Phytoprotection

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Volume 83, numéro 1, 2002

URI : https://id.erudit.org/iderudit/706228ar
DOI : https://doi.org/10.7202/706228ar

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Éditeur(s)

Société de protection des plantes du Québec (SPPQ)l

ISSN

0031-9511 (imprimé)
1710-1603 (numérique)

Découvrir la revue

Citer cet article

Papadopoulos, Y., Kimpinski, J., McRae, K., Christie, B., Gallant, C. & Fillmore, S. (2002). Invasion and reproduction by rootlesion nematode (*Pratylenchus penetrans*) differs among selected lines of red clover (*Trifolium pratense*). *Phytoprotection*, *83*(1), 41–45. https://doi.org/10.7202/706228ar

Résumé de l'article

Dans une étude en serre, les réponses relatives à l'inoculation par le nématode des lésions racinaires (*Pratylenchus penetrans*) ont été évaluées pour dix-huit cultivars et lignées pour l'amélioration du trèfle rouge (*Trifolium pratense*). La fréquence d'envahissement des plantes et la concentration des nématodes dans les racines étaient généralement correlées, mais il y avait des exceptions. La fréquence d'envahissement des plantes était faible pour un cultivar (Florex), mais la concentration des nématodes dans les racines des plantes envahies était élevée. Trois des plantes testées (CRS 15, CRS 5 et CRS 11) avaient une faible fréquence d'envahissement et une faible concentration de nématodes dans les racines. Un cultivar, AC Kingston, a été évalué comme étant très sensible, alors que la fréquence d'envahissement et la concentration des nématodes dans les racines variaient pour les autres plantes testées.

Short communication / Communication brève

Invasion and reproduction by root-lesion nematode (*Pratylenchus penetrans*) differs among selected lines of red clover (*Trifolium pratense*)

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Received 2001-06-22; accepted 2002-03-26

PHYTOPROTECTION 83 : 41-45

Eighteen cultivars and breeding lines of red clover (*Trifolium pratense*) were evaluated in a greenhouse study for their relative response to inoculation by the root-lesion nematode (*Pratylenchus penetrans*). The incidence of plants invaded and the nematode concentration in the roots were generally related but not always. One cultivar (Florex) had a low incidence of plants being invaded, but a high concentration of nematodes in the roots of invaded plants. Three entries (CRS 15, CRS 5, and CRS 11) displayed a low incidence of plants being invaded and a low concentration of nematodes in the root. One cultivar, AC Kingston, was judged to be highly susceptible, while the remaining entries had differing invasion incidences and differing concentrations of root-lesion nematodes in the roots.

[Variations dans l'établissement et la reproduction du nématode des lésions racinaires (*Pratylenchus penetrans*) entre des lignées choisies de trèfle rouge (*Trifolium pratense*)]

Dans une étude en serre, les réponses relatives à l'inoculation par le nématode des lésions racinaires (*Pratylenchus penetrans*) ont été évaluées pour dix-huit cultivars et lignées pour l'amélioration du trèfle rouge (*Trifolium pratense*). La fréquence d'envahissement des plantes et la concentration des nématodes dans les racines étaient généralement correlées, mais il y avait des exceptions. La fréquence d'envahissement des plantes était faible pour un cultivar (Florex), mais la concentration des nématodes dans les racines des plantes envahies était élevée. Trois des plantes testées (CRS 15, CRS 5 et CRS 11) avaient une faible fréquence d'envahissement et une faible concentration de nématodes dans les racines. Un cultivar, AC Kingston, a été évalué comme étant très sensible, alors que la fréquence d'envahissement et la concentration des nématodes dans les racines variaient pour les autres plantes testées.

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Although red clover (*Trifolium pratense* L.) is a widely adapted and productive forage legume species in the Canadian Maritime provinces, commercial cultivars do not persist well in pasture. This is largely attributed to the nature of the species, particularly its poor winter hardiness and/or its susceptibility to numerous root and crown diseases. The root-lesion nematode (*Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans Stekhoven) affects forage legumes in the Maritime region, especially red clover crops, and may play a major role in reducing persistence (Thompson and Willis 1970; Willis and Thompson 1973; Willis *et al.* 1971, 1982). In a greenhouse study, *P. penetrans* was shown to reduce forage yields of birdsfoot trefoil (*Lotus corniculatus* L.), red clover, and alfalfa (*Medicago sativa* L.) (Thompson and Willis 1970; Willis and Thompson 1969, 1973). Although field applications of nematicides can be used to increase yields of forage, potato, carrot, strawberry, and vegetable crops (Kimpinski and Thompson 1990; Thompson and Willis 1970; Willis and Thompson 1973; Willis *et al.* 1982), chemical control methods are expensive and can be damaging to the environment. An alternative method of improving the persistence of forage legume species is to identify and adopt nematode-resistant cultivars (Kimpinski and Thompson 1990). In this study we assessed a wide range of red clover lines for resistance to the root-lesion nematode.

