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Invasion and reproduction of *Pratylenchus penetrans* in birdsfoot trefoil cultivars

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Greenhouse trials were conducted to determine the levels of invasion of birdsfoot trefoil *(Lotus corniculatus)* cultivars and lines by the root-lesion nematode *(Pratylenchus penetrans).* Numbers of nematodes in roots grown in 50-cm^3 polystyrene starter pots were determined 6 weeks after planting. Nematodes were detected in the roots of all cultivars and lines, though the degree of invasion varied significantly. In the first screening trial, carried out in 1994 on 23 cultivars and lines, NB90-104, Upstart, and Viking harbored the lowest population densities of nematodes with levels below 1 000 g^{-1} of dry root, while Fergus and EPF had population densities over 30 000 g^{-1} of dry root. In the second screening trial conducted in 1995, all nine cultivars and lines tested, including NB90-104, Upstart, and Viking, had nematode levels greater than 7 900 g^{-1} of dry root. The results indicated that the cultivars and lines tested in this study exhibited wide genetic variability for invasion by root-lesion nematodes.

[Envahissement et reproduction du *Pratylenchus penetrans* dans des cultivars de lotier corniculé]

Des essais en serre ont été menés afin de déterminer les niveaux d'envahissement de cultivars et de lignées du lotier corniculé *(Lotus corniculatus)* par le nématode des lésions racinaires *(Pratylenchus penetrans).* Le nombre de nématodes présents dans les racines a été déterminé 6 semaines après la plantation dans des pots de démarrage de polystyrène de 50 cm^3. Des nématodes ont été trouvés dans les racines de tous les cultivars et lignées, cependant les degrés d'envahissement différaient significativement. Dans le premier essai de triage, effectué en 1994 avec 23 cultivars et lignées, NB90-104, Upstart et Viking contenaient les plus faibles densités de population de nématodes dans les racines avec des niveaux inférieurs à 1 000 g^{-1} de matière sèche, alors que Fergus et EPF avaient des densités de population supérieures à 30 000 g^{-1} de matière sèche. Dans le deuxième essai de triage, réalisé en 1995, les neuf cultivars et lignées étudiés, y compris NB90-104, Upstart et Viking, avaient des

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niveaux de nématodes supérieurs à 7 900 g^{-1} de matière sèche. Les résultats montrent que les cultivars et lignées examinés dans cette étude ont une grande variabilité génétique face à l'envahissement par le nématode des lésions racinaires.

INTRODUCTION

The root-lesion nematode *Pratylenchus penetrans* (Cobb) parasitizes forage legumes in the Maritime region of Canada (Willis *et al.* 1971), and nematicide treatments in the field have resulted in higher forage yields (Thompson and Willis 1970; Willis and Thompson 1973; Willis *et al.* 1982). In greenhouse tests, *P. penetrans* reduced yields of birdsfoot trefoil *(Lotus corniculatus* L.*)*, red clover *(Trifolium pratense* L.*)*, and alfalfa *(Medicago sativa* L.*)* (Thompson and Willis 1970; Willis and Thompson 1969, 1973).

Nematicides are expensive for nematode control in forage crops, and there are also concerns that residues may contaminate groundwater, soil, and plant products (Good 1972; Marshall 1985). Development of nematode-resistant lines is an attractive alternative to chemicals for management of nematodes in forage legumes. Genetic resistance in alfalfa to root-lesion nematodes has been reported (Christie and Townshend 1992; Nelson *et al.* 1985), and two germplasms with partial field resistance have been released (Barnes *et al.* 1990).

No information is available on tolerance or resistance of birdsfoot trefoil to *P. penetrans.* Resistance to root-lesion nematodes is needed to ensure the long-term persistence of birdsfoot trefoil in the Maritime region and in other temperate regions of North America. This study was conducted to evaluate birdsfoot trefoil cultivars and lines for resistance to root-lesion nematodes in the greenhouse.

MATERIALS AND METHODS

The nematodes used in this study were obtained from a soybean [*Glycine max* (L.) Merr.] field at the Harrington Farm, Agriculture and Agri-Food Canada Research Centre, Charlottetown (lat. 46°21' N, long. 63°9' W). Two hundred female specimens were selected at random and examined under a compound microscope (1000 X). Of these, 195 were identified as *P. penetrans* and the rest resembled *P. crenatus.*

Greenhouse trial, 1994

One hundred plants from each of 23 cultivars or lines were started from seed and grown individually in 50-cm^3 polystyrene starter pots. Due to the size of the trial (2 300 plants), inoculations were staggered and each of three cultivars or lines were planted on 2, 7, 10, 15, 20, 23, 28 June and two on 4 July 1994. Each pot contained 40 g of field soil (70% sand, 20% silt, 10% clay, 2.5% organic matter; pH range of 5.8-6.0) that was carefully mixed by hand in an attempt to approach a uniform inoculum density. The density in each pot was estimated at 4.0 ± 3.37 SE (n = 20) root-lesion nematodes g^{-1} of soil. Nematode extractions were initiated 6 wk after planting, and three procedures were used for evaluations.

