PHENOTYPIC VALIDATION OF CORN HYBRIDS WITH LOW REPRODUCTION FACTORS FOR *PRATYLENCHUS BRACHYURUS*

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ABSTRACT

Aquino, L. T., L. N. R. Lima, M. C. G. Oliveira, A. P. Calderon, S. A. Silva, and A. C. Z. Machado. 2024. Phenotypic validation of corn hybrids with low reproduction factors for *Pratylenchus brachyurus*. Nematropica 54:158-165.

In Brazil, corn is primarily grown in succession to soybeans. However, certain nematodes, such as *Pratylenchus brachyurus* multiply on corn, increasing in density and impacting subsequent crops. There is currently no standardized methodology for phenotyping corn for host response to *P. brachyurus*, and some hybrids have shown inconsistent results in the field. The objective of this study was to evaluate the reproduction factor (RF = final nematode density/initial nematode density) of *P. brachyurus* in eight different corn hybrids, previously identified as having low RF values for this nematode, across two different time points, in two experiments. Eight corn hybrids were sown and maintained in a greenhouse under controlled conditions (28°C ± 2°C). At the first vegetative growth stage, each plant was inoculated with 500 *P. brachyurus*. Roots were collected at 76 and 97 days after inoculation (DAI), then washed, weighed, and nematodes extracted. The fresh root weight (FRW) and the number of nematodes and eggs were used to calculate the number of nematodes per gram of roots (Nema/g) and the RF values for each hybrid. The experiment was conducted in a completely randomized design with an 8x2 factorial arrangement and 7 replicates each. A significant difference in RF values was observed between the two evaluation dates. At 97 DAI, none of the hybrids had low RF values, differing from the evaluation at 76 DAI, in which the majority of hybrids were classified as resistant. The findings of this study suggest that assessing the resistance or susceptibility of corn hybrids to *P. brachyurus* shortly after inoculation can produce inconsistent results. This variability could hinder effective nematode management in the field, as these hybrids may still contribute to increasing soil nematode densities, negatively affecting subsequent crops in the rotation.

Key words: Low RF; root lesion nematode; *Zea mays*

RESUMO

Aquino, L. T., L. N. R. Lima, M. C. G. Oliveira, A. P. Calderon, S. A. Silva, and A. C. Z. Machado. 2024. Validação da fenotipagem de híbridos de milho com baixo fator de reprodução para *Pratylenchus brachyurus*. Nematropica 54:158-165.

No Brasil, o milho é cultivado principalmente em sucessão à soja. No entanto, alguns nematoides, como *Pratylenchus brachyurus*, multiplicam-se no milho, aumentando as populações no solo e impactando as culturas subsequentes. Atualmente, não há uma metodologia padronizada para fenotipar o milho quanto à sua resposta a *P. brachyurus*, e alguns híbridos têm mostrado resultados inconsistentes em campo. O objetivo deste trabalho foi avaliar o fator de reprodução (FR) de *P. brachyurus*, em oito híbridos de milho diferentes, previamente identificados como tendo baixo FR para esse nematoide, em dois períodos de avaliação, em dois experimentos distintos. Oito híbridos de milho foram semeados e mantidos em casa de vegetação sob condições controladas (28°C ± 2°C). No primeiro estádio de crescimento vegetativo, cada planta foi inoculada com 500 *P. brachyurus*. As raízes foram coletadas aos 76 e 97 dias após a inoculação (DAI), lavadas, pesadas e avaliadas para extração de nematoides. A massa fresca da raiz (MFR) e o número de nematoides foram utilizados para calcular o número de nematoides por grama de raiz (Nema/g) e o FR para cada híbrido. O experimento foi conduzido em delineamento inteiramente casualizado com arranjo fatorial 8x2 e 7 repetições cada. Observou-se uma diferença significativa nos valores de FR entre as duas datas de avaliação. Aos 97 DAI, nenhum dos híbridos apresentou baixo FR em ambos os experimentos, diferindo da avaliação aos 76 DAI, na qual a maioria dos híbridos foi classificada como resistente. Os resultados deste estudo sugerem que avaliar a resistência ou suscetibilidade dos híbridos de milho a *P. brachyurus* logo após a inoculação pode produzir resultados inconsistentes. Essa variabilidade pode dificultar o manejo eficaz dos nematoides em campo, pois esses híbridos ainda podem contribuir para o aumento das populações de nematoides no solo, afetando negativamente as culturas subsequentes na rotação.

