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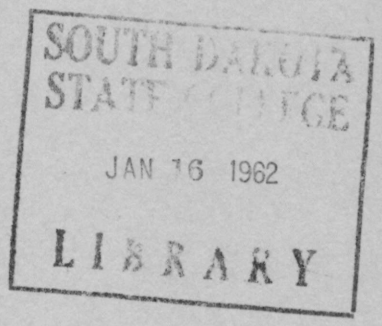

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Report of Progress: South Dakota Experiment Station Project #302, contributing to and supported by NC-37.

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The Genetics of Reaction of Alfalfa to Diseases of the Blackstem Complex

I. Introduction

Following the establishment in 1957 of Regional Project NC-37 entitled "Improvement of Alfalfa and Other Forages Through Basic Studies in Genetics and Pathology", the South Dakota Agricultural Experiment Station developed a contributing project directed toward the investigation of the inheritance of resistance to diseases of the blackstem complex of alfalfa. The objectives of the contributing project were described as follows:

1. To determine the gentic nature of reaction to the foliar diseases represented by the blackstem complex, utilizing principally diploid alfalfa.
2. To originate and use methods of breeding by which disease resistance may be incorporated into improved strains of alfalfa.

The procedures by means of which the objectives were to be attained are cited below.

a. Diploid Medicago falcata and M. sativa from several geographic sources will be carefully screened and progeny-tested in order to identify genotypes that are resistant and susceptible to the major components of the blackstem disease complex (Phoma herbarum var. medicaginis and Cercospora zebrina).

b. These clones will be selfed and diallely crossed to produce sets of all possible F_1's which will then be artificially inoculated and scored for their disease reactions.

c. Biometrical analysis designed for data from diallel crosses will be used to elucidate the genetic nature of the factors conditioning disease reactions.

d. Tentative hypotheses will be developed and tested in advanced generations of some of the crosses.

e. Selected plants from the diploid level will be raised to the tetraploid level and their disease reactions observed in order to generalize the hypotheses and extend them to the tetraploid case.

This report will serve to review chronologically the results which have been obtained at South Dakota prior to and since the initiation of the contributing project and to interpret the results in terms of the initial objectives.

II. Exploratory Research

(1954-1956)

The forage legume breeding project of the South Dakota Agricultural Experiment Station began with an investigation of the possibility of selecting for resistance to alfalfa blackstem in 1954. The research was conducted by M. W. Adams, G. Semeniuk, and H. Geise and culminated in a thesis by H. Geise[1/]. Much of the subsequent research employed host genotypes selected during the period 1954 - 1956.

The plant materials were derived from ten geographic sources and varied in degree of ploidy, origin, and phenotype. These populations are identified and described in table 1.

Forty plants were selected at Brookings from these ten populations to provide a range of expression of crown diameter and observed field reaction to specified leaf and stem diseases. The characteristics of the forty clones are cited in tables 2 and 3.

1/ Geise, Harry A. Reaction of Certain Diploid and Tetraploid Alfalfas to some Phytopathogens Inducing the Blackstem Disease. M. Sc. thesis. South Dakota State College. Brookings, South Dakota. 1957.

Table 1. Identification of plant materials

IDENTITY	ACCESSION NUMBER	DEGREE OF PLOIDY	SPECIES	SOURCE OF MATERIAL	ORIGIN
Alaskan	S.P.I. 24452 (S.D. 42)	2N=16	Medicago falcata	Alaska Agr. Exp. Sta.	Collected by N. E. Hansen near Obb, Tomsk Province, Siberia
Caucasus	Ultuna #109	2N=16	Medicago falcata	Univ. of Alberta	Collected by Dr. Vasiljtzenka from Northwest Caucasus region of the U.S.S.R.
Don	S.P.I. 20725 (S.D. 46)	2N=16	Medicago falcata	Univ. of British Columbia	Collected by N. E. Hansen in Don.Prov. of Lower Volga region of Southeast Russia.
Don-S	---	2N=16	Medicago falcata	S. Dak. Agr. Exp. Sta.	Seed obtained from herbarium specimen of Don
AF	---	2N=16	Medicago falcata	S. Dak. Agr. Exp. Sta.	Seed collected by D. D. Harpstead near Hohenfels, Bavaria, in Southern Germany
S33-1	---	2N=16	Medicago falcata	Univ. of Sasketchewan	Origin unknown
Iran	Ultuna #206	2N-16	Medicago sativa	Univ. of Alberta	Collected near Kashan, Iran
S2128	Saskatoon #2128	2N=16	Medicago sativa	Univ. of Sasketchewan	Collected at Botanical Gardens, Acad of Sci., Armenian S.S.R. at Erevan, Kanaku, U.S.S.R.
Turkey	Iowa #1976 Edmonton#252	2N=16	Medicago sativa	Univ. of Alberta	Collected originally in Turkey
ClCk	---	4N-32	From crosses involving M. sativa and M. falcata	Domminion Exp. Sta. Swift Current, Sasketchewan	Selections of crosses involving Siberian and Ladak which were collected by N. E. Hansen.

Table 2. Characteristics for which initial plants were selected

Plant Selection	Crown diameter in inches	Cercospora Blackstem (1-10 score)	Cercospora Leafspot (1-10 score)	Pseudopeziza Leaf spot (1-5 score)	
Tetraploid selections					
ClCk 1	34	2.3	4.2	4.4	
ClCk 2	34	2.3	3.4	2.8	
ClCk 4	26	3.1	5.0	3.3	
ClCk 6	30	2.7	3.4	1.2	
ClCk 11	30	3.0	3.5	1.0	
ClCk 13	35	1.8	3.8	5.0	
ClCk 14	32	2.4	3.4	4.7	
ClCk 16	32	2.0	2.6	4.0	
ClCk 18	31	2.4	3.3	1.0	
ClCk 19	34	2.1	3.6	1.0	
ClCk 20	41	1.1	4.3	1.0	
ClCk 21	42	1.2	3.3	3.6	
ClCk 22	44	4.0	4.1	3.8	
ClCk 23**	--	5.0	4.2	1.4	
Diploid selections					
Iran*	--	--	--	*No previous information available on these lines	
Turkey*	--	--	--		
Caucasus*	--	--	--		
Alaska*	--	--	--		
Don	20	1.0	1.0		
Don S-1	9	4.0	5.0	**this plant is not a spreading type	
Don S-3	8	8.0	1.0		
Don S-4	9	9.0	2.0		
S2128-1	9	8.0	5.0		
S2128-2	7	8.0	5.0		
S2128-4	7	9.0	2.0		
S2128-6	7	10.0	1.0		
S2128-8	6	9.0	5.0		
S2128-9	8	9.0	3.0		
S2128-10	8	9.0	5.0		
S2128-11	10	10.0	5.0		
S33-1-7	10	8.0	2.0		
S33-1-12	13	7.0	3.0		
S33-1-14	20	1.0	1.0		
HF-1	14	1.0	5.0		
HF-2	13	1.0	6.0		
HF-6	13	1.0	1.0		
HF-7	16	3.0	1.0		
HF-9	16	2.0	2.0		
HF-11	15	1.0	3.0		
HF-12	10	4.0	3.0		

Table 3. Genera of Fungi isolated from stems or leaves of sixteen diploid and one tetraploid alfalfa grown in the field, 1954.

GENUS OF FUNGI	CERCOSPORA(3)		PHOMA(4)		COLLETOTRICHUM		PLEOSPORA		PHOMA		FUSARIUM		RHIZOCTONIA		ALTERNARIA	
Plant Identity	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf
Don	x		x	x	x	x										
Don-S-1	x										x	x			x	x
Don-S-4	x		x	x	x	x					x		x		x	x
S2128-1	x		x			x					x	x		x		x
S2128-4	x	x	x	x							x		x	x		x
S2128-6					x	x		x								
S2128-10	x	x	x													x
S2128-11	x	x	x		x											
S33-1-7	x		x		x	x					x				x	x
S33-1-14			x			x		x			x	x			x	x
HF-1			x			x					x	x		x	x	x
HF-6			x		x	x		x				x	x			x
HF-7	x	x									x		x			x
HF-12	x	x	x		x	x		x				x			x	x
Caucasus		x							x							
Iran			x	x					x							
ClCk(2)			x	x					x							

Notes: (1) The presence of x's does not imply complete susceptibility, nor does the absence of x's imply immunity.

(2) ClCk is tetraploid (4N=32).

(3) Cercospora zebrina

(4) Phoma herbarum

Vegetative propagation of these plants permitted the establishment of ten replications of three cuttings per clone in the greenhouse during the fall of 1954. The parent clones were cross- and self-pollinated to derive F_1 and S_1 progenies. These progenies and four replications of the parent plants were transplanted into the field during the spring of 1955. In the following summer, vegetative cuttings of the S_1 progenies in the field were rooted to provide a similar array of genotypes in the greenhouse.

All plants in the greenhouse were grown in soil in benches or flats and inoculated in situ after sufficient growth was made to insure a leaf area suitable for disease evaluation. Pathogenic cultures of isolates of fungi indigenous to hosts grown in the vicinity of Brookings were macerated, diluted, and sprayed on test plants by means of a De Vilbiss sprayer connected to an electrically driven pressure pump. The benches were covered with plastic sheets for 3 to 5 days following inoculation. During this incubation period, the relative humidity of the space over the plants was maintained between ninety and one hundred per cent by the use of humidifiers. Host plants were subjectively scored for the amount of leaf area infected on a 1-5 scale with a score of 1 indicating the absence of lesions.

P. herbarum:

The analysis of variance of the results of the bench tests of the reaction of the forty selected clones to P. herbarum is presented in table 4. It is evident that differences existed among both diploid and tetraploid clones in these trials. Although the two isolates of the pathogen differed in virulence, the ranking of the clonal reactions were similar for both isolates. In both instances, tall erect tetraploid plants tended to be more resistant than diploid or prostrate plants.

Table 4. Analysis of reaction of forty clones of alfalfa to infection by P. herbarum. (Greenhouse 1954-1955)

Source	D.F.	Mean Square
Total	479	- - - - - -
Clones	39	24.95 **
2N vs. 4N	1	130.00 **
Among diploids	25	15.58 *
Among tetraploids	13	34.89 **
Isolates	1	236.60 **
Clones x isolates	39	7.63
Blocks	1	.75
Pooled error	79	8.71
Within plots	320	.71

* Significant at 5% level. ** Significant at 1% level.

Table 5. Analysis of regression of S_1 progeny performance on diploid parents for reaction to P. herbarum. (Field 1956)

Source	D.F.	Mean Square
Total	16	- - - - - - -
Regression	1	3.2330 **
Deviations from regression	15	.2191

b = .5954 ** r = .7042 **

The regression analysis of S_1 progeny performance on diploid parents, table 5, showed that the observed differences among the field reactions of the parent genotypes were highly heritable. Similar effects were evident among the open-pollinated progenies of the same parents when rated in the field (table 6) as well as in open-pollinated progenies of other diploid plants evaluated in the greenhouse (tables 7 and 8). The obtained heritability estimate indicated that 35 per cent of the mean superiority of selected families would be transmitted to their open-pollinated offspring.

C. zebrina:

Field and greenhouse evaluation of the reactions of the parent clones and their S_1 and open-pollinated progenies are summarized in the analyses presented as tables 9-14. The data were interpreted as supporting the existence of significant heritable differences in the reactions of the genotypes to C. zebrina. The three isolates of this pathogen could not be distinguished on the basis of the response of the host genotypes tested.

Colletotrichum trifolii:

Genetic differences in the reaction of both diploid and tetraploid clones to C. trifolii were observed (tables 15-19). Furthermore, the tests indicated that the genetic differences were highly heritable. On the basis of the obtained estimate of $H^2 = .7940$ it was assumed that about 79 per cent of the mean superiority of selected families would be transmitted to their open-pollinated progeny.

Pseudomonas medicaginis:

Data pertaining to the reaction of alfalfa to P. medicaginis were only available for the extent of leaf spot and stem blackening of S_1 families of 12 tetraploid clones. These analyses are presented in tables 20 and 21. Genetic differences in reaction existed but estimates of heritability were not made.

