

# Inheritance of Resistance to *Pratylenchus penetrans* in Alfalfa<sup>1</sup>

JUDY A. THIES,<sup>2</sup> DANIEL BASIGALUP,<sup>3</sup> AND DONALD K. BARNES<sup>4</sup>

**Abstract:** Fifty-two alfalfa (*Medicago sativa* L.) clones, randomly selected from the cultivar Baker and the experimental line MNGRN-4, were evaluated for resistance (based on nematode reproduction) to *Pratylenchus penetrans* in growth chamber tests (25 C). Twenty-five clones, representing the range of nematodes and eggs per plant, were selected and retested. Four moderately resistant and two susceptible alfalfa clones were identified. Inheritance of resistance to *P. penetrans* was studied in these six clones using a diallel mating design. The S<sub>1</sub>, F<sub>1</sub>, and reciprocal progenies differed for numbers of nematodes and eggs per g dry root and for shoot and root weights ( $P < 0.05$ ). Resistance, measured as numbers of nematodes in roots, was correlated between parental clones and their S<sub>1</sub> families ( $r = 0.94$ ), parental clones and their half-sib families ( $r = 0.81$ ), and S<sub>1</sub> and half-sib families ( $r = 0.88$ ). General combining ability (GCA) effects were significant for nematode resistance traits. Both GCA and specific combining ability (SCA) effects were significant for plant size traits, but SCA was more important than GCA in predicting progeny plant size. Reciprocal effects were significant for both nematode resistance and plant size traits, which may slow selection progress in long-term selection programs. However, the GCA effects are large enough that breeding procedures that capitalize on additive effects should be effective in developing alfalfa cultivars with resistance to *P. penetrans*.

**Key words:** alfalfa, inheritance of resistance, *Medicago sativa*, *Pratylenchus penetrans*, nematode, nematode resistance, root-lesion nematode.

Root-lesion nematodes (*Pratylenchus penetrans* [Cobb] Filipjev Stekhoven) limit the establishment, yield, and longevity of alfalfa (*Medicago sativa* L.) (6,11,12,15-17). Fallowing, nematicides, and seeding dates have been used to increase alfalfa stand establishment and yield in the presence of *P. penetrans*. Eight weeks after alfalfa was spring sown into winter wheat (*Triticum aestivum* L.) fields in Kentucky, alfalfa stands were severely damaged, and alfalfa

seedlings had >100,000 root-lesion nematodes/g fresh root (6). However, fall seeding of alfalfa in the same fields, after summer fallow, improved stands and limited root injury, with only about 1,000 root-lesion nematodes/g fresh root (6). Fallowing for one growing season may reduce *P. penetrans* populations and increase alfalfa yields (19). Delayed seeding (later June-early July) after 7 weeks of fallow increased alfalfa stands and reduced alfalfa root populations of *P. penetrans* at Grand Rapids, Minnesota (15).

Nematicides also have been used to reduce root-lesion nematode populations and increase alfalfa establishment and forage yields (12,15-17,19). Applications of carbofuran resulted in reduced population densities of *P. penetrans* in alfalfa roots 17 and 69 weeks after application and increased forage yields in the seeding year in eastern Canada (16). Carbofuran reduced numbers of *P. penetrans* in alfalfa roots in the seeding and following year at Grand Rapids (12). This compound increased alfalfa stand establishment (21%) and reduced *P. penetrans* population densities in alfalfa roots (37%) in the seeding year in other research (15).

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<sup>2</sup>Research Plant Pathologist (Nematology), USDA ARS, U.S. Vegetable Laboratory, 2875 Savannah Highway, Charleston, SC 29414.

<sup>3</sup>Research Assistant, Department of Agronomy and Plant Genetics, University of Minnesota. Present address: Plant Breeder INTA, Manfredi, Argentina.

<sup>4</sup>Research Geneticist, USDA ARS, Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55414.

