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PLANT PATHOLOGY AND NEMATOLOGY

Pathogenicity of *Pratylenchus brachyurus* on Cotton Plants

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ABSTRACT

Although the root-lesion nematode *Pratylenchus brachyurus* is frequently reported in cotton fields, its pathogenicity is still in question. Two greenhouse trials were carried out to evaluate the pathogenic effect of *P. brachyurus* on cotton cultivar Delta Opal. Four initial population densities were utilized: uninoculated, 12,000, 30,000 and 75,000 nematodes/400 cm^3 of soil. Cotton growth parameters evaluated showed that *P. brachyurus* damaged plants only at the two highest initial population densities. Also, nematode reproduction data suggest that cotton is a suitable host for *P. brachyurus*. We therefore conclude that cotton is tolerant to *P. brachyurus*, as it did not cause severe symptoms in infected plants and only slightly affected plant growth. This information is important for management programs of this nematode utilizing crop rotation.

Cotton yield losses due to attack by phytonematodes are significant in Brazil and worldwide (Koenning et al., 2004; Machado, 2005). In Brazil, three species are economically important: races 3 and 4 of the root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood, the reniform nematode, *Rotylenchulus reniformis* Linford & Oliveira, and the root-lesion nematode, *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurm. Stekh. The species *P. brachyurus* has been reported as the most widespread nematode in cotton fields of Brazil throughout Mato Grosso, Mato Grosso do Sul, Goiás, and Bahia, states accounting for about 95% of the national production (Asmus, 2004; Gielfi et al., 2003; Santos et al., 2009 Silva et al., 2004; Zambiasi et al., 2007).

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Associations of *P. brachyurus* with cotton have been reported in both Brazil and United States (Lordello and Arruda, 1957; Martin et al., 1951; Robbins et al., 1989), and the widespread distribution of the lesion nematode is a concern to Brazilian cotton growers. However, the potential pathogenic effect of the nematode on cotton has not been adequately assessed (Inomoto et al., 2001; Machado et al., 2006; Starr and Mathieson, 1985). For example under greenhouse conditions, high population densities of *P. brachyurus* caused necrosis in cotton roots and resulted in suppressed root growth (Inomoto et al., 2001; Machado et al., 2006; Starr and Mathieson, 1985). However, under field conditions, Machado et al. (2006) did not observe a high level of damage from this nematode, and suggested that lack of damage was probably due to the low population densities that were present.

The objectives of this research were to evaluate 1) the pathogenicity of *P. brachyurus* on cotton under greenhouse conditions, and 2) the suitability of cotton cultivar Delta Opal as a host for *P. brachyurus*. Pathogenicity here is defined as the ability of the nematode to suppress the growth of cotton plants and suitability is defined as the reproductive potential of the nematode on cotton (Roberts, 2002).

MATERIALS AND METHODS

Two greenhouse experiments were conducted at Escola Superior de Agricultura “Luiz de Queiroz” (ESALQ), Piracicaba (22º42′S; 47º38′W), S*ão* Paulo, Brazil. Cotton seeds of the cultivar Delta Opal were sown directly into plastic pots containing 400 cm^3 of soil (sandy soil with 74-76% sand, 6% silt, 18-20% clay, 1.0-1.7% organic matter, pH 5.9-6.1) previously disinfested with methyl bromide (150 cm^3/m^3) (Great Lakes Chemical Corporation; West Lafayette, IN). Seedlings were thinned to one per pot 3 wk after germination.

Pratylenchus brachyurus population Pb20 (Machado et al., 2006) was cultured on okra. To obtain inoculum, okra roots were processed by a modified Baermann funnel (Southey, 1986). A suspension composed of mixed life stages was poured into the soil into two 2- to 4-cm-deep holes beside the root system of 17-day-old seedlings. Four initial population densities (Pi) of Pb20 were used to evaluate the damage threshold of the nematode: uninoculated (0), 12,000, 30,000, and 75,000 nematodes/400 cm^3 of soil. A nutrient solution ($N:P_2O_5:K_2O:Ca:Mg:S$ – formula 15:15:20:1:4:0.4) was applied 15 d after inoculation.