Table 6. Analysis of regression of open-pollinated progeny performance on diploid parents for reaction to P. herbarum and blackstem. (Field 1956)

Source	D.F.	Mean Square
Total	12	
Regression	1	1.822 **
Deviations from regression	11	.0807
b = .5714 **	r = .8201 **	

Table 7. Analysis of variance for reaction of sixty open-pollinated families of diploid alfalfa to P. herbarum and blackstem. (Greenhouse 1955-1956)

Source	D.F.	Mean Square
Total	1757	
Between families	59	3.2286 **
Within families	1698	.1827

Table 8. Analysis of variance for reaction of forty open-pollinated families of diploid alfalfa (Alaskan) to P. herbarum leaf spot. (Greenhouse 1955-1956)

Source	D.F.	Mean Square
Total	1190	
Between families	39	6.397 **
Within families	1151	.218
H^2 = .3507		

Table 9. Analysis of Reaction of Forty Clones of Alfalfa to Infection by C. zebrina.
(Greenhouse 1954-1955)

Source of Variation	D.F.	M.S.
Clones		
2N vs 4N	1	662.020**
Among Diploids	25	13.582
Among Tetraploids	13	34.100**
Isolates	2	31.510
Clones x Isolates	78	5.916
Blocks	1	24.200
Pooled Error	119	10.884
Within Plots	480	0.060
Total	719	

Table 10. Analysis of Variance for Reaction of Twenty-six Diploid Alfalfa Clones to Infection by C. zebrina.
(Greenhouse 1954-1955)

Source of Variation	D.F.	M.S.
Clones	25	13.582**
Blocks	5	19.844**
Error	125	5.003
Total	155	

Table 11. Analysis of Regression of S_1 Progeny Performance on Diploid Parents for Reaction to Blackstem (C. zebrina).
(Field, October 1955)

Source of Variation	D.F.	M.S.
Regression	1	6.4677**
Deviation from Regression	16	.4783
Total	17	

b = .6808** r = .6767** r^2 = .4579

Table 12. Analysis of Variance for Reaction of Fourteen Tetraploid Alfalfa Clones to Infection by C. zebrina. (Greenhouse 1954-1955)

Source of Variation	D.F.	M.S.
Clones	13	34.100 **
Blocks	5	18.782
Error	65	8.677
Total	83	

Table 13. Analysis of Variance for Reaction of Twelve S_1 Tetraploid Families of Alfalfa to Infection by C. zebrina Blackstem. (Field 1956)

Source of Variation	D.F.	M.S.
Between Families	12	2.070**
Within Families	564	.187
Total	576	

Table 14. Analysis of Regression of Open-pollinated Progeny Performance on Diploid Parents for Reaction to Blackstem (C. zebrina).

(Field 1955)

Source of Variation	D.F.	M.S.
Regression	1	5.013 **
Deviations from Regression	23	.396
Total	24	

b = .37 ** r = .59 **

Table 15. Analysis of Variance for Reaction of Sixty O.P. Families of Diploid Alfalfa to Colletotrichum trifolii Blackstem. (Greenhouse 1955-1956)

Source of Variation	D.F.	M.S.
Between Families	59	1.3215 **
Within Families	1701	.1570
Total	1760	

Table 16. Analysis of Variance for Reaction of Forty O.P. Families of Diploid Alfalfa (Alaskan) to Colletotrichum trifolii Blackstem. (Greenhouse 1955-1956)

Source of Variation	D.F.	M.S.
Between Families	39	1.436 **
Within Families	1152	.294
Total	1191	

Table 17. Analysis of Variance for Reaction of Sixty O.P. Families of Diploid Alfalfa to Colletotrichum trifolii Leaf Infection. (Greenhouse 1955-1956)

Source of Variation	D.F.	M.S.
Between Families	59	1.8071 **
Within Families	1701	.2219
Total	1760	

Table 18. Analysis of Variance for Reaction of Twelve S_1 Tetraploid Families of Alfalfa to Infection by Colletotrichum trifolii Leafspot (Greenhouse 1956)

Source of Variation	D.F.	M.S.
Between Families	12	.300 **
Within Families	502	.132
Total	514	

Table 19. Analysis of Variance for Reaction of Forty O.P. Families of Diploid Alfalfa (Alaskan) to Colletotrichum Trifolii leafspot. (Greenhouse 1955-1956)

Source of Variation	D.F.	M.S.
Between Families	39	2.18 **
Within Families	1151	.26
Total	1190	

Table 20. Analysis of Variance for Reaction of Twelve S_1 Tetraploid Families of Alfalfa to Infection by Pseudomonas medicaginis Bacterial Leafspot (Field 1956)

Source of Variation	D.F.	M.S.
Between Families	12	2.080 **
Within Families	505	.382
Total	517	

Table 21. Analysis of Variance for Reaction of Twelve S_1 Tetraploid Families of Alfalfa to Infection by Pseudomonas medicaginis Blackstem (Field 1956)

Source of Variation	D.F.	M.S.
Between Families	12	1.822 **
Within Families	504	.278
Total	516	

Discussion:

At the conclusion of this series of tests it was known that heritable differences in the response of alfalfa clones to various pathogens of the blackstem complex did exist. Clonal reactions varied over a range of highly resistant to susceptible and appeared to be moderately to highly heritable. At no time, however, was either a completely susceptible or completely resistant family observed. With one bimodal exception, variability in all of the families appeared to be unimodal.

An association of the responses of plants to P. herbarum and C. zebrina sufficient to produce a significant positive correlation coefficient of .36 was noted. While this value was too small to assume that selection can be made for resistance to both pathogens at the same time, it did suggest that multiple resistance was not a physiological impossibility.

III. Establishment of Diallel Populations

(1957 - 1958)

Following the initial research with diploid alfalfa populations, Dr. M. W. Adams established two sets of diallel crosses with 8 and 12 parent clones, respectively. However, 25 of the F_1 and 2 of the selfed families were severely decimated by the occurrence of chlorophyll deficient seedlings. A portion of the available plant material was tested even though family sizes were too small to warrant complete diallel analysis. These trials were conducted in the greenhouse in the manner previously described.

P. herbarum:

The analysis of variance for the parent clones, the female array means and the male array means are presented in tables 22, 23, and 24, respectively. Significant genetic differences were observed in all cases.

Table 22. Analysis of variance of reaction of 9 diploid clonal parents to infection by P. herbarum.

Source	D.F.	Mean Square
Between clones	8	1.76**
Within clones	52	.406
Total	60	---

Table 23. Analysis of variance of female array means for reaction to infection by P. herbarum.

Source	D.F.	Mean Square
Between female arrays	8	1.1606 **
Within female arrays	72	.3327
Total	80	----

Table 24. Analysis of variance of male array means for reaction to infection by P. herbarum.

Source	D.F.	Mean Square
Between male arrays	8	1.1079**
Within male arrays	72	.3386
Total	80	----

Examination of the data provided by the 43 F_1 families in which family size was sufficiently large to assure reliability led to the following four observations.

(1) Four crosses showed complete dominancae for the resistance level of the more resistant parent in each cross.

(2) Three crosses showed complete dominance for the resistance level of the more susceptible parent in each cross.

(3) 18 crosses showed little or no dominance toward either parent but tended to cluster at the mid-parent level.

(4) 18 crosses showed greater susceptibility than the more suscep-

tible parent in each cross.

Although a factorial interpretation of the results was impossible, the data suggested rather striking gene interactions. These included dominance for resistant effects in a few crosses, dominance for susceptibility in certain crosses, simple additive effects, and non-allelic interactions promoting susceptibility.

C. zebrina:

A similar test with C. zebrina was conducted on the same plants. The analysis of variance are included in tables 25 and 26.

Table 25 Analysis of variance of female array means for reaction to infection by C. zebrina.

Source	D.F.	Mean Square
Between female arrays	8	5.5697 **
Within female arrays	72	.1656
Total	80	----

Table 26. Analysis of variance of male array means for reaction to infection by C. zebrina.

Source	D.F.	Mean Square
Between male arrays	8	5.4340 **
Within male arrays	72	.1807
Total	80	----

The following observations were based upon a total of 47 cross families with adequate numbers of plants per family to assure reliability.

(1) 4 crosses showed a mean reaction comparable to the mean of the more susceptible parent.

(2) 10 crosses showed a mean reaction comparable to the mean of the more resistant parent.

(3) 8 crosses showed a mean reaction equal to the mid-parent.

(4) 25 crosses showed a mean reaction of resistance greater than

the more resistant parent.

These observations also seemed to suggest an array of genic effects similar to those mentioned for the P. herbarum inoculation.

A simple correlation for joint reaction of 43 cross-families to infection by Phoma and Cercospora was found to be .2913.

IV. Analysis of Intact Diallels (1959 - 1961)

Because of the difficulty in obtaining and maintaining sufficient numbers of plants in the progenies of certain of the diploid clones, these clones were deleted from the diallel populations. This permitted the establishment and testing of the responses of genotypes in two 8 x 8 diallel sets designated Set I and Set II. The latter was to be used primarily for research into the nature of resistance to P. herbarum whereas the first was to be used for a detailed analysis of the genetics of resistance fo both P. herbarum and C. zebrina. Since the techniques utilized for the two series of tests were different, the data will be discussed independently.

A. Diallel Set II.

1. Materials and methods.

An 8 x 8 set of diallel crosses among heterozygous parental diploid clones and the selfed progenies of these clones was available for this investigation. An outline of the parental designations, sources, and species follows:

Parent Clone	Source	Species
1 A	Iran	M. sativa
9 (Don 1)	Don Province	M. falcata
10 (HF-11)	Hohenfels, Bavaria	M. falcata
12 (HF-12)	Hohenfels, Bavaria	M. falcata

16 (Don S-3)	Don Province	M. falcata
17 (Don S-4)	Don Province	M. falcata
21 (HF-2)	Hohenfels, Bavaria	M. falcata
38 (S2128-2)	Armenian SSR	M. sativa

The source populations had previously been tested for response to the pathogen studied. The four sources tended to be susceptible and only rarely were resistant clones present. Selected plants showing a spectrum of reaction types were retained as parents of the diallel.

Individual plants as cuttings or seedlings were set in soil in greenhouse benches at 2.5 inch intervals. The number of plants representing a genotype or genotypic group varied considerably. A maximum of 15 plants to estimate an F_1 family mean was possible in each trial. The average number of plants per family was somewhat less than this value. Parental families were obtained by vegetative propagation of the parental clones.

An aqueous spore suspension to which a wetting agent had been added was sprayed on the alfalfa when the plants were in vigorous vegetative growth and had attained a height of approximately 5 inches. Two single spore isolate cultures of the pathogen were plated on malt agar to provide the inoculum. Isolate 30 had previously been determined to be more virulent than isolate number 17[2/]. Following application of the Waring-blended pathogenic suspension, the beds were covered with polyethylene plastic sheets for 4 days to maintain a desirable environment for infection. Two such trials were conducted for each of the two isolates tested.

Readings were made on a 1 - 5 scale with 1 indicating no infection and 5 indicating extreme susceptibility. No attempt was made to further

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2/ The isolates of Phoma were obtained through the courtesy of B. L. Renfro, Department of Plant Pathology and Botany, University of Minnesota, and were so evaluated by him.

define the reactions as to lesion density or size. The scale was a subjective composite of both of these characters and was found to be adequately reproducible by the person making the evaluation. The results were largely analyzed by the methods established for diallels with heterozygous parentage.

A combined analysis was accomplished by the construction of a diallel table with each family represented by its most susceptible rating irrespective of the isolate inciting the reaction. Such a procedure is believed to more closely simulate the phenotype expected following inoculation by mass culture of the two isolates than would simply averaging the scores obtained from separate tests with these isolates.

Variance and covariance estimates were obtained for these data as follows:

V_{p_1} = variance of the parental clones

V_{p_2} = variance of the selfed families

$\bar{V}_r$ = mean variance of the arrays

V_r = variance of array means

$\overline{W}_{p_1/r}$ = mean covariance of the arrays with P_1

$\bar{W}_{p_2/r}$ = mean covariance of the arrays with P_2

W_{p_1/p_2} = covariance of P_1 with P_2

Genetic parameters of the host population were estimated by the use of these statistics.

2. Results and discussion.

A satisfactory level of infection was attained in all four trials. Mean scores of 2.5 and 3.6 were obtained with isolates 17 and 30, respectively. The difference between isolates was significant. Analysis of variance (table 27) demonstrated that the means of the 8 arrays, averaged

over the four trials were dissimilar. This was likewise true of the reactions of diallel family means within arrays. The corresponding interactions of the array means and family means within arrays sources of variation with isolates also proved to be highly significant. Thus the reaction of progenies of these heterozygous diploid alfalfa clones to P. herbarum was dependent upon the particular culture of the pathogen as well as the genotype of the host.

Table 27. Analysis of variance of the reaction of families of the 8 x 8 diploid alfalfa diallel Set II to two isolates of P. Herbarum.

Source	D.F.	Mean Square
Total	287	
Isolates	1	88.01 **
Trials in isolates	2	10.08 **
Between families	71	.87 **
Parental families	7	.71
Diallel families	63	.87 **
Between arrays	7	2.97
Within arrays	56	.61 **
Parents vs. diallel families	1	1.59
Isolates x families	71	.32 **
Isolates x parental families	7	.22
Isolates x diallel families	63	.28 **
Isolates x between arrays	7	.83 **
Isolates x within arrays	56	.21 **
Isolates x parents vs. diallel	1	3.71
Trials in isolates x families	142	.11
Trials x parental families	14	.20
Trials x diallel families	126	.08
Trials x between arrays	14	.12
Trials x within arrays	112	.07
Trials x parents vs. diallel	2	1.18

** Significant at 1% level.

Various variance and covariance estimates were derived for each isolate and for the constructed diallel. These were corrected for environmental effects where necessary and are presented in table 27. In all cases the response to isolate 17 was less variable than to isolate 30. Combining the data for the two cultures resulted in a general increase in the magnitude of the variances and covariances.

The statistics were used to develop the additive, dominance, and intra-

locus additive x dominance components cited in table 28. The genetic differences in reaction of alfalfa to the two isolates are readily apparent. The magnitude of F_I and F_{II}, the additive x dominance components, is somewhat surprising. In all cases they are larger than individual estimates of the additive or dominance components.

