Genetics of Host-Parasite Interactions Between Alfalfa and *Peronospora trifoliorum*

D. Z. Skinner and D. L. Stuteville

Research assistant and professor, respectively, Department of Plant Pathology, Kansas State University, Manhattan 66506.
Portion of a thesis submitted by the first author in partial fulfillment of the requirements for the M.S. degree, Kansas State University, Manhattan.
Contribution 84-229-J, Department of Plant Pathology, Kansas Agricultural Experiment Station, Manhattan.
Accepted for publication 5 July 1984.

ABSTRACT

Skinner, D. Z., and Stuteville, D. L. 1985. Genetics of host-parasite interaction between alfalfa and *Peronospora trifoliorum*. Phytopathology 75:119-121.

All possible populations derived from self- and cross-pollinations and some F_2 populations of three diploid alfalfa clones (P1, P5, and P6) were inoculated with conidia of three monoconidial isolates (I-5, I-7, and I-8) of *Peronospora trifoliorum*. Segregation ratios suggested a gene-for-gene relationship involving at least five corresponding gene pairs, each capable of preventing conidial production. Clones P5, P6, and P1 were postulated to have four, three, and no genes conditioning resistance, respectively. Four, five, and two corresponding genes conditioning avirulence were postulated for isolates I-5, I-7, and I-8, respectively. F_2 data suggested the presence of many other genes with additive effects leading to increased susceptibility.

Additional key words: downy mildew, inheritance of resistance, *Medicago sativa*.

Reports have differed on the inheritance of resistance in tetraploid alfalfa (*Medicago sativa* L.) to *Peronospora trifoliorum* d By., causal fungus of alfalfa downy mildew. Probably based on work by Jones and Torrie (7), Jones and Smith (6) stated that susceptibility behaved as an incompletely dominant character. However, Pedersen and Barnes (10) indicated that resistance was conditioned by one tetrasomically inherited gene with incomplete dominance. Backcross and self-pollination data obtained by Stanford (14) also suggested a degree of dominance for resistance. All reports except Stanford's (14), were based on naturally occurring downy mildew in the field and Stanford used bulk inoculum from the field. Therefore, the possible effect of pathogenically different isolates, which exist (15), was not considered.

Alfalfa is a naturally cross-pollinated, highly heterozygous, and heterogeneous crop. The flower is perfect and some plants are partially self-fertile. However, inbreeding depression renders progeny sterile after one or two cycles of selfing; thus, the production of homozygous lines by selfing is not possible. Most alfalfa plants are autotetraploid and produce diploid gametes which further complicates their genetics (1). However, diploid alfalfa plants occur (8).

In this paper, we report on the inheritance of resistance in three diploid alfalfa plants to three pathogenically different monoconidial isolates of *P. trifoliorum*.

MATERIALS AND METHODS

P. trifoliorum isolates I-5 and I-7 were collected from plants in Kansas. Isolate I-8 was collected by W. F. Lehman from the Imperial Valley Field Station, El Centro, CA.

Monoconidial isolates were derived by spraying a dilute aqueous conidial suspension 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 4-day-old alfalfa seedling. About 10 plants (one per 2.5-cm^2 diameter pot) were inoculated. Each pot was enclosed in a small plastic bag and placed in darkness in a growth chamber at 20 C. After a 24-hr infection period, the bag was removed and 98.2 $\mu E \cdot s^{-1} \cdot m^{-2}$ of continuous cool-white fluorescent lighting was provided. After 5 days, each pot again was enclosed in a plastic bag and placed in darkness for 16 hr to permit conidial production. Conidia thus produced were used to repeat the process 11 times with isolates I-5 and I-7, and once with isolate I-8. Progenies of the final cycle of single-sporing were used in this study.

Plants P1 and P5 were individual plants grown from seed lots PI172984 and PI172989, respectively, of diploid *M. sativa* supplied by W. H. Skrdla, U.S. Department of Agriculture, North Central Regional Plant Introduction Station, Ames, IA. They were selected at the cotyledonary stage, based on their reaction 1 wk after inoculation with conidia of isolate I-5. Plant P1 had heavy conidial production, whereas plant P5 was free of symptoms. Plant P5 produced variegated flowers and sickle-shaped seed pods, indicating *Medicago falcata* ancestry.

Plant 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). It was selected because it was free of symptoms 1 wk after inoculation with a mixture of conidia of isolates I-5, I-7, and I-8.

These three plants were also selected for relatively high levels of self-fertility. They were cloned by rooting stem cuttings in sand and were maintained in greenhouses or growth chambers. Ploidy level was confirmed with chromosome counts on root meristem cells.

