A GENE-FOR-GENE RELATIONSHIP BETWEEN ALFALFA AND *PERONOSPORA TRIFOLIORUM*

by

DANIEL ZOLEK SKINNER

B. A., St. Cloud State University, 1978

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Plant Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1983

Approved by:

Donald L. Stuteville
Major Professor

TABLE OF CONTENTS

Page

Tables

Figures

Reports have differed on the inheritance of resistance in alfalfa (Medicago sativa L.) to Peronospora trifoliorum d By., causal fungus of alfalfa downy mildew. Probably based mostly on work reported by Jones and Torrie (6), Jones and Smith (7) stated that susceptibility behaved approximately as a dominant character. However, Pedersen and Barnes (16) indicated that resistance was conditioned by one tetrasomically inherited gene with incomplete dominance. Backcross and self-pollination data obtained by Stanford (18) during development of 'Caliverde' also suggested a degree of dominance for resistance.

Since pathogenically different isolates of the fungus have been identified (19), I investigated the possibility that the discrepancies in the inheritance of mildew resistance reported may, in part, stem from host-parasite interactions inherited differently. As a result, in this paper I propose that alfalfa and P. trifoliorum share a gene-for-gene relationship (3,10) involving genes with a major effect and a polygene-for-polygene relationship (15) involving genes with small, additive effects.

MATERIALS AND METHODS

Monoconidial isolates of Peronospora trifoliorum were used. Isolates I5 and I7 were from alfalfa plants collected from fields in Kansas. I8 was isolated from an alfalfa plant sent from El Centro, California, by W. F. Lehman, Imperial Valley Field Station, El Centro, California 92243.

To derive monoconidial cultures a dilute aqueous conidial suspension was sprayed onto water agar in petri dishes. With the aid of a dissecting microscope and a small spatula, a block of agar 2-3 mm^2 was cut around an isolated conidium, removed and inverted onto a cotyledon

of a highly susceptible 4-day-old alfalfa seedling. About 10 plants (one per 2.5 cm^2 pot) were inoculated. Each pot was enclosed in a small plastic bag and placed in dark growth chamber at 20 C. After a 24-hr infection period, the bag was removed and 7,300 lux of continuous fluorescent lighting was provided. Five days later each pot was again enclosed in a plastic bag and placed in dark for 16 hr to permit conidium production. Conidia thus produced were used to repeat the process 11 times with I5 and I7, and once with I8 prior to this study.

Sources of plants. Diploid plants P1, P2 and P3 were grown from Medicago sativa seed lots PI172984, PI206286, and PI172983, respectively. P4 and P5 were grown from seed lot PI172989. Seed was supplied by W. H. Skrdla, Plant Introduction Station, Ames, IA 50010. P5 produced variegated flowers and sickle-shaped seed pods indicating some Medicago falcata ancestry.

P6 was a yellow-flowered diploid plant grown from seed lot Wis 72-23 (about 50% M. falcata background) supplied by E. T. Bingham, Department of Agronomy, University of Wisconsin, Madison 53706.

Pollination. Plants were kept either in growth chambers or a greenhouse. They were selfed and/or crossed with toothpicks (1). Plants crossed were emasculated with ethanol (21).

Screening of seedlings. To improve uniformity of seedling emergence, each seed was scarified by cutting through the seed coat with a razor blade. Seeds were planted about 8 mm deep in autoclaved masonry sand in 24.5 x 14.0 x 7.0 cm aluminum pans (bread pans). The pans were placed in a growth chamber at 20 C and about 7,300 lux of continuous cool white fluorescent lighting. The sand was sprinkled daily with distilled water to settle it around the emerging seedlings to aid

uniform emergence. Five days after seeding, the plants (at the cotyledonary stage) were inoculated as previously described (4).

Conidium production was induced on the evening of the sixth day following inoculation by placing two pans of plants per darkened 35 x 26 x 16 cm plastic sweater box. Conidium production on the cotyledons was evaluated 15 hr later under 12X magnification.

Interaction (aegricorpus (8)) phenotypes were rated on a zero to five scale where 0 = no conidium production and 5 = very heavy conidium production. Those rated zero were classified as low infection type (LIT) (9). Those rated 1-5 were classified as high infection type (HIT).

Besides conidium production, downy mildew symptoms on the cotyledons included a wide range of chlorosis and chlorotic flecking which was rated on a scale of zero (no chlorosis) to five (severe chlorosis). This rating was similar to the sporulation rating for most plants but being less objective was considered less reliable than sporulation as a measure of disease severity.

Host and pathogen genes and interaction genotypes were named according to the method proposed by Loegering (9) except lr was used to symbolize recessive alleles of Lr genes.

Segregation ratios were tested for goodness of fit to theoretical ratios with chi-square tests including a continuity correction (17).

RESULTS AND DISCUSSION

Infection types produced by interaction of P5 S_1 plants and I5, I7 and I8 gave a good fit for LIT:HIT segregation ratios of 63:1, 255:1 and 3:1 suggesting three, four and one corresponding Lp/Lrlr gene pair(s), respectively (Table 1). Interaction of P6 S_1 plants and I5, I7 and I8

fit LIT:HIT ratios of 15:1, 63:1 and 15:1 indicating two, three, and two corresponding Lp/Lrlr gene pairs, respectively (Table 1). Each gene pair was individually capable of preventing sporulation and each was expressed in the category IV interaction (11).

Reciprocal crosses were completed with all plants. No maternal influence was evident. Infection types produced by interaction of P5 X P6 F_1 plants and I5, I7 and I8 fit LIT:HIT ratios of 31:1, 127:1 and 7:1, respectively (Table 2). This indicated that five dominant heterozygous Lr genes were involved (Table 3).

Only HITs were produced by interaction of P1 S_1 plants and I5, I7 and I8 indicating no corresponding Lp/Lr gene pairs were involved.

