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Cereal Cyst Nematode (*Heterodera avenae*) on Oats. II. Early Root Development and Nematode Tolerance[1]

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Abstract: The effect of *Heterodera avenae* infestation on early seminal and lateral root growth was examined in four oat genotypes differing in tolerance to *H. avenae.* Recently emerged seminal roots were inoculated with a range of *H. avenae* larval densities, then transferred to a hydroponic system to remove the effect of later nematode penetration on root development. Intolerance to *H. avenae* was assessed in terms of impairment of seminal root extension resulting in fewer primary lateral roots emerging from the seminal root below the zone of juvenile penetration. Tolerant plants infested with *H. avenae* had longer lateral root systems than infested intolerant plants. The decline in lateral root growth below the penetration zone was partly offset by increased growth above. This did not contribute to tolerance, however, as there were no differences between cultivars for this feature. Nematodes induced earlier nodal root emergence in all cultivars. Nodal root development was most advanced on the most tolerant cultivar.

Key words: Avena sativa, cereal cyst nematode, *Heterodera avenae,* nematode tolerance, oat, root extension.

The cereal cyst nematode, *Heterodera avenae* Woll., is found in most cereal growing regions of the world (12) and is regarded as the most serious root pathogen of cereal crops in the southern wheatbelt of Australia (4). Invasion of seedling roots by *H. avenae* slows root extension and reduces root tip numbers (10,16,17). Radial thickening and profuse short lateral root formation at the invasion site also are typical (8). Impaired root development is the ultimate cause of yield decline of *H. avenae*-infected plants (14,17).

Yields of some oat and wheat cultivars are affected less than others by *H. avenae* and are said to be tolerant (2,6). Root systems of infected, tolerant plants have been found to be less stunted than intolerant plants (5; Volkmar, unpubl.). This may be because roots of tolerant plants are less sensitive to stunting caused by nematode invasion or because growth of similarly infected root systems is more vigorous in tolerant than in intolerant plants.

The objective of this study was to determine whether plants gain their tolerance through insensitivity to damage caused by invading nematodes or through stimulated root growth following invasion.

MATERIALS AND METHODS

Oat (*Avena sativa* L.) seeds were sterilized in 1.0% NaClO for 5 minutes and pregerminated at 20 C for 36 hours on water agar plates after 6 days incubation at 2 C. Seedlings with three emerged roots were planted in plastic electrical conduit (5.4 × 13.0 cm) containing John Innes soil mix composed of equal parts steam-sterilized coarse sand and medium loam amended with fertilizer (g m^{-3}: blood meal, 600; K_2SO_4; KH_2PO_4, 550), but containing no peat. Plants were grown in a controlled environment cabinet maintained at 20-C day and 15-C night temperatures with a 16-hour photoperiod. Light was supplied by a combination of high pressure sodium (60 W) lamps, "Cool White" fluorescent tubes, and incandescent bulbs, to provide a total irradiance of 590 $\mu E/m^2/sec$ at canopy level.

One day after planting, 100, 400, or 900 second-stage juveniles (J2) of *H. avenae* suspended in 0.5 ml water were applied to each plant. Control plants remained uninoculated. After 4 days, six plants of each treatment were taken for assessment of growth and larval population. The remaining plants were removed from their tubes, rinsed lightly to remove sand particles, and laid on a 45-degree inclined sur-

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face overlaid with Whatman chromatography paper which served as a wick supplying aerated half-strength Hoagland's (9) nutrient solution. A layer of black polyester fibre mat was placed over the roots and this, in turn, was covered with clear plastic to reduce moisture loss. The apparatus facilitated the assessment of the effect of larval invasion on extension of the infested root and on subsequent development of the uninfested root system. Plants were returned to the growth cabinet for 16 days. Samples were taken at 4-day intervals following transplanting.

Measurement of individual seminal root lengths was obtained with a ruled line that had distances marked every 0.5 cm. After staining with 0.1% cotton blue for 24 hours, each seminal root was laid straight on a glass plate and its length measured. It was then scanned with a dissecting microscope at 250× magnification to detect sites of nematode penetration. The effects of localized infection on root growth in uninfested parts of the root were determined for each of the three seminal roots. Each seminal root was examined separately for first order lateral root number and first and higher order lateral root length proximal to (zone 1), within (zone 2), and distal to (zone 3) the infected region of the seminal root. A zone commencing 2 cm below the stem base and extending 2 cm in uninfected plants was used for comparison. Root length was estimated using the gridline intersect method (13).

