

Phytonematodes infesting common bean fields in Brazil, and pathogenicity tests with *Pratylenchus brachyurus*

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ABSTRACT

Phytonematodes are among the main pathogens of the common bean. In general, control practices that aim to control these pathogens have not been regularly adopted in Brazil. The objectives of the present study were to evaluate the occurrence of phytonematodes in dry bean fields in Paraná and São Paulo states and estimate the pathogenicity of *Pratylenchus brachyurus* to dry bean in glasshouse experiments. Root and soil were sampled for nematode extraction, identification and estimation of population density. Four glasshouse experiments with different initial population densities were carried out to evaluate the effect of *P. brachyurus* on the growth of dry bean plants. Six species of phytonematodes were detected, namely *Helicotylenchus dihystera*, *P. brachyurus*, *Pratylenchus crenatus*, *Meloidogyne javanica*, *Meloidogyne incognita* and *Rotylenchulus* sp. The spiral nematode *H. dihystera* was found in all samples and was the most abundant species in both states. The lesion nematode *P. brachyurus* was also frequent, reaching 94% in Paraná and 100% in São Paulo. The root-knot nematodes and *Rotylenchulus* sp. were reported only in São Paulo fields (45% and 18% frequency, respectively), and *P. crenatus* only in Paraná (12%). The most abundant nematode in the soil was *H. dihystera*, and in roots was *P. crenatus*. It was demonstrated that densities of 6.66 specimens of *P. brachyurus* per cm³ of soil provoke visible and measurable root decay. In conclusion, survey data showed low phytonematode densities and pathogenicity tests demonstrated that densities above 6.67 specimens of *P. brachyurus* per cm³ of soil cause damage to the dry bean roots.

Keywords: *Phaseolus vulgaris*; *Helicotylenchus dihystera*; *Meloidogyne* spp.; *Rotylenchulus* sp.; root-lesion nematodes.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an herbaceous annual plant widely cultivated for consumption as dry grain (dry bean) or unripe pods (green bean or snap bean). *Phaseolus vulgaris* is a major source of proteins in various countries, mainly in Brazil, China, India, Kenya, Mexico, Myanmar, Tanzania and USA (MIRANDA, 2013). Plant-parasitic nematodes, mainly root-knot nematodes (*Meloidogyne* spp.), are major pathogens of common bean in many producing countries. High occurrence (86%) of these nematodes was reported in dry bean plantations of Kenya (KIMENJU et al., 1999). In California (USA), other species of phytonematodes were reported in dry bean plantations, but only the root-knot nematodes are reported to cause damage (WESTERDAHL, 2014).

Brazil is the first American and third world producer of common dry beans, but it is not a significant producer of green beans (FAO, 2019). In 2020/2021, the estimated area for the production of dry beans was 2,938,400 ha, with a total production of 2,884,900 tonnes in Brazil (CONAB, 2021). São Paulo and Paraná states produced 173,300 and 534,000 tonnes of dry beans in 2020/2021 (CONAB, 2021). In a survey carried out in bean fields from the states of Goiás and

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Minas Gerais, in 2005–2007, root-lesion nematodes, mainly *Pratylenchus brachyurus* (Godfrey), were found in all samples, whereas root-knot nematodes were found in 78% of the samples (MACHADO; LOBO JUNIOR, 2007). These results suggested that *P. brachyurus* is a potential threat for dry bean crops in Brazil, as the estimated production for those states was respectively 353,900 and 529,300 tonnes of dry bean in 2020/2021 (CONAB, 2021). However, surveys in other Brazilian states are not available, and the role of *P. brachyurus* as a bean pathogen was not yet properly evaluated. A single glasshouse experiment, conducted to evaluate the effect of *P. brachyurus* on two bean cultivars, was not conclusive because the results between the cultivars were divergent (MACHADO et al., 2014). Therefore, further studies are necessary to estimate the damage caused by *P. brachyurus* to the bean.

The objectives of the present study were: (i) to determine the nematode species associated with bean plants in ten municipalities of Paraná and São Paulo states; (ii) and to evaluate the pathogenicity of *P. brachyurus* on dry bean plants under glasshouse conditions.

MATERIAL AND METHODS

Climatic characterization of localities

According to the Köppen climatic classification, the localities sampled in Paraná and São Paulo states belong to climatic type Cfb (subtropical temperate/temperate oceanic climate) and Cwa (subtropical warm/monsoon-influenced humid subtropical climate), respectively. Both climatic types are subtropical, but the former is temperate with rainy winter, and the latter is hot with dry winter (ROLIM et al., 2007).

Nematode sampling and extraction

Three small nematode surveys were conducted in the main bean producing municipalities of Paraná state (Cantagalo, Goioxim, Guarapuava, Pinhão, Guamiranga and Prudentópolis) and São Paulo state (Angatuba, Itaí, Itapeva and Paranapanema). Sixteen composite samples were collected in Paraná state (February 2011, corresponding to mid-summer in the Southern Hemisphere), and forty composite samples in São Paulo state (September 2011 in São Paulo, corresponding to late winter in the Southern Hemisphere). An additional survey was conducted from November 2012 to January 2013 (corresponding to mid-spring until mid-summer, in the Southern Hemisphere) in two municipalities of São Paulo state (Itaí and Itapeva), in which 13 composite samples were collected, exclusively to obtain additional specimens of *Meloidogyne* spp.

A composite sample was taken from each field of variable sizes (1 to 86 ha), consisting of ten randomly collected subsamples of roots and soil. Each root subsample was composed of roots from ten (up to 1-month-old) or five (at least 2-months-old) bean plants. All plants were collected at the vegetative phase (V4) or the beginning of the reproductive phase (R6). Soil from each sampled plant's rhizosphere (0–20 cm depth) constituted a soil subsample. Each of the composite samples of roots and soil was placed separately in plastic bags. In the laboratory, the root system was cut into 2-cm-length pieces and mixed thoroughly. Then, a subsample of 10 g was taken for nematode extraction by blender centrifugal-flotation method (COOLEN; D'HERDE, 1972) with kaolin and sucrose solution ($d = 1.15 \text{ g}\cdot\text{mL}^{-1}$), using a centrifuge with four 138-cm³-tubes (4 cm diameter and 11 cm height) at 1,800 rpm and 580 g. Similarly, the soil was mixed systematically, and a subsample of 250 cm³ was taken and processed by the centrifugation-flotation method (JENKINS, 1964).