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tally safe control of the root-lesion nematode would be use of resistant cultivars. Genetic variability for the reaction to *P. penetrans* in alfalfa has been reported (11, 14, 18). Some alfalfa populations were resistant (supported low numbers of *P. penetrans* in the roots) and others were tolerant (supported moderate numbers of nematodes without a reduction in shoot and root weights) (11). Two alfalfa germ-plasms, MNGRN-2 and MNGRN-4, that showed superior performance in fields infested with large populations of *P. penetrans* are available (3), but resistant cultivars have not been developed. Moderate resistance to *P. penetrans* in MNGRN-4 enhanced stand establishment (35%) and yield (22%) and reduced numbers of *P. penetrans* per g fibrous roots (37%) compared to the susceptible cultivar Baker (15). However, the inheritance of resistance to *P. penetrans* has not been determined.

The objectives of this research were to: i) identify alfalfa clones differing in resistance and susceptibility to *P. penetrans* based on nematode reproduction and ii) characterize the inheritance of resistance to *P. penetrans* in selected alfalfa clones.

#### MATERIALS AND METHODS

*Nematode inoculum: Pratylenchus penetrans* inoculum for all clonal experiments was increased on 'Pioneer hybrid 3720' corn (*Zea mays* L.) or 'Astro' oat (*Avena sativa* L.), grown in a steam-pasteurized mixture of equal parts Waukegan loamy sand soil and fine-washed river sand in the greenhouse at approximately 25 C. Inoculum for the inheritance experiments was increased on alfalfa callus tissue (10) incubated at 25 C.

*Clonal selection test:* Thirty clones from Baker (B) (an alfalfa cultivar susceptible to *P. penetrans* [11, 15]) and 22 clones from MNGRN-4 (M) (an experimental alfalfa line selected for field resistance to the root-lesion nematode [3]) were chosen at random for this test. *Pratylenchus penetrans*-infested soil and infected corn roots were removed from pots, and roots were

chopped into 1–2 cm pieces. Root pieces and soil were mixed and placed in polypropylene flats (36 cm × 52 cm × 87.5 cm deep). Soil was inoculated with *Rhizobium meliloti* Dang. Rooted ramets of 52 clones/replicate were transplanted into the soil on 3-cm centers (two replicates/flat). Border rows of ramets were planted around the outside edges of each flat. Initial nematode population density was approximately nine *P. penetrans*/plant. The plants were grown in a growth chamber at 25 ± 2 C with a 12-hour photoperiod provided by fluorescent lights. The experiment was designed as a randomized complete block replicated four times. Eight weeks after transplanting, shoots were clipped to a height of 2.5 cm. Twelve weeks after transplanting, plants were removed from the soil and the roots were washed. Plants were clipped at the top of the root and roots were weighed. The entire root system of each plant was stained (5), and numbers of nematodes and eggs determined.

*Clonal test 1:* Twenty-five alfalfa clones (18 from Baker and 7 from MNGRN-4), representing the range of root weights and numbers of nematodes and eggs per plant, were selected from the initial clonal selection test (Fig. 1A,B) and retested. Rooted ramets were grown in the greenhouse for about 7 weeks. Immediately before transplanting, the roots were washed, and shoots and roots were uniformly trimmed to 2.5 cm and 7.5 cm, respectively.

Nematode inoculum was extracted from corn and oat roots for 4 days using a modification of the shaker method (7). The roots were cut into 1–2 cm pieces, and then 1–2 g of the root tissue was placed in 10 cm × 2.5 cm petri dishes containing 20 ml tap water, and agitated on a horizontal shaker for 48 hours. Then the liquid containing nematodes was decanted from the roots, and this inoculum was applied at a rate of about 400 *P. penetrans* per alfalfa plant. Inoculum was distributed by spraying 150 ml of nematode suspension onto each of three successive layers of soil mix (described in *Nematode inoculum* section) in polypropylene flats. The soil was inoculated with *R.*