Certain similarities, however, in reaction to the two isolates are deducible from the properties of these components. For equal allele frequencies, $q = y$, $H_I = H_{II}$, $H_{III} = H_{IV}$, and $F_I = F_{II} = 0$. This was not true in the present investigation for either of the cultures or for the combined analysis of the data. Also, the weighted mean level of dominance given by the expression $\sqrt{\frac{\frac{1}{2}(H_I + H_{IV})}{D_I}}$ approximated complete dominance in all trials. Values of 1.00, .95, and .87 were obtained for isolates 17, 30, and the combined analysis, respectively. Because all the F_I and F_{II} components were positive and the mean reaction scores were quite high, an excess of dominant alleles which increase reaction scores is indicated. This would mean, of course, that resistance to the pathogen is an attribute primarily conditioned by recessive genes which occur at relatively low frequencies in the host populations.

Table 28. Genetic variances and covariances derived from the reaction of diploid alfalfa to isolates of P. herbarum.

	Phoma Isolate		
Parameter Estimated	17	30	Combined Analysis
V_{p_1}	.18	.29	.22
V_{p_2}	.35	.57	.75
$\bar{V}_r$	.18	.21	.25
$V_{\bar{r}}$	.09	.15	.13
$\bar{W}_{p_{1/r}}$	.11	.12	.09
$\bar{W}_{p_{2/r}}$	.15	.27	.33
$W_{p_{1/p2}}$	.18	.25	.30

Table 29. Genetic components of variation of the reaction of diploid alfalfa to isolates of P. herbarum.

Component	Phoma Isolate 17	30	Combined Analysis
D_I	1.96	3.14	4.04
H_I	3.28	2.92	2.96
H_{II}	1.44	.96	1.92
H_{III}	2.72	5.76	5.92
H_{IV}	.64	2.72	3.20
F_I	3.84	5.84	7.04
F_{II}	5.44	10.88	13.12

There were some indications for a lack of unidirectional dominance. Such indications were associated with differential reactions to the two single spore isolates of P. herbarum. The dominance order of the parents was established by their point representations along the obtained regression lines in the variance-covariance graphs presented in figures 1, 2, and 3. This was then compared to their ranking based upon mean reaction scores as parental clones and the corresponding array means. The responses of parents 9 and 17 were most interesting. Parent 9 was found to be both susceptible to isolate 17 and recessive (figure 1). However, the array mean of the same parent was the most resistant of the 8 arrays when the plants were inoculated with isolate 30. Figure 2 clearly shows that clone 9 was the most dominant parent in these trials. A somewhat similar situation existed with respect to parent clone 17 and its progeny. It was, however, resistant and dominant in its reaction to culture 17 and resistant and recessive in its reaction to culture 30. Thus a reversal of response and apparent dominance was present. In certain instances, therefore, resistance to this blackstem organism may be conditioned by dominant genes.

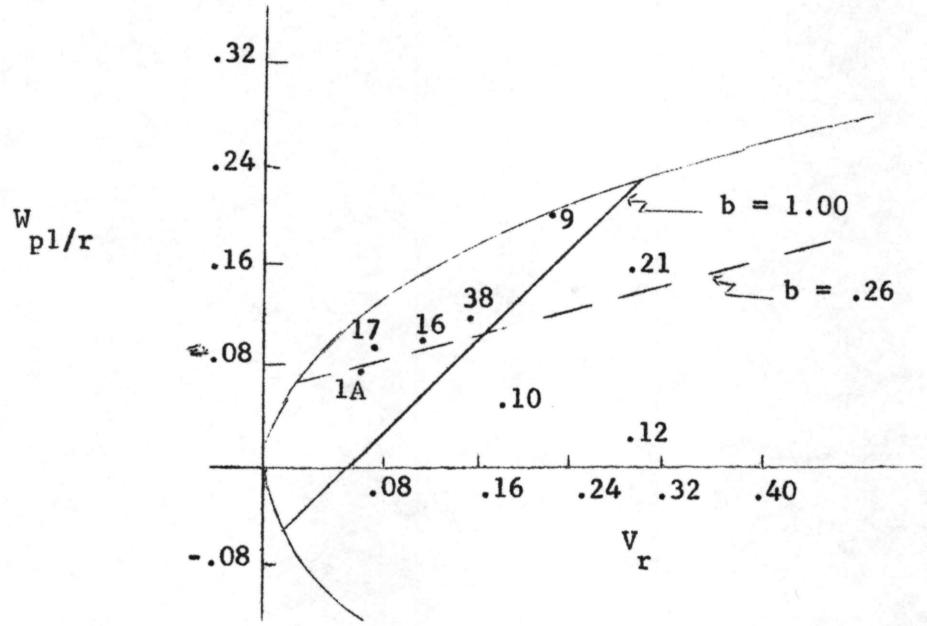

Figure 1. The variances of the arrays (V_r) and the covariances of the family means of the arrays and the parent clones ($W_{pl/r}$) for the reaction of diploid alfalfa to P. herbarum isolate 17.

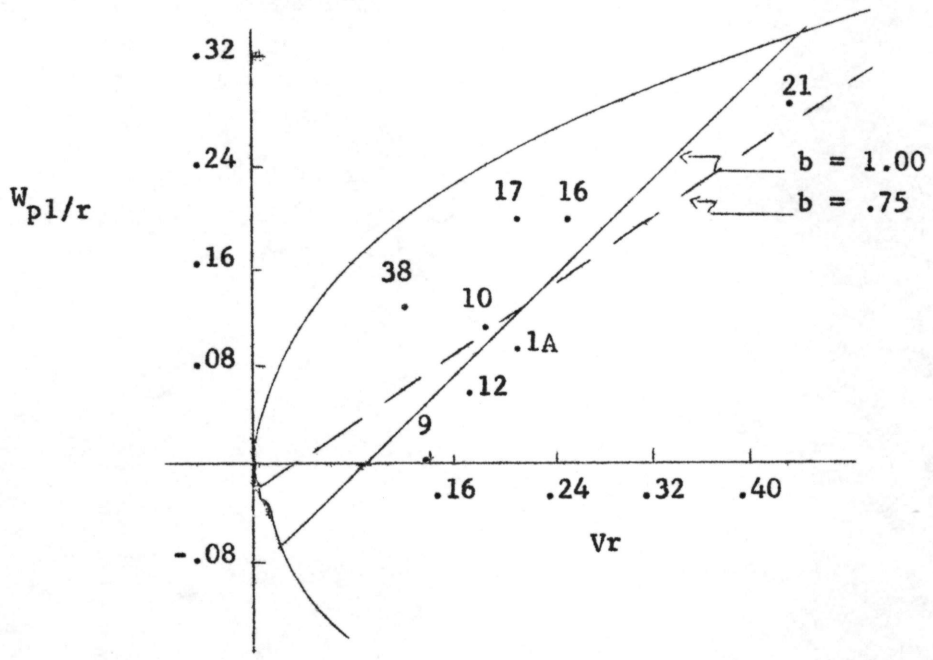

Figure 2. The variances of the arrays (V_r) and the covariances of the family means of the arrays and the parent clones ($W_{p1/r}$) for the reaction of diploid alfalfa to P. herbarum isolate 30.

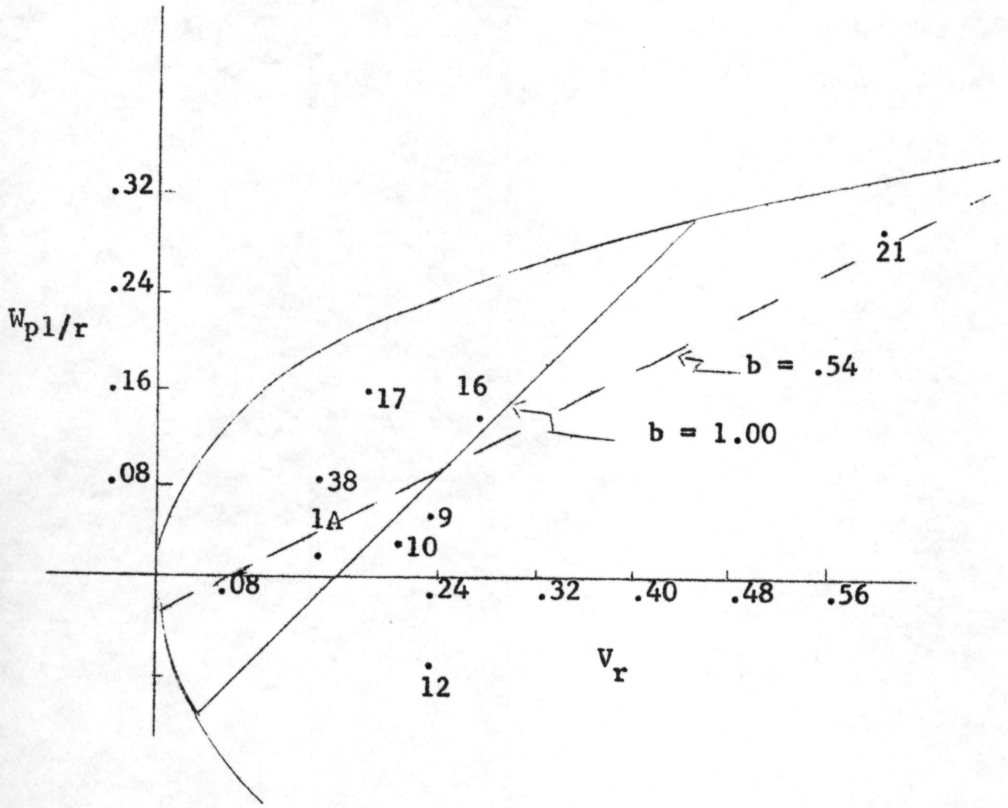

Figure 3. The variances of the arrays (V_r) and the covariances of the family means of the arrays and the parent clones ($W_{p1/r}$) for the combined analysis of the reaction of diploid alfalfa to P. herbarum isolates 17 and 30.

The number of loci exhibiting dominance was estimated by the expression $\frac{16\ (\bar{F}_I - \bar{P}_2)^2}{H_{II}}$. Minimum values of 4, 2, and 1 loci were obtained with cultures 17 and 30, and with the combined data. The number of loci and the heterozygosity of the parent clones may account partially for the reduction in slope of the regression functions from that anticipated for diallels of homozygous origin. Dickinson and Jinks 3/ have calculated that with 2 loci, complete dominance, and all possible genotypes represented as parents, the regression slope would be .92 even though the model excluded non-allelic interactions.

In this investigation, epistatic control of disease reaction was indicated for certain of the parents. The appearance of points 10 and 12 below and to the right of the regression line in Figure 1 suggests this possibility. In figure 2, epistatic genetic effects are not readily discernable. Figure 3, for the combined data, demonstrates the involvement of epistasis in the responses of the progenies of parent clone 12.

Estimates of heritability in the broad sense for the means of families were derived from components of variance in the analysis of parental scores. Values of .59, .37, and .56 were calculated for isolates 17, 30, and the average scores, respectively.

3. Summary

An 8 x 8 set of diallel crosses amoung heterozygous diploid alfalfa clones was used to deduce the genetic basis of reaction to two isolates of P. herbarum. Significant genotype x isolate interactions were detected for some of the arrays. Susceptibility was conditioned primarily by genes operating at the level of complete dominance. Recessive genes inducing resistance were present at a low frequency in the populations studied. Epistasis and a lack of unidirectional dominance for resistance were also indicated.

3/ Dickinson, A. G. and Jinks, J. L. A generalized analysis of diallel crosses. Genetics 41: 65-78. 1956.

B. Diallel Set L.

1. Materials and methods.

The diploid alfalfa plants used as parents for the development of diallel Set I were related to those of diallel Set II. Their origin is described below.

Parent Clone	Source	Species
1 B	Iran	M. sativa
2	Turkey	M. sativa
3	Caucasus	M. falcata
7 (S2128-9)	Armenian SSR	M. sativa
11 (Don S-1)	Don Province	M. falcata
14 (S33-1-7)	Origin unknown	M. falcata
20 (Alaska)	Tomsk Province	M. falcata
40 (HF-9)	Hohenfels, Bavaria	M. falcata

The parents were progagated vegetatively, intercrossed in diallel combination, and selfed to provide 72 families for testing. Two replications of 10 plant plots were established in soil in the greenhouse. Individual plants were spaced 2.5 inches apart between and within rows.

The evaluation of host reaction was based upon the detached leaf technique.[4/] Three unblemished leaves of comparable size and age were selected from each plant for each test. They were floated upon a 2 per cent sucrose solution in a syracuse watch glass. Inoculum was applied as uniformly as possible by means of a hand atomizer. An incubation period

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4/ Ward, C. H The detached-leaf technique for testing alfalfa clones for resistance to blackstem. Phytopathology 49: 690-696. 1959.

of six days at 68^{o}-72^{0} F. in the dark preceded the scoring of host reaction. The ratings were on a 0-10 scale with 0 indicating no infection, and 10 indicating extreme susceptibility.

The pathogenic treatments were as follows:

(1) Phoma herbarum

(2) Cercospora zebrina

(3) A mixture of P. herbarum and C. zebrina

(4) P. herbarum followed after 48 hours by C. zebrina

(5) C. zebrina followed after 48 hours by P. herbarum

The available facilities did not permit the simultaneous evaluation of all host plants for more than one of the pathogenic treatments at any one time. Therefore, intervals of various durations separated the tests involving the different treatments. Each pathogenic treatment was utilized in the sequence cited above on both replications of hosts. A complete sequence of all-treatments will be referred to as constituting a trial. Two such trials were conducted. All analytical procedures were based upon the plot means in any specified replication, treatment, or trial.