All cross- and self-pollinations were made by hand. Flowers used as female were first emasculated with ethanol (18).

Procedures from the standard test to characterize downy mildew resistance in alfalfa (16) were used. To improve uniformity of seedling emergence, each seed was scarified with a razor blade. Seeds were planted about 8 mm deep in autoclaved masonry sand in aluminum bread pans. The pans were placed in a growth chamber set at 20 C and 98.2 $\mu E \cdot s^{-1} \cdot m^{-2}$ of continuous cool-white fluorescent lighting. The sand was sprinkled daily with distilled water to settle it around the emerging seedlings and aid uniform emergence. Five days after seeding, the plants (at the cotyledonary stage) were inoculated by spraying a suspension of conidia (adjusted to 10^5 conidia per milliliter of water) onto the plants.

On the evening of the sixth day after inoculation, pans of plants were placed in plastic boxes covered with aluminum foil to produce the darkness and near 100% relative humidity required for conidial production (3). Conidial production on the cotyledons was evaluated 15 hr later under 12× magnification.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

Intensity of conidial production on the cotyledons was rated on a 0–5 scale in which 0 = no conidial production and 5 = copious conidial production.

Plants that did not support conidial production were of particular interest because of the practical considerations of identifying resistance. They were classified as resistant. Those that supported the production of any conidia were classified as susceptible.

TABLE 1. Segregations in S_1 populations of three diploid alfalfa plants inoculated with three monoconidial isolates of *Peronospora trifoliorum*

Plant	Isolate	No. of plants[a] Resistant	Susceptible	Suggested ratio	*P*-value[b]
P1	I-5	0	82	0:1	...
	I-7	0	76	0:1	...
	I-8	0	177	0:1	...
P5	I-5	107	2	63:1	>0.90
	I-7	368	2	255:1	>0.90
	I-8	44	12	3:1	0.50–0.70
P6	I-5	77	2	15:1	0.20–0.40
	I-7	128	3	63:1	0.70–0.90
	I-8	113	7	15:1	0.80–0.90

[a] Resistant = no conidia produced; susceptible = conidia produced.
[b] Determined with chi-square tests, including a continuity correction.

TABLE 2. Segregations in F_1 populations of three diploid alfalfa plants inoculated with three monoconidial isolates of *Peronospora trifoliorum*

Cross	Isolate	No. of plants[a] Resistant	Susceptible	Expected ratio	*P*-value[b]
P1 × P5	I-5	22	35	7:1	<0.001
	I-7	59	66	15:1	<0.001
	I-8	41	44	1:1	0.50–0.70
P1 × P6	I-5	127	28	3:1	0.50–0.70
	I-7	130	14	7:1	0.20–0.50
	I-8	78	36	3:1	0.10–0.30
P5 × P6	I-5	157	8	31:1	0.20–0.50
	I-7	297	5	127:1	0.10–0.30
	I-8	135	15	7:1	0.30–0.50

[a] Resistant = no conidia produced; susceptible = conidia produced.
[b] Determined with chi-square tests, including a continuity correction.

TABLE 3. Reaction to *Peronospora trifoliorum* isolate I-8 by diploid alfalfa S_1 populations of P5 × P6 F_1 plants that remained free of symptoms after inoculation with isolate I-8

F_1 plant	No. of F_2 plants Resistant[a]	Susceptible[a]	Chi-square values[b] for segregation ratio 3:1	15:1
1	100	0	...	...
2	43	0	...	...
3	52	0	...	...
4	76	7	11.28**	0.35
5	22	1	4.19*	0.14
6	66	4	12.88**	0.03
7	45	2	9.71**	0.07
8	72	5	13.10**	0.01
9	86	6	15.78**	0.01
10	66	17	0.68	26.31**
11	51	13	0.52	19.27**
12	90	23	1.06	35.99**
13	72	16	1.83	19.39**

[a] Resistant = no conidia produced; susceptible = conidia produced.
[b] Calculated with a continuity correction, values followed by one asterisk were significant at $P = 0.05$, two asterisks indicate significance at $P = 0.01$.

Segregation ratios were tested for goodness of fit to theoretical ratios with chi-square tests, including a continuity correction where appropriate (13).

Postulated host resistance genes were symbolized as *PtR* for the dominant allele and *ptr* for the recessive allele, each followed by a numeric subscript. Postulated parasite virulence genes were symbolized as *Pta* if they conditioned avirulence and *Ptv* if they conditioned virulence, each followed by a numeric subscript corresponding to the host gene conditioning resistance to that parasite gene. This terminology is similar to that proposed by Sidhu (12), but because we did not work with parasite gene segregation, we know nothing of the dominance relationships of the parasite genes. We have adjusted the gene symbols accordingly.