Interactions of F_1 plants of P1 X P6 and I5, I7 and I8 segregated in accordance with the expected LIT:HIT ratios of 3:1, 7:1 and 3:1, respectively. This supported the presence of three dominant Lr genes in P6 and confirmed their absence in P1.

Interaction of F_1 plants of P1 X P5 and I5 or I7 did not segregate in accordance with expected ratios (Table 2). This indicated P5 alleles conditioning reaction to I5 and/or I7 were not completely dominant to P1 alleles. The infection type expressed in the category IV interaction (8-11) was therefore other than zero and LITs were not recorded.

As evidenced by the monogenic segregation ratio seen in the F_1 progeny, the P5 allele conditioning the low reaction response to I8 was completely dominant to P1 alleles (Table 2). Therefore, the Lp_2/Lr_2 interaction was epistatic to all other interactions and thus expressed in the category IV interaction (8-11).

Conidium production by HIT interactions of P5 and P6 S_1 and F_1 populations interacting with any isolate was not consistent, suggesting

TABLE 1. Infection type data obtained by inoculating S_1 populations of six diploid alfalfa plants with three monoconidial *Peronospora trifoliorum* isolates.

Plant	Isolate	No. infection types		Suggested ratio	P value
		Low[a]	High[a]		
P1	I5	0	82	0:1	—
	I7	0	76	0:1	—
	I8	0	117	0:1	—
P2	I5	3	69	1:15	>0.50
	I7	6	166	1:15	>0.10
	I8	1	82	1:63	>0.70
P3	I5	105	0	1:0	—
	I7	29	90	1:3	>0.80
	I8	32	43	7:9	>0.90
P4	I5	42	5	15:1	>0.25
	I7	43	2	15:1	>0.80
	I8	87	10	15:1	>0.10
P5	I5	107	2	63:1	>0.90
	I7	368	2	255:1	>0.90
	I8	44	12	3:1	>0.50
P6	I5	77	2	15:1	>0.20
	I7	128	3	63:1	>0.70
	I8	113	7	15:1	>0.80

[a]Low infection type = no conidia produced; High = conidia produced.

TABLE 2. Infection type data obtained by inoculating F_1 populations of six diploid alfalfa plants with three Peronospora trifoliorum isolates

Cross	Isolate	No. infection types[a] Low	High	Expected ratio[b]	P value
P1 X P2	I5	0	170	0:1	—
	I7	21	371	0:1	—
	I8	3	85	0:1	—
P1 X P3	I5	63	26	1:0	—
	I7	0	88	0:1	—
	I8	0	78	1:1	<0.001
P1 X P4	I5	66	99	3:1	<0.001
	I7	103	53	3:1	<0.050
	I8	87	92	3:1	<0.001
P1 X P5	I5	22	35	7:1	<0.001
	I7	59	66	15:1	<0.001
	I8	41	44	1:1	>0.500
P1 X P6	I5	127	28	3:1	>0.050
	I7	130	14	7:1	>0.250
	I8	78	36	3:1	>0.100
P2 X P3	I5	73	21	1:0	—
	I7	6	80	0:1	—

TABLE 2. Continued

Cross	Isolate	No. infection types[a] Low	High	Expected ratio[b]	P value
	I8	0	101	0:1	—
P2 X P4	I5	42	47	13:3	<0.001
	I7	51	42	13:3	<0.001
	I8	58	33	25:7	<0.005
P2 X P5	I5	46	24	7:1	<0.001
	I7	115	37	7:1	<0.001
	I8	78	69	1:1	>0.500
P2 X P6	I5	74	15	3:1	>0.100
	I7	74	11	7:1	>0.500
	I8	54	29	3:1	<0.050
P3 X P4	I5	105	28	1:0	<0.001
	I7	99	36	3:1	>0.500
	I8	69	76	3:1	<0.001
P3 X P5	I5	127	6	1:0	—
	I7	61	13	15:1	<0.001
	I8	40	35	1:1	>0.500
P3 X P6	I5	85	4	1:0	—
	I7	71	16	7:1	>0.050

TABLE 2. Continued.

Cross	Isolate	No. infection types[a] Low	High	Expected ratio[b]	P value
	I8	49	42	7:1	<0.001
P4 X P5	I5	74	10	31:1	<0.001
	I7	79	6	63:1	<0.001
	I8	72	12	7:1	>0.500
P4 X P6	I5	151	7	15:1	>0.250
	I7	160	11	31:1	<0.025
	I8	130	30	15:1	<0.001
P5 X P6	I5	157	8	31:1	>0.200
	I7	297	5	127:1	>0.100
	I8	135	15	7:1	>0.300

[a]Low infection type = no conidia produced; High = condidia produced.

[b]Assuming complete epistasis of suggested dominant Lr genes.

TABLE 3. Proposed genotypes of selected diploid alfalfa plants and *Peronospora trifoliorum* isolates

Proposed genotype					
Plant[a]			Isolate[b]		
P1	P5	P6	I5	I7	I8
lr_1lr_1	Lr_1lr_1	lr_1lr_1	Lp_1	Lp_1	Hp_1
lr_2lr_2	Lr_2lr_2	Lr_2lr_2	Lp_2	Lp_2	Lp_2
lr_3lr_3	lr_3lr_3	Lr_3lr_3	Lp_3	Lp_3	Lp_3
lr_4lr_4	Lr_4lr_4	lr_4lr_4	Lp_4	Lp_4	Hp_4
lr_5lr_5	Lr_5lr_5	Lr_5lr_5	Hp_5	Lp_5	Hp_5

[a] Lr = low reaction allele, lr = high reaction allele.