Since it proved impossible to visually distinguish lesions due to P. herbarum from those due to C. zebrina, the ratings for treatments (3) - (5) above were gross measurements of lesion density and size irrespective of the inciting organism.

2. Results and discussion.

Satisfactory levels of infection were obtained in all tests with P. herbarum. However, differences between trials with this pathogen were detected (table 30.). This variation was not of sufficient magnitude

to preclude the detection of differential responses between parent clones and between the constant parent arrays of the diallel.

Unfortunately, the results with C. zebrina were not as reliable as those with P. herbarum. Table 31 shows that significant ($P < .05$) differences were not detected among the families when their responses were averaged over both trials. Although excellent infection and differential responses were obtained in trial 1, the results in the subsequent trial were disappointing. The mean score for the latter test was low and genetic differences among the parents and among their progenies were not in evidence.

Similar effects were noted when C. zebrina was used prior to, in mixture with, or following inoculum of P. herbarum. This resulted in mean square estimates for trials in tables 32, 33, and 34 which were of approximately the same magnitude as that for C. zebrina alone. Although genetic differences are apparent in these analyses, it is difficult to assess the extent to which they were caused by each of the two species of pathogens.

Table 30. Analysis of variance of the reaction of families of the 8 x 8 diploid alfalfa diallel Set I to culture 42 of P. herbarum.

Source	D.F.	Mean Square
Total	287	
Trials	1	18.7834 **
Replications in trials	2	14.1008 **
Families	71	1.6940 **
Parental families	7	3.3430 **
Diallel families	63	1.5297 *
Between arrays	7	3.6249 *
Within arrays	56	1.2678
Parents vs. diallel families	1	.5059
Trials x families	71	.8567 *
Trials x parents	7	.4264
Trials x diallel families	63	.9080 *
Trials x between arrays	7	.8877
Trials x within arrays	56	.9106 **
Trials x parents vs. diallel	1	.6325
Replications x families	142	.5404
Replications x parents	14	.5038
Replications x diallel families	126	.5384
Replications x between arrays	14	.6909
Replications x within arrays	112	.5194
Replications x parents vs. diallel	1	.9195

Table 31. Analysis of variance of the reaction of families of the 8 x 8 diploid alfalfa diallel set I to C. zebrina.

Source	D.F.	Mean Square
Total	287	
Trials	1	310.3578 **
Replications in trials	2	.8374
Families	71	1.6248
Parental families	7	2.6513
Diallel families	63	1.4780
Between arrays	7	3.6428
Within arrays	56	1.2074
Parents vs. diallel families	1	3.6880
Trials x families	71	1.2672 **
Trials x parents	7	2.0752
Trials x diallel families	63	1.1060 **
Trials x between arrays	7	2.4302
Trials x within arrays	56	.9405 **
Trials x parents vs. diallel	1	5.7690 **
Replications x families	142	.6543
Replications x parents	14	.9847
Replications x diallel families	126	.6274
Replications x between arrays	14	1.6179
Replications x within arrays	112	.5036
Replications x parents vs. diallel	2	.0356

Table 32. Analysis of variance of the reaction of families of the 8 x 8 diploid alfalfa diallel Set I to a mixture of inoculum of P. herbarum and C. zebrina.

Source	D.F.	Mean Squares
Total	287	
Trials	1	360.2599 **
Replications in trials	2	1.7154
Families	71	1.9761 **
Parental families	7	5.3093
Diallel families	63	1.4944 **
Between arrays	7	1.9889
Within arrays	56	1.4326 **
Parents vs. diallel families	1	8.9949
Trials x families	71	.9416 *
Trials x parents	7	1.9154 *
Trials x diallel families	63	.8069
Trials x between arrays	7	.7029
Trials x within arrays	56	.8199
Trials x parents vs. diallel	1	2.6114
Replications x families	142	.6087
Replications x parents	14	.5986
Replications x diallel families	126	.6003
Replications x between arrays	14	.8508
Replications x within arrays	112	.5690
Replications x parents vs. diallel	2	1.2107

Table 33. Analysis of variance of the reaction of families of the 8 x 8 diploid alfalfa diallel Set I to inoculum of P. herbarum succeeded after 48 hours by inoculum of C. zebrina.

Source	D.F.	Mean Square
Total	287	
Trials	1	432.5286 **
Replications in trials	2	66.7182 **
Families	71	2.4917 *
Parental families	7	6.3839
Diallel families	63	2.0053 *
Between arrays	7	5.1612
Within arrays	56	1.6108
Parents vs. diallel families	1	5.8876
Trials x families	71	1.5834 **
Trials x parents	7	4.4000 **
Trials x diallel families	63	1.2809
Trials x between arrays	7	2.0245
Trials x within arrays	56	1.1879
Trials x parents vs. diallel	1	.9233
Replications x families	142	.9577
Replications x parents	14	.7948
Replications x diallel families	126	.9832
Replications x between arrays	14	1.6212
Replications x within arrays	112	.9035
Replication x parents vs. diallel	2	.4858

Table 34. Analysis of variance of the reaction of families of the 8 x 8 diploid alfalfa diallel Set I to inoculum of C. zebrina succeeded after 48 hours by inoculur of P. herbarum.

Source	D.F.	Mean Square
Total	287	
Trials	1	147.6335 **
Replications in trials	2	47.0470
Families	71	1.4464 *
Parental Families	7	2.2279
Diallel Families	63	1.3791 **
Between arrays	7	4.9648 **
Within arrays	56	.9309
Parents vs. diallel families	1	.2201
Trials x families	71	.8821 *
Trials x parents	7	1.7954
Trials x diallel families	63	.6975
Trials x between arrays	7	.3411
Trials x within arrays	56	.7421
Trials x parents vs. diallel	1	6.1203
Replications x families	142	.5792
Replications x parents	14	.8173
Replications x diallel families	126	.5519
Replications x between arrays	14	1.0727
Replications x within arrays	112	.4868
Replications x parents vs. diallel	2	.6345

A comparison of the F_1 family means with the parental means and midparent mean of each cross proved of interest. These data are summarized in table 35. The five phenotypic classes have approximate genetic bases as follows:

Class I : Heterosis for resistance.
Class II : Dominance for resistance.
Class III : No dominance.
Class IV : Dominance for susceptibility.
Class V : Heterosis for susceptibility.

The first and last classes contain those progenies which exhibited heterotic responses and may be accounted for by (1) the additive effects of dominant or partially dominant loci, (2) overdominance, or (3) the interaction of non-alleles. In excess of 40 percent of the progenies reacted in this manner. The frequency distributions for the five pathogenic treatments were quite consistent.

An analysis of the data by variance and covariance techniques was attempted. It was realized that the data did not meet some of the assumptions, such as that of unidirectional dominance, required for complete validity of application of the technique.

Family means averaged over both trials were used to compute the

phenotypic variances and covariances. These were corrected for the environmental components E_2 and E_3[5/] wherever necessary. The resulting

Table 35. Frequency distribution (%) of F_1 family means relative to the performance of the parents in each cross in diallel Set I.

Treatment	Phenotypic Class[1/]					Heterotic
	I	II	III	IV	V	Segregants
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

1/ Phenotypic classes were established as follows:

Class I F_1 more resistant than most resistant parent

Class II F_1 equal to the most resistant parent

Class III F_1 equal to the midparent

ClasssIV F_1 equal to the most susceptible parent

Class V F_1 more susceptible than most susceptible parent

genetic variances and covariances are shown in table 36. Similar estimates were computed within each trial. From these, the genetic parameters included

5/ $E_2 = \dfrac{\frac{V_e}{rt} + \frac{V_{tf}}{t}}{N}$ and $E_3 = \dfrac{E_2}{N}$

Where V_e is the mean square for replications in trials x families

V_{tf} is the trials x families variance component

r is the number of replications per trial

t is the number of trials

N is the number of families in one array or the number of arrays in the diallel

in tables 37 and 38 were estimated.

The within trial estimates, table 37, indicate the fluctuation in magnitude of the various sources of genetic variance which may be expected in tests conducted in differing environments. However, all of the dominance ratios, h/d, were well into the range of overdominance. Similarly, all of the estimates of the number of loci exhibiting dominance were less than 2. The values obtained from the combined analysis, table 38, approximated those obtained in the separate trials but resulted in higher dominance ratios.

Spurious overdominance may arise in the diallel analysis from non-allelic interaction. Plotting of the covariances and variances is useful to detect epistatic effects of this nature. The consistent appearance of points to the right and below the regression line is indicative of genic interaction for these arrays. An examination of figures 4 - 13 revealed that the point representations of constant parents 14 and 20 are more frequently situated below and to the right of the second order regression line than above and to the left of the line. Parent 2 is below the line in two of the figures and parents 1, B, 3, 7, and 11 are below the line in three of the figures. Epistasis is very likely involved in the reaction of some of the genotypes but it is difficult to assess the magnitude of the influence of such interactions upon the dominance estimates which were obtained.

Table 36. Genetic variances and covariances obtained from diploid alfalfa diallel Set I for the indicated treatments.

Statistic	Pathogenic Treatment				
	Phome	Cercospora	Mixture	Phoma - Cer.	Cer. - Phoma
V_{P1}	.8064	.6245	1.2987	1.5411	.5304
V_{P2}	.4895	.2322	.7603	.2416	.3454
$\bar{V}_{r_1}$	.3594	.3159	.3784	.5450	.3365
$\bar{V}_{r_2}$	.3748	.2832	.3357	.4377	.3380
$V_{\bar{r}_1}$	.0460	.1157	.0513	.0745	.0197
$V_{\bar{r}_2}$	.0240	.0867	.0524	.0826	.0204
$\bar{W}_{p_{1/r}}$	.2137	.1380	.1992	.2703	.0959
$\bar{W}_{p_{2/r}}$	-.0021	.1372	.1635	.0348	.1866
$W_{p_{1/P2}}$	.2760	.1830	.9147	.6050	.3119

Table 37. Estimates of genetic parameters obtained from trials I and II of the diploid alfalfa diallel Set I.

Parameter Estimated	Pathogenic Treatment									
	Phoma		Cercospora		Mixture		Phoma-Cercospora		Cercospora-Phoma	
	I	II	I	II	I	II	I	II	I	II
					P_1 in array					
D_I	13.3862	.9164	7.3388	.3630	4.6952	2.8344	5.7660	2.1558	7.9244	.7098
H_I	35.3068	9.4648	23.4352	1.8492	22.0240	5.7408	41.5992	9.5340	14.6504	6.0388
H_{II}	7.4256	5.7936	8.6896	1.3824	13.8976	2.1136	17.6448	4.2416	12.5424	3.1392
H_{III}	28.0064	6.3344	34.6464	.4496	22.6752	3.8848	46.6688	8.3696	22.6432	4.8144
H_{IV}	1.0040	1.5408	14.8000	.0128	12.2368	.0792	16.9536	2.7264	12.6016	.3680
F_I	53.6600	3.8320	21.6192	1.1352	14.8144	9.0784	31.1408	7.4808	15.3520	3.6824
F_{II}	51.1472	4.5568	40.4096	.9472	19.9200	7.5664	41.2832	8.5120	30.6464	4.4192
h/d	1.1646	2.4505	1.6150	1.6015	1.9101	1.0133	2.2533	1.6863	1.3113	2.1244
Loci	1.2120	.3781	.0974	.7234	1.3175	.7752	.8709	.4621	.9879	.0413
					P_2 in array					
H_I	37.7068	9.0124	21.0048	1.9836	18.3104	6.5712	37.0008	8.8118	14.7176	5.4900
H_{II}	9.0864	5.9136	7.9280	1.4400	9.8192	2.9328	14.8848	3.7728	12.1712	2.7248
h/d	1.2025	2.4030	1.5629	1.6583	1.8036	1.0832	2.1630	1.6364	1.3151	2.0314
Loci	.9904	.3704	.1068	.6944	1.8309	.5586	1.0324	.5195	1.0180	.0476

Table 38. Estimates of genetic parameters obtained from the combined analysis of the diploid alfalfa diallel Set I.