The oat cultivars used were designated as either tolerant or intolerant and susceptible or resistant to *H. avenae,* based on their performance in the field (2) and greenhouse (Volkmar, unpubl.). The cultivar New Zealand Cape (NZC) is resistant and tolerant, Sual is resistant and intolerant, and Stout is susceptible and intolerant. Wild oat species *Avena fatua* was also tested to determine if general disease tolerance observed in wild relatives of cultivated species (3) is also present in oats. Treatments were replicated six times. The experiment was analysed as a completely randomized design. Analyses of variance were

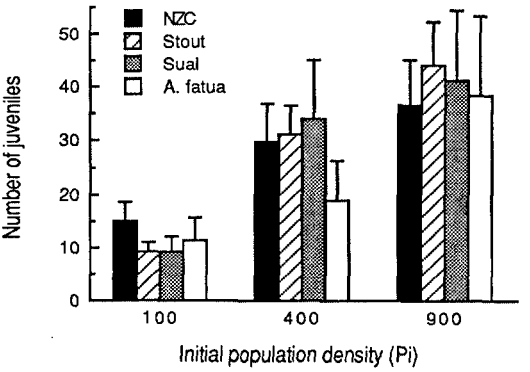


FIG. 1. Effect of initial population density (Pi) of *Heterodera avenae* juveniles on the number of juveniles in seminal roots of three oat cultivars and a wild oat species (*Avenae fatua*) 3 days after inoculation. Vertical bars denote LSD ($P = 0.05$). NZC = New Zealand Cape.

conducted for all variables with least significant differences (LSD) between treatments determined where the F-test was significant.

Root system nomenclature conforms to that used by Hackett (7). A root produced from the base, seed, or stem of the plant is an axis; those arising from the axis are termed first order or primary laterals; those arising from first order laterals are called second order or secondary laterals, and so on. Seminal roots arise from initials present in the ungerminated embryo, whereas nodal axes develop subsequently from nodes on the shoot.

RESULTS

Nematode infection was confined to a zone starting about 2 cm below the base of the stem and extending another 2 cm down the root. The number of *H. avenae* J2 found in roots increased with increasing Pi, with no differences between cultivars (Fig. 1).

Total seminal root lengths (sum of three seminal roots per plant) for NZC and Stout were significantly ($P = 0.05$) greater than those for Sual and *A. fatua* 19 days after inoculation (Table 1). Seminal roots inoculated at Pi of 400 J2 or more attained maximum extension rates later than those inoculated at lower Pi (Fig. 2), resulting in shorter total seminal root length on the

TABLE 1. Total seminal root length (cm) of three oat cultivars and a wild oat species (*Avena fatua*) 22 days after germination and 19 days after inoculation with different population densities of *Heterodera avenae* juveniles (Pi).

Pi	New Zealand Cape	Stout	Sual	*A. fatua*	Mean	LSD ($P = 0.05$)
0	112	106	93	83	98	9
100	101	100	86	66	90	9
400	76	65	52	42	59	7
900	54	42	40	38	43	8
Mean	86	78	68	59		
LSD ($P = 0.01$)	11	12	10	11		

more heavily infested plants. Pi effects were not significant among cultivars.

The effect of Pi on the number of lateral roots in each of the arbitrarily designated root zones after 19 days was variable (Table 2). While lateral roots in zone 1 appeared later on Sual and *A. fatua* than on NZC and Stout, by day 19 cultivar differ-