Palavras-chave: Baixo FR, nematoide das lesões radiculares, *Zea mays*

INTRODUCTION

Pratylenchus brachyurus is the second most damaging plant-parasitic nematode in Brazil, affecting a wide range of host plants like soybean, corn, and cotton (Favoreto *et al*., 2019). In soybean (*Glycine max*), Brazil´s most widely cultivated grain, *P. brachyurus* can cause yield losses exceeding 30% in infested fields (Oliveira *et al*., 2022).

According to Syngenta (2023), *Pratylenchus* spp. were found in approximately 76% of the 21,000 soil samples analyzed from the major grain-producing regions of Brazil. In addition to the direct damage caused by this migratory endoparasitic nematode, which feeds on plant cells and creates root lesions as it moves through the tissue, these lesions also provide entry points for fungal and bacterial infections. This can further compromise plant health and, in severe cases, lead to plant death (Ferraz and Brown, 2016).

Controlling nematodes often requires targeted and expensive management practices, with *P. brachyurus* posing particular challenges due to its biology and wide host range, which complicate genetic and cultural control strategies (Bellé *et al*., 2017). Unlike sedentary nematodes, *P. brachyurus* is a migratory endoparasite, entering host roots to feed and reproduce while freely moving through plant tissue rather than remaining in one place (Castillo and Vovlas, 2007), making breeding for resistance to *P. brachyurus* more challenging. The resistance to *Pratylenchus* spp. is typically polygenic, linked to post-infection biochemical events or to diverse morphological features between resistant and susceptible cultivars (Peng and Moens, 2003). Consequently, resistance to migratory root-parasitic nematodes is relatively rare, and it generally only reduces nematode reproduction rather than completely preventing infection (Peng and Moens, 2003).

Currently, no soybean cultivars are known to be resistant to this nematode. The resistance mechanism, where present, is post-infection: the plant responds by causing the attacked cell or region to deteriorate, forcing the nematode to leave. However, the nematode often reattacks another part of the root or nearby plants. Plants with this type of resistance tend to produce less in infested soils because their energy is diverted to nematode defense rather than photosynthesis. Even if soybean cultivars with post-infection resistance were available, their use would be impractical, necessitating alternative management methods (Lima and Sousa, 2018).

Therefore, using crops that are resistant or moderately resistant, with a low reproduction factor (RF = final nematode density/initial nematode density) values for *P. brachyurus* is crucial in infested areas before soybean cultivation. Species such as *Crotalaria spectabilis*, *C. juncea*, and *Cajanus cajan* cv. IPR 43 are known to be antagonistic to *P. brachyurus*. Despite their benefits, these species are not commonly grown because they do not provide direct income to farmers (Santana-Gomes *et al*., 2019).

As a result, many farmers choose to cultivate corn (*Zea mays*) as an economic crop during the soybean off-season. The National Supply Company (Conab, 2023) estimated that 95 million tons of corn were produced in 2022-2023. Although the losses caused by *P. brachyurus* in corn are not well documented, the nematode´s ability to multiply rapidly in this crop raises concerns about increasing soil population densities and affecting subsequent crops.

To address this, genetic breeding companies are testing corn genotypes for host status to nematodes based upon RF values. Several hybrids, like GNZ 2005 and P30K75, have been released as resistant or moderately resistant (low RF) for *P. brachyurus*, indicating that these cultivars have a reduced potential for nematode reproduction (Inomoto, 2011; Mendonça Filho *et al*., 2012). However, in some cases, RF values were measured under field conditions (Mendonça Filho *et al*., 2012), which do not provide reliable results due to the inconsistent assessment of soil nematode population densities, or were evaluated shortly after inoculation (Inomoto, 2011). This study aimed to validate phenotyping methods to select corn genotypes that reduce *P. brachyurus* population densities and have the potential the impact on soybean.

MATERIALS AND METHODS

Eight corn hybrids (DKB 390, 2B688PW, DKB 177, FS 500, P30K75, P4285VYHR, SYN7205, TMG987VIP3) were evaluated in two experiments under greenhouse conditions at 28°C ± 2°C to assess their response to *P. brachyurus*. Experiments were conducted in the municipality of Londrina, Paraná State, Brazil (latitude 23°24’23” S; longitude 51°24’83”W, altitude 610 m), from November 2023 to March 2024 and from March to July 2024. The experiments were conducted in a completely randomized design with seven replicates per treatment. Experiment 2 was a replica of Experiment 1.