[b] Lp = low pathogenicity and Hp = high pathogenicity. Capital letters do not indicate dominance.

that P5 and P6 possessed genes capable of influencing these interactions. To investigate the nature of some of these genes, 10 plants, representing the range of infection types formed with I8, were selected from the F_1 population of P5 and P6 and self pollinated. The resulting F_2 populations were inoculated with I8. The mean rating of the HITs in a given F_2 population and the percent of that F_2 population exhibiting HIT upon interaction with I8 were significantly positively correlated ($r = 0.88$, $P > 0.95$, Table 4). This indicated that genes with additive effects conditioned high infection types. Whether some of these genes were Lr genes which had been overcome by Hp genes (Table 3), similarly proposed for the wheat/Erysiphe graminis tritici pathosystem (12,13), or all were additional genes, was not investigated.

Interactions of P2 and P3 S_1 plants and any isolate suggested dominant host genes were involved in HIT except I5/P3 S_1 interactions (Table 1). Interactions of P4 S_1 plants and any isolate suggested recessive host genes were involved in HIT (Table 1). However, segregation in all possible F_1 populations of P1-P4 intercrossed or crossed with P5 or P6 was generally not in accordance with the expected ratios (Table 2). This suggested polygenes were involved.

To investigate the nature of some of these polygenes, F_1 plants of P1 X P2 which had interacted with I7 to yield the range of interaction phenotypes, were self pollinated. The F_2 populations thus produced were inoculated with I7. A significant ($P > 0.95$) positive correlation of the percent of HITs in the individual F_2 populations and the mean rating of the HITs of that population when inoculated with I7 ($r = 0.76$, Table 5) indicated host genes with additive effects conditioned high infection types. A range of 68.8 to 85.7% of the S_1 progeny of plants from LIT

TABLE 4. Reaction to Peronospora trifoliorum isolate I8 by selected P5 X P6 F_1 plants of diploid alfalfa and mean infection types of the $I8/F_2$ HIT interactions

F_1 plant	Reaction[a]	% F_2 plants forming HIT[b]	Mean reaction of F_2 plants forming HIT[b]
1	0	1.8	1.0
2	0	19.4	2.2
3	1	13.8	1.8
4	1	14.4	1.7
5	2	49.9	2.5
6	2	26.4	2.0
7	3	79.3	2.8
8	3	92.8	2.6
9	4	72.1	2.8
10	4	78.4	2.6

[a]0 = no conidium production; 5 = much conidium production.

[b]HIT = high infection type, conidia produced.

TABLE 5. Reaction to Peronospora trifoliorum isolate I7 by selected P1 X P2 F_1 plants of diploid alfalfa and mean infection types of the I7/F_2 HIT interactions

F_1 plant	Reaction[a]	% F_2 plants forming HIT[b]	Mean reaction of F_2 plants forming HIT[b]
1	0	68.8	1.9
2	0	70.2	2.1
3	0	81.4	2.8
4	0	82.6	2.4
5	0	85.7	3.5
6	1	56.8	2.2
7	1	51.5	2.2
8	1	47.2	2.1
9	2	55.2	2.8
10	2	78.6	2.5
11	2	80.0	3.2
12	2	87.8	3.3
13	3	64.3	2.6
14	3	75.7	2.9
15	3	93.2	2.6
16	4	100.0	3.7
17	5	93.6	3.9
18	5	100.0	4.0

[a]0 = no conidium production; 5 = much conidium production.

[b]HIT = high infection type, conidia produced.

interactions (Nos. 1-5, Table 5) formed HIT interactions with I7. This and the entire F_1 population of P1 and P2 inoculated (392 plants), about 15/16 of which formed HIT interactions with I7 (Table 2), indicated that most genes favored conidium production. However, one parent (No. 8, Table 5) was from an HIT interaction yet produced an S_1 population which formed 52.8% LIT interactions with I7. This indicated that some genes conditioning a lower infection type were partially dominant.

To further investigate the nature of the genes involved, infection severity data from all possible crosses and selfs of P1-P6 were analysed as a heterozygous diallel cross with the method devised by Hayman (5) and generalized by Dickinson and Jinks (2). A diallel data table from a six-parent cross may be partitioned into six five-parent tables, 15 four-parent tables, and 20 three-parent tables. Each of these possible data tables was individually analysed for each isolate. The generalized analysis (2) requires data from the parental generation as well as the S_1 generation. The inoculation method I used allowed evaluation of a plant's interaction with only one isolate (20). Therefore, a complete analysis was possible with only I5 infection severity data, the isolate used to inoculate the parents. However, plotting the Vr, Wr graph (5) (V_r, $Wp_{2/r}$ graph (2)) was possible for each isolate and revealed the relative proportion of dominant genes in each parent (2).

In a diallel cross of homozygous parents there are two indicators of mean direction of dominance. These are the sign of the difference of the mean of the parental array subtracted from the mean of their progeny ($M_{L1}-M_{LO}$ (5)) and the sign of the correlation coefficient of the parental S_1 array (Yr) and the order of dominance, as determined by Wr + Vr, if significant (5). Heterozygosity and non-allelic gene interaction

will confound these measurements but they will nevertheless indicate the direction of dominance realized in the crosses.

The generalized analysis (2) of I5 infection severity data of the diallel cross of all six parents indicated the degree of dominance was 0.88. The level of heterozygosity was 0.55 indicating about half of the loci showing dominance were in the heterozygous condition in the parents. An estimate of gene number was less than unity, indicating gene effects were not equal and/or dominance was bidirectional (2).