In the first procedure, root systems of five seedlings from each cultivar or line from each planting date were stained in a solution of 0.05% methyl blue in lactic acid (Hooper 1986b). After 24 h, the roots were washed in water and destained in 10% lactic acid solution for 24 h. Individual root systems were placed between glass plates (6 cm square, 3 mm thick) and examined under a stereomicroscope.

The second procedure utilized a modification of Young's (1954) incubation method. The root systems of five seedlings from each cultivar or line were removed from the soil, rinsed, and placed individually in polyethylene bags containing 15 mL of sterile tap water. After incubation for 7 d at room temperature (23 ± 2° C), the water containing

nematodes that had exited the roots was poured into a counting dish.

In the third procedure, the remaining 15 plants from each cultivar or line were bulked into three sets of five plants and placed on a fine screen in the mist chamber (Hooper 1986a) for 7 d at 23° C. Nematodes that exited the roots were collected in test tubes in 10 to 15 mL of water and poured into counting dishes.

Greenhouse trial, 1995

Nine cultivars or lines from the 23 used in 1994 were chosen for further study. One hundred plants from each of the nine cultivars or lines were started from seed and grown individually in 50-cm^3 polystyrene pots. Each pot contained 40 g of hand-mixed field soil with an inoculum density of 7.6 ± 3.96 SE (n = 20) root-lesion nematodes g^{-1} of soil. The soil was from a soybean field in the same location at Harrington that was used in the 1994 screening trial.

Due to the size of the trial (900 plants), replicates 1, 2, 3, and 4 of each cultivar or line were planted on 8, 14, 23, and 30 June 1995, respectively. Six wk after planting, nematodes were extracted from individual root systems using the mist chamber. In addition, two stem cuttings were taken from each plant in which nematode counts in roots were zero. The cuttings were treated with rooting hormone (Kerigrow®) and placed individually in 50-cm^3 polystyrene pots containing 50 g of the same field soil at the same nematode density as in the 1995 trial. After 8 wk, root systems were processed in the mist chamber. The root staining technique and the incubation method were not utilized in 1995.

Nematodes and eggs in stained roots or live specimens in counting dishes were examined under a stereomicroscope at 20 to 70 magnification. After numbers of nematodes or eggs were determined in roots, tissue samples were dried for 24 h at 100°C and data expressed as numbers g^{-1} of dry root.

Experimental designs and statistical analyses

The experimental design in each trial was a randomized complete block with four replicates in each treatment. Nematode data were transformed by $\log_{10}$ (X+1) prior to the analyses of variance (Genstat 1993) to achieve homogeneity of variance. Tukey's Honestly Significant Difference test and Duncan's Multiple Range test (SAS Institute Inc. 1985) were used to make comparisons among means.

RESULTS

In the 1994 greenhouse trial, the analysis of variance on log-transformed data indicated that the numbers of nematodes extracted from roots in the mist apparatus and the incubation method, and the numbers of nematodes detected in stained roots were similar (Table 1). Therefore, data from the three methods was combined for the analysis of variance to determine cultivar differences.

Invasion by root-lesion nematodes varied significantly ($P \geq 0.001$) among all the cultivars and lines tested in 1994 and 1995 (Table 2). For the 23 entries tested in the greenhouse in 1994, the heritability (broad sense) was 70% (Becker 1985). In this trial, NB90-104, Upstart and Viking had densities of root lesion nematodes at levels below 1 000 g^{-1} of dry root, while Fergus and EPF had densities over 30 000 g^{-1} of dry root. In the 1995 greenhouse trial, all

Table 1. Comparison of methods for determining root-lesion nematodes in birdsfoot trefoil roots, 6 weeks after planting in greenhouse trial (1994)

Method	No. of nematodes g^{-1} of dry root[a]
Stain[b]	3.80 a (6 310)
Mister	3.89 a (7 760)
Incubation	3.73 a (5 370)
Standard error of the mean	0.282

[a] $\log_{10}$ means with geometric means in parentheses; means followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference test ($P < 0.05$).

[b] Includes eggs as well as juveniles and adults.

Table 2. Numbers of root-lesion nematodes in birdsfoot trefoil cultivars and lines in the greenhouse.