Nematode identification and estimation of population density

Nematodes were heat-killed at 55 °C and mounted on slides in 2% formaldehyde solution, and examined under a light microscope (Leitz Laborlux L, Wetzlar, Germany, and Leica DMLB, Wetzlar, Germany) at 1,250 or 1,000× magnification. *Helicotylenchus*, *Pratylenchus* and *Rotylenchulus* were identified to species level using identification keys (SHER, 1966; CAFÉ FILHO; HUANG, 1989; SIDDIQI, 2000), and morphological studies for *Helicotylenchus* (FORTUNER et al., 1981; FORTUNER, 1984; SUBBOTIN et al., 2015).

The values of body length (L), stylet length (St) and relative distance from anterior end to the vulva (V%) were measured in 26 females of *Helicotylenchus* from all samples of Paraná state. Seven qualitative characters were examined in 100 females and juveniles of *Helicotylenchus* from all samples of Paraná state and 200 females and juveniles of *Helicotylenchus* from

all samples of São Paulo state: lip region, the shape of stylet knobs, shape of the spermatheca, phasmid location relative to anus, areolation of outer bands of lateral fields, inner lines of lateral fields and tail shape.

L, St and V% were measured in 96 females of *Pratylenchus* from Paraná state (1–16 females per composite sample, except for three samples without females) and 125 females of the same genus from São Paulo state (4–11 females per composite sample).

Juveniles, females and males of *Meloidogyne* were detected in samples from São Paulo state, but species identification was not possible due to the low number of specimens. In the subsequent sampling, only two root samples exhibited visible galls. Therefore, the roots and soil from each sample were potted in a 1,000-cm³-pot (10.75 cm diameter and 11.0 cm height), and a 2-week-old seedling of tomato ‘Rutgers’ was transplanted in each pot and was maintained for four months to obtain females for the correct identification. Galled roots were observed in five samples from Itaí and two from Itapeva. Females were extracted from the tomato galls using a needle under a stereomicroscope at 40× magnification. Species identification was based on α -esterase phenotype (CARNEIRO et al., 1996). Eleven to 26 females of each sample were placed individually in the extraction solution buffer and macerated with a piston. The resulting homogenates were used for α -esterase electrophoresis in a Bio-Rad III apparatus, with stacking (4%) and separating (7%) polyacrylamide gels. The whole system was subjected to 100 V for 15 min and 200 V for 45 min at 4 °C (CARNEIRO; ALMEIDA, 2001). Staining was conducted with a solution of α -naphthyl acetate and Fast Blue RR Salt at 37 °C for 30 min (CARNEIRO; ALMEIDA, 2001). Phenotype J3 of *M. javanica* was used as a reference.

Additionally, slides with perineal sections of *Meloidogyne* females were mounted in dehydrated glycerin for species identification (EISENBACK, 1985). Six to eight specimens were prepared per sample of each field, when possible. The perineal sections were examined under a light microscope at 1,000× magnification and compared with patterns available in the literature (EISENBACK, 1985; JEPSON, 1987).

Glasshouse experiments

Although the survey demonstrated higher densities of *H. dihystra* than *P. brachyurus*, the latter was chosen for the pathogenicity test because it is currently a major pest for cash crops in Brazil, mainly soybean (DEBIASI et al., 2011), contrary to *H. dihystra*. The soil was collected in a cotton field from Sapezal, Mato Grosso state, Brazil, to obtain the *P. brachyurus* population (Pb23). This soil was potted, and corn (*Zea mays* L.) was sown and grown for two months. The nematodes were recovered from maize roots by blender centrifugal-flotation (COOLEN; D’HERDE, 1972), and females were picked under a stereomicroscope. A water suspension containing 30 to 65 *P. brachyurus* females was inoculated in okra seedlings cropped in 8-L clay pots containing 6 L of steam-treated soil (121 °C for 2 h).

This population has been maintained for 12 years in pots cultivated alternately with okra (*Abelmoschus esculentus* L.), maize (*Zea mays* L.), grain sorghum (*Sorghum bicolor* (L.) Moench) and cotton (*Gossypium hirsutum* L.) plants. The molecular diagnostic of Pb23 was confirmed by a species-specific primer (MACHADO et al., 2007). In addition, specimens of each population were periodically extracted from the plants and checked under a light microscope (1,000× magnification) to confirm the species identity based on morphological features.

The *P. brachyurus* inocula used in the glasshouse experiments were extracted from grain sorghum roots infected by Pb23. Roots were washed, cut into 2–3 cm-length pieces and processed for nematode extraction using a blender with 300 mL of tap water, at low speed for 1 min. The suspension containing root debris, eggs, juveniles and adults of *P. brachyurus* was poured through a 60-mesh sieve (0.250 mm aperture) placed over a 500-mesh sieve (0.025 mm aperture). The solid material retained in the 500-mesh sieve was recovered and put on two sheets of absorbent kitchen paper in a 13-cm plastic sieve over an 18-cm flat container containing a small amount of water (HOOPER, 1986). This container was stored in a chamber at 28 °C for 48 h to obtain the motile forms (juveniles and females) of *P. brachyurus*. The nematode suspension was recovered in 500-mL beakers, and the nematodes were counted in a Peters’ slide with a light microscope.

Four experiments, referred to as experiments 1, 2, 3, and 4, were carried out at glasshouse, with temperature control at the Escola Superior de Agricultura “Luiz de Queiroz”, in the municipality of Piracicaba, São Paulo state. The first two experiments were carried out to determine the effect of different initial population densities of *P. brachyurus* on resistance parameters evaluated for common bean, using the cultivars Pérola and Campeiro. Experiment 3 was conducted to estimate the potential of *P. brachyurus* in damaging common bean ‘Pérola’, and experiment 4 was a replicate of experiment 3.