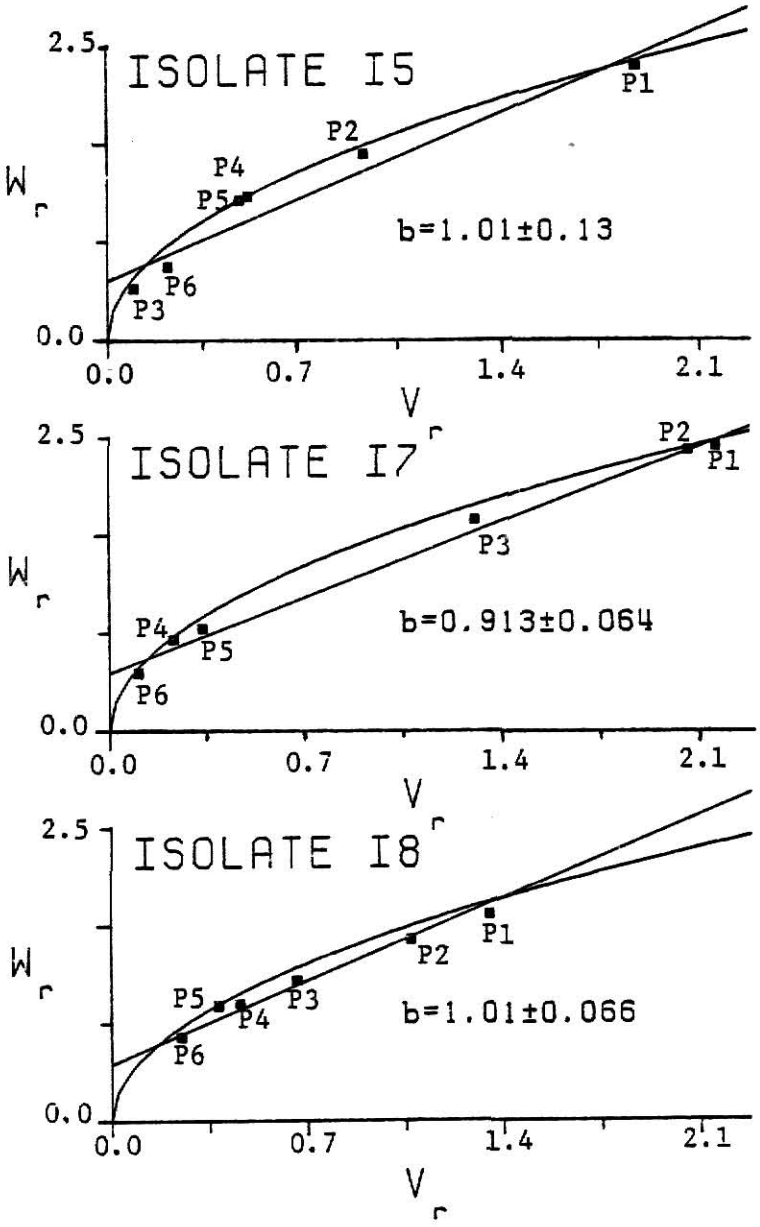
The Vr, Wr plot revealed that P3 had mostly dominant genes, P1 had very few dominant genes, P4-P6 had many dominant genes, while P2 behaved as an intermediate (Fig. 1).

Analysis of the I5 infection severity data table including only P1, P2 and P3 yielded a significant positive correlation of Yr and Wr + Vr ($r = 1.00$) and a negative $M_{L1} - M_{L0}$. Both indicated negative genes (i.e. genes conditioning a lower infection type) were mostly dominant.

Analysis of the I5 infection severity data table including only P4, P5 and P6 yielded a significant negative correlation of Yr and Wr + Vr ($r = -0.998$) and a positive $M_{L1} - M_{L0}$. Both indicated positive genes were mostly dominant.

Analysis of the complete six-parent data table revealed a significant positive correlation of Yr and Wr + Vr ($r = 0.930$) and a negative $M_{L1} - M_{L0}$, indicating negative genes were mostly dominant. Therefore, it is evident that some plants possess recessive negative genes and some possess dominant negative genes. This was also deduced from S_1 data (Table 1). From the diallel analyses it is apparent that dominant genes conditioning a lower infection type tended to predominate in these six plants.

Fig. 1. Vr, Wr plots of six-parent diallel crosses of diploid alfalfa interacting with three isolates of Peronospora trifoliorum.