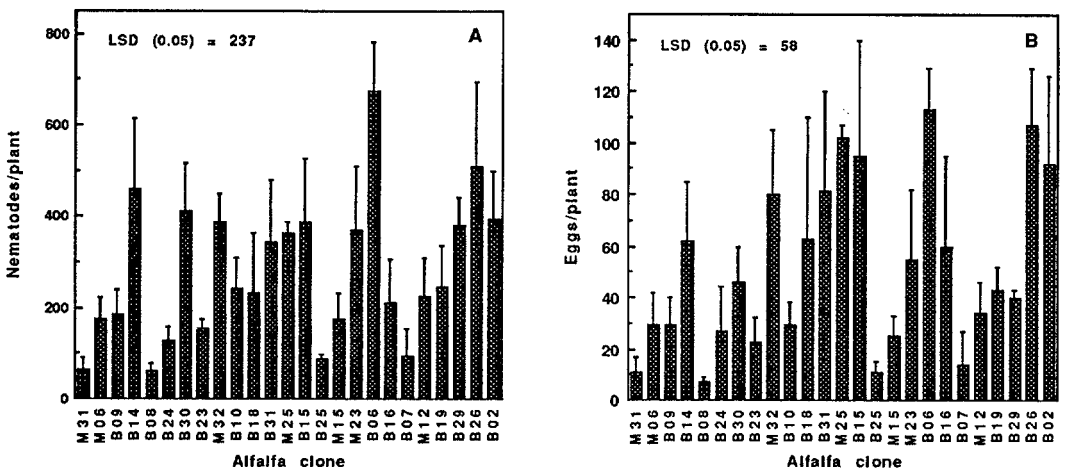


FIG. 1. Numbers of *Pratylenchus penetrans* in roots of 25 selected alfalfa clones evaluated in a growth chamber test, 12 weeks after inoculation. (A) Adults and juveniles per plant. (B) Eggs per plant. Values are means of four replicates, Clonal Selection Test. Pi = 9 nematodes per plant. Vertical bars = 1 SE.

*meliloti* and gently mixed before planting. Then, the ramets were transplanted on 3-cm centers (as previously described) into flats containing the nematode-infested soil.

The experiment was designed as a randomized complete block with 3 blocks, 2 replicates/block, and the 25 selected alfalfa clones/replicate. Each block of two replicates was placed in a separate growth chamber at  $25 \pm 2$  C, with a 14-hour photoperiod provided by fluorescent lights. Six weeks after inoculation, plants were harvested, roots washed, and numbers of nematodes and eggs were determined as described earlier.

**Clonal test 2:** Rooted ramets of the same 25 clones evaluated in Clonal Test 1 were grown and trimmed as previously described. Each ramet was transplanted into a polyethylene Super Cell Cone-tainer (19 cm long  $\times$  3.8-cm-d, Stuewe & Sons, Corvallis, OR) containing approximately 160 cm<sup>3</sup> soil mix (described earlier) that had been inoculated with *R. meliloti*. Plants were grown in the greenhouse for 4 days at about 25 C before inoculation with *P. penetrans*. Six ml of a nematode-tap water suspension (about 50 nematodes/ml) were injected into the soil surrounding each root system using a 10-ml glass syringe with a 10-cm long cannula. The plants

were grown under the same conditions used in previously described tests.

The experiment was designed as a randomized complete block with seven replicates. Six weeks after inoculation, plants were removed from the cone-tainers, roots washed, and numbers of nematodes and eggs were determined as previously described.

**Inheritance test:** Six of the 25 clones evaluated in Clonal Tests 1 and 2 were selected as parents based on their reaction to *P. penetrans*. The six clones were chosen as parents because they reflected differences in numbers of nematodes and eggs per plant: clones B02 and B26 were susceptible, and clones B09, B14, M06, and M31 were moderately resistant (Fig. 2A,B,C).

Seed of S<sub>1</sub> was produced on each parental clone by gently rolling racemes between the fingers. The clones also were crossed in a complete diallel mating design to produce 15 F<sub>1</sub> crosses and reciprocals. Flowers used in making cross-pollinations were emasculated using a suction method (2). Two scarified seeds per entry were planted in cone-tainers containing about 160 cm<sup>3</sup> soil mix (previously described) that had been inoculated with *R. meliloti*. Seedlings were randomly thinned to one per cone-tainer, 1 week after emergence. Two weeks after seedling emergence, 4 ml of a