Parameter Estimated	Pathogenic Treatment				
	Phoma	Cercospora	Mixture	Phoma - Cer.	Cer. - Phoma
			P_1 in arrays		
D_I	3.3208	1.6426	1.3622	.1750	1.3288
H_I	15.8784	6.1572	6.7340	12.2812	3.6048
H_{II}	5.0144	3.2032	5.2336	7.5280	5.0688
H_{III}	11.9024	7.8512	3.6736	9.1632	4.0320
H_{IV}	.7904	3.5192	2.5008	3.7208	3.1992
F_I	16.7696	4.3880	3.4040	3.9112	.8784
F_{II}	15.3184	8.6384	1.2032	3.3488	4.5680
h/d	1.5842	1.7162	1.8411	6.7615	1.6001
Loci	1.0734	.3122	2.0058	.9541	.6973
			P_2 in arrays		
H_I	16.1248	5.6340	6.0508	10.5644	3.6288
H_{II}	5.6128	3.1440	4.5328	5.6816	5.0816
h/d	1.5959	1.6692	1.7717	6.3888	1.6029
Loci	.9590	.3181	2.3159	1.2642	.6955

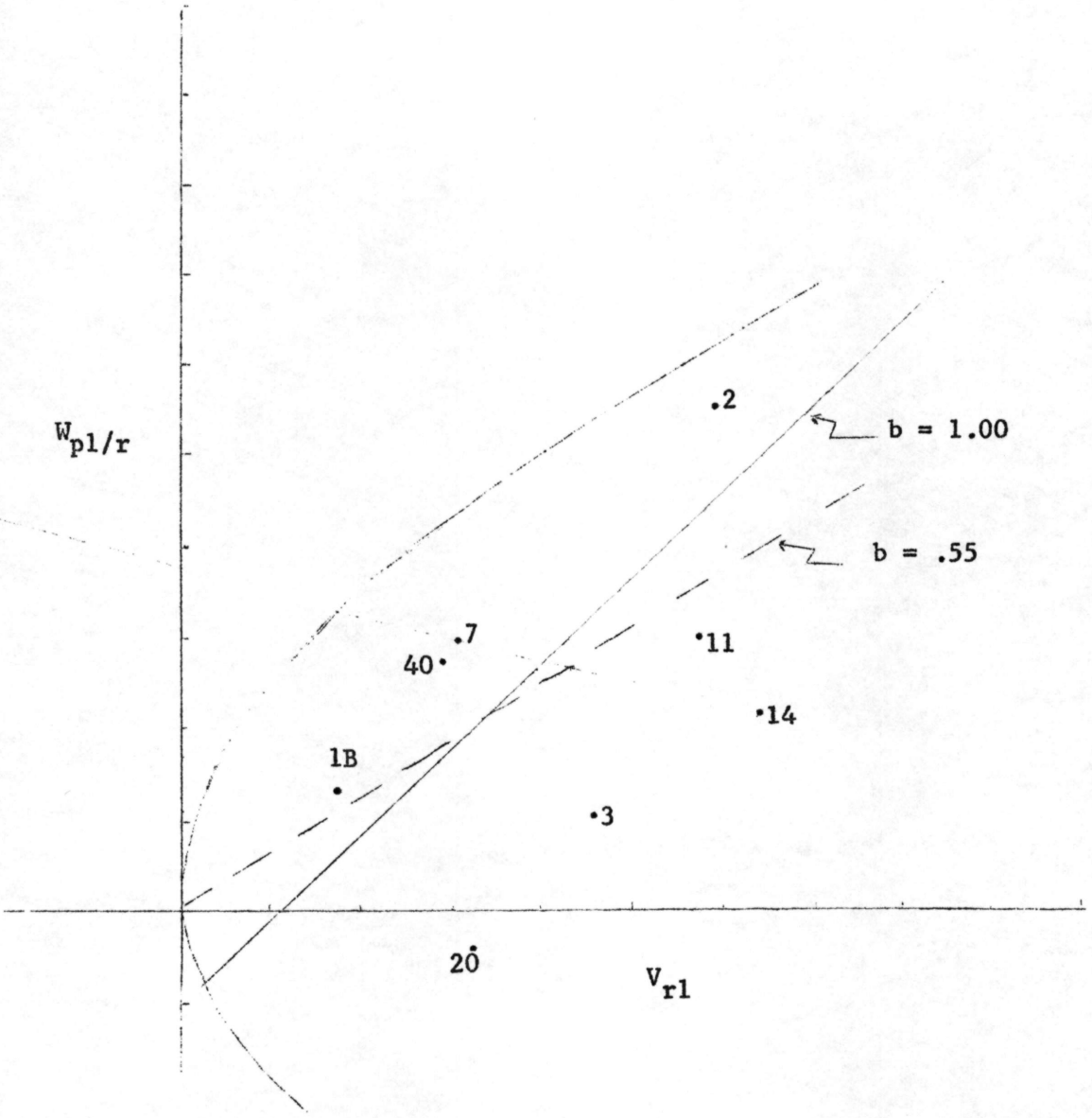

Figure 4. The variances of the arrays (V_r) and the covariances of the family means of the arrays and the parent clones ($W_{p1/r}$) for the reaction of diploid alfalfa diallel Set I to P. herbarum when P_1 is included in the arrays.

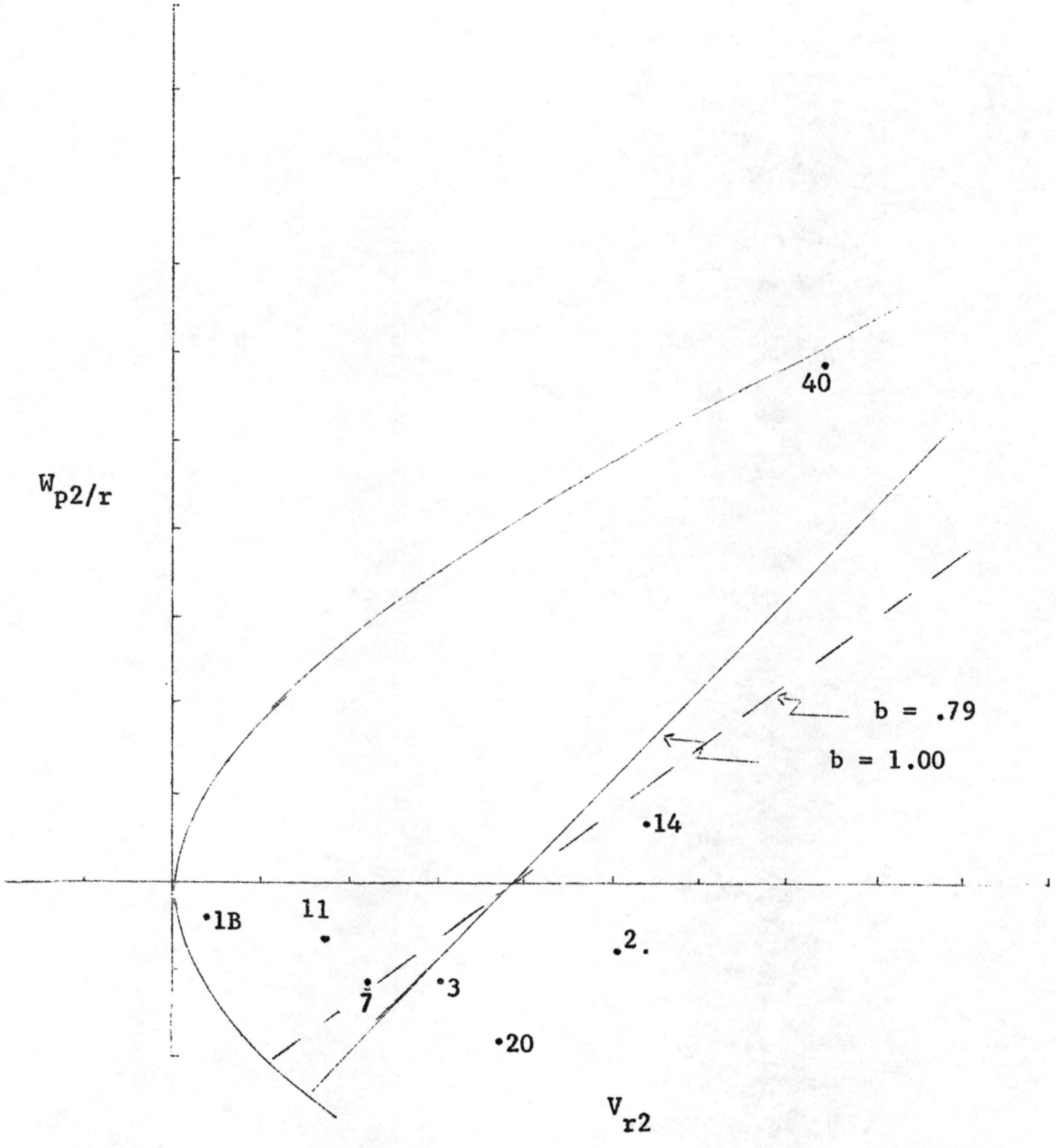

Figure 5. The variances of the arrays (V_r) and the covariances of the family means of the arrays and the selfed family means ($W_{p2/r}$) for the reaction of diploid alfalfa diallel Set I to P. herbarum when P_2 is included in the arrays.

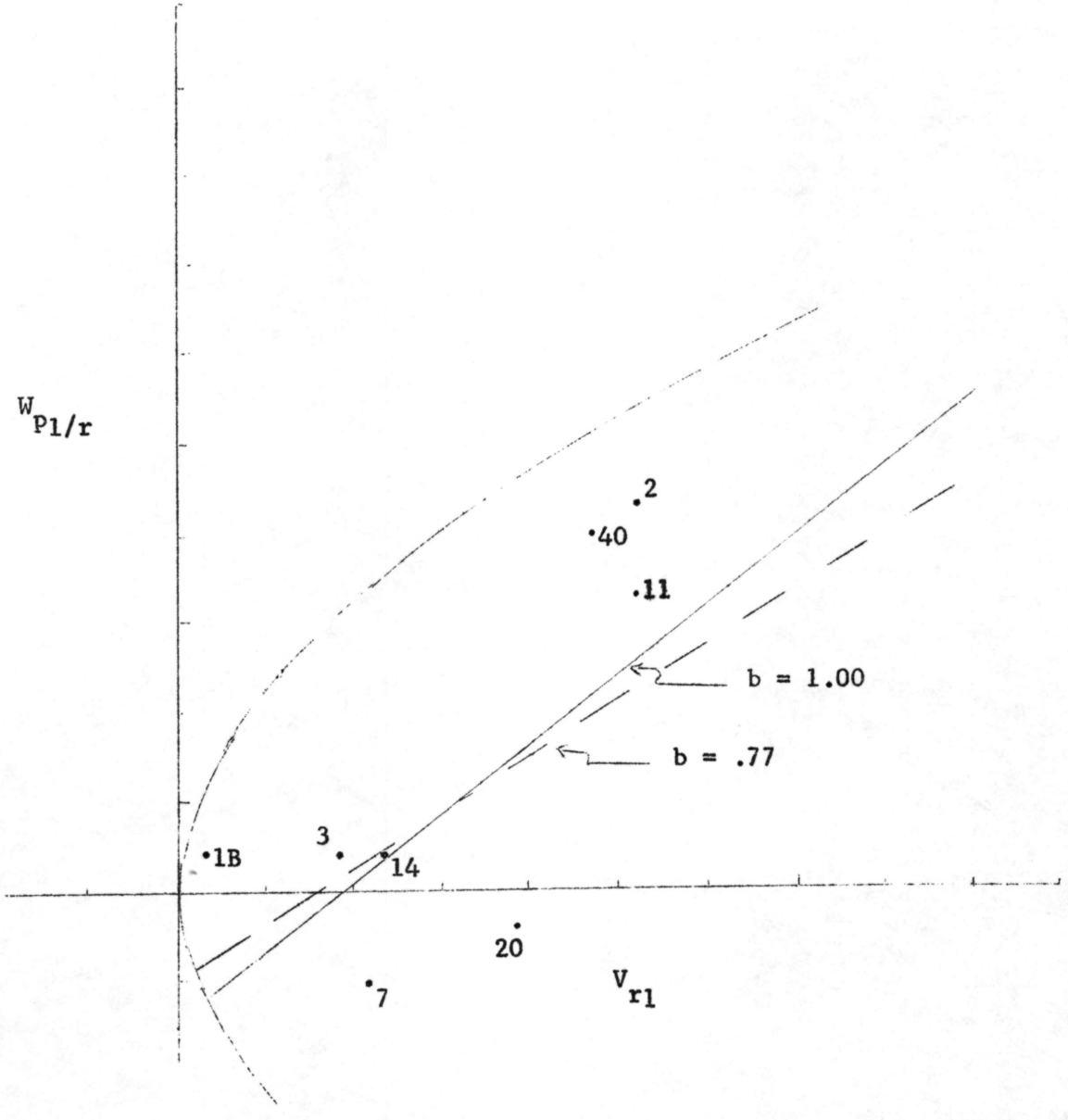

Figure 6. The variances of the arrays (V_r) and the covariances of the family means of the arrays and the parent clones ($W_{p1/r}$) for the reaction of diploid alfalfa diallel Set I to C. zebrina when P_1 is included in the arrays.