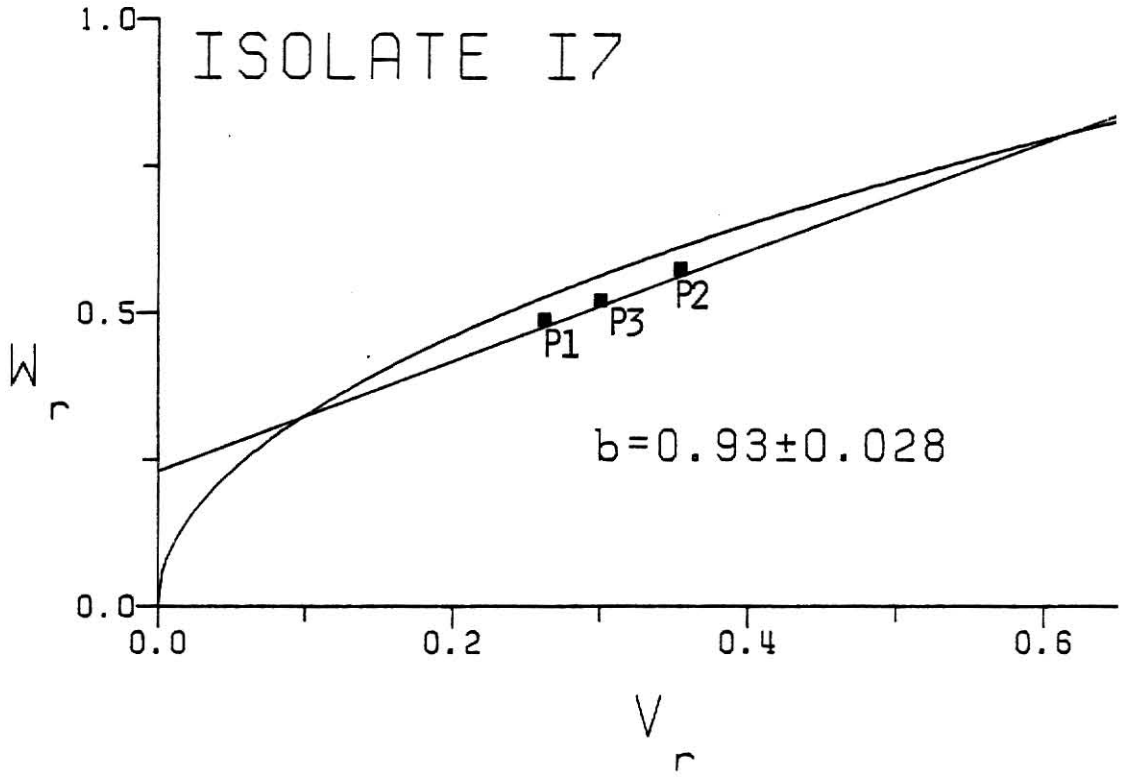

ISOLATE I5
b=1.01±0.13
P1
P2
P3
P4
P5
P6
ISOLATE I7
b=0.913±0.064
ISOLATE I8
b=1.01±0.066
2.5
0.0
0.7
1.4
2.1
W r
V r

S_1 data suggested P1 and P3 were homozygous for genes conditioning response to I5 (Table 1). Estimates of heterozygosity levels in the three-parent crosses involving P1, P3 and P5 or P6 and the four parent cross of those plants were all less than 1%. Therefore, these plants were homozygous or nearly so. Estimates of heterozygosity levels for the five-parent crosses of P1, P3, P5, P6 and P2 or P4 were 56% and 9%, respectively. Therefore, P2 was highly heterozygous and P4 was somewhat less heterozygous than P2.

Analysis of the six-parent diallel table of I7 infection severity data revealed that P4-P6 had mostly dominant genes, P1 and P2 mostly recessive genes, and P3 behaved as an intermediate (Fig. 1). Correlation of Yr and Wr + Vr was positive and significant ($r = 0.966$) and $M_{L1} - M_{L0}$ was negative indicating negative genes were mostly dominant. Of the 41 diallel tables including less than six parents of I7 infection severity data, 39 corroborated these conclusions. However, analysis of the three-parent data table including only P1, P2 and P3 indicated that P1 had more dominant genes than P2 or P3 (Fig. 2). This was opposite the indications from analysis of any other I7 infection severity data table including these parents. Also, $M_{L1} - M_{L0}$ was positive indicating positive genes (i.e. genes conditioning a higher infection type) were mostly dominant. Correlation of Yr and Wr + Vr was negative but not significant ($r = -0.541$). These results suggested complementary genes conditioned susceptibility. The F_1 progeny of P2 and P3 had a higher mean infection severity score than the S_1 progeny of either parent indicating the complementary genes were in P2 and P3.

Analysis of the I7 infection severity data table including P2-P4 yielded a Vr, Wr plot with the regression line slope less than unity (b

Fig. 2. V_r, W_r plot of three-parent diallel cross of diploid alfalfa plants P1, P2 and P3 interacting with Peronospora trifoliorum isolate I7.


ISOLATE I7
1.0
0.5
0.0
W r
P1
P3
P2
b=0.93±0.028
0.0
0.2
0.4
0.6
V r

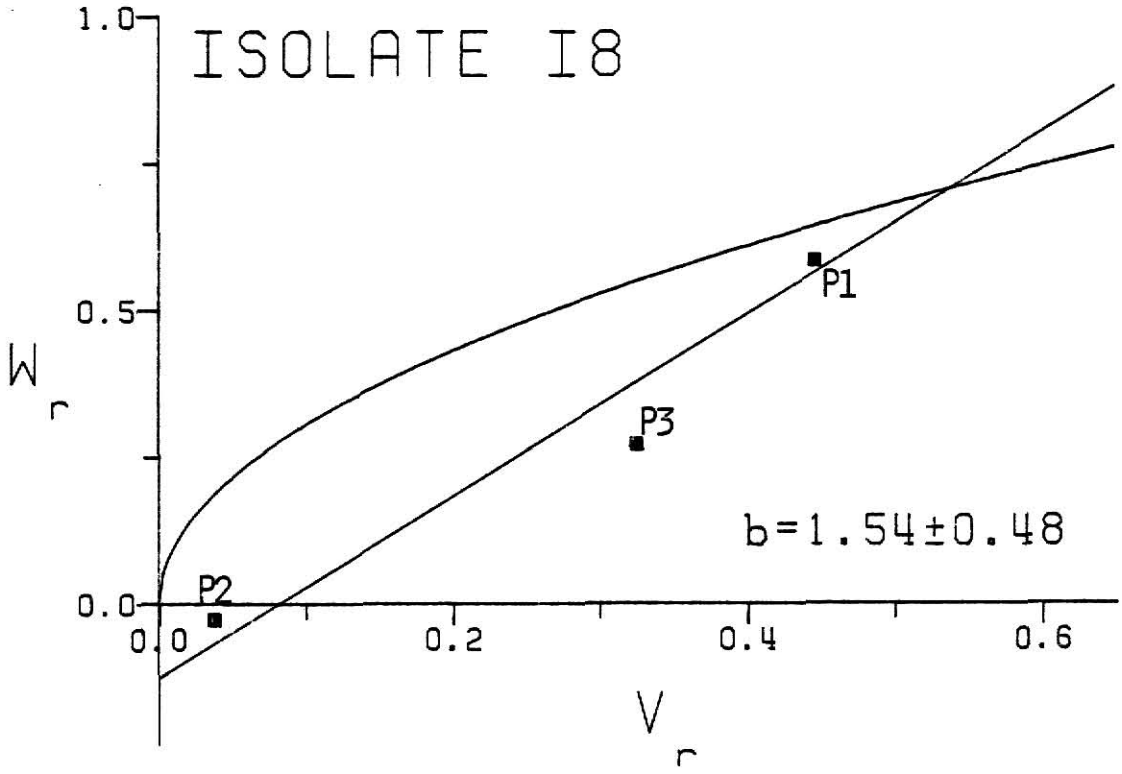
= 0.85 ± 0.025), indicating complementary gene action (2). This further indicated complementary action of genes in P2 and P3. Analysis of the data tables including P2, P3 and P6 or P5 yielded Vr, Wr plots with regression line slopes less than unity but not significantly so; b = 0.86 ± 0.17 and b = 0.87 ± 0.14, respectively.