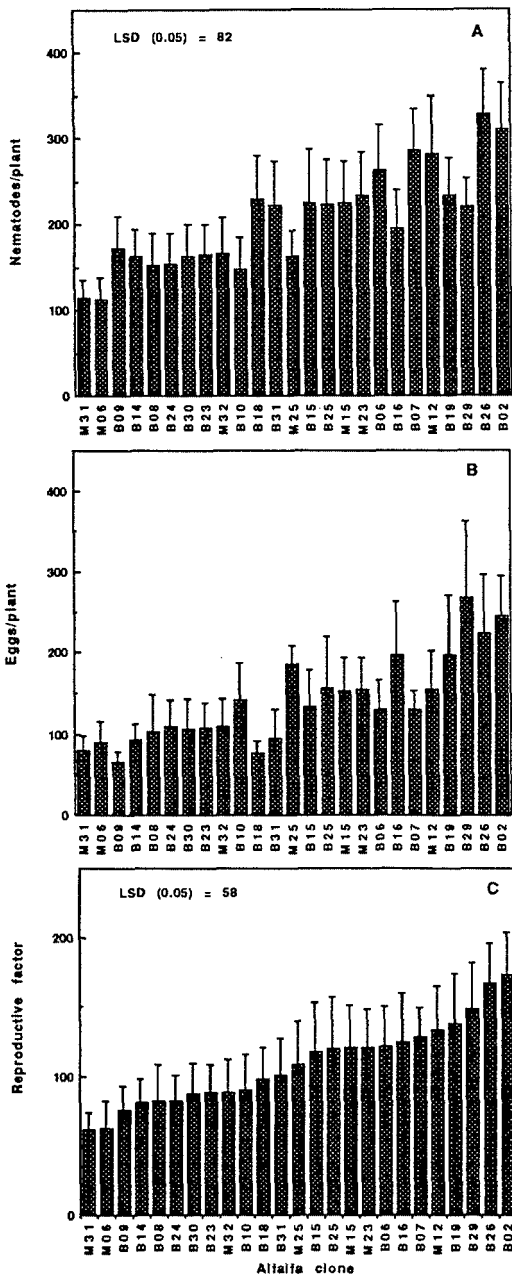


FIG. 2. Numbers of *Pratylenchus penetrans* in roots of 25 alfalfa clones evaluated in growth chamber tests, 6 weeks after inoculation. (A) Adults and juveniles per plant (B) Eggs per plant. (C) Reproductive factor. Values are means of Clonal Tests 1 and 2 (13 replicates). Each plant was inoculated with 400 (Clonal Test 1) or 300 (Clonal Test 2) nematodes per plant. Vertical bars = 1 SE.

nematode-tap water suspension (approximately 10 nematodes/ml) were injected into the soil surrounding each root system.

The plants were grown in a growth chamber at  $24 \pm 1$  C with a 14-hour photoperiod.

The experimental design was a randomized complete block with three replicates. Forty entries (15  $F_1$  crosses, 15 reciprocals, 6  $S_1$  families, and 4 checks) were evaluated. Each replicate had six plants per entry. The four checks and their reactions to *P. penetrans* were as follows: Baker (susceptible), WL-219 (low resistance), MNGRN-4 (moderate resistance) (15) and MNGRN-14 (moderate resistance) (Thies, unpubl. data). MNGRN-14 represents a second cycle of field selection from MNGRN-4 germplasm. Six weeks after inoculation, plants were removed from the cone-tainers, roots were washed, and shoots were separated from roots by clipping at the crown. Root and shoot fresh weights were recorded and adjusted on a dry matter basis.

Two nematode traits were used to characterize plant resistance to *P. penetrans*: nematodes per g dry root and eggs per g dry root. Two plant traits were used to characterize plant size: shoot and root dry weights.

**Data analysis:** Data from all tests were subjected to analysis of variance (ANOVA), and means were separated using Fisher's least significant difference or Duncan's multiple-range test. Nematode and egg numbers were not transformed because the 25 clones (Clonal Selection Test) and the parental clones were selected based on actual data. A nematode reproductive factor was calculated for alfalfa clones in Clonal Tests 1 and 2; reproductive factor = final population of nematodes and eggs per plant/initial population of nematodes and eggs/plant =  $P_f/P_i$ . In the inheritance test, Griffing's Method 3, Model I (fixed effects) (9) was used to analyze the genetic effects for each trait. Variance components due to crosses were partitioned into general combining ability (GCA), specific combining ability (SCA), and reciprocal effects. The importance of GCA relative to SCA was estimated by comparing variances of effects (for a model with fixed effects) as suggested by Baker (1) in which

