The *Journal of Cotton Science 11:288–292 (2007)*
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PLANT PATHOLOGY AND NEMATOLOGY

Response of Cotton Cultivars to Two Brazilian Populations of *Pratylenchus brachyurus* (Godfrey) Filipjev & Sch. Stekh.

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ABSTRACT

***Pratylenchus brachyurus* (Godfrey) Filipjev & Sch. Stekh. is widespread in Brazilian cotton fields and is presumed to cause crop losses. Little information is available concerning its relationship with other crop species. Some economically important crops, such as soybean and maize, are known to be susceptible to *P. brachyurus*, and there is no resistance reported in these plant species to this nematode. Three greenhouse trials were conducted to assess the response of some of the most commonly cultivated and economically important cotton cultivars grown in Brazil to two populations of *P. brachyurus*. The initial population density (Pi) was 1,000 nematodes/plant. Final nematode population data (Pf) was obtained through extraction of nematodes from the growth medium and roots. Nematode reproduction factors (Rf) were determined by dividing the final population (Pf in substrate + Pf in roots) by Pi. All cultivars tested were susceptible or slightly resistant. Rf values varied greatly. These results demonstrate that management of *P. brachyurus* in cotton fields by cultivar selection is not feasible. In addition, the two populations of *P. brachyurus* tested were different in their aggressiveness to the cotton cultivars.**

Phytonematodes cause significant yield losses to cotton in Brazil (Machado, 2005). Although the root-lesion nematode (*Pratylenchus brachyurus*) is widespread across all cotton producing areas of Brazil (Lordello & Arruda, 1957; Asmus, 2004; Silva et al., 2004) and the United States (Robbins et al., 1989; Wrather et al., 1992; Kinloch & Sprenkel, 1994; Baird et al., 1996; Koenning et al., 1999; 2004; McLean & Lawrence, 2000), its importance as a pathogen of cotton has not been extensively assessed under greenhouse or field conditions (Inomoto et al., 2001; Machado et al., 2006). Under greenhouse conditions, high population densities (Pi = 12,000) of *P. brachyurus* caused necrosis in cotton roots and stunted root growth (Inomoto et al., 2001; Machado et al., 2006), which demonstrates that this nematode is pathogenic to cotton. In field trials, Machado et al. (2006) could not demonstrate the same level of pathogenicity for *P. brachyurus* that was observed under greenhouse conditions. This was probably because of the low population densities encountered in the field. The authors predicted that rotation of cotton with highly susceptible host crops could allow the nematode population to increase above the damage threshold.

The host range of *P. brachyurus* includes maize and soybean. These are important crops for production systems used in cotton producing states of Brazil. These cropping systems make management of *P. brachyurus* by crop rotation difficult. The use of cotton cultivars with resistance or moderate resistance to *P. brachyurus* could be an option; however, information on the response of cotton cultivars to *P. brachyurus* is not available. Three greenhouse experiments were conducted to assess the response of 15 cotton cultivars commonly cultivated in Brazil to two populations of *P. brachyurus*.

MATERIALS AND METHODS

Inoculum preparation. Two populations of *P. brachyurus* (Pb20 and Pb22) were used to test for the presence of intraspecific variation in this species, which has been observed in previous research (Machado et al., 2006; Siqueira & Inomoto, 2006; Payan & Dickson, 1988). Pb20 was obtained from okra (*Abelmoschus esculentus* Moench.) roots in Seropédica, Rio de Janeiro state, and Pb22 from cotton roots in Campo Verde, Mato Grosso state (Machado et al., 2006). The nematode was cultured on okra plants and extracted from the roots by a modification of the Baermann method (Southey, 1986). The resulting

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suspension, containing mobile stages of *P. brachyurus*, was used as inoculum for each experiment.