Cotton growth parameters (plant height, dry shoot weight, and fresh root weight), and final nematode populations (Pf) were determined at 54 and 62 d after inoculation in experiments 1 and 2, respectively. Average monthly temperatures ranged from 24.9 to 26.5 °C, in experiment 1, and from 18.8 to 22.6 °C, in experiment 2. Plant shoots were dried at 60 °C for 72 hr to obtain the dry shoot weight. After the shoots were removed, pots were immersed in a bucket containing 4 L of water to separate roots from soil. Soil remaining in the pot and soil obtained from roots were processed using the centrifugal-flotation technique (Jenkins, 1964). Roots were washed with tap water, dried on absorbent paper, cut in 1-cm^2 pieces, and weighed. The entire root system of each replicate was processed for nematode extraction by blender and centrifugal-flotation technique (Coolen and D'Herde, 1972). Nematode Pf was estimated by counting mixed life stages from parasitized roots and soil with the aid of a Peter's slide. The reproductive factor (Rf = Pf/Pi) and nematodes per gram of root tissue were calculated for each replicate.

Experiments were arranged in a completely randomized design, with four treatments (nematode levels) and 10 replicates. Data obtained from plant growth, Rf, and nematodes $(g\ root)^{-1}$ were transformed by $\ln(x + 1)$ prior to analysis of variance and treatments were compared by Fisher´s Least Significant Difference (LSD) Test ($P \leq 0.05$) using SAS software (version 9.1; SAS Institute, Cary, NC, USA). In addition, data from plant growth were submitted to regression analysis and fitted on a linear equation using the same statistical program.

RESULTS AND DISCUSSION

The infected cotton plants did not exhibit any particular symptom in either of the two experiments, except for a slight growth reduction. Plant height was suppressed in both experiments at Pi levels of 30,000 and 75,000 nematodes relative to the uninfested control (Table 1). Dry shoot weight was also lower in experiment 1 at 30,000 and 75,000 nematodes, but not at 12,000. No differences in dry shoot weight among treatments were seen in experiment 2. Similarly, fresh root weights were lower at both 30,000 and 75,000 nematodes in experiment 1, but not in experiment 2. Irregularly elongate lesions were observed on the secondary and tertiary roots of plants inoculated with the two higher Pi. Tap roots were generally free of lesions.

Rf and numbers of nematodes in the roots showed that the Delta Opal cultivar was susceptible to *P. brachyurus* (Table 1), as previously reported on other cotton cultivars (Goulart et al., 1997; Hussey and Roncadori, 1978; Machado et al., 2007). Rf values were low, possibly due to the relatively short experimental periods used. However, the Rf values showed that reproduction of *P. brachyurus* followed an inverse relationship with initial population densities (Table 1), which might be due to intraspecific competition for feeding sites (Inomoto et al., 2001).

Table 1. Effect of *Pratylenchus brachyurus* on the growth of Delta Opal cotton, reproductive factor (Rf), and number of nematodes per gram of roots [Nem $(g\ roots)^{-1}$].

	Plant height (cm)		Dry shoot weight (g)		Fresh root weight (g)		Rf		Nem $(g\ roots)^{-1}$	
Pi^z	Exp. 1^y	Exp. 2^y	Exp. 1^y	Exp. 2^y	Exp. 1^y	Exp. 2^y	Exp. 1^y	Exp. 2^y	Exp. 1^y	Exp. 2^y
0	35.56 a	29.39 a	5.02 a	2.79 a	6.84 a	13.37 a	-^{x}	-^{x}	-^{x}	-^{x}
12,000	32.50 ab	27.66 ab	4.01 ab	2.52 a	7.36 a	15.86 a	1.18 a	1.00 a	2,405.7 a	900.6 b
30,000	30.99 b	27.30 b	3.35 bc	2.35 a	3.82 b	16.52 a	0.27 b	0.69 b	2,907.8 a	1623.6 ab
75,000	30.39 b	25.96 b	2.96 c	2.24 a	3.22 b	13.24 a	0.09 b	0.33 c	2,771.3 a	2281.8 a

^{z} Initial population of nematodes.

^{y} Means followed by the same letter in the columns did not differ at the 5% significance level, according to Fisher's least significant difference (LSD) test.

^{x} Uninoculated control.

Data of plant height, fresh root weight, and dry shoot weight (Table 1) suggest that, at least under greenhouse conditions, cotton is a tolerant host of *P. brachyurus*, because slight effects on plant growth were observed only under high initial population densities. This could be verified, especially in experiment 2, where a reduction of 11.67% in the plant height was observed at the highest Pi (75,000), and no effect was detected in the dry shoot or fresh root weights. This population density is high and is not likely to occur under field conditions. It is possible that plants in experiment 2 appeared more tolerant than those in experiment 1 because they had larger root systems and consequently, fewer nematodes per total root system. Some previous reports also found that high population densities of *P. brachyurus* were necessary to cause damage to cotton under greenhouse conditions (Hussey and Roncadori, 1978; Inomoto et al., 2001; Starr and Mathieson, 1985).