In experiments 1 and 2, respectively, the seeds ‘Pérola’ and ‘Campeiro’ were surface-sterilized in sodium hypochlorite 0.5% for 5 min and kept for five days in a plant growth incubator (Scientific Precision Dual model 818, Thermo Scientific, Waltham MA) adjusted to 28 °C. In each experiment., the common bean seedlings were transplanted individually to 200 cm³ pots, with approximately 180 cm³ of sterilized (121 °C for 2 h) soil (20% silt, 7% clay and 73% sand). Nine days

after transplanting, with the first pair of true leaves completely extended, plants in each treatment were inoculated with their respective initial populations ($P_i = 150$, $P_i = 450$, $P_i = 1,350$ motile forms). The inoculum was deposited in two holes (2- and 4-cm deep), made in the soil, at about 1 cm from the stem of plants and covered with vermiculite. Experiments 1 and 2 were carried out between August and November 2011, simultaneously, in a completely randomized design with four treatments (three initial populations and a check non-inoculated) and ten replications.

After six days from inoculation, the plants and the entire soil content (200 cm³) were transferred to 1,200-cm³ clay pots, with 1,000 cm³ of sterilized soil (120 °C for 2 h). The P_i of each treatment corresponded to 0.15, 0.45, 1.35 and 0 nematodes-cm⁻³ (check non-inoculated), respectively. The plants were watered daily until entering the reproductive stage (R5). From the R5 onwards, plants were watered when necessary.

The mineral fertilization (5 mL of a solution containing 0.1 g·mL⁻¹ of a nutrient formula with 20% N, 10% P₂O₅, 20% K₂O, 0.05% B, 0.005% Co, 0.05% Cu, 0.2% Fe, 0.05% Mn, 0.005% Mo, 0.1% Zn; Polifertil Fertilizantes, São Paulo, Brazil) was done when the plants showed the first symptoms of nutritional deficiency.

The evaluations took place at the end of the life cycle of each cultivar, after the senescence of each plant. The average life cycle of 'Campeiro' was about 75 days, while for 'Pérola' was about 74 days, and the average time from inoculation to evaluation for both cultivars was 61 days and 60 days, respectively. The nematodes were extracted from the roots by the method described by COOLEN; D'HERDE (1972). The resulting suspensions of nematodes were calibrated to 10 mL. The final population (P_f), the numbers of nematodes per g of roots (Nem/g) and the reproduction factor ($RF = P_f/P_i$) were estimated. Fresh root weight (FRW), shoot dry weight (TDW), pod number (PN), grain number (GN), grain/pod (G/P) and grain weight (GW) were also evaluated.

For experiments 3 and 4, seeds of common bean 'Pérola' were surface-sterilized with 0.5% sodium hypochlorite and stored at 28 °C. After three days, the seeds were planted in 1,700-cm³ plastic pots (11.0 cm diameter and 17.9 cm height) with 1,500 cm³ of steam-treated sandy clay loam soil (120 °C for 2 h; 22% clay, 3% silt, 75% sand). Inoculation was done by pouring the nematode suspension into the holes where the seeds had just been placed. The experimental design was completely randomized, with three treatments $P_i = 0$; 10,000 specimens per pot (6.66-cm⁻³); and 30,000 specimens per pot (20.0-cm⁻³) and 16 replications. These P_i values were used because previous experiments demonstrated that $P_i \geq 8$ specimens of *P. brachyurus* per cm³ of soil in a 3,000-cm³ container ($\geq 24,000$ specimens per container with one common bean plant) was sufficient to reduce the growth of 'Pioneiro' and that P_i of 8 and 16 specimens did not affect the growth of 'Campos Gerais' (MACHADO et al., 2014).

The plants were fertilized 15 days after transplanting/inoculation, by the application of 5 mL of a solution containing 0.1 g·mL⁻¹ of a nutrient formula (20% N, 10% P₂O₅, 20% K₂O, 0.05% B, 0.005% Co, 0.05% Cu, 0.2% Fe, 0.05% Mn, 0.005% Mo, 0.1% Zn) and irrigated daily. Soil temperature on the surface of an experimental pot was monitored with a data logger (TagTemp; Novus Automation).

Nematode evaluation was carried out in plant harvest, 85 days after transplanting/inoculation. The following variables were measured: fresh root weight (FRW), plant height (PH), pod number (PN), grain number (GN) and grain weight (GW). The weight of leaves was not measured because most of them had fallen by the time of evaluation. The entire root system was processed by the blender centrifugal flotation method (COOLEN; D'HERDE, 1972). Using Peters' slide, eggs, J2, J3, and J4 and adult females were quantified under a light microscope at 100× magnification. Final nematode population (P_f), nematodes per gram of roots (Nem/g) and reproduction factor (RF) were also calculated for each plot.

For all experiments, the average of each variable was calculated for every treatment. Box-Cox transformation ($(y^\lambda - 1)/\lambda$) (BOX; COX, 1964) was used to find the optimal values (λ) for the transformation of the variables Nem/g and R (CRAWLEY, 2007). Then, these variables were submitted to normality (Shapiro-Wilk), and variances homogeneity (Bartlett test) tests before submitting the data to one-way analysis of variance (ANOVA) (VENABLES; RIPLEY, 2002). Means were compared by Scott-Knot test for $\alpha = 0.05$ (BARBIN, 2003; SILVA, 2010) using the R 2.15.0 program (R DEVELOPMENT CORE TEAM, 2008).

RESULTS

Nematode identification and estimation of population densities

Only three species of phytonematodes were detected in the samples from Paraná state: *H. dihystra*, *P. brachyurus* and *P. crenatus* (Table 1).

Table 1. Occurrence of phytonematodes in dry common bean plantations in Paraná and São Paulo states, Brazil.