Analysis of the I7 infection severity data table including P4-P6 yielded a positive $M_{L1} - M_{L0}$ suggesting positive genes were mostly dominant. Correlation of Yr and Wr + Vr was negative but not significant at the 5% level ($r = -0.918$, $0.4 > P > 0.2$). As with I5 data, it appeared there were dominant negative genes and dominant positive genes involved in reaction to I7. Dominant negative genes again appeared to predominate in the complete diallel.

Analysis of the I8 infection severity data table of the six-parent diallel cross indicated an order of dominance similar to that seen with I7 except P4 and P5 were reversed (Fig. 1). Correlation of Yr and Wr + Vr was positive and significant ($r = 0.976$) and $M_{L1} - M_{L0}$ was negative indicating negative genes were mostly dominant.

Analysis of the I8 infection severity data table including only P1-P3 indicated P2 was the most dominant parent (Fig. 3). This was opposite the indications from analysis of any other data table including these three parents and suggested non-allelic interaction. This probably was a complementary effect of genes in P2 and P3. The F_1 population of P2 and P3 was more severely diseased than the S_1 population of either parent but was much more similar to the S_1 population of P2. This caused the apparent abundance of dominant genes in P2 (Fig. 3). $M_{L1} - M_{L0}$ in this three-parent analysis was positive indicating the mean F_1 severity score exceeded the mean S_1 severity

Fig. 3. V_r, W_r plot of three-parent diallel cross of diploid alfalfa plants P1, P2 and P3 interacting with Peronospora trifoliorum isolate I8.


ISOLATE I8
1.0
0.5
0.0
W r
P1
P2
P3
b=1.54±0.48
0.0
0.2
0.4
0.6
V r

score. This obviously was influenced by the complementary effect. Correlation of Yr and Wr + Vr was positive but not significant (r = 0.549).

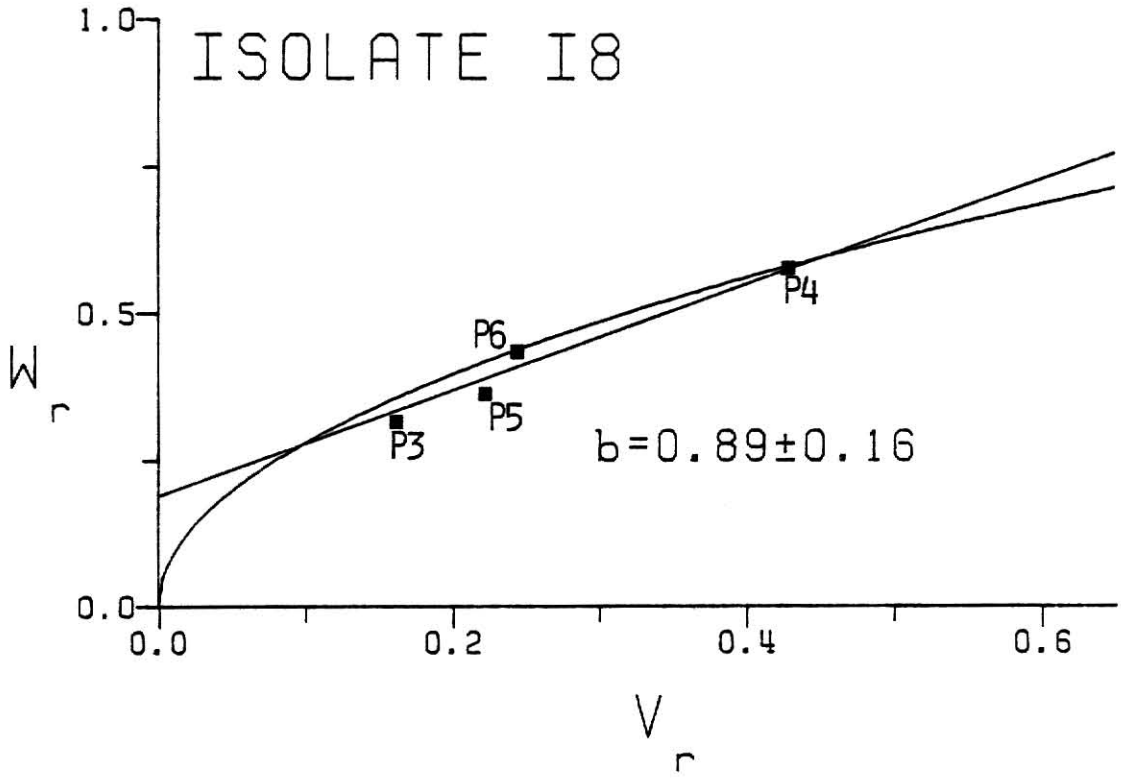
Analysis of the I8 infection severity data table including P3-P6 indicated P3 was the most dominant parent (Fig. 4). P3 was the most dominant parent in the analyses of all of the three-parent combinations involving P3-P6, but not if P1 or P2 was included.

$M_{L1} - M_{L0}$ was positive and correlation of Yr and Wr + Vr was negative (but nonsignificant) in all possible three-parent crosses of P3-P6 indicating positive genes were mostly dominant. None of the F_1 population disease severity scores exceeded the S_1 severity scores although all were well in excess of the midparent. It appears nonallelic interaction, either complementary or epistatic, promoted higher infection types in crosses of P3-P6.