Entries chosen for this study included five cultivars from the recommended list for the Atlantic Provinces (Atlantic Provinces Agricultural Services Coordinating Committee 2001) and 12 other breeding lines (selected for their diverse genetic background from the red clover breeding program at Agriculture and Agri-Food Canada, Crops and Livestock Research Centre, Charlottetown, Prince Edward Island, Canada). Also included in this investigation was an older cultivar, Florex, which was used as a reference standard in previous evaluation trials conducted at our research center (Atlantic Provinces Agricultural Services Coordinating Committee 2001).

The nematodes used in the study were obtained from soil (70% sand, 20% silt, 10% clay, 2.5% organic matter; pH range of 5.8-6.0) taken from a soybean (*Glycine max* (L.) Merr.) field at the Harrington Farm, Crops and Livestock Research Centre, (lat. 46°21'N, long. 63°9'W). The root-lesion nematode density was estimated at 5.5 ± 2.63 SE ($n = 34$) g^{-1} of soil.

In the greenhouse with a randomized complete block design, 20 plants from each of the 18 entries were grown singly in pots containing 40 g of nematode-infested soil. Experimental units, consisting of five pots of each entry, were randomized to positions within each of the four replicates on the greenhouse bench. Pots were watered using a mister with an electronic timer, which released 7 L of water over an area of 11 m^2 on a greenhouse bench five times per d. Six wk after planting, seedlings were removed from the soil and top growth was removed and discarded. To extract the nematodes, roots were rinsed with tap water and placed on support screens in funnels over 100-ml test tubes, then placed in a mist chamber (Hooper 1986). The temperature in the mister was 24°C and the roots were kept moist with fine mist for 45 s every 10 min. After 7 d, the test tubes were removed from the mist chamber, the liquid suspensions were left to settle for 1 h, and then the supernatant in each tube siphoned to obtain a 20-mL suspension. A 10-mL sub-sample was poured over a microscope grid and the nematodes were counted. After extraction, the roots were left to dry for at least 24 h at 98°C to determine the concentration of nematodes per gram of root dry matter.

A discontinuity on the measurement scale for the nematode concentration in the roots required statistical attention. No nematodes were counted on the microscope slide for some plants, but most plants had a concentration in the hundreds, thousands, or ten thousands of nematodes per gram of dry root. The uneven differences in the magnitude of the concentrations precludes an analysis of the full data set on a continuous scale with ANOVA or linear regression.

Two aspects of the data can be described with common statistical methods. We estimated the plant incidence, or probability, of invasion for each cultivar from the fraction of plants in each replicate with zero counts. From the invaded plant data, we determined the average nematode concentration in the roots on a dry matter basis, i.e., that due to invasion and reproduction.

The plant incidence data were binomial in nature but more variable. The probability of plants being invaded for each entry was estimated by a generalized linear model (GLM) with the variance, or extra dispersion, estimated from among replicates (McCullagh and Nelder 1989). Differences among entries in the GLM linear predictor were identified by Student's two-sided *t*-statistic (α = 0.05).

Nematode concentration in the roots of infected plants were transformed by $\log_{10}$ (X) prior to the analyses of variance to stabilize the variance. Entries were compared by Student's two-sided *t*-statistic (α = 0.05) on the logarithmic scale, but back-transformed means were calculated for presentation.

A combined GLM analysis of the plant incidence and the nematode concentration data was calculated for the ordinal scale data in Table 1. Four categories for plants were defined for 1 g of dry root of invaded plants: 0, zero nematodes; 10^2+, from 100 up to 999 nematodes; 10^3+, from 1000 up to 9999 nematodes; and10^4+, from 10 000 up to 100 000 nematodes. A fifth possible category, for counts from 1 to 99, was excluded from the analysis because it contained zero counts. In the proportional-odds model for this GLM analysis, a multinomial distribution was assumed with cumulative probabilities over the four increasingly severe categories, i.e., not invaded, and three categories of increasing concentrations. The cultivar/breeding line effect is average shift in the log-odds ratio [log ((Prob. of being in a higher category than *i*) / (Prob. of being in category *i* or lower)) for *i* in the first three categories] relative to the reference entry, CRS 15. The odds-ratio between any two entries is assumed to be constant over the first three categories. The extent of extra dispersion was estimated from the data. Differences among entries were compared using Student's two-sided *t*-statistic (α = 0.05). All the statistical analyses were conducted with Genstat 5 (Genstat 5 Committee 1993).