RESULTS

Downy mildew symptoms on the cotyledons included a wide range of conidial production and chlorosis development. The intensity of each was similar for most plants.

Progeny resulting from the self-pollination of clone P5 (S_1 generation) segregated to fit ratios for three, four, and one gene in clone P5 conditioning resistance to isolate I-5, I-7, and I-8, respectively (Table 1). Segregation in the S_1 generation of clone P6 indicated that two, three, and two genes in clone P6 conditioned resistance to isolate I-5, I-7, and I-8, respectively (Table 1). All S_1 plants of clone P1 were susceptible to the three isolates (Table 1).

Reciprocal crosses among all clones indicated no maternal cytoplasmic influence.

Segregation of F_1 plants from P1 × P6 crosses and F_1 plants from P5 × P6 crosses fit the expected resistant:susceptible ratios (Table 2). F_1 plants of clones P1 and P5 segregated in accordance with the expected ratios in response to inoculation with conidia of isolate I-8, but not isolate I-5 or I-7 (Table 2).

Thirteen F_1 plants resistant to isolate I-8, from the P5 × P6 crosses, were selfed and the resulting progeny were inoculated with conidia of isolate I-8. Three of those F_2 populations did not segregate, four segregated 3 resistant : 1 susceptible, and six segregated 15 resistant : 1 susceptible (Table 3). This was not

TABLE 4. Reaction to *Peronospora trifoliorum* isolate I-8 by susceptible[a] F_1 plants from crosses of diploid alfalfa clones P5 and P6 and the frequency and mean ratings of susceptible F_2 plants

F_1 generation Plant no.	Rating[b]	Susceptible F_2 plants Frequency (%)	Mean rating[b]
1	1	13.8	1.8
2	1	14.4	1.7
3	2	49.9	2.5
4	2	26.4	2.0
5	3	79.3	2.8
6	3	92.8	2.6
7	4	72.1	2.8
8	4	78.4	2.6

[a] Susceptible = conidia produced.
[b] Zero to five scale in which 0 = no conidial production and 5 = copious conidial production.

TABLE 5. Proposed genotypes of three diploid alfalfa plants and three monoconidial isolates of *Peronospora trifoliorum*

Plant[a]			Isolate[b]		
P1	P5	P6	I-5	I-7	I-8
ptr_1ptr_1	PtR_1ptr_1	ptr_1ptr_1	Pta_1	Pta_1	Ptv_1
ptr_2ptr_2	PtR_2ptr_2	PtR_2ptr_2	Pta_2	Pta_2	Pta_2
ptr_3ptr_3	ptr_3ptr_3	PtR_3ptr_3	Pta_3	Pta_3	Pta_3
ptr_4ptr_4	PtR_4ptr_4	ptr_4ptr_4	Pta_4	Pta_4	Ptv_4
ptr_5ptr_5	PtR_5ptr_5	PtR_5ptr_5	Ptv_5	Pta_5	Ptv_5

[a] *PtR* = allele conditioning resistance, and *ptr* = allele conditioning susceptibility in alfalfa.
[b] *Pta* = allele conditioning avirulence, and *Ptv* = allele conditioning virulence in *P. trifoliorum*, toward the corresponding host gene.

significantly different from the expected two not segregating, three segregating 3 resistant : 1 susceptible, and two segregating 15 resistant : 1 susceptible ($\chi^2 = 3.13$, $0.10 < P < 0.05$).

Eight F_1 plants from the P5 × P6 crosses, susceptible to isolate I-8 and representing the range of conidial production, were selfed. They were expected to produce only susceptible progeny. Instead, they produced populations that ranged from 13.8 to 92.8% susceptible (Table 4). The F_1 plants that had the lowest disease ratings produced the F_2 populations with the lowest frequency of susceptible plants (Table 4). Also, the incidence and the mean rating of the susceptible plants in the F_2 populations were positively correlated ($r = 0.93$, $P > 0.95$, Table 4).

DISCUSSION

In some cases, host genes that conditioned resistance to one isolate did not condition resistance to a second isolate (Tables 1 and 2). Therefore, the effects of a host gene were not expressed unless one or more specific genes were present in the parasite.

The simplest hypothesis to account for these data is that one gene in the parasite was necessary for one gene in the host to be expressed. We suggest, therefore, that alfalfa and *P. trifoliorum* share a gene-for-gene relationship (2,9). We suggest that five *PtR* genes distributed among clones P5 and P6 were matched by five *Pta* genes distributed among the three isolates (Table 5). The presence of any one of these corresponding gene pairs was sufficient to completely inhibit conidial production in most cases.