$$RI_{GCA} = \frac{(\sum g_i^2/df_G)/(\sum g_i^2/df_G + \sum \sum s_{ij}^2/df_S)}{1}$$

where  $RI_{GCA}$  is the relative importance of GCA compared to SCA, and

$$\sum g_i^2/df_G = (MS_G - MS_E)(p - 1)/[2b(p - 2)]$$

and

$$\sum \sum s_{ij}^2/df_S = (MS_S - MS_E)(p)(p - 3)/4b;$$

$MS_G$ ,  $MS_S$ , and  $MS_E$  are the mean squares for GCA, SCA, and the error term, respectively; and  $b$  is the number of replications and  $p$  is the number of parents. According to Baker (1), the closer this ratio is to 1, the greater the predictability of progeny performance is based on GCA alone.

## RESULTS

*Variability among alfalfa clones:* The 52 alfalfa clones evaluated in the Clonal Selection Test differed ( $P < 0.05$ ) for numbers of nematodes and eggs per plant and ranged from 39 nematodes and 6 eggs per plant to 675 nematodes and 113 eggs per plant. Twenty-five clones representing the range of numbers of nematodes and eggs per plant were selected for further study (Fig. 1A,B). Clones from both Baker and MNGRN-4 varied in reaction to the nematode.

*Identification of clones with resistance:* Clones differed for numbers of nematodes per plant ( $P < 0.01$ ), but not for numbers

of eggs per plant (Fig. 2A,B). Clones M31 and M06 had the smallest number of nematodes per plant (113 and 114, respectively), and B02 and B26 had the largest (312 and 329 nematodes per plant, respectively). Clones M31, M06, B09, and B14 had the lowest reproductive factor (61, 62, 76, and 81, respectively), and clones B26 and B02 had the highest reproductive factor (167 and 173, respectively) (Fig. 1C).

*Inheritance of resistance:* The  $S_1$  progenies differed for numbers of *P. penetrans* adults and juveniles per g dry root, and for shoot and root dry weights ( $P < 0.05$ ). The  $S_1$  progenies of clones M31, M06, B14, and B09 had lower ( $P < 0.05$ ) numbers of adults and juveniles per g dry root than clone B26, but nematode root population densities of these four clones did not differ significantly from the checks (Table 1). The correlation between  $S_1$  family means (nematodes per g dry root) and parental clones (nematodes per plant) was  $r = 0.94$  ( $P < 0.01$ ). Among the checks, the susceptible cultivar Baker had the highest number of adults, juveniles, and eggs per plant, while the moderately resistant population MNGRN-14 had the lowest (Table 1).

Significant GCA effects were observed for all traits (Table 2). Specific combining ability was significant for plant traits but not for nematode resistance traits. The relative importance of GCA compared to

TABLE 1. Numbers of *Pratylenchus penetrans* and eggs and plant weights for six alfalfa  $S_1$  families and four check entries evaluated in a growth chamber test, 6 weeks after inoculation.

Entries	Adults and juveniles (no./g dry root)†	Eggs (no./g dry root)†	Shoot dry weight (g)†	Root dry weight (g)†
$S_1$ progenies				
M31	890 a	152 a	0.22 abcd	0.12 bcd
M06	807 a	122 a	0.30 e	0.13 c
B14	1,260 a	162 a	0.19 ab	0.07 a
B09	1,272 a	302 a	0.25 bcde	0.11 bc
B02	1,621 ab	347 a	0.25 bcde	0.12 bcd
B26	2,215 b	340 a	0.17 a	0.10 b
Checks‡				
MNGRN-14 (MR)	751 a	125 a	0.20 abc	0.13 bcd
MNGRN-4 (MR)	1,130 a	135 a	0.26 de	0.15 d
WL-219 (LR)	1,112 a	158 a	0.26 de	0.15 d
Baker (S)	1,308 a	175 a	0.28 e	0.12 bcd

† Each value is an average of 18 plants. Means followed by the same letters within a column are not different according to Duncan's multiple-range test ( $P \leq 0.05$ ).