Nematodes	Paraná state (16 fields)				São Paulo state (40 fields)			
	Frequency (%)		Density ^a (in 250 cm ³ or 1 g)		Frequency (%)		Density ^a (in 250 cm ³ or 1 g)	
	Soil	Roots	Soil	Roots	Soil	Roots	Soil	Roots
<i>Helicotylenchus dihystrera</i>	100	100	200 ± 119	78 ± 82	100	95	611 ± 510	45 ± 54
<i>Pratylenchus brachyurus</i>	94	94	4 ± 5	52 ± 56	65	100	24 ± 48	40 ± 34
<i>Pratylenchus crenatus</i>	12	12	38	96	0	0	0	0
<i>Meloidogyne</i> spp. ^b	0	0	0	0	0	45	0	9 ± 20
<i>Rotylenchulus</i> sp.	0	0	0	0	18	18	0	1 ± 3

^a Each value is the mean ± standard deviation.

^b *Meloidogyne incognita* and *Meloidogyne javanica*.

Source: Elaborated by the authors.

One hundred *Helicotylenchus* females from Paraná were examined, and all exhibited the typical features of *H. dihystrera*: lip region hemispherical with 4–5 transversal annules; stylet with bulbs generally projected laterally and anteriorly, forming an indentation, but sometimes rounded; small spermatheca offset without sperm; phasmids anterior to anal level; outer bands of lateral field areolated; inner lines of the lateral field fused on the tail in Y- or V-shaped pattern; tail asymmetric and dorsally curved, with a short projection (SHER, 1966; UZMA et al., 2015).

Of the 96 *Pratylenchus* females examined, 80 were *P. brachyurus*. The following features of *P. brachyurus* were used for the identification: lip region angular at the lateral view, with two distinct annuli; stylet strong with rounded bulbs; tail conoid and with smooth tip; small spermatheca without sperm.

Another *Pratylenchus* species was detected in two samples from Guarapuava, and identified as *P. crenatus* Loof, 1960 by BONFIM JUNIOR et al. (2016). In one sample it was the only nematode species found, whereas in the other it was found in association with *P. brachyurus*.

Five species were found in the samples from São Paulo state: *H. dihystrera*, *P. brachyurus*, *Rotylenchulus* sp., *Meloidogyne incognita* and *M. javanica* (Table 1). The females of *Helicotylenchus* were examined, and all exhibited the typical *H. dihystrera* qualitative morphological features (SHER, 1966).

The *Rotylenchulus* species could not be identified because of the scarcity of specimens, particularly immature females, which are essential for morphological identification (DASGUPTA et al., 1968). However, *R. reniformis* is the only species of reniform nematode undoubtedly occurring in Brazil. This species is frequently cited as a potential pathogen of common bean (SIKORA et al., 2005).

The α -esterase phenotypes demonstrated the occurrence of *M. incognita* (phenotypes I1 [$Rm = 1.00$] and I2 [$Rm = 1.00, 1.03$]) in three samples from Itai and two from Itapeva, and the occurrence of *M. javanica* (phenotype J3 [$Rm = 1.00, 1.16, 1.26$]) in two samples from Itai (Fig. 1). Examination of perineal patterns confirmed the electrophoretic identification, except for one sample from Itai, in which the identification of *M. javanica* was based exclusively on the α -esterase phenotypes.

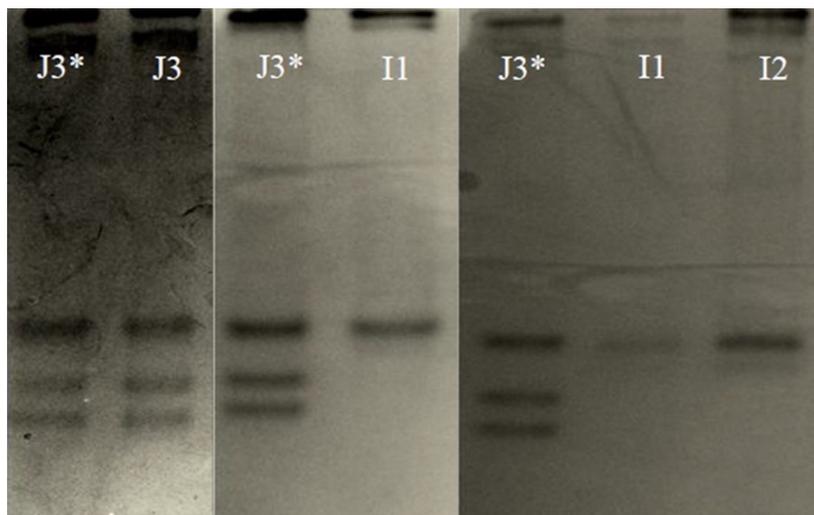


Figure 1. Typical α -esterase phenotypes of *Meloidogyne javanica* (J3) and *Meloidogyne incognita* (I1, I2) specimens collected in dry common bean growing fields of São Paulo state.

Source: Elaborated by the authors.

Helicotylenchus dihystera occurred in all samples from Paraná state, being the most abundant species (Table 1), reaching densities of 4,545 specimens per 250 cm³ of soil in a sample from Goioxim and 336 specimens per gram of roots in a sample from Pinhão. *Pratylenchus brachyurus* occurred in all samples, except one, but typically at low densities. Of 16 samples collected in Paraná state, 13 soil samples contained less than ten specimens per 250 cm³, and four root samples contained less than ten specimens per gram. In three soil samples, the densities were over ten specimens per 250 cm³, and in four root samples, over 100 specimens per gram.

Helicotylenchus dihystera also occurred in all samples from São Paulo state, except for three root samples, and was the most abundant species (Table 1), reaching 1,640 specimens per 250 cm³ of soil in a sample from Itapeva and 189 specimens per gram of roots in a sample from Itaí. *Pratylenchus brachyurus* was ubiquitous in root samples but usually at low densities. From 40 root samples collected in São Paulo state, eight contained less than ten specimens per gram and only two contained over 100 specimens per gram.

Root-knot nematodes were not found in soil samples, but were found in almost half of root samples, usually at low densities, in Angatuba (one sample), Paranapanema (one sample), Itaí (eight samples) and Itapeva (eight samples). From 18 root samples positive for root-knot nematodes, only one, from Itaí, contained over 100 specimens per gram.