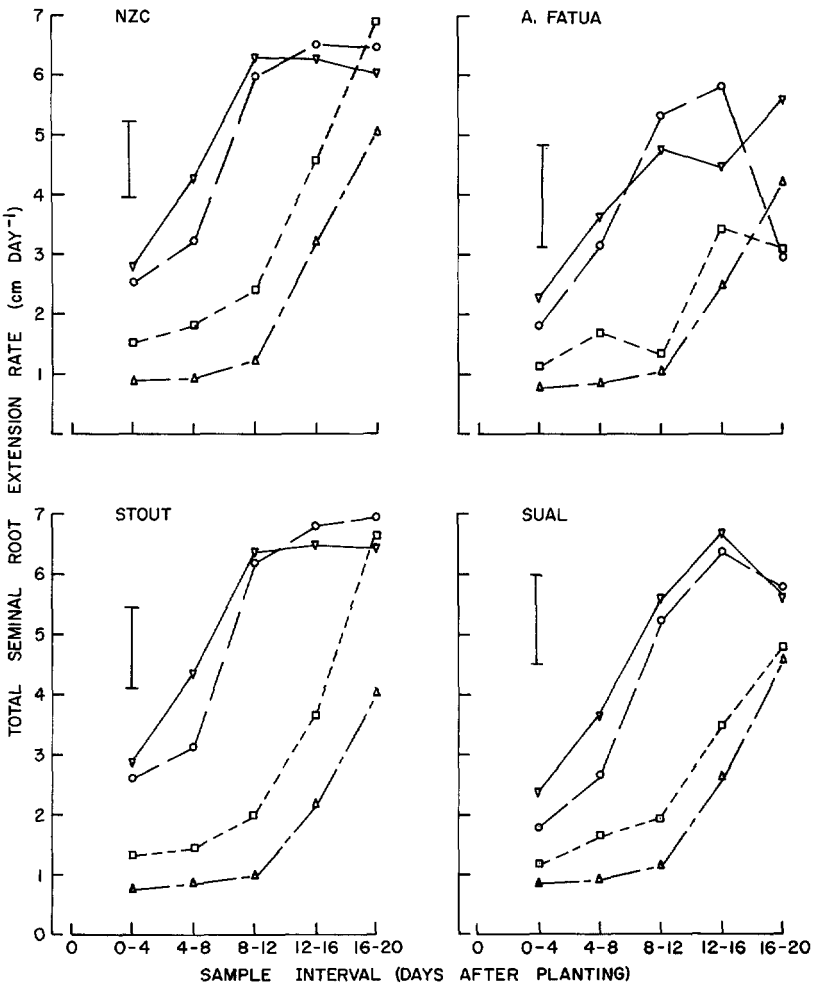
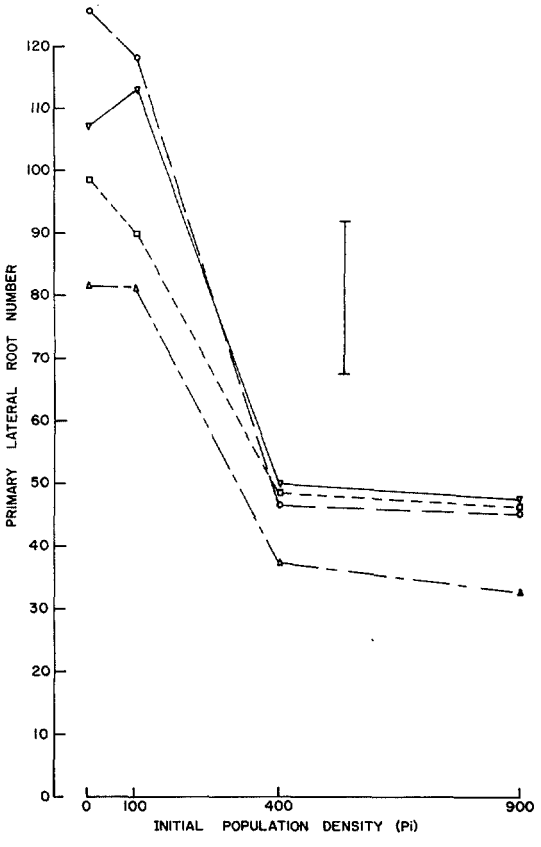


FIG. 2. Effect of initial population density (Pi) of *Heterodera avenae* juveniles on the combined total rate of extension of three seminal roots on three oat cultivars and a wild oat species (*Avenae fatua*) 4–20 days after planting. Pi: ▽——▽ = 0; ○— — —○ = 100; □- - -□ = 400; △—·—△ = 900. Vertical bars denote LSD ($P = 0.05$). NZC = New Zealand Cape.

