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SCREENING OF ALFALFA CULTIVARS TO THE LESION NEMATODE *PRATYLENCHUS PENETRANS* FOR COMMERCIAL RELEASE

by
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Summary. In three separate experiments in a glasshouse 37 alfalfa cultivars were evaluated for their tolerance to the lesion nematode *Pratylenchus penetrans* compared with the susceptible cultivar 'Baker'. Dry weight and fresh weight of foliar and root system along with soil, root and total nematode population were considered for tolerance level of alfalfa. In the first (13 cultivars) and second (11 cultivars) experiment total and root population of all the tested cultivars were significantly lower as compared to the susceptible cv Baker. In two cultivars, ZN 9541 and ZN 9651 a significant reduction in root and total nematode population were observed with an increase in shoot dry weight. In two cultivars, ZM 9421 and ZB 9546, fresh shoot weight significantly increased as compared to the susceptible cultivars. In the third experiment among the 13 cultivars tested, a significant reduction in nematode population with an increase in fresh and dry weight of root along with fresh shoot weight was observed in ZC 9751A. However, in two cultivars although there was no significant change in nematode population, fresh shoot weight and dry root weight increased.

Plant parasitic nematodes are one of the major constraints on irrigated alfalfa production in the USA. Among all, root lesion nematodes *Pratylenchus* spp. are predominantly distributed in Pacific northwest regions (Hafez and Mousa, 1997). The most economically important species of lesion nematode is *Pratylenchus penetrans*, however, relatively uncommon in Idaho. The most common species in Idaho are *P. neglectus* and *P. thornei* (Hafez, 1995). Plants infected with root lesion nematodes do not show above ground symptoms that can positively aid in nematode identification. Above-ground symptoms are more general, and can include stunting and nutrient deficiencies (Griffin, 1991, 1995). Secondary infections of roots by other bacterial and fungal pathogens commonly occur after root lesion nematode invasion. Direct damage to alfalfa roots leads to susceptibility of the plant to other pathogenic bacteria and fungi. Lesion nematode infection not only reduces alfalfa seed and hay yield, but also shows major impact on other crops grown in rotation with alfalfa.

Success of lesion nematode control involves utilization of rotational crops, application of chemicals (Thies *et al.*, 1992) and manipulation of resistant varieties (Barnes *et al.*, 1990; Christie and Townshend, 1992; Thies *et al.*, 1995) in the effective management strategy. However, since lesion nematodes have a very wide host range, and more than one species may occur in a field (O'Bannon and Esser, 1988; Mani *et al.*, 1997) crop rotation is not as effective as resistant cultivars. Chemical management is a common practice (Thies *et al.*, 1992) but environmental concern, health hazards and cost of the chemical made the availability of registered

chemicals to the minimum. Hence development of alfalfa cultivars with resistance to one or more *Pratylenchus* species will probably be the best means of controlling lesion nematode. Though, alfalfa cultivars have been identified and developed with resistance to lesion nematodes, exploitation of resistance are not yet commercially available.

Materials and methods

In the first experiment, tolerance level of 14 alfalfa (*Medicago sativa* L.) cultivars to *P. penetrans* (Cobb, 1917) Filipjev *et* Schuurmans Stekhoven, 1941 were tested under glasshouse conditions. Cones of 150 cc capacity were filled with sterilised soil and seeds of each of alfalfa cultivar were planted in each cone. After germination, seedlings were thinned to three per cone and inoculated with *P. penetrans*, obtained from callus culture, at the rate of 160 nematodes per container. After one week, each container was inoculated with 240 nematodes. Seven weeks after planting, fertilizer (20-20-20) was applied and other cultural practices were carried out regularly. Fifteen weeks after planting plants were uprooted and data on fresh weight of root and shoot were recorded. Nematode population in the root and total population, including root and soil populations, were estimated.