Trials 1, 2, and 3. The trials were conducted under greenhouse conditions at the Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ), Piracicaba (22°42′ S; 47°38′ W; 546 m altitude), Brazil, from 28 Oct. to 18 Dec. 2003 (51 d; Trial 1), 13 Feb. to 29 Apr. 2004 (75 d; Trial 2), and from 12 Mar. to 27 May 2004 (76 d; Trial 3). Seeds were sown directly into plastic pots containing 400 cm^3 of soil 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. The inoculum was distributed into two holes made in the substrate close to the stem. The initial population density (Pi) was 1,000 nematodes/plant. After the inoculation, the seedlings were maintained in a shaded room for 2 d to avoid heat stress on the nematode, and then were transferred to the greenhouse. A nutrient solution ($N:P_2O_5:K_2O$:Ca:Mg:S - formula 15:15:20:1:4:0.4) was used 15 d after inoculation. Thiamethoxan (Syngenta Crop Protection UK Limited; Whittlesford, Cambridge) was used twice, 15 and 30 d after inoculation, to control whiteflys (*Bemisia tabaci* Gennadius).

The cotton cultivars tested were Coodetec 405 (Cooperativa Central de Pesquisa Agrícola; Cascavel, Brazil), FMT Saturno (Fundação Mato Grosso; Rondonópolis, Brazil), Delta Opal (Delta and Pine Land Co.; Scott, MS), BRS Ipê (Empresa Brasileira de Pesquisa Agropecuária; Campina Grande, Brazil), BRS Cedro (Empresa Brasileira de Pesquisa Agropecuária), Makina (Syngenta Seeds; Sao Paulo, Brazil), and Fabrika (Syngenta Seeds) in Trial 1; and Acala 90 (Delta and Pine Land Co.), Delta Penta (Delta and Pine Land Co.), DP4049 (Delta and Pine Land Co.), BRS Aroeira (Empresa Brasileira de Pesquisa Agropecuária), IAC 24 (Instituto Agronômico de Campinas; Campinas; São Paulo, Brazil), ITA 90 (Centro Nacional de Pesquisa Algodoeira; Campina Grande, Brazil), and São Miguel (Fazenda; Mato Grosso, Brazil) in Trial 2. Trial 3 was comprised of some cultivars previously tested in Trials 1 and 2 (Acala 90, Delta Opal, Delta Penta, Coodetec 405, BRS Ipê, BRS Cedro, Makina, and IAC 24) plus Fibermax 966 (Bayer CropSciences; Research Triangle Park, NC).

Pf was obtained 51, 75, and 76 d after inoculation in Trials 1, 2, and 3, respectively. The pots were initially immersed in a bucket containing 4 L of water for separating roots and substrate. This suspension of water and substrate was used for nematode extraction by the centrifugal-flotation technique (Jenkins, 1964). The roots were first gently washed with tap water, dried on absorbing paper, weighed to determine the number of nematodes/g of root, and a 10-g sample was processed for nematode extraction (Coolen & D'Herde, 1972). Nematode final population was estimated by counting nematodes extracted from the substrate and roots, and calculating the total population (Pf = Pf substrate + Pf roots). The reproduction factor (Rf = Pf/Pi) and number of nematodes per gram of fresh root (nematodes/g root) were calculated for each replicate.

Data analysis. Treatments consisted of a factorial arrangement of two populations (Pb20 and Pb22), and seven (Trials 1 and 2) or nine (Trial 3) cultivars, in a completely randomized design, with seven (Trial 1) or five (Trials 2 and 3) replicates. Pf and nematodes/g were transformed using $ln\ (x + 1)$ and analyzed using SANEST software (Departamento de Matemática e Estatística, ESALQ/USP; Piracicaba, Brazil). Means of Rf and nematodes/g were compared with Tukey's Honestly Significant Difference Test ($P = 0.05$).

RESULTS AND DISCUSSION

Values of Rf were numerically smaller in Trial 1 (Rf = 1.33 to 2.34) than in Trials 2 and 3 (Rf = 4.85 to 18.75). Since the values of nematodes/g of root were similar in the three trials (Tables 1, 2, and 3), root growth must have been reduced. A similar variation in the Rf data determined for *P. brachyurus* was reported by Timper & Hanna (2005), which assessed the nematode reproduction on two pearl millet hybrids and on some selected cultivars of cotton, corn, and peanut. In their work, Rf values varied greatly between two similar greenhouse trials. The cotton cultivar DP 5415, Rf was 2.4 in the first trial, but it was less than 1.0 in the second trial.

