, epistatic gene action seemed to play a major role in inducing the genetic variations in the host population tested. Epistatic gene action was reported to appear as heterosis in the F_1 families (3,23) and as h/d ratio in excess of 1.0 (11). In this experiment more than 40 per cent of the F_1 families exhibited heterosis for resistance and susceptibility, and in all analyses an h/d ratio of more than 1.0 has been obtained. Other evidence for the epistatic gene action was derived from the graphs in Figures I to V, where in each graph at least three of the eight points appeared below and to the right side of the regression line. Removal of four of these parents from the Phoma data and reanalysis resulted in a drop of the h/d ratio from 1.2553 to 0.9028, and in an increase in number of loci showing dominance from 0.9139 to 31.9489.

The consistent and high percentage of F_1 families that exhibited heterosis for resistance and susceptibility between the five pathogenic treatments requires explanation. From the graphs in Figures I to V it was evident that the reactions of parents 14 and 20 and their arrays were conditioned by epistatic gene action. Parent 14, which was moderately susceptible to both pathogens, produced the most resistant P_2 family, while parent 20, which was the most resistant parent to both pathogens, produced a moderately susceptible P_2 family. This may indicate that the interacting loci in these two parents were not the same, since the breakdown of these loci by inbreeding resulted in somewhat different phenotypes. If this was the case, then there are at least

two sets of interacting loci, which may have accounted for the more than 40 per cent heterotic segregants.

One of the interesting findings in this experiment was the close association between the reaction of the 72 families to P. herbarum and to C. zebrina. A genetic correlation coefficient of 0.8839 between the reactions of the host plants to the two pathogens was obtained. Selection of the parental clones and/or the wide crosses may have biased this correlation coefficient upward. However, the similarity in distribution of points on the graphs in Figures I and II, and rank-correlation coefficients between scores on the parents, selfed progenies and the arrays in the Phoma and the Cercospora treatments suggested the genetic factors which controlled the reaction to both pathogens were closely related if not identical. Geise[5] obtained a positive correlation coefficient of 0.36, which was significant at the 5 per cent level between the reaction of a group of alfalfa plants to both pathogens. He reported that, although this value was too small, it did suggest that selection for resistance to both pathogens at the same time was feasible, and "multiple resistance is not a physiological impossibility." These observations may indicate that genes with pleiotropic effects have conditioned the reaction of the host plants to both pathogens.

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[5]Geise, H. A. Reaction of certain diploid and tetraploid alfalfas to some phytopathogens inducing the blackstem disease. Unpublished information from Master of thesis, at South Dakota State College, March, 1957.

SUMMARY

Eight diploid alfalfa clones were selected, crossed in every possible way and clonally propagated to establish a diallel set. The 72 families were space planted at 2.5 inches between and within rows in two greenhouse benches, each bench constituted a replication. Each family was represented by a maximum of 10 plants per replication.

One P. herbarum and two C. zebrina isolates were used to inoculate the plants, using the detached-leaf technique described by Ward (46). The pathogenic treatments and the order in which they were used were as follows: (a) Phoma, (b) Cercospora, (c) mixture, (d) Phoma-Cercospora and (e) Cercospora-Phoma. This order of inoculations was repeated in two trials.

The objectives of this investigation were (1) to determine the ecological relationship between the two pathogens, and (2) to obtain some genetic information regarding the mechanism or mechanisms which condition the reaction of the host population to the two pathogens.

Since it was difficult to distinguish between the lesions produced by each pathogen parasitizing the same leaf-tissue, the gross infection was used as basis for determining the relationship between the two fungi. The comparison between the magnitude of level of infection produced by the five treatments revealed that such relationship was not neutral. The possibility that C. zebrina has an antagonistic effect on P. herbarum was proposed.

Analysis of diallel crosses showed that dominant and recessive genes were involved in controlling resistance in the host plants to

both pathogens. Dominant genes, however, were more frequent than recessive. Dominance was not unidirectional. Lack of unidirectional dominance resulted in underestimation of number of loci showing dominance. It was evident, however, that at least two loci were involved.

Epistatic gene action seemed to play a major role in controlling the reaction of these plants to both pathogens. This non-allelic interaction appeared as h/d ratio in the range of overdominance and as heterosis in the F_1 families for resistance and susceptibility. The extent to which this epistatic gene action was operational in relation to dominance was not determined.

Genetic and rank correlation of the order of dominance indicated that the genetic system which controlled the reaction of the host population to P. herbarum was similar to that which conditioned the reaction to C. zebrina. The possibility that genes with pleiotropic effects were controlling the reaction to both pathogens was proposed.

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