Glasshouse experiments

Experiments 1 and 2 showed the detrimental effect of the high Pi (1,350) on *P. brachyurus* reproduction (Table 2), probably because roots were insufficient to support this nematode density. None of the Pi (150, 450 and 1,350) provoked any measurable damage on dry bean shoots or roots. Higher Pf, Nem/g and RF values were detected when the Pi = 450 was used in both experiments. Despite this, there was no significant difference between the RF values of Pi = 150 and Pi = 450. On the other hand, the RF of Pi = 1,350 was the lowest and significantly different from the others. With regard to Nem/g, significant differences between the Pi = 450 and Pi = 150 were observed in both experiments. However, in Experiment 1, the Nem/g of the Pi = 1,350 was not different from that of the Pi = 450.

Table 2. Final population (Pf), the number of nematodes per gram of fresh roots (Nem/g), reproduction factor (R), fresh root weight (FRW), top dry weight (TDW), pod number (PN), grain number (GN), grains/pod (G/P) and grain weight (GW) at the end of the cycle (75 days) of the cultivars Campeiro and Pérola inoculated with a different initial population (Pi) of *Pratylenchus brachyurus*.

Experiments	Pi	Pf	Nem/g	R	FRW (g)	TDW(g)	PN	GN	G/P	GW (g)
1 (Campeiro)	0	-	-	-	3.42 ^{ns}	0.57 ^{ns}	2 ^{ns}	9 ^{ns}	4 ^{ns}	1.33 ^{ns}
	150	672	184 b	4.48 a	3.44	0.63	3	9	3	1.36
	450	3079	644 a	6.84 a	4.93	0.59	3	9	3	1.21
	1,350	1635	511 a	1.21 b	3.65	0.51	3	10	4	1.53
2 (Pérola)	0	-	-	-	2.96 ^{ns}	0.46 ^{ns}	3 ^{ns}	8 ^{ns}	3 ^{ns}	1.28 ^{ns}
	150	921	268 b	6.14 a	3.09	0.61	3	8	3	1.49
	450	3241	958 a	7.20 a	3.57	0.81	3	11	4	1.84
	1,350	964	317 b	0.71 b	3.02	0.68	3	9	4	1.42

*Means of ten replicates (one plant). ^{ns} not significant according to Scott-Knot test (p=0.05).

Source: Elaborated by the authors.

When the Pi of 1,350 was inoculated in 'Campeiro', there was a short increase in the populational level at the end of the experimental period (RF = 1.21). In 'Pérola', there was a reduction in the populational level when inoculated with the higher Pi (RF = 0.71).

It was observed that in both treatments (Pi = 150 and Pi = 450), the Nem/g and RF were higher in 'Pérola' than in 'Campeiro'. In the Pi = 1,350, the Nem/g and the RF values were lower in 'Pérola' than in 'Campeiro'. The FRW of the treatments did not differ statistically among the treatments.

In experiments 3 and 4, despite the complete senescence of the aerial part of the plants at the time of the evaluation, the roots of the check plants looked healthy, with surface homogeneously cream or beige, without any sign of damage. Conversely, roots infected by *P. brachyurus* exhibited extensive decaying tissues areas, with a dark brown or black surface, mainly on secondary roots. In some plants, the main root was completely destroyed except the more proximal portion, from which some secondary roots emerged. The densities of *P. brachyurus* reached very high levels, 1,601 and 2,572 specimens per gram of roots for Pi of 10,000 and 30,000 in experiment 1, and 12,345 and 8,129 specimens per gram of roots for Pi of 10,000 and 30,000 in experiment 2. However, RF values were low, ranging from 0.85 to 7.16, probably due to the high Pi values (Table 3).

In experiment 3, as a consequence of root decay, the two Pi of *P. brachyurus* caused a reduction in FRW values, but PH, PN, GN and GW were not affected. In experiment 4, a significant reduction in FRW was observed only between Pi 10,000 and check treatments (Table 3).

Table 3. Effect of different initial population densities (Pi) of *Pratylenchus brachyurus* on final population density (Pf), the number of nematodes per gram of roots (Nem/g), reproduction factor (R), fresh root weight (FRW), plant height (PH), pod number (PN), grain number (GN) and grain weight (GW) of common bean 'Pérola' at the end-of-life cycle (85 days) of common bean plants.

Experiments	Pi (Nem·cm ⁻³ soil)	Pf	Nem/g	R	FRW(g)	PH	PN	GN	GW(g)
3 (Pérola)	0 (0)	0	0	-	15.2 a	101.00 ^{ns}	5 ^{ns}	21 ^{ns}	5.24 ^{ns}
	10,000 (6.66)	17,022	1,601	1.70	11.0 b	115.88	6	22	5.38
	30,000 (20)	25,595	2,572	0.85	10.1 b	116.19	6	21	5.86
4 (Pérola)	0 (0)	0	0	-	8.2 a	- ¹	-	-	-
	10,000 (6.66)	71,624	12,345	7.16	5.5 b	-	-	-	-
	30,000 (20)	60,967	8,129	2.03	7.4 ab	-	-	-	-

Each value is the mean of 16 replications. Means followed by the same letter in column did not differ according to Scott-Knot test ($p = 0.05$).

¹ Not measured.

Source: Elaborated by the authors.

DISCUSSION

Survey

Species of *Helicotylenchus* and *Pratylenchus* were found in soil and roots collected from fields representing the main growing regions of Paraná state. MACHADO; LOBO JUNIOR (2007) also found these species in a previous study conducted in 12 municipalities of Goiás and three of Minas Gerais state. In these states, three genera were detected, but at high frequencies: *Helicotylenchus* (71% in soil and 84% in root samples), *Pratylenchus* (71 and 100%) and *Meloidogyne* (46 and 78%). The maximum density occurred for *Pratylenchus* spp. (500 specimens·g⁻¹ of the root) contrasted with that found in the present work for both regions sampled (142 and 170 specimens·g⁻¹ of the root). Additionally, *Meloidogyne* spp. were not found in growing regions of Paraná state.