Removal of P3 from the six-parent cross resulted in a clear indication of mostly dominant negative genes ($M_{L1} - M_{L0} < 0$ and r(Yr, Wr + Vr) = 0.985) and an order of dominance as occurred with the six-parent cross (Fig. 1).

Analysis of the three-parent tables of I8 infection severity data including P1-P3 or P2-P4 indicated positive genes were mostly dominant. However, analysis of the diallel cross of P1-P4 or P1, P2 and P4 indicated negative genes were mostly dominant. From S_1 data (Table 1), it is apparent the genotypes of P1-P3 favored conidium production. The S_1 progeny of P4 generally did not allow sporulation by I8 (Table 1) but this effect was largely lost upon crossing with P3 (Table 2). Therefore, the realized direction of dominance with these four plants was positive although non-allelic interaction was certainly an important

Fig. 4. V_r, W_r plot of four-parent diallel cross of diploid alfalfa plants P3-P6 interacting with Peronospora trifoliorum isolate I8.


ISOLATE I8
1.0
0.5
0.0
0.0
0.2
0.4
0.6
W r
V r
P3
P4
P5
P6
b=0.89±0.16

factor.

Dominant negative genes were abundant in P5 and P6 and were generally expressed in F_1 progeny (Table 2, Fig. 1). However, an effect promoting higher infection type was apparent upon crossing to P3 (Table 2, Fig. 4).

The many genes involved in this pathosystem can be classified as either major or minor genes. Major host genes have individually discernible effects and completely inhibit conidium production (Table 3). Their expression is dependent upon interaction with a specific isolate. Therefore, it is likely host major genes interacted with pathogen genes in a gene-for-gene relationship (3,10).

Host minor genes contribute to the interaction phenotype but do not have individually discernible effects. Their expression is also dependent upon interaction with a specific isolate. Most likely host minor genes and pathogen minor genes interacted in a gene-for-gene manner. Therefore, the additive and/or epistatic gene action described above occurred at the interorganismal level among Category III interactions, not among host genes *per se* (10). Such a system has been proposed and its selective advantage explained (15).

Nelson has suggested that a given gene can behave either as a minor or a major gene depending on what other genes are present (14). A rigorous investigation of this possibility with the present pathosystem has not been done, however, segregation ratios of various F_1 populations support this hypothesis. For example, P6 appeared to possess two major genes in the heterozygous state (Lr_2 and Lr_3) matched by two Lp genes in I8 (Table 3). Segregation ratios in the S_1 population and the F_1 populations of P6 X P1 and P6 X P5 were as expected (Table 2). However,

when crossed with P2, P3 or P4, this plant no longer appeared to possess major genes. This indicated the genes of P2, P3 or P4 modified or negated the effects of Lr_2 and Lr_3. They were no longer functioning as major genes. Whether they had any effect on phenotype is a matter for speculation but seems highly likely as Nelson suggested (14).

The minor genes involved in this pathosystem tended to behave in a manner contrary to expectations. P4, P5 and P6 produced S_1 populations which were largely not infected by any isolate (Table 1). Yet, the mean disease severity of the F_1 progeny of these three parents was greater than the mean disease severity of the respective S_1 progeny, regardless of the isolate. The correlation coefficient of Yr and Wr + Vr in the analyses of the diallel cross of these three parents was less than -0.9 with each isolate but was significant at the 5% probability level only with I5.

These results indicated that the minor genes of P4, P5 and P6 ("resistant" plants) favored conidium production. On the other hand, these same plants imparted a great deal of resistance to the F_1 progeny produced with P1, P2 or P3 (Table 2) which was largely dominant (Fig. 1). Therefore, the apparent positive direction of dominance in the F_1 progeny of P4-P6 indicated an excess of diseased F_1 individuals and/or extraordinarily severely diseased F_1 individuals. The former was clearly evident in F_1 progeny of P4 and P5 or P4 and P6 (Table 2). This was interpreted as an indication of linkage of host genes conditioning a higher infection type. With the inheritance of many such genes as a unit, the frequency of their expression (and thus HITs) would be greater than would occur with random segregation. A single gene with a large effect could accomplish the same feat but F_2 data (Table 4) clearly

indicated many genes with additive effects were involved.

Such a system would allow rapid progress from selection for susceptibility in a largely resistant population. This did occur with selection of plants susceptible to I8 in the F_1 population of P5 and P6. The four most severely diseased plants (Nos. 7-10, Table 4) produced F_2 populations ranging from 72.1 to 92.8% susceptible, reflecting gains of from 62.1 to 82.8%. Clearly a preponderance of genes conditioning a higher infection type had been inherited by these plants from their largely resistant parents.

Response to selection for resistance could be expected to be rapid but not as rapid as response to selection for susceptibility. Selection of plants resistant to I7 ("0" rating) in the F_1 population of P1 and P2 yielded F_2 populations ranging from 14.3 to 31.2% resistant (Nos. 1-5, Table 5), reflecting gains of from 9.0 to 25.9%, much less than realized gains from selection for susceptibility.

Selection for resistance in a broader gene base would be expected to yield more dramatic results due to the greater variety of genotypes capable of overcoming the effects of genes conditioning high infection types, and the tendency for resistance to predominate in a series of crosses (Fig. 1). Stanford (18) made rapid progress from selecting resistant plants from 'California Common', a largely susceptible variety.

The results of this investigation help explain the previous conflicting reports on the inheritance of resistance in alfalfa to P. trifoliorum (6,7,16). Partially dominant susceptibility is certainly evident in some plants such as P1 and P2 S_1 progeny when interacting with any isolate. Investigation of these plants alone would lead one to

the conclusion reached by Jones and Smith (7), that susceptibility is essentially dominant.

Partially dominant resistance was evident in other plants such as P4, P5 and P6 S_1 progeny when interacting with any isolate. Investigation of these plants alone would have led to the conclusion reached by Pedersen and Barnes (16), that resistance is partially dominant. However, my data indicate that many genes are involved, rather than one as they proposed (16).