TABLE 2. Total number of first order laterals on root systems of three oat cultivars and *Avena fatua* above (zone 1), at (zone 2), and below (zone 3) the infected area 22 days after germination and 19 days after inoculation with different population levels of *Heterodera avenae* juveniles (Pi).

Pi	New Zealand Cape	Stout	Sual	*A. fatua*	Mean	LSD ($P = 0.05$)
			Zone 1			
0	19	21	23	16	20	5
100	20	18	20	18	19	6
400	14	19	17	17	17	5
900	17	17	19	14	17	4
Mean	18	19	20	16		
LSD ($P = 0.05$)	6	5	6	6		
			Zone 2			
0	2	1	2	2	2	1
100	3	2	3	3	3	1
400	4	5	4	3	4	2
900	5	6	5	5	5	2
Mean	3	4	4	3		
LSD ($P = 0.05$)	2	2	2	2		
			Zone 3			
0	87	106	73	64	83	18
100	90	99	67	60	79	17
400	31	23	26	17	24	7
900	24	18	19	13	19	6
Mean	58	61	46	38		
LSD ($P = 0.05$)	17	19	17	15		



ences in root number were not significant. Pi had no effect on lateral root number in zone 1. Juvenile invasion in zone 2 significantly increased ($P = 0.05$) lateral root number of all cultivars similarly. In zone 3, nematode infection reduced the number of lateral roots of all cultivars at Pi of 400 or more. Root numbers on uninfected *A. fatua* were significantly less ($P = 0.01$) than on NZC, Stout, and Sual after 19 days.

Taken over the entire root system, nematode infection significantly reduced ($P = 0.01$) the number of primary lateral roots on all cultivars at Pi of 400 J2 or more. In the absence of nematode infection, *A. fatua* had significantly fewer ($P = 0.05$) laterals than the other cultivars (Fig. 3).

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FIG. 3. Effect of initial population density (Pi) of *Heterodera avenae* juveniles on the total number of first order lateral roots of three oat cultivars and a wild oat species (*Avenae fatua*) 4–20 days after planting. ▽——▽ = New Zealand Cape; ○— — —○ = Stout; □- - -□ = Sual; △—·—△ = *A. fatua*. Vertical bars denote LSD ($P = 0.05$).

TABLE 3. Total lateral root length (cm) in the infected area (zone 2) of three oat cultivars and *Avena fatua* 22 days after germination and 19 days after inoculation with different population levels of *Heterodera avenae* juveniles (Pi).

Pi	New Zealand Cape	Stout	Sual	*A. fatua*	Mean	LSD ($P = 0.05$)
0	104	117	72	68	89	13
100	81	68	89	65	76	11
400	27	13	23	21	21	7
900	12	15	8	10	12	4
Mean	56	5	48	40		
LSD ($P = 0.05$)	12	14	11	14		

Lateral root length within zone 1 increased steadily with time as a result of continuous extension and branching from the primary lateral roots (Fig. 4A). Pi effects were not significant, although nematode infection tended to increase lateral root length of all cultivars. Zone 3 lateral root length increased with time (Fig. 4B). At Pi of 400 or more, zone 3 lateral root length was reduced ($P = 0.01$). At Pi above 100 J2, root growth within zone 2 was strongly retarded, comprising at most 4.5% of the total lateral root length (Table 3). The ratio of total lateral root length over total seminal root length after 19 days was not influenced by Pi or cultivar (data not shown).

Cultivar and Pi effects on nodal root number were not significant, but total length of nodal root axes of *A. fatua* was less ($P = 0.05$) than that of the other cultivars (Table 4). Nematode infection enhanced nodal root axis lengths of all cultivars. Primary and secondary lateral root development on nodal axes was advanced only in NZC. The length of primary and secondary nodal roots of all cultivars generally increased with Pi. This was also the pattern of growth of the total nodal root system.

Nematode infection significantly reduced total root lengths of all cultivars (Table 5). After 19 days, root lengths of Sual, Stout, and *A. fatua* were still less than those of controls at the highest Pi.