Two seeds of each hybrid were planted in 950 mL styrofoam pots containing soil (80% sand, 5% silt, 15% clay) previously sterilized (160°C for 5 hr). After germination, the seedlings were thinned to retain only one plant per pot.

Plants with the first leaf with a visible collar at the base of the leaf and rounded tip, corresponding to the V1 growth stage (Ciampitti *et al*., 2011), were inoculated with 500 *P. brachyurus* (a mixture of eggs + juveniles + adults). The inoculum was extracted from okra cv. Santa Cruz 47 roots maintained in a purified inoculum bank, using the blender-sieving methodology described by Boneti and Ferraz (1981), without the addition of sodium hypochlorite. The collected nematode suspension was calibrated to obtain the desired density used as inoculum.

The evaluation periods were selected according to the results obtained in the study by Inomoto (2011). At 76 and 97 days after inoculation (DAI), the plants were cut just above the soil level and roots were carefully washed and weighed to determine the fresh root weight (FRW). Nematodes were extracted using the blender-sieving method described by Boneti and Ferraz (1981). The final number of nematodes (Pf) was counted using a Peters slide (Astel) under an Alphaphot-2 YS2 optical microscope (Nikon, Melville, NY).

The number of nematodes per gram of roots (Nema/g) was calculated by dividing the Pf by the FRW. Additionally, the reproduction factor (RF) was determined by the ratio of Pf to the initial population (Pi), as proposed by Oostenbrink (1966) and described by Cook and Evans (1987).

Experiments were arranged in an 8x2 factorial scheme, with factors corresponding to the corn hybrids and evaluation dates, respectively. The data were first checked for residual normality using the Shapiro-Wilk test (1965), followed by analysis of variance (ANOVA). The means were grouped by the Scott-Knott test at a 5% significance level, using the statistical software R version 4.1.2 (R Core Team, Vienna, Austria) using the packages MASS (Venables and Ripley 2002) and ExpDes (Ferreira *et al*., 2013).

RESULTS

There was significant variation in both the RF values and Nema/g variables (Tables 1 and 2). Consequently, each experiment was analyzed separately. For RF values, all hybrids except DKB 177 and P4285VYHR exhibited higher RF values in Experiment 2 compared to Experiment 1 at 76 DAI (Table 1). Conversely, at 97 DAI, only hybrids 2B688PW, FS 500, and P30K75 displayed higher RF values in Experiment 1 compared to

Table 1. Reproduction factor (RF) values of *Pratylenchus brachyurus* in corn hybrids at 76 and 97 days after inoculation (DAI), in Experiments 1 (RF 1) and 2 (RF 2) (N=7)[y].

Hybrids	RF 1 76 DAI	RF 2 76 DAI	RF 1 97 DAI	RF 2 97 DAI
DKB 390	0.66 b[z]	12.96 a	53.22 a	82.56 a
2B688PW	0.60 b	3.24 a	26.58 a	11.28 b
DKB 177	0.60 a	1.80 a	11.04 a	15.72 a
FS 500	0.30 b	3.24 a	13.44 a	4.80 b
P30K75	0.72 b	2.88 a	18.42 a	7.80 b
P4285VYHR	1.08 a	1.56 a	24.00 a	28.32 a
SYN7205	1.02 b	5.52 a	36.30 a	22.92 a
TMG987VIP3	0.78 b	5.04 a	11.88 a	9.52 a

[y]Mean values from seven replicates.
[z]Means followed by the same letter in the columns did not differ at the 5% significance level, according to Scott-Knott Test.

Table 2. Number of nematodes per gram of roots (Nema/g) of *Pratylenchus brachyurus* in corn hybrids at 76 and 97 days after inoculation (DAI), in Experiments 1 (Nema/g 1) and 2 (Nema/g 2) (N=7)[y].

Hybrids	Nema/g 1 76 DAI	Nema/g 2 76 DAI	Nema/g 1 97 DAI	Nema/g 2 97 DAI
DKB 390	119 b[z]	1,195 a	2,060 b	7,003 a
2B688PW	71 a	239 a	497 a	419 a
DKB 177	84 a	131 a	149 b	1,021 a
FS 500	32 a	243 a	160 a	191 a
P30K75	96 a	183 a	478 a	703 a
P4285VYHR	102 a	171 a	629 a	1,290 a
SYN7205	115 a	225 a	989 a	1,167 a
TMG987VIP3	109 b	739 a	211 a	374 a

[y]Mean values from seven replicates.
[z]Means followed by the same letter in the columns did not differ at the 5% significance level, according to Scott-Knott Test.