‡ S = susceptible; LR = low resistance; MR = moderate resistance.

TABLE 2. Mean square values for general combining ability (GCA), specific combining ability (SCA), and reciprocal effects (RE) when six alfalfa clones were crossed in a diallel mating design and evaluated in a growth chamber test for resistance to *Pratylenchus penetrans*.

Genetic effects†	df	Adults and juveniles (no./g dry root)	Eggs (no./g dry root)	Shoot dry weight (g)	Root dry weight (g)
GCA	5	1,846,542.00**	167,709.76**	0.0070**	0.0081**
SCA	9	407,162.00 NS	59,961.53 NS	0.0051**	0.0022**
Reciprocals	15	543,873.47*	51,784.45 NS	0.0076**	0.0028**
Error	58	245,953.63	35,094.69	0.0011	0.0004

† Genetic effects as estimated by Griffing's Method 3, Model 1.

\*, \*\* Significant at 0.05 and 0.01 probability level, respectively.

SCA was estimated to be about 0.17 and 0.37 for shoot dry weight and root dry weight, respectively. Significant reciprocal effects were observed for numbers of adults and juveniles per g dry root and for both plant traits (Table 2).

Half-sib family means differed for numbers of adults and juveniles per g dry root and eggs per g dry root, and for shoot and root dry weights ( $P < 0.01$ ) (Table 3). Half-sib family means for numbers of adults and juveniles per g dry root and eggs per g dry root of clone M31 were less ( $P < 0.05$ ) than those of clones B02 and B26. Half-sib family means for numbers of adults and juveniles per g dry root and eggs per g dry root of clone M06 also were less ( $P < 0.05$ ) than those of clone B26. The correlation between half-sib family means and  $S_1$  family means for adults and juveniles per g dry root was  $r = 0.88$  ( $P < 0.05$ ), and the correlation between half-sib family means (adults and juveniles per g dry root) and parental clones (adults and juveniles per

plant) was  $r = 0.81$  ( $P < 0.05$ ). Numbers of nematodes in the roots were not correlated with shoot or root weights for parental clones,  $S_1$  families, or half-sib families (data not shown).

The mean values for adults and juveniles per g dry root and eggs per g dry root for the 15 possible crosses among the six parental clones are presented in Table 4. The moderately resistant  $\times$  moderately resistant (MR  $\times$  MR) crosses had the lowest numbers of nematodes and eggs in the roots (mean = 840 adults and juveniles/g dry root, 132 eggs/g dry root) and the susceptible  $\times$  susceptible (S  $\times$  S) cross had one of the highest (1,995 adults and juveniles/g dry root, 482 eggs/g dry root). The means of the MR  $\times$  S crosses were 1,091 adults and juveniles/g dry root and 208 eggs/g dry root.

## DISCUSSION

Although 'Baker' is susceptible and MNGRN-4 is moderately resistant to *P.*

TABLE 3. Half-sib family means for two nematode resistance and two plant size traits for six alfalfa clones used either as a female or a male parent in an inheritance study of resistance to *Pratylenchus penetrans*.

Clone	Resistance traits						Plant size traits					
	Adults and juveniles (number/g dry root)			Eggs (Number/g dry root)			Shoot dry weight (g)			Root dry weight (g)		
	♀	♂	$\bar{x}$	♀	♂	$\bar{x}$	♀	♂	$\bar{x}$	♀	♂	$\bar{x}$
M31	718 a	696 a	707 a	90 a	86 a	88 a	0.28 b	0.28 a	0.28 ab	0.18 d	0.16 a	0.17 c
M06	681 ab	1,006 a	844 ab	104 a	191 a	147 ab	0.34 c	0.28 a	0.31 c	0.18 d	0.15 a	0.17 c
B14	987 ab	1,099 a	1,043 abc	188 a	248 a	218 bc	0.29 b	0.28 a	0.28 ab	0.14 b	0.14 a	0.14 a
B09	1,207 bc	1,136 a	1,171 bc	195 a	202 a	198 abc	0.24 a	0.30 b	0.27 a	0.11 a	0.15 a	0.13 a
B02	1,015 ab	1,241 a	1,128 bc	197 a	251 a	224 bc	0.29 b	0.30 b	0.29 b	0.14 bc	0.17 a	0.15 b
B26	1,592 c	1,024 a	1,308 c	382 b	180 a	281 c	0.28 b	0.28 a	0.28 ab	0.16 c	0.15 a	0.16 c

Each value is an average of 18 plants. Means followed by the same letters within a column are not different according to Duncan's multiple-range test ( $P \leq 0.05$ ).

