

RESEARCH NOTES

Penetration of Alfalfa Roots by Different Stages of *Pratylenchus penetrans* (Cobb)¹

SUEBSAK SONTIRAT AND R. A. CHAPMAN²

It has been stated generally that all stages of *Pratylenchus* spp. penetrate roots of many plants. In our laboratory, however, during studies of rates of penetration of roots of alfalfa (*Medicago sativa* L.) by *P. penetrans* (Cobb), it appeared that the composition of inocula containing mixtures of stages affected results. Kable and Mai (3) suggested that only fourth-stage larvae and adults of *P. penetrans* invade alfalfa because only these stages were found in roots incubated for three and seven days with inocula containing mixed stages. With two different species of *Pratylenchus*, *P. zaei* Graham and *P. brachyurus* (Godfrey), Southards (6) reported more rapid population increases from inocula containing only adults than from those containing only larvae.

This paper reports the results of our investigation to determine whether the different stages of *P. penetrans* differ in penetrativeness.

Nematodes were collected from *P. penetrans* cultured in alfalfa callus maintained on Krusberg's medium (4). Single 3 to 4-day-old 'Atlantic' alfalfa seedlings, placed on cellulose acetate discs in 50 mm-diameter petri dishes (2), were inoculated with 25 hand-picked specimens of a single stage in 1.5 ml water. The inoculated seedlings were incu-

bated 48 hours in darkness at 20–25 C. After incubation the roots were fixed and stained 48 hr in 10 ml 1:1 (v/v) absolute ethyl alcohol and glacial acetic acid containing one drop of 0.25% aqueous acid fuchsin, then cleared 4 min in concentrated aqueous chloral hydrate (1g/ml). This is another modification of the widely used method initially developed by McBryde (5). After clearing, the roots were placed in lactophenol, destained by the method of Alves and Bergeson (1), and mounted in lactophenol for observation. The results are summarized in Table 1.

No increased penetration by second-stage larvae occurred when numbers were increased (25, 50, or 100/1.5 ml), when the amount of water containing 25 larvae was reduced (1.5, 1.0, or 0.5 ml), or when adults (5, 10, or 25 males or females) were added to 1.5 ml suspensions of 25 larvae.

TABLE 1. Number of *Pratylenchus penetrans* in roots of alfalfa seedlings each inoculated with 25 females (F), males (M), fourth-stage larvae (L4), or second-stage larvae (L2). The incubation period was 48 hr and the data are means of four replicates.

Expt. No.	Seedling age (hr)	Nematodes in roots			
		F	M	L4	L2
1	72	22.0 ^{a†}	13.5 ^{b**}	17.5 ^{ab}	0.8 ^{c**}
2	72	19.0 ^a	16.5 ^a	15.0 ^a	4.3 ^{b**}
3	72	18.8 ^a	16.5 ^a	16.0 ^a	
4	72	16.0 ^a	15.8 ^a		0.8 ^{b**}
5	72	19.0 ^a	13.0 ^{b*}		
6	96	18.3 ^a			1.0 ^{b**}
7	96		7.3 ^a	2.3 ^a	

† Means with the same superscript do not differ significantly.

* Difference significant at the 5% level (t-test).

** Difference significant at the 1% level (t-test).

Received for publication 25 November 1969.

¹ From a thesis submitted by the senior author for the M.S. in Agriculture at the University of Kentucky. Published with permission of the Director of the University of Kentucky Agricultural Experiment Station as Journal Article No. 69-11-147.

² Graduate Student and Professor of Plant Pathology, University of Kentucky, Lexington, Kentucky 40506. Present address of the senior author: Northeastern Agricultural Research Center, Tha Pra, Khon Kaen, Thailand.

These results confirm the observation of Kable and Mai (3). They also point up the necessity for knowing quality as well as quantity in inocula used for studying penetration and population development of this nematode. Inocula containing high proportions of males and/or larvae, especially second stage larvae, would be inferior to those containing high proportions of females.

LITERATURE CITED

1. ALVES, L. M., and G. B. BERGESON. 1967. A quick destaining procedure for showing contrast between nematodes and root tissue. *Plant Dis. Rep.* 51:511.
2. CHAPMAN, R. A., and M. J. EASON. 1969. A technique for studying penetration of roots of plants by endoparasitic nematodes. *J. Nematol.* 1:279-280.
3. KABLE, P. F., and W. F. MAI. 1968. Influence of soil moisture on *Pratylenchus penetrans*. *Nematologica* 14:101-122.
4. KRUSBERG, L. R. 1961. Studies on the culturing and parasitism of plant parasitic nematodes, in particular *Ditylenchus dipsaci* and *Aphelenchoides ritzemabosi* on alfalfa tissues. *Nematologica* 6:181-200.
5. MCBRYDE, M. C. 1936. A method of demonstrating rust hyphae and haustoria in unsectioned leaf tissue. *Amer. J. Bot.* 23:686-689.
6. SOUTHARDS, C. J. 1968. The influence of the stage of development of lesion nematodes on population dynamics and subsequent host response. *Nematologica* 14:15-16 (Abstr.).