In the second experiment 12 cultivars of alfalfa were tested for their tolerance to *P. penetrans* under glasshouse conditions. The experimental design was a completely randomised block design with four replications of ten containers each. Each container was planted with ten alfalfa seeds and later thinned to 5-7 seedlings per container. Seven days after planting each container was inoculated with *Rhizobium meliloti* D. Two and three weeks after planting each of the containers were inoculated with 45 and 40 *P. penetrans* per ml of soil, respectively. Eleven weeks after last inoculation, plants were harvested and fresh and dry weight were recorded. Soil from each container was subjected to nematode analysis and population was assessed. Roots were removed from the container and nematode population was assessed.

In the third experiment tolerance of 14 alfalfa selections against *P. penetrans* was compared under glasshouse conditions. Soil cones of 150 ml capacity were filled with sterilised soil mixture and each cone contained a final population of three plants. The experimental design was a completely randomised block design with 14 cultivars of four replications. Each replication consisted of seven cones and inoculated with 100 specimens of *P. penetrans* obtained from microplot culture. Hundred days after inoculation, plants were harvested, fresh and dry weight of shoots were determined. After determining the fresh root weight, roots were placed under the mist chamber and the nematodes were extracted and egressed nematodes from the roots were counted. Root dry weight was also determined.

Results and discussion

In the first and second experiment (Tables I and II) root and total nematode population densities of all the tested cultivars were significantly lower as compared to the susceptible cv. Baker. Resistance and susceptibility of alfalfa cultivars to *P. penetrans* were indicated by root and total nematode population. In ZN 9541 and ZN 9651 a significant reduction in root and total nematode population were observed with an increase in shoot dry weight (Table I). On the other hand, in the second experiment fresh shoot weight significantly increased while root population and total population decreased in two cultivars ZM 9421 and ZB 9546 as compared to the susceptible cultivar 'Baker' (Table II). Reduction in nematode population in the root and increase in the shoot and root weight of the cultivars indicated the existence of a relationship between these two parameters. Os-

TABLE I - *Mean shoot weight, root weight and nematode population density of alfalfa cultivars inoculated with* Pratylenchus penetrans *and harvested 15 weeks after planting (I experiment).*

Alfalfa cultivar	Shoot weight (g)		Root weight (g)		*P. penetrans* population	
	Fresh	Dry	Fresh	Dry	per g root	Total (soil+root)
ZN 9531	45.4	21.8 a	55.1 abcde	9.5	93 bcd	1045 bc
ZN 9530	49.1	21.6 abc	53.1 bcde	9.9	112 bcd	1323 b
ZN 9533	47.2	21.7 ab	50.0 de	10.0	111 bcd	1310 b
ZN 9540	45.5	21.3 abcd	51.0 cde	9.9	119 b	1350 b
ZN 9541	48.5	21.8 a	50.0 de	10.0	116 bc	1265 bc
ZN 9537	44.4	20.7 cde	52.9 bcde	10.3	84 bcd	1000 bc
ZN 9539	46.6	21.3 abcd	51.8 cde	9.5	60 d	748 c
ZN 9650	49.1	21.7 ab	54.7 abcde	10.6	62 cd	858 bc
ZN 9651	49.2	21.9 a	59.0 ab	10.8	57 d	760 c
ZN 9640	46.3	20.4 de	49.8 de	10.0	69 bcd	848 bc
MNGRN-14	46.4	20.7 cde	56.0 abcd	10.4	92 bcd	1120 bc
MNGRN-16	46.9	20.1 e	59.0 ab	10.0	84 bcd	975 bc
MNGRN-4	46.3	21.3 abcd	59.5 abc	9.3	105 bcd	1145 bc
Baker (susc)	44.6	20.8 bcde	59.6 a	10.0	200 a	2188 a

Values within column followed by the same letter are not significant according to Duncan's Multilple Range Test (P=0.05).