Regression analysis confirmed that cotton is a tolerant host of *P. brachyurus*, because a significant effect on plant growth was observed only under high Pi (Figs. 1and 2). One of the most noticeable effects observed in the infected plants, especially at the highest Pi in experiment 1, was the reduction in dry shoot weight (P < 0.001; Fig. 1), fresh root weight (P = < 0.001; Fig. 2), and plant height (P = 0.005). In experiment 2, a negative effect (P = 0.002) on plant height was also observed, but Pi did not significantly affect the dry shoot weight (P = 0.068; Fig. 1) or fresh root weight (P = 0.065; Fig. 2). The linear equation obtained for the relationship between the dry shoot weight and the Pi of the nematode in experiment 1 indicated that a high Pi such as 30,000 nematodes might decrease the dry shoot weight by only 0.7 g. This value represents 15.7% of the estimated weight of uninfected plants, evidence for tolerance in cultivar Delta Opal to *P. brachyurus*.

In summary, Delta Opal cotton appeared to be a suitable host for *P. brachyurus* and that the nematode might suppress cotton growth at extremely high initial population densities. Regression analysis demonstrated the growth reduction of Delta Opal in the greenhouse was moderate, but these results might underestimate damage under field conditions where other biotic and abiotic factors are likely to enhance plant damage. Therefore, further research is needed to determine the likelihood of damage occurring under field conditions. The lack of aboveground symptoms in infected plants could mask the presence of *P. brachyurus* in the field, thereby compromising subsequent susceptible crops such as soybeans. Therefore, *P. brachyurus* should be managed in cotton to avoid nematode population increase. In a management program, tolerance combined with resistance is more desirable than tolerance alone, because the large, healthy root systems of tolerant plants allow nematode populations to increase. This population increase then creates problems for subsequent susceptible crops. However, the use of tolerant crops may be important in well-managed cropping systems where strategies to control nematodes are integrated or combined.

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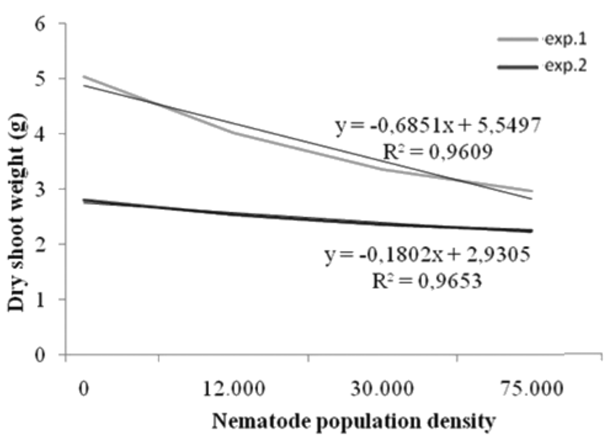

Figure 1. Effect of inoculum densities of Pratylenchus brachyurus on the dry shoot weight of cotton cultivar Delta Opal in experiments 1 and 2.

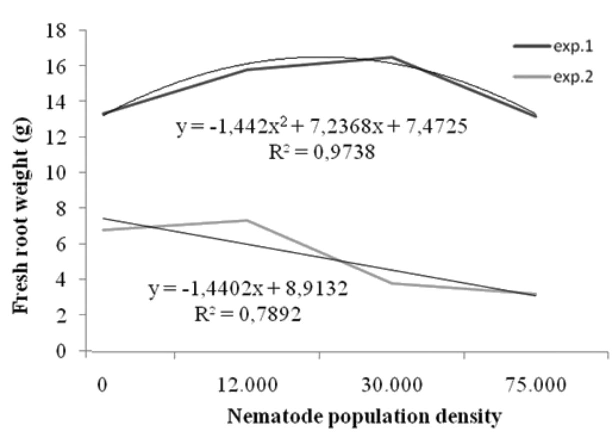

Figure 2. Effect of inoculum densities of Pratylenchus brachyurus on the fresh root weight of cotton cultivar Delta Opal in experiments 1 and 2.

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