TABLE 4. Mean numbers of *Pratylenchus penetrans* and eggs per g dry root for 15 crosses in a six-alfalfa clone diallel, evaluated in a growth chamber test, 6 weeks after inoculation.

Cross	Resistance classification†	Adults and juveniles (no./g dry root)	Eggs (no./g dry root)
M31 × M06	MR × MR	594 a	94 a
M31 × B26	MR × S	679 a	76 a
M31 × B14	MR × MR	699 a	90 a
M31 × B09	MR × MR	732 a	67 a
M31 × B02	MR × S	827 a	113 a
M06 × B02	MR × S	840 a	154 a
M06 × B14	MR × MR	873 a	224 ab
M06 × B26	MR × S	913 a	171 a
B09 × B02	MR × S	983 a	121 a
B09 × M06	MR × MR	996 a	90 a
B09 × B14	MR × MR	1,147 ab	227 ab
B14 × B26	MR × S	1,228 ab	244 ab
B14 × B02	MR × S	1,263 ab	300 abc
B02 × B26	S × S	1,724 bc	428 bc
B09 × B26	MR × S	1,995 c	482 c

† MR = moderately resistant; S = susceptible.

Each value is an average of 18 F<sub>2</sub> progenies. Means followed by the same letters within a column are not different according to Duncan's multiple-range test ( $P \leq 0.05$ ).

*penetrans*, plants from both populations were among the most susceptible and most resistant. Townshend and Baenziger (18) reported that alfalfa clones derived from the cultivars Angus and Algonquin varied for numbers of *P. penetrans* per g fresh root weight. Individual plants within most alfalfa cultivars differ for many inherited characteristics, including disease, nematode, and insect resistances (8,13). The cross-pollinated, tetraploid-inheritance characteristics of alfalfa contribute to the existence of this type of variability.

Resistance and susceptibility of alfalfa clones to *P. penetrans* were indicated by numbers of nematodes per plant and reproductive factor in both Clonal Tests 1 and 2. Clones M31, M06, B09, and B14 were among the most resistant, and B26 and B02 were among the most susceptible. The reproductive factor of the four resistant clones was less than one-half that of the two susceptible clones.

In general, the resistance classification of the checks agreed with those of previous studies (11,15). The high correlation between parental clone means and S<sub>1</sub> progeny means for numbers of nematodes in the roots indicates good agreement between the studies for classification of resis-

tance among parental clones and S<sub>1</sub> progenies. This correlation also indicates that resistance to *P. penetrans* is conditioned by additive gene action (4).

General combining ability was most important in predicting progeny performance for nematode resistance traits among crosses of the six parental clones in this experiment. The significant reciprocal effects for one nematode resistance trait and for both plant size traits may hinder selection progress in long-term selection programs. Specific combining ability effects were more important than GCA effects for predicting progeny performance for plant size traits. However, there was no apparent effect of plant size on nematode numbers, indicated by the lack of correlation between nematode resistance traits and plant size traits. Therefore, large SCA effects for plant size should not affect selection for nematode resistance. The high correlations between half-sib family means and S<sub>1</sub> family means and between half-sib family means and parental clones for nematodes per g dry root indicate the importance of additive effects in the inheritance of resistance to *P. penetrans* for these parental alfalfa clones.

Because these six clones may not be rep-

representative of alfalfa genotypes as a whole, inferences regarding GCA, SCA, and reciprocal effects need to be made with caution. The large GCA effects for nematode resistance traits should allow significant progress from selection using methods that utilize GCA effects. Breeding procedures that capitalize on additive effects should be effective in developing alfalfa cultivars with resistance to *P. penetrans*.

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