For absolute proof of this hypothesis by "Flor's criterion" (11), it would be necessary to observe segregation of parasite genes conditioning avirulence in exact correspondence with host resistance gene segregation for each gene involved. We cannot currently do this because we cannot germinate *P. trifoliorum* oospores (4).

The F_1 population of clones P1 and P5 did not segregate in accordance with the expected ratios in response to inoculation with conidia of isolate I-5 or I-7 (Table 2). Apparently, the genes from clone P1, or a combination of genes from clones P1 and P5, resulted in susceptibility that overcame the effects of the proposed corresponding gene pairs of clone P5 and isolate I-5 or I-7 that would have otherwise resulted in expressed resistance. Similar instances have been found in the pathogen-host systems *Helminthosporium turcicum*/ *Zea mays* (5) and lettuce mosaic virus/ *Carthamus tinctorius* (safflower) (17).

The significant positive correlation of incidence and mean rating of susceptible plants in F_2 populations of clones P5 and P6 on interaction with isolate I-8 (Table 4), indicated that additive gene action was involved. The F_1 plants that were selfed were susceptible to isolate I-8, yet produced some resistant S_1 progeny (Table 4). Therefore, some genes that could not individually condition a resistant reaction, could do so in concert with other genes. We have further investigated this phenomenon in the clones included here and in three additional clones (*unpublished*).

LITERATURE CITED

1. Busbice, T. H., Hill, R. R., Jr., and Carnahan, H. L. 1972. Genetics and breeding procedures. Pages 283-318 in: Alfalfa Science and Technology. C. H. Hanson, ed. American Society of Agronomy, Madison, WI. 812 pp.
2. Flor, H. H. 1956. The complementary genic systems in flax and flax rust. Adv. Genet. 8:29-54.
3. 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.
4. Hodgden, L. D. 1978. I. Environmental effects on *Peronospora trifoliorum* oospore production in seedlings of two alfalfa clones. II. Attempts to germinate *Peronospora trifoliorum* oospores. M.S. thesis. Kansas State Univ., Manhattan. 42 pp.
5. Hooker, A. L. 1963. Monogenic resistance in *Zea mays* L. to *Helminthosporium turcicum*. Crop Sci. 3:381-383.
6. 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, Washington, DC. 972 pp.
7. Jones, F. R., and Torrie, J. H. 1946. Systemic infection of downy mildew in soybean and alfalfa. Phytopathology 36:1057-1059.
8. Lesins, K., and Gillies, C. B. 1972. Taxonomy and cytogenetics of *Medicago*. Pages 53-86 in: Alfalfa Science and Technology. C. H. Hanson, ed. American Society of Agronomy, Madison, WI. 812 pp.
9. Loegering, W. Q. 1978. Current concepts in interorganismal genetics. Annu. Rev. Phytopathol. 16:309-320.
10. Pedersen, M. W., and Barnes, D. K. 1965. Inheritance of downy mildew resistance in alfalfa. Crop Sci. 5:4-5.
11. Sidhu, G. S. 1975. Gene-for-gene relationships in plant parasitic systems. Sci. Prog. 62:467-485.
12. Sidhu, G. S. 1977. A proposal for standardizing the nomenclature of resistance and virulence genes operative in plant parasitic systems. Crop Improv. 3:60-63.
13. Snedecor, G. W., and Cochran, W. G. 1980. Statistical Methods. Seventh ed. The Iowa State Univ. Press, Ames. 507 pp.
14. Stanford, E. H. 1952. Transfer of disease resistance to standard varieties. Proc. 6th Int. Grassland Cong. 6:1585-1590.
15. Stuteville, D. L. 1973. Pathogenic specialization in *Peronospora trifoliorum*. (Abstr. 0715) Abstracts of Papers. 2nd Int. Cong. Plant Pathol. 5-12 September. University of Minnesota, Minneapolis. (unpaged).
16. Stuteville, D. L. 1974. Evaluating downy mildew resistance. Pages 15-16 in: Standard Tests to Characterize Pest Resistance in Alfalfa Varieties. Publication ARS-NC-19, U.S. Department of Agriculture, U.S. Government Printing Office. 23 pp.
17. Thomas, C. A. 1981. Inheritance of resistance to lettuce mosaic virus in safflower. Phytopathology 71:817-818.
18. Tysdal, H. M., and Garl, J. R. 1940. A new method for alfalfa emasculation. J. Am. Soc. Agron. 32:405-407.