

## RESEARCH NOTES

### Technique for Quantifying Injury to Seedling Soybeans by *Pratylenchus penetrans* Without Sacrificing the Plant<sup>1</sup>

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Lesion nematodes (*Pratylenchus* spp.) are abundant in soybean fields in Illinois and Indiana (2, 3) and are capable of causing damage to soybeans (1, 5). Use of nematicides to protect soybeans is not economical. Breeding resistant varieties may be a more logical approach, but a technique which might prove adequate for fast and effective screening of soybean lines has not been available. A technique is described herein which might be satisfactory.

Seeds of soybean (*Glycine max* L. 'Clark 63') were soaked 30 min in 10% commercial bleach solution (0.5% NaOCl) and rinsed in sterile water, and four seeds placed hilum down

in a diSPo® growth pouch over holes punched in the trough of the paper wick (diSPo pouches are made of clear plastic, 16.5 X 17.5 cm, and contain a paper wick folded at the top to form a trough). Preliminary tests showed that it was not necessary to sterilize the pouches (5). The charged pouches were placed at 25-27 C until the germinating tap root was 0.5-3 cm long (18-36 hr). Seeds that failed to germinate were replaced with a supplemental supply of seedlings.

*Pratylenchus penetrans*, obtained from callus culture, were used as inoculum. From 1000 to 40,000 total specimens in water suspension were added/pouch in 3, 5, or 10

TABLE 1. Results from tests designed to establish procedures for inoculation of soybean seedlings with lesion nematodes at 21 C.

Length of test <sup>a</sup> (days)	Inoculation with <i>Pratylenchus penetrans</i>				Growth parameters <sup>b</sup> exhibiting significant differences (t-test)			
	Total nemas/ seedling <sup>c</sup>	No. inoculations to apply nematodes	Interval between inoculations	Vol. fluid/ inoculation	RW	TRL	LLL	TNL
			hr	ml				
14	250	5	24	4				
14	4000	5	24	4	**	*		*
10	8500	3	24	3 <sup>d</sup>	**		**	*
9	6500	5	24	4		**	**	*
9	6500	10	12	4		**	**	
9	8000 <sup>e</sup>	5	24	2	**	**	**	**
9	8000 <sup>f</sup>	5	24	2	**	**	**	**
11	10,000	5	24	2	**	**	**	**

<sup>a</sup> An uninoculated control treatment was included in each test.

<sup>b</sup> RW = root weight; TRL = tap root length; LLL = length of longest lateral root; TNL = total number of lateral roots; \*\* differences between inoculated and control significant at 1% level; \* 5% level.

<sup>c</sup> Divided equally among inoculations, except as noted.

<sup>d</sup> The first inoculation was 1 ml/pouch.

<sup>e</sup> Number applied per pouch of four seedlings at each of the five inoculations was 16,000; 4000; 4000; 4000; and 4000; respectively.

<sup>f</sup> Number applied per pouch of four seedlings at each of the five inoculations was 10,000; 8000; 6000; 4000; and 4000; respectively.

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inoculations with an interval of 12 or 24 hr. The level of nematodes per inoculation was held constant in some trials and varied with time in others (Table 1). A maximum of 2 ml of fluid/inoculation was found to diminish loss of inoculum to the bottom of the pouch. Pouches without inoculum served as controls.

All pouches were placed in a growth chamber at  $21 \pm 1$  C with a 16-hr photoperiod for 9-14 days. Duration of a test was from start of seed preparation to termination.

Seedling growth was determined by fresh weight of roots (blotted to remove moisture), length of tap root, length of longest lateral root, and total number of lateral roots. An estimate of nematode penetration was made by determining the number of specimens which emerged from root systems incubated individually for 1 week in 100 ml of continuously aerated deionized water. In eight tests, an average of 7% of the inoculum was recovered.

Growth reduction of the inoculated root system (when compared to the uninoculated system) was taken as the criterion for a successful test system. Consistent responses to lesion nematodes for all four root measurements could be obtained using a total of 8000 or 10,000 nematodes/seedling applied over 5 days in five inoculations. Since significant reductions in growth were apparent using all four parameters (Table 1), sacrifice of the plant to obtain root weight data is not necessary.

Although large numbers of nematodes are required for this test, it is still practical in terms of inoculum and time required. According to Riedel and Foster (4), very large numbers (30,000 to 40,000/test tube) of *P. penetrans* are easily produced.

#### LITERATURE CITED

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