In Trial 1, Coodetec 405 and Delta Opal had higher mean Rfs than the other cultivars. Coodetec 405, Delta Opal, FMT Saturno, and Makina supported higher levels of nematodes/g root than Fabrika (Table 1). In Trial 2, DP 4049 and Delta Penta had significantly higher mean Rf values than BRS Aroeira, Acala 90, Ita 90, or IAC 24 (Table 2). In Trial 3, Delta Opal and Delta Penta had significantly higher mean Rf values than Fibermax 966, Makina, BRS Cedro, or BRS Ipê (Table 3). In Trials 2 and 3, the cultivars could be separated into groups based

on nematodes/g root that corresponded to groups based Rf values. DP 4049 and Delta Penta had higher numbers of nematodes/g root than Ita 90, BRS Aroeira, and Acala 90 in Trial 2. Delta Opal, Delta Penta, IAC 24, Acala 90, and Coodetec 405 had more nematodes/g root than Makina and BRS Ipê in Trial 3.

Although none of the 15 cultivars tested were highly resistant to *P. brachyurus*, the results of the three trials showed that BRS Cedro, Makina, and BRS Ipê, which had the lowest values for Rf and nematodes/g root, were the most resistant. On the other hand, Delta Opal and Delta Penta were susceptible to *P. brachyurus*.

These results demonstrated that all cultivars tested were either susceptible or had only low levels of resistance to *P. brachyurus.* This makes management of PB on cotton exclusively by cultivar selection difficult. From a practical point of view, if any of these cultivars are grown in a monoculture in an infested area, a progressive increase in the population density of the nematode is expected to occur. Since some of these cultivars have excellent agronomic performance and yield well, they often are the most preferred by Brazilian growers. Therefore, a rotation of these cotton cultivars, which are susceptible to the nematode, and poor hosts of the nematode, as *Crotalaria spectabilis* Roth and *C. breviflora* DC.,

Table 1. Reproduction factor (Rf) and number of nematodes per gram of root (nematodes/g) for populations Pb20 and Pb22 of *Pratylenchus brachyurus* on cotton cultivars in Trial 1

Cultivar	Rf (Pf[z]/Pi)			Nematodes/g		
	Pb_{20}	Pb_{22}	Mean	Pb_{20}	Pb_{22}	Mean
Coodetec 405	1.87	2.82	2.34 a	805	1,467	1,136 a
Delta Opal	2.17	2.23	2.20 a	1,135	1,149	1,142 a
Makina	1.14	2.22	1.68 b	734	1,254	994 ab
Fabrika	1.41	1.73	1.57 b	750	917	833 bc
BRS Cedro	1.07	1.59	1.33 b	740	718	729 c
BRS Ipê	1.19	1.78	1.48 b	440	808	624 d
FMT Saturno	1.09	1.64	1.36 b	656	1,342	999 ab
Mean	1.42 B	2.0 A		751 B	1,093 A	

[z] Pf (final population) is the total number of nematodes recovered from soil and roots. Each value is the mean of seven replicates. Means within a column followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference Test ($P = 0.05$). Plants were inoculated with 1,000 nematodes/plant (Pi).

Table 2. Reproduction factor (Rf) and number of nematodes per gram of roots (nematodes/g) of populations Pb20 and Pb22 of *Pratylenchus brachyurus* on cotton cultivars in Trial 2

Cultivar	Rf (Pf[z]/Pi)			Nematodes/g		
	Pb_{20}	Pb_{22}	Mean	Pb_{20}	Pb_{22}	Mean
DP 4049	14.3	23.2	18.75 a	1,354	2,599	1,976 a
Delta Penta	12.8	20.2	16.50 a	1,464	2,201	1,832 ab
São Miguel	11.2	18.0	14.60 ab	1,153	1,828	1,490 abc
Ita 90	10.4	10.6	10.50 bc	817	794	805 cd
BRS Aroeira	6.5	14.2	10.35 bc	650	1,394	1,022 cd
IAC 24	9.3	8.9	9.10 bc	1,240	901	1,070 bcd
Acala 90	9.6	8.4	9.00 c	938	727	832 d
Mean	10.6 B	14.8 A		1,088 B	1,492 A	

[z] Pf (final populations) is the total number of nematodes recovered from soil and roots. Each value is the mean of five replicates. Means within a column followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference Test ($P = 0.05$). Plants were inoculated with 1,000 nematodes/plant (Pi).

is essential to achieve any level of management of *P. brachyurus*.