Owners of the 16 fields evaluated in Paraná state in this study did not consider phytonematodes major dry common bean pests, probably due to the absence of root-knot nematodes, which are easily distinguished by the root galling symptom and usually cause severe crop losses. Field experiments have not demonstrated a correlation between the initial population (at sowing) or final population of *H. dihystra* and the yield of snap bean (MCSORLEY, 1980); however, no information is available about the damage potential of *H. dihystra* and *P. brachyurus* on dry bean yield.

In Paraná state, a double-cropping system (two successive crops in the same year) is a usual practice. Typically, the dry bean is double-cropped with black and white oat (*Avena strigosa* Schreb. And *A. sativa* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and soybean (*Glycine max* L.). Black oat and barley are poor hosts for *P. brachyurus* (RF < 1), but white oat, wheat and soybean are good hosts (BORGES et al., 2010; ENDO, 1959). Consequently, there is a potential for *P. brachyurus* to reach high densities in fields where dry beans are double-cropped with white oat, wheat or soybean in the long run. In other Brazilian states, crop losses caused by *P. brachyurus* on dry bean have not been reported, but plant wilting attributed to the presence of this nematode was reported in a field cultivated to this crop for over ten years in the municipality of Santa Fé de Goiás (Goiás state) (SILVEIRA; RAVA, 2004).

Root-knot nematodes are widespread in dry bean production fields of São Paulo state, but, from the 40 sampled fields, these nematodes were controlled by furrow application of terbufos in a 51-ha field in Itaí. In 17 other fields, damage by root-knot nematodes was not detected, perhaps because of the low densities, 9 ± 20 J2 in root samples and absence in soil samples. In a glasshouse experiment, initial population densities of up to 100 eggs of *M. javanica* per 1,000 grams of soil did not affect the growth of 'Roxinho' dry bean 52 days after inoculation, whereas densities of 1,000 and 10,000 eggs per 1,000 g of soil caused reduction of 43 and 80% in stem weight, respectively (SHARMA, 1982).

No information is available about root-knot nematode densities in dry bean fields of other Brazilian states. However, there was a 4-fold yield increase of 'Pérola' dry bean, a cultivar extensively cultivated in Brazil, with the adoption of practices

to control *M. incognita* in Minas Gerais state (DUTRA; CAMPOS, 2003). In São Paulo state, the dry bean is double-cropped with cotton (*Gossypium hirsutum* L.), soybean, corn, potato and barley, good hosts (RF > 1) for *M. incognita* and *M. javanica* (JOHNSON; MOTSINGER, 1989; SIKORA et al., 2005) (except cotton for *M. javanica*). Additionally, these crops are also hosts for *P. brachyurus* (BORGES et al., 2010; ENDO, 1959). Therefore, particular attention should be paid to nematode densities in São Paulo dry bean-producing areas in the long run.

Little information is available about the effect of *Rotylenchulus* species on this crop. However, field experiments have demonstrated a negative correlation between *R. reniformis* population level at harvesting time and snap bean yield, but no correlation between *R. reniformis* or *H. dihystra* population at sowing time and the snap bean yield, or between the final population of *H. dihystra* and the snap bean yield (MCSORLEY, 1980).

Pathogenicity tests

The glasshouse experiments demonstrated that *P. brachyurus* reproduces, causing severe damage to dry bean roots, as seen in experiment 4. It had been experimentally demonstrated that high initial densities of *P. brachyurus* (eight and 16 specimens per cm³ of soil, or 24,000 and 48,000 specimens per plant), comparable to the densities used in this work, caused reduced growth of dry bean plants (FRW and shoot fresh weight) of the 'Pioneiro' but not of 'Campos Gerais' (MACHADO et al., 2014). Therefore, the producer's perception of the reduced importance of *P. brachyurus* in dry bean production seems correct, but likely due to the low density of the nematode in dry bean plantations in Paraná and São Paulo states. The maximum densities obtained in this study (0.6 and 1.2 specimens per cm³ soil in Paraná and São Paulo states) are far below the level required to cause growth reduction in glasshouse experiments (6.67 in this work and 8.0 specimens per cm³ soil according to MACHADO et al. (2014). Therefore, if common beans were sown in such high-infested fields, yield losses would probably occur.

In conclusion, pathogenicity tests demonstrated that densities above 6.67 specimens of *P. brachyurus* per cm³ soil cause damage to the dry bean roots. Survey data showed low phytonematodes densities. The low or undetectable crop losses reported by dry bean growers in sampled areas of the state of Paraná and São Paulo might be attributed to the low phytonematode densities here presented. However, growers should adopt production systems to prevent an increase in nematode population, especially of *P. brachyurus*, *M. incognita* and *M. javanica*, which are still low in numbers but could lead to future yield losses, especially if double-cropping systems use suitable hosts for these nematode species continues to be employed.

AUTHORS' CONTRIBUTIONS

Conceptualization: Bonfim Junior, M.F.; Inomoto, M.M. **Data curation:** Bonfim Junior, M.F. **Formal analysis:** Bonfim Junior, M.F.; Araújo Filho, J.V. **Funding acquisition:** Bonfim Junior, M.F.; Inomoto, M.M. **Investigation:** Bonfim Junior, M.F.; Inomoto, M.M.; Araújo Filho, J.V. **Methodology:** Bonfim Junior, M.F.; Inomoto, M.M.; Araújo Filho, J.V. **Project administration:** Bonfim Junior, M.F. **Supervision:** Inomoto, M.M. **Visualization:** Bonfim Junior, M.F.; Inomoto, M.M.; Araújo Filho, J.V. **Writing – original draft:** Bonfim Junior, M.F. **Writing – review & editing:** Bonfim Junior, M.F.

AVAILABILITY OF DATA AND MATERIAL

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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CONFLICTS OF INTEREST

We have no conflict of interest to declare.

ETHICAL APPROVAL

Not applicable.