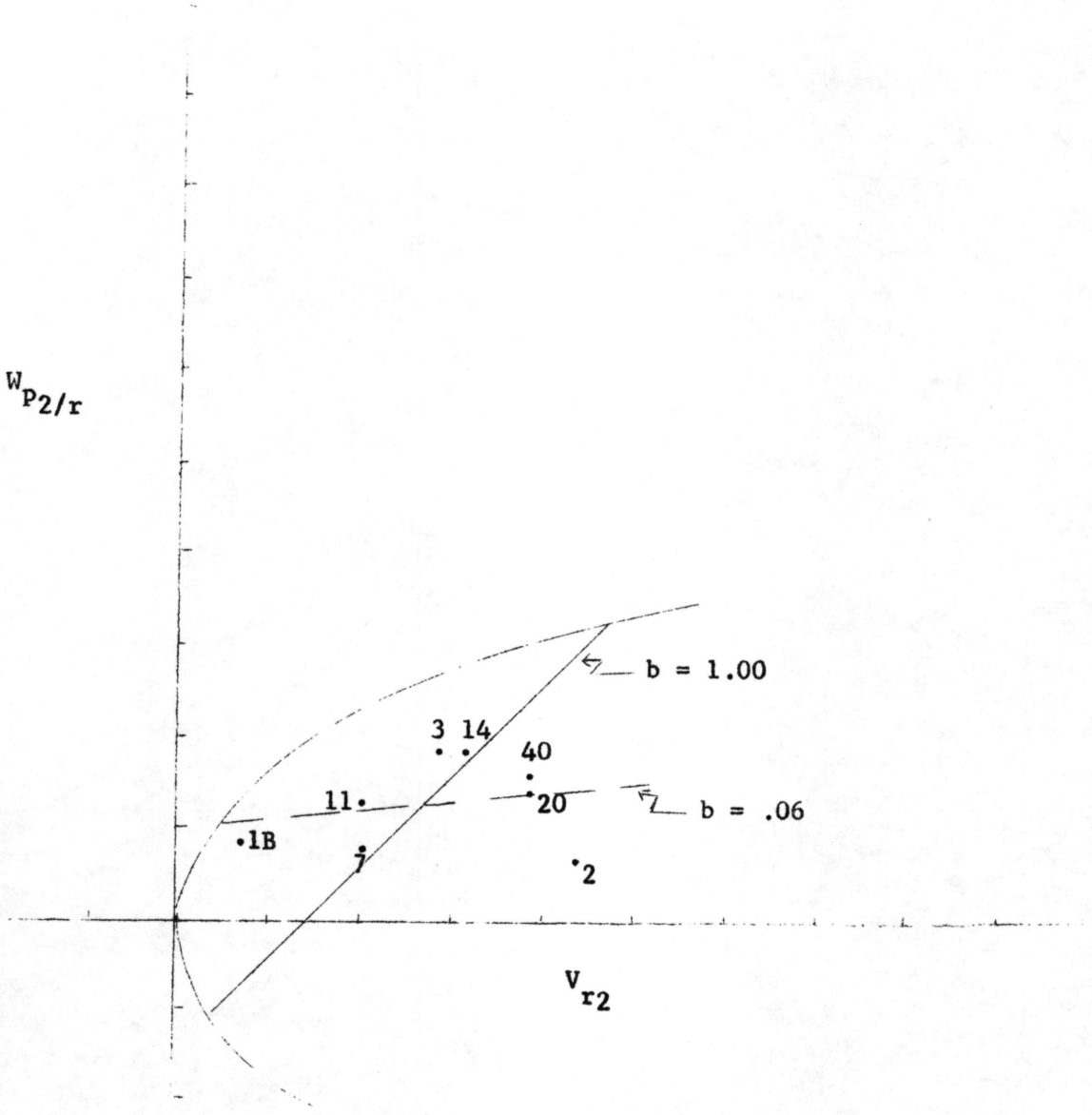

Figure 7. The variances of the arrays (V_r) and the covariances of the family means of the arrays and the selfed family means ($W_{p2/r}$) for the reaction of diploid alfalfa diallel Set I to C. zebrina when P_2 is included in the arrays.

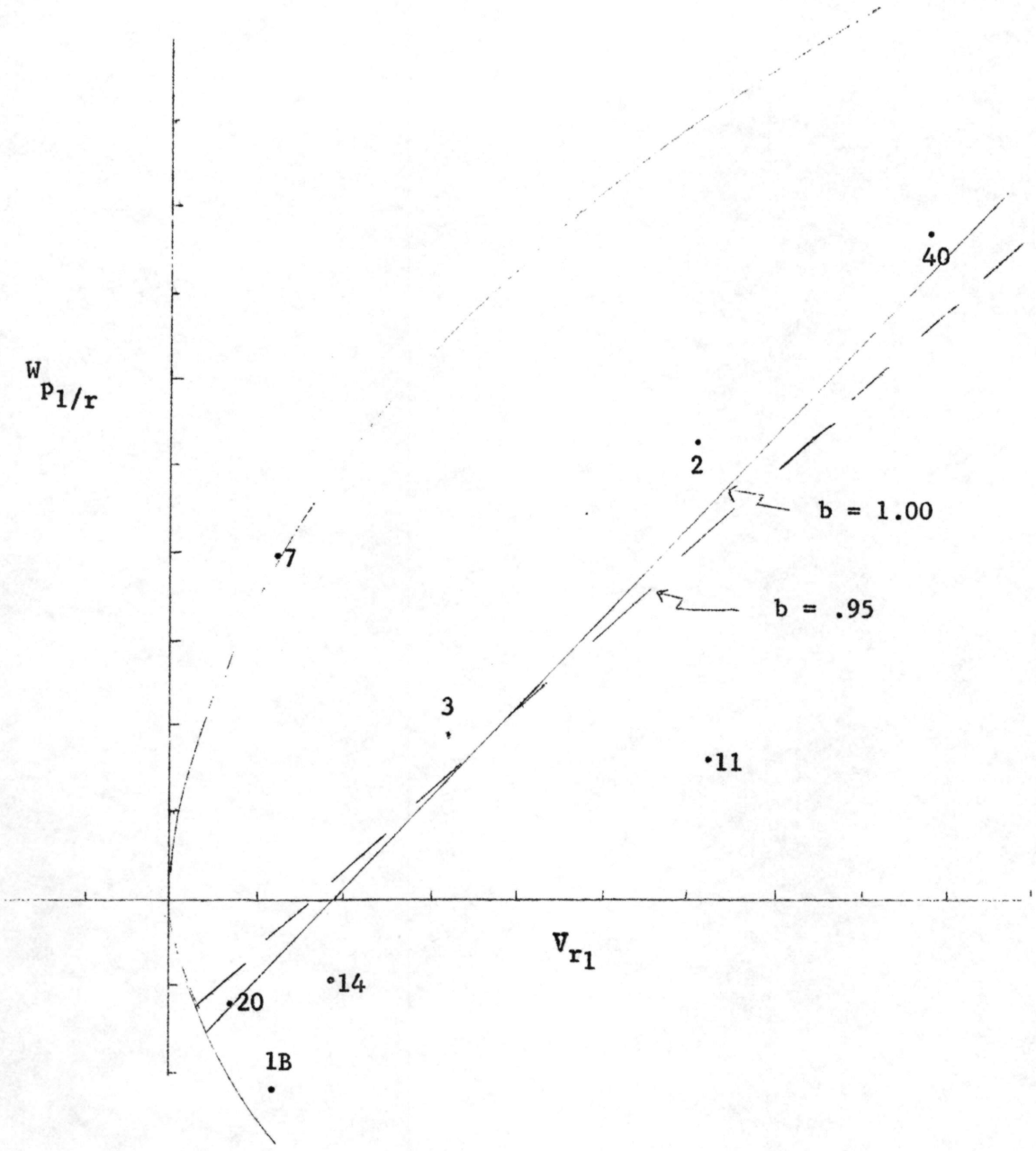

Figure 8. The variances of the arrays (V_r) and the covariances of the family means of the arrays and the parent clones ($W_{P1/r}$) for the reaction of diploid alfalfa diallel Set I to a mixture of inoculum of P. herbarum and C. zebrina when P_1 is included in the arrays.

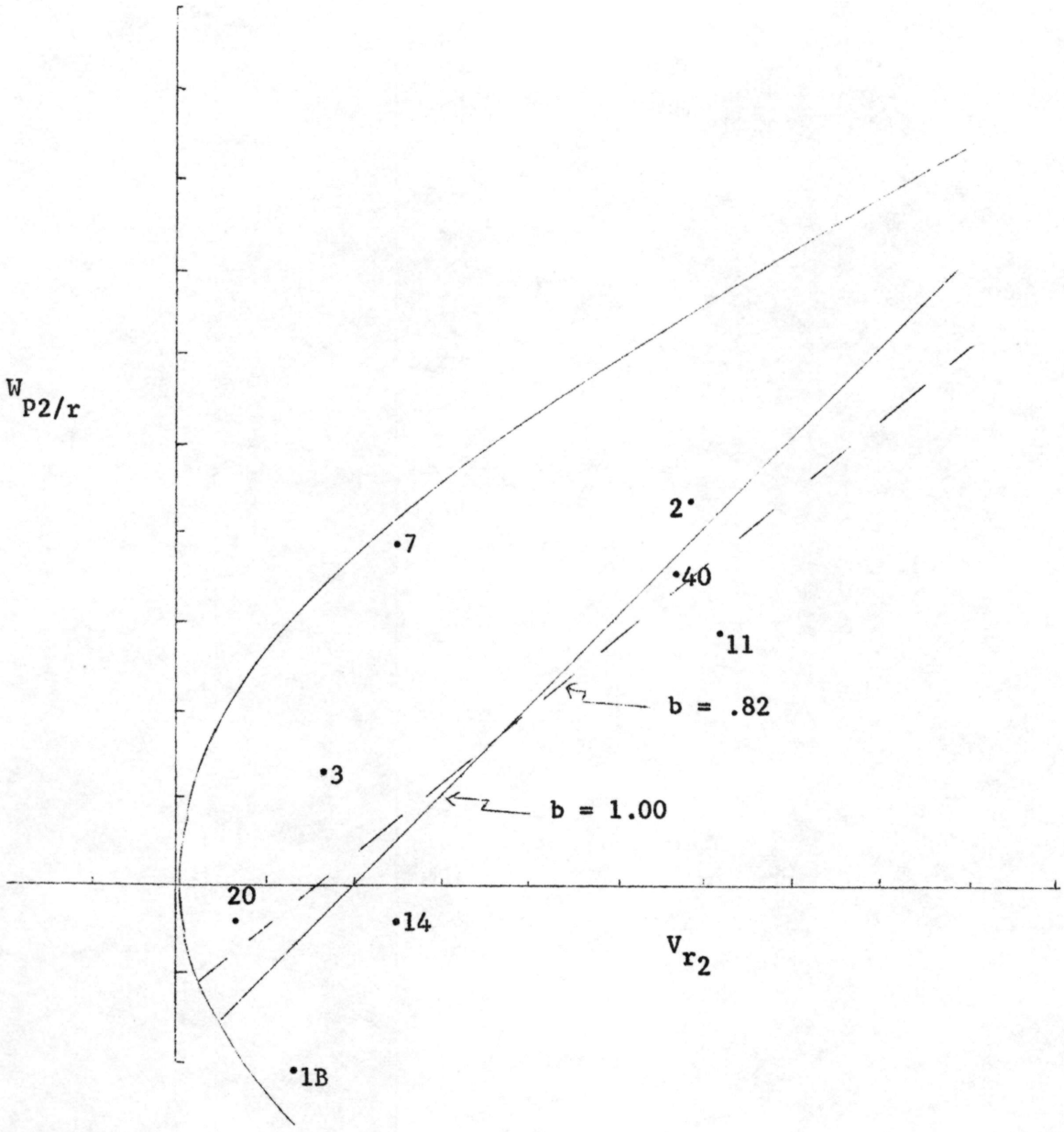

Figure 9. The variances of the arrays (V_r) and the covariances of the family means of the arrays and the selfed family means ($W_{p2/r}$) for the reaction of diploid alfalfa diallel Set I to a mixture of inoculum of P. herbarum and C. zebrina when P_2 is included in the arrays.

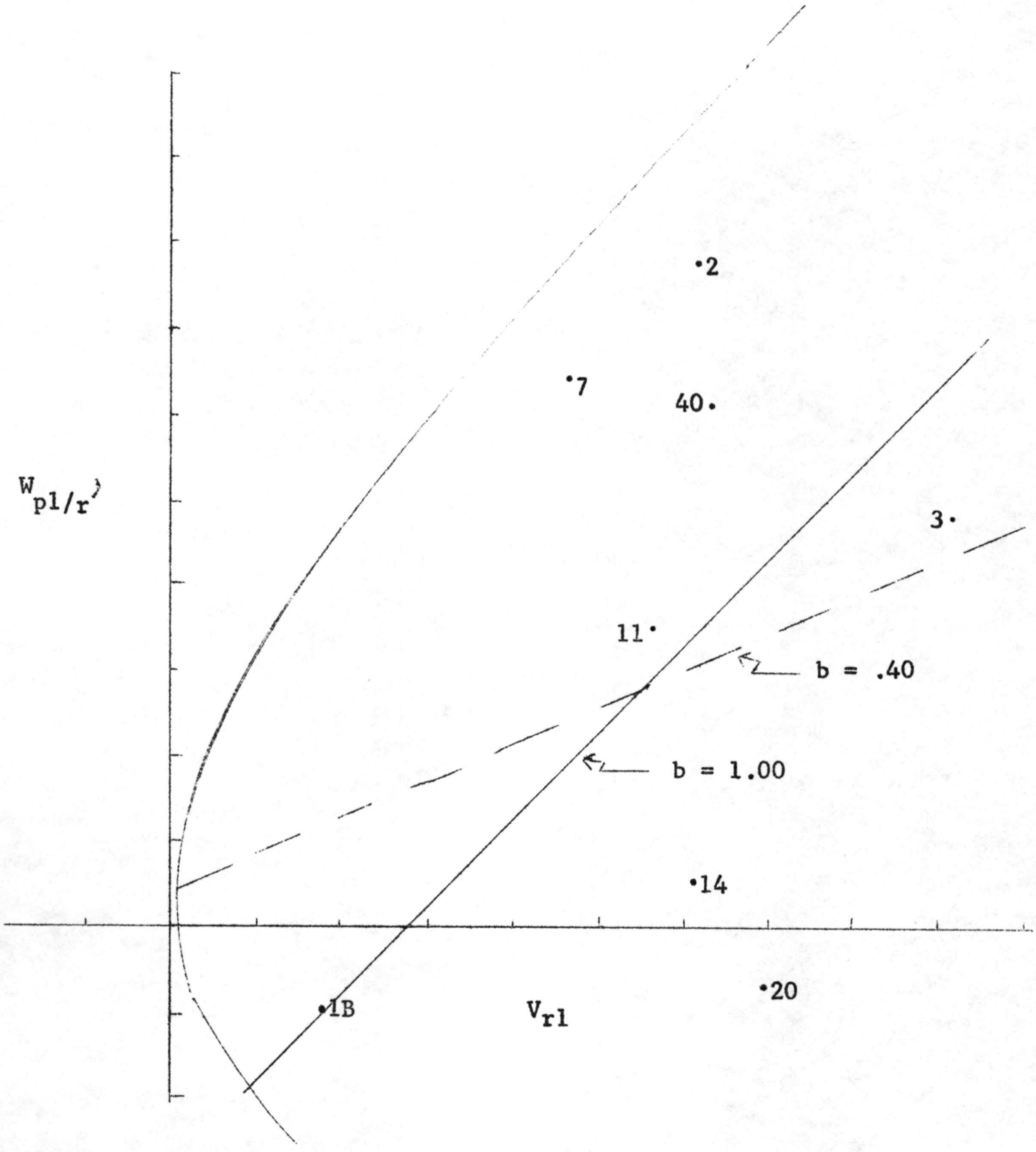

Figure 10. The variances of the arrays (V_r) and the covariances of the family means of the arrays and the parent clones ($W_{p1/r}$) for the reaction of diploid alfalfa diallel Set I to inoculum of P. herbarum succeeded after 48 hours by inoculum of C. zebrina when P_1 is included in the arrays.