Of the 18 entries inoculated in this study, none was identified as being immune to root-lesion invasion, i.e., without nematodes in the roots, but there were significant differences between cultivars (Table 1). The incidence of plants invaded and the nematode concentration in the roots were generally related, except for the cultivar Florex. It had low incidence (55%) with nine plants having zero counts, but in its invaded plants the nematode average concentration was numerically the greatest (9800 nematodes g^{-1} dry root). Three breeding lines from the Charlottetown red clover breeding program (CRS 15, CRS 5, CRS 11) displayed low plant incidence to invasion and a low concentration of nematodes in the roots (Table 1). The GLM analysis distinguishes between the entries to a greater extent than the analysis for plant invasion incidence and the analysis for nematode concentration in the roots; this result is due to the GLM analysis combining both sources of information. At one extreme, the entries were characterized by having a higher probability of their plants being in the '0' and '10^2+' categories, indicating lower susceptibility to plant invasion and lower concentrations of *P. penetrans* in the roots. At the other extreme, the entries had a higher probability of their plants being in the '10^3+' and '10^4+' categories, indicating susceptibility to plant invasion and higher concentrations of *P. penetrans* in the roots. This range in susceptibility of entries has also been reported when evaluating alfalfa reaction to root-lesion invasion (Christie and Townshend 1992).

In conclusion, the results of this study indicate a wide variability among current red clover cultivars and breeding lines in their resistance to root-lesion nematode invasion. Cultivars AC Christie, AC Endure, Marino, AC Charlie and AC Kingston, currently recommend-

Table 1. Relative incidence and concentration of *Pratylenchus penetrans* in the roots of red clover cultivars and breeding lines.

	No. of plants in each category of nematode concentration[a]				GLM[b] analysis for ordinal responses	Incidende of plants with nematodes (%)		Nematode concentration in 1 g of dry root of invaded plants	
Cultivar or Breeding Line	0	10^2+	10^3+	10^4+	Diff. from CRS15	Mean	SE	($\log_{10}$ x)	No. g^{-1} dry root
CRS 15	12	6	2	0	0.0 a[c]	40 a[c]	17	2.88	750 a[c]
CRS 5	11	3	5	1	0.48 ab	45 a	17	3.34	2100 abcd
CRS 11	8	7	5	0	0.73 abc	60 ab	17	3.09	1200 ab
CRS 17	6	4	8	2	1.51 abcd	70 ab	16	3.27	1800 abc
Florex	9	1	4	6	1.6 abcd	55 ab	17	3.99	9800 d
CRS 18	5	4	10	1	1.62 abcd	75 ab	15	3.31	2000 abc
CRS 10	4	6	7	3	1.76 bcde	80 ab	14	3.32	2000 abc
AC Endure	3	5	11	1	1.86 bcde	85 ab	12	3.39	2400 abcd
CRS 12	5	3	8	4	2.01 bcde	75 ab	15	3.48	3000 abcd
Marino	2	5	11	2	2.11 bcde	90 b	10	3.37	2300 abcd
CRS 14	1	6	11	2	2.16 cde	95 b	7	3.34	2100 abcd
AC Charlie	4	5	5	6	2.2 cde	80 ab	14	3.48	3000 abcd
CRS 6	2	5	10	3	2.21 cde	90 b	10	3.37	2300 abcd
CRS 9	4	3	8	5	2.32 cde	80 ab	14	3.67	4600 bcd
AC Christie	4	2	10	4	2.32 cde	80 ab	14	3.65	4400 bcd
CRS 13	1	2	16	1	2.45 de	95 b	7	3.32	2000 abc
CRS 7	4	0	11	5	2.68 de	80 ab	14	3.80	6200 cd
AC Kingston	1	4	5	10	3.45 e	95 b	7	3.73	5300 bcd
				Ave SED	0.825	Ave SED		0.235	

[a] Nematode concentration categories: 0 denotes none; 10^i+ denotes 10^i to 10^{i+1} nematodes g^{-1} dry root, where i=2, 3, or 4.
[b] GLM denotes analysis by a generalized linear model for the plant counts in categories that are ordered but unequally spaced.
[c] Means followed by the same letter are not significantly different by Student's *t*-test at the 5% level.

ed by the Atlantic Provinces Agricultural Services Coordinating Committee (2001), were found to be susceptible to highly susceptible. Breeding lines CRS 15, CR 5, and CRS 11, recently developed at the Crops and Livestock Research Centre, were the least susceptible. How the nematode concentration affects symptom development and plant persistence, and the degree of immunity that red clover genotypes have to root-lesion nematode invasion, are questions requiring further investigation.

ACKNOWLEDGEMENT

The authors thank Carol Banks, Chris Costain, Scott Caldwell, Janet McIsaac, and Robert Sterling for technical assistance, Christina McRae from EditWorks for structural editing of the manuscript, and Jeannie Carpenter for word processing.

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