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REFERENCES

- BARBIN, D. *Planejamento e análise estatística de experimentos agrônômicos*. Arapongas: Midas, 2003. 194p.
- BONFIM JUNIOR, M.F.; CONSOLI, E.A.; INOMOTO, M.M.; OLIVEIRA, C.M.G. First report of *Pratylenchus crenatus* in Brazil. *New Disease Reports*, London, v.34, p.7, 2016. <https://doi.org/10.5197/j.2044-0588.2016.034.007>
- BORGES, D.C.; MACHADO, A.C.Z.; INOMOTO, M.M. Reação de aveias a *Pratylenchus brachyurus*. *Tropical Plant Pathology*, Brasília, v.35, n.3, p.178-181, 2010. Available from: <https://www.scielo.br/j/tpp/a/Sh4zqSf6qmLNv6nMRx9PVxL/?format=pdf&lang=pt>. Access on: 30 Apr. 2014.
- BOX, G.E.P.; COX, D.R. An analysis of transformations. *Journal of the Royal Statistical Society: Series B (Methodological)*, London, v.26, n.2, p.211-252, 1964.
- CAFÉ FILHO, A.C.; HUANG, C.S. Description of *Pratylenchus pseudofallax* n. sp. with a key to species of the genus *Pratylenchus* Filipjev, 1939 (Nematoda: Pratylenchidae). *Révue de Nématologie*, Harpenden, v.12, p.7-15, 1989.
- CARNEIRO, R.M.D.; ALMEIDA, A.R.A.; CARNEIRO, R.G. Enzyme phenotypes of Brazilian isolates of *Meloidogyne* spp. *Fundamental and Applied Nematology*, Saint Andrews, v.19, p.555-560, 1996.
- CARNEIRO, R.M.D.G.; ALMEIDA, M.R.A. Técnica de eletroforese usada no estudo de enzimas dos nematoides de galhas para identificação de espécies. *Nematologia Brasileira*, Brasília, v.25, n.1, p.35-44, 2001.
- CONAB - Companhia Nacional de Abastecimento. *Último levantamento da safra de grãos 2020/21 confirma redução na produção de grãos*. Brasília: Conab. Available from: <https://www.conab.gov.br/ultimas-noticias/4234-ultimo-levantamento-da-safra-2020-21-confirma-reducao-na-producao-de-graos#:~:text=A%20produ%C3%A7%C3%A3o%20total%20%C3%A9%20estimada,mil%20toneladas%20de%20feij%C3%A3o%2Dcaupi>. Access on: 4 Nov. 2021.
- COOLEN, W.A.; D'HERDE, C.J. *A method for the quantitative extraction of nematodes from plant tissue*. Ghent: State Nematology and Entomology Research Station, 1972. 77p.
- CRAWLEY, M.J. *The R Book*. West Sussex: John Wiley & Sons, 2007. 950p.
- DASGUPTA, D.R.; RASKI, D.J.; SHER, S.A. A revision of the genus *Rotylenchulus* Linford and Oliveira, 1940 (Nematoda: Tylenchida). *Proceedings of the Helminthological Society of Washington*, Washington DC, v.35, n.2, p.169-192, 1968. Available from: <http://bionames.org/bionames-archive/issn/0018-0130/35/169.pdf>. Access on: 15 Mar. 2014.
- DEBIASI, H.; MORAES, M.T.; FRANCHINI, J.C.; DIAS, W.P.; SILVA, J.F.V.; RIBAS, L.N. Manejo do solo para controle cultural do nematoide das lesões radiculares na entressafra da soja. In: XXXIII CONGRESSO BRASILEIRO DE CIÊNCIA DO SOLO, 31 July, 2011, Uberlândia. *Proceedings [...]*. Uberlândia: Sociedade Brasileira de Ciência do Solo. Available from: <http://ainfo.cnptia.embrapa.br/digital/bitstream/item/48290/1/debiasi.manejo.PDF>. Access on: 22 Apr. 2014.
- DUTRA, M.R.; CAMPOS, V.P. Manejo do solo e da irrigação como nova tática de controle de *Meloidogyne incognita* em feijoeiro. *Fitopatologia Brasileira*, Brasília, v.28, n.6, p.608-614, 2003. <https://doi.org/10.1590/S0100-41582003000600005>
- EISENBACK, J.D. Diagnostic characters useful in the identification of the four most common species of root-knot nematodes (*Meloidogyne* spp.). In: SASSER, J.N., CARTER, C.C. (ed.). *An advanced treatise on Meloidogyne*. Vol.1. Biology and Control. Raleigh: North Carolina State University Graphics, 1985. chap. 8, p.95-112.
- ENDO, B.Y. Responses of root-lesion nematodes, *Pratylenchus brachyurus* and *P. zaei*, to various plants and soil types. *Phytopathology*, Saint Paul, v.49, p.417-421, 1959.
- FAO – Food and Agriculture Organization of the United Nations. *Top 10 Country Production of Beans, dry*. Rome: FAO, 2019. Available from: https://www.fao.org/faostat/en/#rankings/countries_by_commodity. Access on: 5 Nov. 2021.