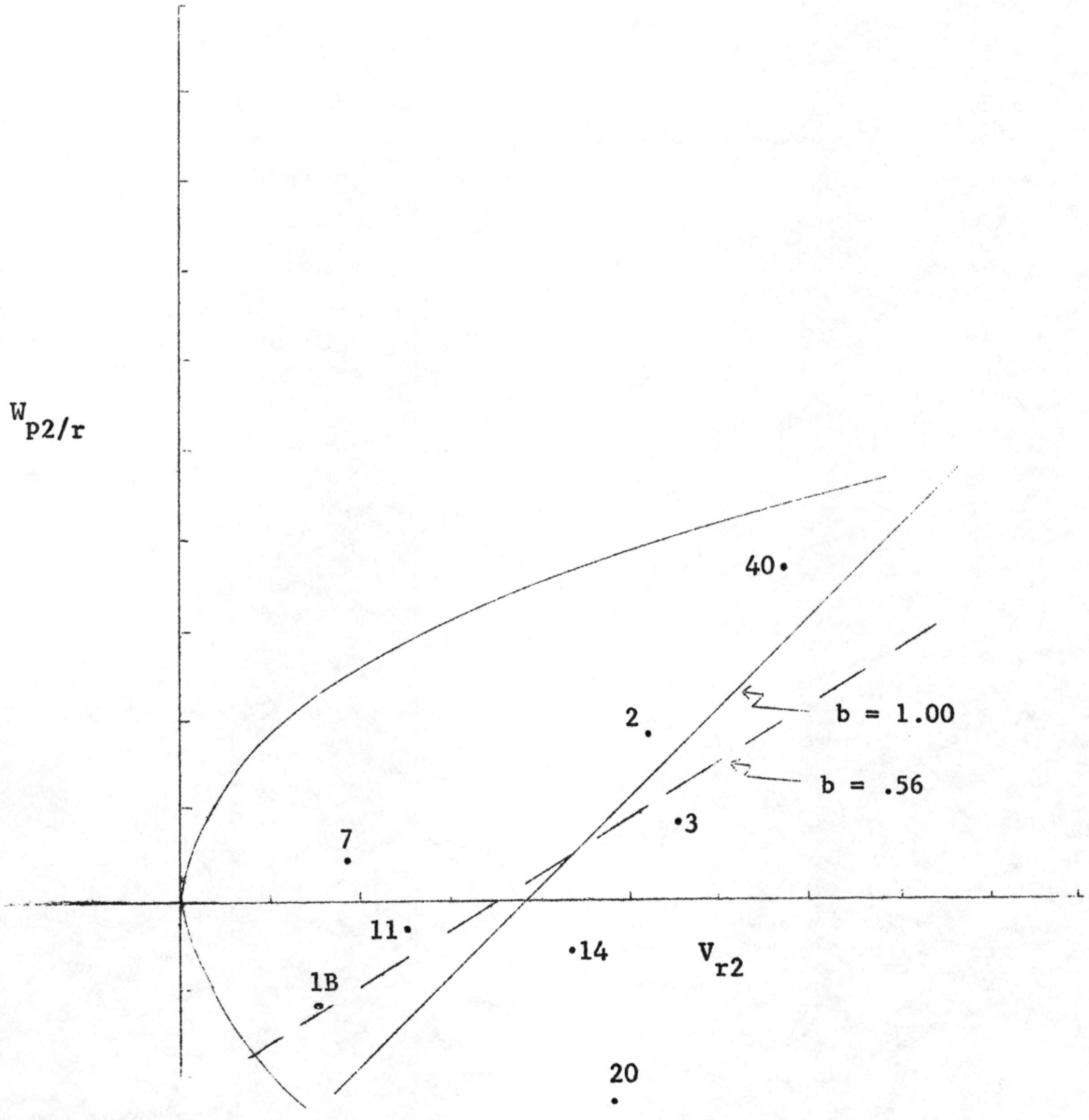

Figure 11. The variances of the arrays (V_r) and the covariance of the family means of the arrays and the selfed family means ($W_{p2/r}$) for the reaction of diploid alfalfa diallel Set I to inoculum of P. herbarum succeeded after 48 hours by inoculum of C. zebrina when P_2 is included in the arrays.

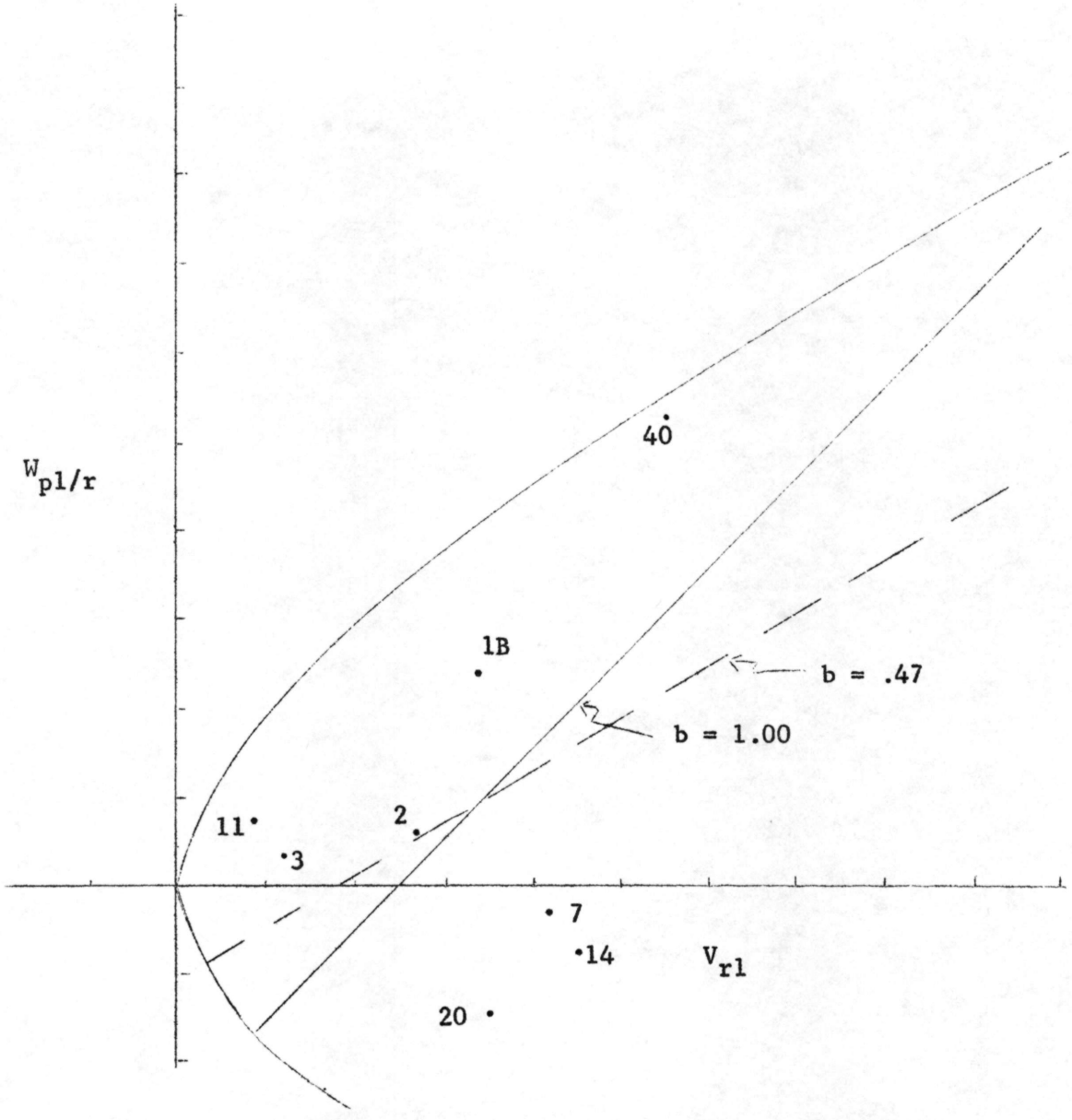

Figure 12. The variances of the arrays (V_r) and the covariances of the family means of the arrays and the parent clones ($W_{pl/r}$) for the reaction of diploid alfalfa diallel Set I to inoculum of C. zebrina succeeded after 48 hours by inoculum of P. herbarum when P_1 is included in the arrays.

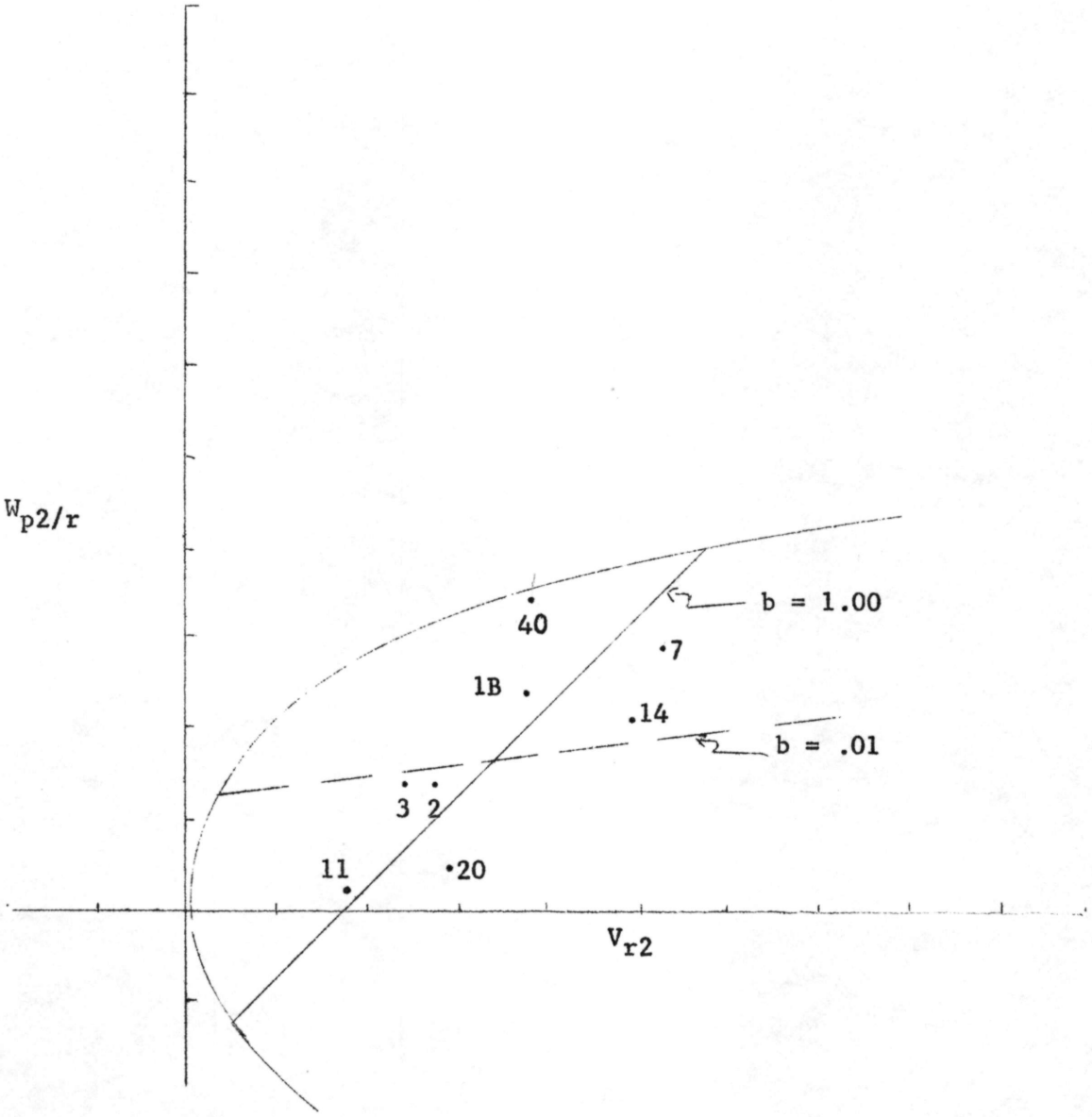

Figure 13. The variances of the arrays (V_r) and the covariance of the family means of the arrays and the selfed family means ($W_{p2/r}$) for the reaction of diploid alfalfa diallel Set I to inoculum C. zebrina succeeded after 48 hours by inoculum of P. herbarum when P_2 is included in the arrays.