DISCUSSION

The main effect of *H. avenae* infestation was to suppress seminal root extension, as already reported (14,16). Unique to this investigation was the finding that cultivars differed in the degree to which seminal root growth was impaired, the extent of growth impairment being related to their level of tolerance to *H. avenae*. Accordingly, seminal root length of NZC, a tolerant cultivar, was reduced less by nematodes than seminal root length of Sual and Stout, cultivars intolerant to *H. avenae*. Significant stunting of *A. fatua* suggested that wild oats was not tolerant to *H. avenae*, at least with respect to root growth.

Reduced total lateral root length of infested plants was attributable to a decline in seminal root growth, as indicated by no Pi effects on the ratio of total lateral to seminal root length. Cultivar differences in final root system length therefore arose indirectly from the effect of seminal root stunting on subsequent growth of first and higher order lateral roots. Shorter seminal roots resulted in fewer primary laterals emerging from the region distal to the infested zone. This in turn resulted in shorter root systems on moderately to heavily infected plants. The decline in lateral root growth within zone 3, the region below the infected zone, was partly offset by lateral root development in zone 1, the region above the infected zone. The uninterrupted development of the lateral root system in zone 1 resulted in only marginal effects of Pi on overall root growth. This usually would not occur, however, under natural conditions where the prolonged exposure of roots to nematodes probably would affect both seminal and nodal root growth. Assuming similar sensitivity to growth im-

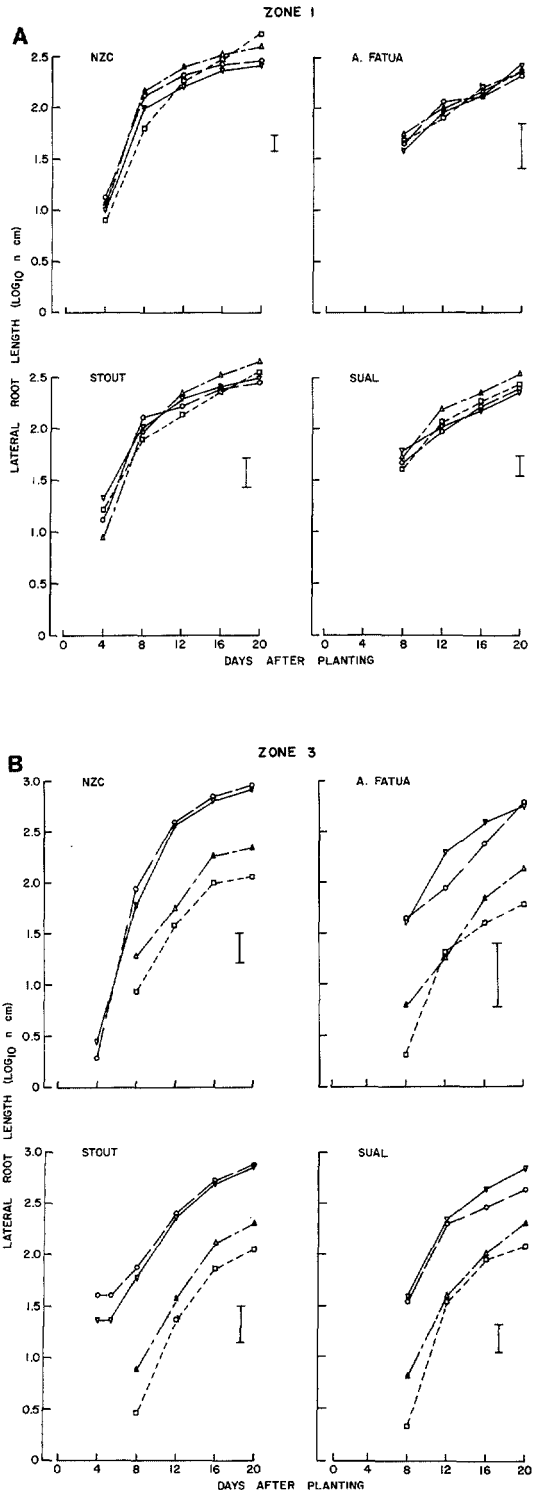

FIG. 4. Effect of initial population density (Pi) of *Heterodera avenae* juveniles on the total length of first and higher order lateral roots of three oat cultivars and a wild oat species (*Avenae fatua*) 4–20 days after planting. A) Above the root infected area (zone 1). B) Below the root infected area (zone 3). Pi: ▽——▽ = 0; ○— — —○ = 100; □- - -□ = 400; △—·—△ = 900. Vertical bars denote LSD ($P = 0.05$).