- FORTUNER, R. Morphometrical variability in *Helicotylenchus* Steiner, 1945. 6: Value of the characters used for specific identification. *Revue de Nématologie*, Harpenden, v.7, n.3., p.245-264, 1984. Available from: https://horizon.documentation.ird.fr/exl-doc/pleins_textes/pleins_textes_5/pt5/nemato/17434.pdf. Access on: 8 Apr. 2014.
- FORTUNER, R.; MERNY, G.; ROUX, C. Morphometrical variability in *Helicotylenchus* Steiner, 1945. 3: Observations on African populations of *Helicotylenchus dihystra* and considerations on related species. *Revue de Nématologie*, Harpenden, v.4, n.2, p.235-260, 1981. Available from: https://www.researchgate.net/publication/32990356_Morphometrical_variability_in_Helicotylenchus_Steiner_1945_3_observations_on_African_populations_of_Helicotylenchus_dihystra_and_considerations_on_related_species#fullTextFileContent. Access on: 27 Mar. 2014.
- HOOPER, D.J. Extraction of free-living stages from soil. In: SOUTHEY, J.F. (ed.). *Laboratory Methods for Work with Plant and Soil Nematodes*. London: Her Majesty's Stationery Office, 1986. p.5-30.
- JENKINS, W.R. A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Disease Reporter*, Wallingford, v.48, n.9, p.692, 1964. Available from: <https://www.cabdirect.org/cabdirect/abstract/19650801105>. Access on: 15 Apr. 2014.
- JEPSON, S.B. *Identification of root-knot nematodes (Meloidogyne species)*. Wallingford: CABI, 1987. 265p.
- JOHNSON, A.W.; MOTSINGER, R.E. Suitability of small grains as hosts of *Meloidogyne* species. *Journal of Nematology*, Karachi, v.21, n.4S, p.650-653, 1989.
- KIMENJU, J.M.; KARANJA, N.K.; MACHARIA, I. Plant parasitic nematodes associated with common bean in Kenya and the effect of *Meloidogyne* infection on bean nodulation. *African Crop Science Journal*, Kampala, v.7, n.4, p.503-510, 1999. <https://doi.org/10.4314/acsj.v7i4.27744>
- MACHADO, A.C.Z.; FERRAZ, L.C.C.B.; OLIVEIRA, C.M.G. de. Development of a species-specific reverse primer for the molecular diagnosis of *Pratylenchus brachyurus*. *Nematropica*, Homestead, v.37, n.2, p.249-257, 2007. Available from: <https://journals.flvc.org/nematropica/article/view/64430>. Access on: 20 Apr. 2014.
- MACHADO, A.C.Z.; MARINI, P.M.; DADAZIO, T.S.; SILVA, S.A. Patogenicidade de *Pratylenchus brachyurus* em feijão. In: CONGRESSO NACIONAL DE PESQUISA DE FEIJÃO, 14, 2014, Londrina. *Proceedings [...]*. Londrina: Iapar, 2014.
- MACHADO, V.O.F.; LOBO JUNIOR, M. Fitonematóides associados à cultura do feijoeiro comum cultivado em áreas de cerrado. *Fitopatologia Brasileira*, Brasília, v.32, p.292, 2007. Supplement.
- MIRANDA, E.E. de. *Agricultura no Brasil do século XXI*. São Paulo: Metalivros, 2013.
- R DEVELOPMENT CORE TEAM. *The R Project for Statistical Computing*. Vienna: R Foundation, 2008.
- ROLIM, G.S.; CAMARGO, M.B.P.; LANIA, D.G.; MORAES, J.F.L. Classificação climática de Köppen e de Thornthwaite e sua aplicabilidade na determinação de zonas agroclimáticas para o estado de São Paulo. *Bragantia*, Campinas, v.66, n.4, p.711-720, 2007. <https://doi.org/10.1590/S0006-87052007000400022>
- SHARMA, R.D. Pathogenicity of *Meloidogyne javanica* to bean (*Phaseolus vulgaris* L.). *Sociedade Brasileira de Nematologia*, Piracicaba, v.5, p.137-144, 1982.
- SHER, S.A. Revision of the Hoplolaiminae (Nematoda) VI. *Helicotylenchus* Steiner, 1945. *Nematologica*, Leiden, v.12, p.1-56, 1966. <https://doi.org/10.1163/187529266X00013>
- SIDDIQI, M.R. *Tylenchida: Parasites of plants and insects*. Wallingford: CABI International, 2000. <https://doi.org/10.1079/9780851992020.0000>
- SIKORA, R.A.; GRECO, N.; SILVA, J.F.V. Nematode parasites of food legumes. In: LUC, M.; SIKORA, R.A.; BRIDGE, J. (ed.). *Plant parasitic nematodes in subtropical and tropical agriculture*. 2th.ed. Wallingford: CABI International, 2005. chap.8, p.259-318. <https://doi.org/10.1079/9780851997278.0259>

SILVA, L.V. Duncan test, Tukey test and Scott-Knott test. R package version 1.0-1. Vienna: R Foundation, 2010. Available from: <http://CRAN.R-project.org/package=laercio>. Access on: 5 May 2010.

SILVEIRA, P.M.; RAVA, C.A. *Utilização de crotalaria no controle de nematoides da raiz de feijoeiro* (Comunicado Técnico 74). Santo Antônio de Goiás: Embrapa Arroz e Feijão. 2004.

SUBBOTIN, S.A.; VOVLAS, N.; YEATES, G.W.; HALLMANN, J.; KIEWNICK, S.; CHIZHOV, V.N.; MANZANILLA-LÓPEZ, R.H.; INSERRA, R.N.; CASTILLO, P. Morphological and molecular characterization of *Helicotylenchus pseudorobustus* (Steiner, 1914) Golden, 1956 and related species (Tylenchida: Hoplolaimidae) with a phylogeny of the genus. *Nematology*, Leiden, v.17, p.27-52, 2015. <https://doi.org/10.1163/15685411-00002850>

UZMA, I.; NASIRA, K.; FIROZA, K.; SHAHINA, F. Review of the genus *Helicotylenchus* Steiner, 1945 (Nematoda: Hoplolaimidae) with updated diagnostic compendium. *Pakistan Journal of Nematology*, Karachi, v.33, n.2, p.115-160, 2015. Available from: https://www.researchgate.net/profile/Shahina-Fayyaz/publication/284277053_Review_of_the_genus_Helicotylenchus_Steiner_1945_Nematoda_Hoplolaimidae_with_updated_diagnostic_compendium/links/56a7270a08ae0fd8b3fde78b/Review-of-the-genus-Helicotylenchus-Steiner-1945-Nematoda-Hoplolaimidae-with-updated-diagnostic-compendium.pdf. Access on: 30 Jan. 2016.

VENABLES, W.N.; RIPLEY, B.D. *Modern applied statistics with S*. New York: Springer, 2002. 498p.

WESTERDAHL, B.B. Dry bean: Nematodes. In: *UC Pest Management Guidelines*. Davis: University of California, 2014.