TABLE II - *Mean shoot weight and nematode population density of alfalfa cultivars inoculated with* P. penetrans *and harvested 14 weeks after planting (II experiment).*

Alfalfa cultivar	Fresh shoot weight (g)	*P. penetrans* population	
		per g root	Total (soil+root)
Baker	25.8 cd	230 a	1030 a
MNGRN-4	20.6 e	140 b	780 ab
ZC 9423	28.8 abc	97 bc	680 bc
ZM 9435	30.6 abc	85 bc	493 bcd
ZM 9421	32.3 a	76 bc	533 bcd
ZC 9525	23.3 de	77 bc	428 cd
ZB 9546	31.9 ab	67 bc	493 bcd
ZC 9420	29.7 abc	64 c	490 bcd
ZM 9431	29.6 abc	57 c	413 cd
ZC 9538	26.80 bcd	50 c	368 cd
ZC 9556	26.0 cd	46 c	310 d
ZN 93DD	25.7 cde	44 c	253 d

Values within column followed by the same letter are not significant according to Duncan's Multilple Range Test (P=0.05).

trander *et al.*, (1992) found that in the presence of *P. scribneri* there was a significant difference in root weight between susceptible and resistant alfalfa cultivars.

In six cultivars viz., ZN 9530, ZN 9533, ZN 9540, ZN 9537, ZN 9539 and ZN 9650 in addition to reduction in nematode population, fresh root weight alone increased as compared to the susceptible cultivar. Differences in the chemical composition of the cultivar roots are one of the factors to decide the resistance/susceptible reaction of alfalfa against *P. penetrans.* Defence response gene transcripts in roots of nematode-resistant and susceptible alfalfa plants differed both constitutively and in inductive responses to nematode infection. Baldridge *et al.* (1998) found that after *P. penetrans* infection, the mRNAs declined over 48 h in resistant roots but rose in susceptible plants during the first 12 h after-infection. Acidic beta-1,3-glucanase mRNA levels were initially similar in both root types but accumulated more rapidly in resistant than in susceptible roots after nematode infection. Constitutive levels of the phytoalexin medicarpin were highest in roots of the resistant plants.

In the third experiment (Table III) among the 14 cultivars tested, a significant reduction in nematode population with an increase in fresh and dry weight of root along with fresh shoot weight increase was observed in ZC 9751A. In two cultivars there was no significant change in nematode population but fresh shoot weight and dry root weight increased. In ZX 9352A reduction in root population with an increase in shoot weight was observed but increase in shoot weight alone was observed in ZC 9850A.

TABLE III - *Mean shoot weight, root weight and nematode population density of alfalfa cultivars inoculated with* P. penetrans *and harvested 15 weeks after planting (III experiment).*

Alfalfa cultivar	Shoot weight (g)		Root weight (g)		*P. penetrans* population	
	Fresh	Dry	Fresh	Dry	per g root	Total (soil+root)
ZC-9842A	26.2 abc	7.7	23.8 cde	4.1 cde	79 a	310
Baker (susc)	22.9 cd	7.4	23.7 cde	4.1 e	63 ab	256
ZC-9741A	28.1 ab	8.1	23.5 de	4.7 bcde	60 ab	295
ZC-9840A	26.5 abc	8.2	26.5 abcd	5.7 ab	54 abc	298
ZC-9750A	27.1 abc	7.8	25.7 bcd	5.1 abcde	48 bcd	228
ZC-9850A	28.5 ab	7.9	25.4 bcde	4.8 bcde	48 bcd	228
ZC-9841A	27.0 abc	7.8	26.4 abcd	5.1 abc	47 bcd	215
ZG-9850	17.8 e	6.7	21.8 e	4.1 de	43 bcd	175
ZX-9852	27.6 ab	7.8	26.7 abcd	6.0 a	42 bcd	263
ZC-9851A	26.8 abc	8.2	26.0 abcd	5.0 abcde	34 bcd	150
ZX-9352	30.1 a	8.7	27.2 abc	5.1 abcd	27 cd	143
ZC-9740A	24.8 bc	7.6	25.8 bcd	5.6 ab	26 cd	138
MNGRN-16	19.9 de	7.7	27.8 ab	5.7 ab	26 cd	145
ZC-9751A	30.0 a	8.5	29.5 a	5.5 ab	23 d	128

Values within column followed by the same letter are not significant according to Duncan's Multilple Range Test (p=0.05).

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