Rf values and recovery of Pb per gram of roots determined for Pb22 were higher in all trials, relative to population Pb20 (Tables 1, 2, and 3). Machado et al. (2004) also reported a variation in terms of Rf of different populations of *P. brachyurus* on Fibermax 966 and Delta Opal, including the populations used in the current work (Pb20 and Pb22). In addition, these results also corroborated those reported by Siqueira & Inomoto (2006), which assessed the suitability of different cowpea cultivars (*Vigna unguiculata* L.) in relation to three *P. brachyurus* populations, specifically Pb20, Pb21, and Pb23 (Pb21 and Pb23 were extracted from cotton roots from Serra do Ramalho, Bahia State, Brazil; and Sapezal, Mato Grosso State, Brazil, respectively). The Rf values of Pb21 and Pb23 were higher than those of Pb20 (Pf/Pi = 9.34 versus 26.23 for Pb21; 8.94 versus 23.54 for Pb23; and 1.70 versus 7.30 for Pb20). All of these results support the idea that *P. brachyurus* populations vary in their aggressiveness in relation to some crops and that the origin of each population can play an important role in this variation. O'Bannon & Tommerlin (1970) first reported the existence of races in *P. brachyurus* based on the large variation observed in the number of nematodes in roots of citrus seedlings inoculated with populations from various localities from the United States. This indicates a general trend that the origin of the population of *P. brachyurus* plays an important role in its level of aggressiveness.

Which nematode population to use in assessing the reaction of cultivars is a crucial point in breeding programs, since plants with higher levels of resistance to nematodes can be identified through the use of aggressive populations (Boerma & Hussey, 1992). In addition, the selection of plants with resistance to various populations of a given nematode species, through the use of a mixture of populations from different geographic regions, is clearly desirable (Hussey & Boerma, 1981).

Payan & Dickson (1988) evaluated the reproduction of four populations of *P. brachyurus*, from different hosts and geographic regions of United States (maize and peanut/ Florida; peanut/ Georgia; and soybean/ North Carolina) on seven crops (alfalfa, citrus, maize, soybean, peanut, common bean, and tomato). In two trials carried out under greenhouse conditions, they reported differences in the behavior among the four populations studied based on the reproductive fitness in the plants tested, assessed by the number of nematodes per gram of root. For example, the populations from peanut (Florida and Georgia) showed higher population densities in common bean roots than the other populations. Differences were also observed in relation to the other hosts studied, except for rough lemon (*Citrus jambhiri* Lush.), which did not allow any nematode population to reproduce.

In conclusion, considerable effort will be necessary to develop systems allowing the use of resistant cultivars to provide safe and highly efficient control

Table 3. Reproduction factor (Rf) and number of nematodes per gram of roots (nematodes/g) of populations Pb20 and Pb22 of *Pratylenchus brachyurus* on cotton cultivars in Trial 3

Cultivar	Rf (Pf[z]/Pi)			Nematodes/g		
	Pb_{20}	Pb_{22}	Mean	Pb_{20}	Pb_{22}	Mean
Delta Opal	10.5 b	16.8 c	13.65 a	969	1,590	1,279 a
Delta Penta	9.2 b	14.4 c	11.80 a	930	1,353	1,141 ab
Acala 90	8.8 b	11.4 ab	10.10 ab	770	1,173	971 ab
IAC 24	8.9 b	10.9 ab	9.90 ab	907	1,157	1,032 ab
Coodetec 405	6.9 b	12.8 a	9.85 abc	761	1,363	1,062 ab
Fibermax 966	5.8 b	6.7 c	6.25 bcd	742	850	796 abc
Makina	5.9 b	6.5 bc	6.20 cd	464	585	525 c
BRS Cedro	5.6 b	6.6 a	6.10 bcd	602	782	692 bc
BRS Ipê	4.8 a	4.9 a	4.85 d	417	502	459 c
Mean	7.37 B	10.07 A		729 B	1,039 A	

[z] Pf (final populations) is the total number of nematodes recovered from soil and roots. Each value is the mean of five replicates. Means within a column followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference Test ($P = 0.05$). Plants were inoculated with 1,000 nematodes/plant (Pi).

of *P. brachyurus* in cotton fields. Cotton breeding programs should consider the genetic variability of different populations of this nematode when conducting screening tests for *P. brachyurus*.

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