Removal of those parents and progenies which exhibit epistasis in the intact diallel has been done by some authors to provide dominance estimates in the absence of non-allelic interaction. In the diallel for reaction to P. herbarum (Figure 4) it was necessary to remove the four parents situated below the regression line in order to obtain an h/d ratio equivalent to partial dominance. The ratio for the four remaining parents was estimated to be .8383. At the same time, the estimate of number of loci exhibiting dominance increased to 127. Although estimates of this sort may have theoretical interest because they reveal spurious overdominance, reduction of the diallel may preclude extension of the results to naturally occuring populations of alfalfa.

The dominance order of the parents of the arrays may be established from the variance - covariance figures. The most dominant parent is situated closest to the intersection of the regression line and the lower curvature of the limiting parabola. Similarly, the most recessive parent is situated closest to the intersection of the regression line and the upper curvature of the parabola. Plants 2 and 40 were found to be more recessive than the other six clones. Also, parent 7 was situated most often in a position which indicated that it might be the most heterozygous clone of the eight used to form the diallel. Rank correlation coefficients for the order of dominance of the parents for the five pathogenic treatments are shown in table 29. Fair agreement in the rankings was obtained with the exception of the Cercospora - Phoma treatment.

Within trial rank correlations were calculated for various combinations of the following measurements: (1) parental score, (2) array mean score, (3) s_1 mean score, and order of dominance on the (4) V_{r1} and (5) V_{r_2} graphs.

Table 39. Rank correlation coefficients for the order of dominance of parents of the diploid alfalfa diallel Set I inoculated with the indicated pathogenic treatments.

		Pathogenic Treatment				
		Phoma	Cercospora	Mixture	phoma-Cer.	Cer.-Phoma
				V_{r_1} Graph		
	Phoma	---	.5000	.4544	.4524	-.5714
V_{r_2} Graph	Cercospora	.7143*	---	.6429*	.5243	-.2619
	Mixture	.3095	.3571	---	.6667 *	.0000
	Phoma-Cer.	.8571**	.8095*	.4286	---	-.4524
	Cer.-Phoma	.2381	-.1667	-.3095	-.0476	---

These values are shown in tables 40 - 44. With the exception of trial 1 for inoculation with C. zebrina, resistance is positively associated with dominance. The average magnitude of the coefficients are presented in table 45. A major point of interest in these data is that the mean reaction of the selfed progeny of a clone provides a better measure of prepotency of the clone in a population than does the parental score.

Most of the data which have been presented indicate an association between the reactions of the host population to P. herbarum and C. zebrina. Phenotypic correlations based upon the mean scores for the 72 families tested are included in table 46. Although none of the coefficients approach 1, they are all positive and significant. The genetic correlation between response to Phoma and response to Cercospora was estimated as:

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

where $C_{f_p f_c}$ is the between families mean product component from the analysis of covariance and V_{f_p} and V_{f_c} are the between families mean square components from the analysis of variance. A coefficient of .8839 was obtained.

Pleiotropy or linkage of genes conditioning host responses is therefore indicated. In view of the similarities in magnitude of the genetic parameters estimated and the point positions on the variance - covariance graphs, it is believed that pleiotropy at one or more loci is probable.

Table 40. Within trial rank correlation coefficients (r_s) for diploid alfalfa diallel Set I when inoculated with P. herbarum. (1=most resistant or most dominant)

		Trial 1				
		Parent Score	Array Mean Score	S_1 Score	Vr_1 Dominance	Vr_2 Dominance
Trial 1	Parent Score	---	.1190	.3333	.5476	.7381*
	Array Mean Score	.4524	---	.3095	.2143	-.0714
	S_1 Score	.6429*	.6429*	---	.0952	.0952
	V_{r_1} Dominance	.0000	.0476	.3333	---	.6310
	V_{r_2} Dominance	.0238	.1429	.3333	.8571**	---

* P less than .10
** P less than .02

Table 41. Within trial rank correlation coefficients (r_s) for diploid alfalfa diallel Set I when inoculated with C. zebrina. (1=most resistant or most dominant)

		Trial 1				
		Parent Score	Array Mean Score	S_1 Score	V_{r_1} Dominance	Vr_2 Dominance
Trial 1	Parent Score	---	.1905	.1905	.1429	.3571
	Array Mean Score	.2143	---	.7619*	-.5238	-.5238
	S_1 Score	.7857*	.5714	---	-.4524	-.5000
	V_{r_1} Dominance	.6667*	.6667*	.7143 *	---	.9524**
	V_{r_2} Dominance	.6071	.4762	.5476	.8810**	---

* P less than .10
** P less than .02

Table 42. Within trial rank correlation coefficients (r_s) for diploid alfalfa diallel Set I when inoculated with a mixture of P. herbarum and C. zebrina. (1=most resistant or most dominant)

		Trial 1				
		Parent Score	Array Mean Score	S_1 Score	Vr_1 Dominance	Vr_2 Dominance
Trial 1	:Parent Score	---	.3095	.6667 *	.3333	.1905
	:Array Mean Score	.6905*	---	.7857*	.3571	.3333
	:S_1 Score	.7619*	.7381*	---	.4524	.3810
	:V_{r_1} Dominance	.7857*	.7381*	.5238	---	.9762**
	:V_{r_2} Dominance	.7143*	.9286**	.7381*	.8810**	---

* P less than .10
** P less than .02

Table 43. Within trial rank correlation coefficients (r_s) for diploid alfalfa diallel Set I when inoculated with Phoma - Cercospora. (1=most resistant or most dominant)

		Trial 1				
		Parent Score	Array Mean Score	S_1 Score	Vr_1 Dominance	Vr_2 Dominance
Trial 1	:Parent Score	---	.4762	.3571	.1905	.1190
	:Array Mean Score	.2616	---	.4048	.0952	.0238
	:S_1 Score	.4048	.3571	---	.9048**	.7857*
	:V_{r_1} Dominance	-.0714	.5476	.4048	---	.9524**
	:V_{r_2} Dominance	-.0714	.5476	.4048	1.0000**	---

*P less than .10
**P less than .02

Table 44. Within trial rank correlation coefficients (r_s) for diploid alfalfa diallel Set I when inoculated with Cercospora - Phoma.

(1=most resistant or most dominant)

		Trial 1				
		Parent Score	Array Mean Score	S_1 Score	Vr_1 Dominance	Vr_2 Dominance
Trial 1	Parent Score	---	.3571	.5952	.6905*	.2381
	Array Mean Score	.2857	---	.6190	.6905*	-.1905
	S_1 Score	.4524	.6429*	---	.3810	-.2143
	Vr_1 Dominance	-.1429	.6667*	.1429	---	.4048
	Vr_2 Dominance	-.0952	.6667*	.2857	.8810**	---

Table 45. Rank correlation coefficients averaged over trials and treatments and the per cent of correlations showing significance in Set I.
(1 = most resistant or most dominant)

Traits Correlated		Average Correlation (r_s)	Per cent of Correlations Significant (%)
Parent Score	- Array Mean Score	.3357	10
	- S_1 Score	.4619	40
	- V_{r_1} Dominance	.3143	30
	- V_{r_2} Dominance	.2821	20
Array Mean Score	- S_1 Score	.5833	50
	- V_{r_1} Dominance	.3500	40
	- V_{r_2} Dominance	.2333	20
S_1 Score	- V_{r_1} Dominance	.3500	20
	- V_{r_2} Dominance	.2857	20
V_{r_1} Dominance	- V_{r_2} Dominance	.8417	80

Table 46. Phenotypic correlations for the responses of 72 families of diploid alfalfa to five pathogenic treatments.

	Cercospora	Mixture	Phoma - Cercospora	Cercospora -Phoma
Phoma	.482**	.593**	.674**	.632**
Cercospora		.340**	.357**	.447**
Mixture			.602**	.576**
Phoma-Cercospora				.635**

** P less than .01

Estimates of heritability in the broad sense (H) for mean score of a parental clone were obtained from analysis of variance.

$$H = \frac{V_p}{V_p + \frac{V_e}{r}}$$

Where V_p is the mean square variance component for between parental clones,

V_e is the mean square for replications x parental clones, and

r is the number of replications.

Values of H for the five treatments in each trial are shown in table 47. These estimates are based upon the total genetic variance and may be considered to establish a ceiling for estimates of heritability in the narrow sense.

The analysis of variance for the data within one trial may be described as follows:

Source of Variation	D.F.	Mean Square Expectation
Replications	r-1	
Between males	p-1	$V_e + rV_f + rpV_m$
Females in Males	p(p-1)	$V_e + rV_f$
Pooled error	(r-1)(p-1)p	V_e

If it is then assumed that gene frequency equals .5, the mean square compo-

nents have the following genetic interpretations:

$V_m = \frac{1}{4}$ the additive genetic variance,

$V_f = \frac{1}{4}$ the total genetic variance, and

$$h^2 = \frac{4V_m}{4V_f + \frac{V_e}{r}}$$

Estimates of heritability in the narrow sense were computed and are included in table 48. With one exception, the value of 7.7285 for Cercospora - Phoma trial 2, the estimates are approximately of the general order of magnitude usually encountered for quantitative data. It should be realized, however, that gene frequency may not be .5 in the diallel population tested. Indeed, for equal allele frequencies it is expected that $H_I = H_{II}$, $H_{III} = H_{IV}$, and $F_I = F_{II} = 0$. This was not true in this series of experiments.(table 37).

The fact that dominance was not unidirectional and that epistasis was present in all treatments of the diallel necessitates certain reservations as to the accuracy of the estimates of the genetic parameters cited in this report. These estimates may be accepted as the best available approximations of the parameters until the difficulties caused by the failure of the biological population to fit the assumptions underlying the analytical procedures are resolved.

Table 47. Estimates of heritability in the broad sense (H) for the reaction of the parent clones of diploid alfalfa diallel Set I to five pathogenic treatments.

Treatment	H	
	Trial I	Trial II
P. Herbarum	.4775	.6973
C. Zebrina	.5504	-.3045
Mixture	.8497	.7357
Phoma - Cercospora	.9120	.6157
Cercospora - Phoma	.7176	.1928

Tables 48. Estimates of heritability in the narrow sense (h^2) for the mean reactions of progenies in diploid alfalfa diallel Set I to five pathogenic treatments.

Treatment	h^2	
	Trial I	Trial II
P. herbarum	.0617	.5117
C. zebrina	.4273	.2167
Mixture	.0177	.1375
Phoma - Cercospora	.3587	.6324
Cercospora - Phoma	.3973	7.7285*

* Unrealistic estimate due to negative variance component for females in males.

3. Summary

An analysis of the reactions of a 8 x 8 set of diallel crosses among diploid alfalfa clones revealed the following:

(a) heterosis for both resistance and susceptibility to Phoma herbarum var. medicaginis and Cercospora zebrina occured in this diploid alfalfa population,

(b) dominance for reaction to the two pathogenes was not unidirectioal,

(c) epistasis was involved in the genetic control of reaction to the two pathogenic species in some arrays of the hosts,

(d) at least two loci exhibited dominance, and

(e) dominance ratios in the overdominant range were obtained. These ratios may have been inflated by the effects cited in (b) and (c) above.

(f) resistance to Phoma and Cercospora were genetically correlated.

V. Future Research

Several years of research into the nature of the genetic determination of the reaction of diploid alfalfa to the inciting pathogens of blackstem have defined the complexity of the problem. A simple uncomplicated factorial gene analysis has not been achieved and perhaps is not possible. The approach which has been used has been beset with difficulties in obtaining and maintaining the desired populations of the host genotypes, in maintaining the cultures of the pathogens, and in uniformly reproducing the desired test environments.

However, information has been derived with respect to heritability, number of loci, level of dominance, direction of dominance, epistatic relationships, and association of resistance to the two pathogens. The results to date indicate certain lines of research which may now be fruitful.

(1) The nature of resistance. If resistance can be further defined in terms of host morphology or physiology, genetic analysis of these elements may be far simpler than that of a gross score for foliage reaction.

(2) Intensive genetic analysis. A few crosses which have exhibited a wide range of gene effects should be selected for intensive analysis. This could be accomplished by obtaining additional generations and applying a partitioning technique to obtain a factorial interpretation of the data.

(3) Variability of the pathogens. The variability of the pathogens needs further study.