There are many isolate-specific alfalfa genes involved in reaction to P. trifoliorum. Some of these genes show partial dominance in the positive direction, i.e. condition higher infection types, while others show partial or complete dominance in the negative direction. In certain genotypes, some of the genes have individually discernible effects, but most genotypes are characterized by additive gene action in which the genes are not individually discernible. Non-allelic interaction promoting higher infection types is evident in some genotypes, and there appears to be linkage of many genes conditioning higher infection types.

LITERATURE CITED

1. Barnes, D. K., Bingham, E. T., Axtell, J. D., and Davis, W. H. 1972. The flower, sterility mechanisms, and pollination control. pages 123-141 in: C. H. Hanson, ed. Alfalfa science and technology. Amer. Soc. Agron. Madison, WI. 812 p.
2. Dickinson, A. G., and Jinks, J. L. 1956. A generalized analysis of diallel crosses. Genetics 41:65-78.
3. Flor, H. H. 1942. Inheritance of pathogenicity in Melampsora lini. Phytopathology 32:653-669.
4. Fried, P. M., and Stuteville, D. L. 1977. Peronospora trifoliorum sporangium development and effects of humidity and light on discharge and germination. Phytopathology 67:890-894.
5. Hayman, B. I. 1954. The theory and analysis of diallel crosses. Genetics 39:789-809.
6. Jones, F. R., and Torrie, J. H. 1946. Systemic infection of downy mildew in soybean and alfalfa. Phytopathology 36:1057-1059.
7. Jones, F. R., and Smith, O. F. 1953. Sources of healthier alfalfa. Pages 228-237 in: Plant Diseases:the Yearbook of Agriculture. U.S. Government Printing Office. 961 p.
8. Loegering, W. Q. 1966. The relationship between host and pathogen in stem rust of wheat. Proc. 2nd Int. Wheat Genet. Symp., Lund, Sweden, 1963, Hereditas (Suppl.) 2:167-177.
9. Loegering, W. Q. 1971. Specificity in plant diseases. Pages 29-41 in: R. T. Bingham, R. J. Hoff, and G. L. McDonald, eds. Biology of rust resistance in forest trees. Proc. NATO-IUFRO Adv. Study Inst. Aug. 17-24, 1969. U.S. For. Ser. Misc. Pub. 1221.
10. Loegering, W. Q. 1978. Current concepts in interorganismal

genetics. Ann. Rev. Phytopath. 16:309-320.

11. Loegering, W. Q., and Powers, H. R. 1962. Inheritance of pathogenicity in a cross of physiological races 111 and 36 of Puccinia graminis f. sp. tritici. Phytopathology 52:547-554.

12. Martin, T. J., and Ellingboe, A. H. 1976. Differences between compatible parasite/host genotypes involving the Pm4 locus of wheat and the corresponding genes in Erysiphe graminis f. sp. tritici. Phytopathology 66:1435-1438.

13. Nass, H. A., Pederson, W. L., MacKenzie, D. R., and Nelson, R. R. 1981. The residual effects of some "defeated" powdery mildew resistance genes in isolines of Chancellor winter wheat. Phytopathology 71:1315-1318.

14. Nelson, R. R. 1978. Genetics of horizontal resistance to plant diseases. Ann. Rev. Phytopathol. 16:359-378.

15. Parlevliet, J. E., and Zadoks, J. C. 1977. The integrated concept of disease resistance; a new view including horizontal and vertical resistance in plants. Euphytica 26:5-21.

16. Pedersen, M. W., and Barnes, D. K. 1965. Inheritance of downy mildew resistance in alfalfa. Crop Sci. 5:4-5.

17. Snedecor, G. W., and Cochran, W. G. 1980. Statistical methods. Seventh ed. Iowa State University Press, Ames, IA. 507 p.

18. Stanford, E. H. 1952. Transfer of disease resistance to standard varieties. Proc. 6th International Grassland Cong. 6:1585-1590.

19. Stuteville, D. L. 1973. Pathogenic specialization in Peronospora trifoliorum. Abstracts of papers. 2nd International Congress of Plant Pathology. Sept. 5-12, 1973. Univ. Minnesota. American Phytopathological Society, St. Paul, MN.

20. Stuteville, D. L. 1974. Evaluating downy mildew. Pages 15-16 in: Standard Tests to Characterize Pest Resistance in Alfalfa Varieties. ARS-NC-19. U.S. Government Printing Office. 1974-651-179/19. 23 p.

21. Tysdal, H. M., and Garl, J. R. 1940. A new method for alfalfa emasculation. J. Amer. Soc. Agron. 32:405-407.

A GENE-FOR-GENE RELATIONSHIP BETWEEN ALFALFA AND PERONOSPORA TRIFOLIORUM

by

DANIEL ZOLEK SKINNER

B. A., St. Cloud State University, 1978

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Plant Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1983

Thirty-six diploid alfalfa families representing all possible S_1 and F_1 populations of six plants (P1-P6) were inoculated with three pathogenically different monoconidial isolates (I5, I7, and I8) of Peronospora trifoliorum. The resulting infection-type data, interpreted in terms of a gene-for-gene relationship, identified four genes individually capable of preventing conidium production in P5, three in P6, and none in P1-P4. I8, I5 and I7 possessed two, four, and five corresponding low pathogenicity genes, respectively. P1-P4 possessed many genes with additive effects. F_2 data indicated P5 and P6 also possessed similar genes in addition to the genes with individually discernible effects. Diallel analysis indicated host and pathogen polygenes operated in a gene-for-gene manner. Resistance to I5 and I7 was largely dominant in all plants, whereas resistance to I8 was largely recessive in P1-P4 but dominant in P5 and P6. Complementary host genes conditioning susceptibility to I7 and I8 were suggested in infection severity data.