TABLE 4. Total nodal root number and lengths of axes, first and second order laterals, and total nodal root systems of three oat cultivars and *Avena fatua* 22 days after germination and 19 days after inoculation with different population levels of *Heterodera avenae* juveniles (Pi).

Pi	Nodal roots	Length (cm) Axes	Laterals	Total
	New Zealand Cape			
0	4.2	57	81	138
100	3.9	56	94	150
400	6.3	113	267	380
900	5.7	156	314	470
Mean	5.0	96	189	285
LSD ($P = 0.05$)	2.5	36	51	73
	Stout			
0	5.6	53	34	87
100	5.2	48	19	67
400	4.8	97	133	233
900	5.1	121	189	310
Mean	5.2	80	140	174
LSD ($P = 0.05$)	2.1	27	43	67
	Sual			
0	3.6	46	0	46
100	5.8	52	0	52
400	4.2	69	54	123
900	4.0	91	113	204
Mean	4.4	65	42	106
LSD ($P = 0.05$)	2.1	33	47	66
	A. fatua			
0	2.1	27	21	48
100	1.8	32	16	48
400	2.9	63	128	191
900	3.4	72	76	148
Mean	2.6	49	60	109
LSD ($P = 0.05$)	1.9	38	46	72
LSD ($P = 0.05$) (cultivar):				
0	2.1	19	16	27
100	2.0	17	29	36
400	2.1	29	51	62
900	2.4	43	67	77
Analysis of variance:				
CV	NS	*	**	*
Pi	NS	**	**	**
CV × Pi	NS	NS	*	*

*, $P = 0.05$; **, $P = 0.01$; NS, not significant.

TABLE 5. Total root system length (cm) of three oat cultivars and *Avena fatua* 22 days after germination and 19 days after inoculation with different population levels of *Heterodera avenae* juveniles (Pi).

Pi	New Zealand Cape	Stout	Sual	*A. fatua*	Mean	LSD ($P = 0.05$)
0	1,437	1,396	1,196	822	1,213	289
100	1,491	1,198	1,005	839	1,133	316
400	1,092	791	735	705	832	243
900	1,156	762	661	501	770	237
Mean	1,284	1,037	899	716		
LSD ($P = 0.05$)	309	291	231	304		

pairment in seminal and lateral roots, probably only NZC would escape serious damage by *H. avenae* at comparable Pi in the field.

No apparent relation existed between the rate of extension of uninfected seminal roots and their sensitivity to root growth impairment. Thus, the rate of seminal root extension of NZC surpassed that of Stout at high Pi despite comparable rates of extension of uninfected roots. These findings suggest that vigorous root growth, in itself, is of little significance in the tolerance response of oats to *H. avenae.* This may not be the case, however, in other plant species–nematode interactions.

Nodal roots are important in the late recovery of plants infested by *H. avenae* (1,10). Nodal roots, which emerged relatively late in this study, contributed significantly to the final root length of infected plants. Because of the large absolute contribution of nodal roots to total root system length in the case of NZC, the overall effect of nematode infection at the highest Pi was to reduce total length by only 13% compared with 43% if nodal roots were excluded. Nodal roots had a similar though less dramatic effect on root length of the remaining cultivars. In Australia, where seedling growth may often precede juvenile emergence by several weeks (11), nodal roots may be of marginal importance in the tolerant response if there is an already well-developed seminal root system extending beyond the upper infested soil layer.

The synchrony between seed germination and J2 emergence, as well as J2 proximity to the root, probably influences the relevance of cultivar differences in root growth to nematode tolerance. Tillage practices that disperse cysts deep into the soil would increase the importance of cultivar differences.

The results of this study indicate that nematode tolerance may be related, at least in part, to the lesser impact of nematode invasion on root extension and to earlier development of nodal roots. Further studies are needed to determine why root growth of tolerant and intolerant cultivars differs in response to nematode invasion. The timing of J2 emergence, tillage practices, and time of root development clearly are factors influencing tolerance and should also be investigated.

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