Cultivar or line	1994[§]	1995[‡]
NB90-104	2.78 (600)a[ψ]	4.20 (15 850)c
Upstart	2.95 (890)a	4.13 (13 490)bc
Viking	2.96 (910)a	4.43 (26 920)d
WIT-II	3.70 (5 010)b	3.94 (8 710)a
CH1	3.71 (5 130)b	4.09 (12 300)bc
NB90-102	3.72 (5 250)b	
NB89-103	3.74 (5 500)b	
NB91-105	3.77 (5 890)bc	
Leo	3.85 (7 080)bcd	4.03 (10 720)ab
Frilo	3.86 (7 240)bcd	
NB91-101	3.86 (7 240)bcd	
MN FR	3.88 (7 590)bcd	4.10 (12 590)bc
Bull	4.00 (10 000)cde	
NB90-101	4.01 (10 230)cde	
NB89-100	4.02 (10 470)de	
NB90-103	4.12 (13 180)e	
Empire	4.14 (13 800)e	
Dawn	4.16 (14 450)e	
Georgia One	4.16 (14 450)e	
NSACB-2	4.18 (15 140)e	
BFT-NY	4.24 (17 380)e	3.90 (7 940)a
Fergus	4.48 (30 200)f	4.12 (13 180)bc
EPF	4.50 (31 620)f	
Standard errors of the mean	0.074	0.053

[§] Combined values from root stains and incubation and mist spray extractions.

[‡] Mist spray extraction.

[ψ] Log_{10} mean with geometric mean in parenthesis; means in a column followed by the same letters are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

nine birdsfoot trefoil cultivars and lines tested had nematode levels greater than 7 900 g^{-1} of dry root. Although NB90-104, Upstart, and Viking had the lowest nematode counts in 1994, they were among the highest counts in 1995 at 15 850, 13 490 and 26 920 nematodes g^{-1} of dry root, respectively. Of the 900 plants examined in 1995, 15 root systems were not invaded by nematodes. However, in a subsequent test, all of the root systems developed from cuttings of these 15 plants were infested at levels ranging from 6 400 to 126 300 nematodes g^{-1} of dry root.

DISCUSSION

In the 1994 trial, significant differences were found among the entries based on nematode numbers in roots. The heritability of 70% indicated that most of the variation was due to genetic differences among the entries. However, in the 1995 trial, the results indicated that the variation was due to a more complex genetic system.

It is generally accepted that a close physiological relationship exists between root-knot nematodes *(Meloido-*

gyne spp.*)* and their hosts, and breeding for resistance for this type of nematode has been quite productive (Sidhu and Webster 1981). On the other hand, root-lesion nematodes, which migrate through and feed on cortical tissue, do not have the same intimate association with the host, so breeding for resistance to these nematodes has not met with the same degree of success (Christie and Townshend 1992).

The reaction of birdsfoot trefoil to *P. penetrans* in previous studies (Barnes *et al.* 1990; Nelson *et al.* 1985) indicated a complex mode of inheritance of resistance to this nematode species. The lack of consistent numbers of root-lesion nematodes in roots in the two trials could be attributed to a number of factors. If the response of birdsfoot trefoil to root-lesion nematodes is controlled by several genes, i.e., quantitative inheritance, then several generations of selection or a combination of selection and progeny testing may be necessary to achieve progress, as suggested by Christie and Townshend (1992). In that study, two generations of divergent selection for resistance or susceptibility resulted in some progress, but there was still considerable overlap between the two populations. In our study, we repeated the evaluation of some cultivars, each of which had considerable genetic variation in the first evaluation. This genetic variation could have contributed to some of the differences between the two evaluations. Another possible reason for the variable results is the genetic diversity within the nematode populations. The populations of root-lesion nematodes used in this study came from the same field location, but they represented samples taken in different years, and there is no information on different races that may have been present. In future, the use of greenhouse populations of root-lesion nematodes within the same genetic pool would help to avoid the genetic heterogeneity of field populations.

Information on root-lesion nematodes in forage legumes such as birdsfoot trefoil is useful for crop and pest management programs. The substitution of a birdsfoot trefoil cultivar that harbors low populations of root-lesion nematodes for a species such as red clover, a good nematode host, might alleviate some of the nematode problems in the Maritime provinces. For example, if a birdsfoot trefoil cultivar was found to be a poor host for *P. penetrans*, it could be used by growers in crop rotations with potatoes, a nematode-susceptible crop.

Our results indicated that there may be wide genetic variability among the birdsfoot trefoil cultivars and lines used in this study. However, information on the implication of invasion levels on symptom development and plant persistence is lacking, but studies to further define this are currently underway at our research facilities. Also, the search for degrees of resistance of birdsfoot trefoil genotypes to root-lesion nematodes is continuing.

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