Experiment 2 (Table 1).

For Nema/g, hybrids DKB 390 and TMG987VIP3 had higher Nema/g values in Experiment 2 at 76 DAI, while at 97 DAI, hybrids DKB 390 and DBK 177 showed lower Nema/g values in Experiment 1 (Table 2). To better clarify the differences between hybrids and evaluation dates within each experiment, the findings are presented under separate subtitles for each experiment.

Experiment 1

At 76 DAI, no significant differences were observed between the corn hybrids regarding RF values. However, six corn hybrids, DKB390, 2B688PW, DKB177, FS500, P30K75, and TMG987VIP3, showed potential resistance to *P. brachyurus*, all with a RF values < 1. Although P4285VYHR and SYN7205 did not demonstrate resistance, the low RF values at this initial evaluation made them statistically comparable to the other hybrids (Table 3). By 97 DAI, hybrids DKB 390, 2B688PW, and SYN7205 exhibited the highest RF values, and none of the hybrids maintained the low RF values observed at 76 DAI (Table 3). As expected, the RF values had significantly increased during this period, revealing differences across all hybrids compared to the first evaluation (Table 3).

For Nema/g, no significant differences were observed between the hybrids at 76 DAI. However, by 97 DAI, DKB 390 exhibited the highest Nema/g value, while the hybrids DKB 177, FS 500, and TMG987VIP3 showed the lowest values, with the remaining hybrids displaying intermediate values

(Table 4). When comparing Nema/g values between the evaluation dates, hybrids DKB 390, 2B688PW, P30K75, P428VYHR, and SYN7205 showed a significant increase in Nema/g values, with higher values at 97 DAI than at 76 DAI.

Experiment 2

At 76 DAI, only hybrid DKB 390 showed a significantly higher RF value compared to the other hybrids. By 97 DAI, DKB 390 maintained the highest RF value, but greater differentiation among the hybrids was observed, with hybrids FS 500 and P30K75 showing the lowest RF values, while the others displayed intermediate values. Regarding differences between evaluation dates, all hybrids exhibited higher RF values at 97 DAI compared to 76 DAI (Table 3).

For Nema/g, at 76 DAI, hybrids DKB 390 and TMG987VIP3 exhibited significantly higher values. By 97 DAI, only DKB 390 maintained the highest value, while hybrids 2B688PW, FS 500, P30K75, and TMG987VIP3 showed the lowest values. Regarding the comparison of evaluation dates, hybrids 2B688PW, FS 500, P30K75, and TMG987VIP3 had Nema/g values that did not differ between the dates, whereas, the other hybrids had significantly higher Nema/g values at 97 DAI compared to 76 DAI.

DISCUSSION

Managing *P. brachyurus* is particularly challenging due to the nematode´s polyphagous nature and the lack of resistant cultivars for major agricultural crops in Brazil (Machado and Araújo

Table 3. Reproduction factor (RF) values of *Pratylenchus brachyurus* in corn hybrids at 76 and 97 days after inoculation (DAI), in Experiments 1 (RF 1) and 2 (RF 2) (N=7)[y].

Hybrids	RF 1 76 DAI	RF 2 76 DAI	RF 1 97 DAI	RF 2 97 DAI
DKB 390	0.66 aB[z]	53.22 aA	12.96 aB	82.56 aA
2B688PW	0.60 aB	26.58 aA	3.24 bB	11.28 cA
DKB 177	0.60 aB	11.04 bA	1.80 bB	15.72 bA
FS 500	0.30 aB	13.44 bA	3.24 bA	4.80 dA
P30K75	0.72 aB	18.42 bA	2.88 bA	7.80 dA
P4285VYHR	1.08 aB	24.00 bA	1.56 bB	28.32 bA
SYN7205	1.02 aB	36.30 aA	5.52 bB	22.92 bA
TMG987VIP3	0.78 aB	11.88 bA	5.04 bA	9.52 cA

[y]Mean values from seven replicates.

[z]Means followed by the same lower case letter in the columns for the comparison between hybrids and capital letters in lines for the comparison between evaluation dates (inside each experiment separately) did not differ at the 5% significance level, according to Scott-Knott Test.

Table 4**.** Number of nematodes per gram of roots (Nema/g) of *Pratylenchus brachyurus* in corn hybrids at 76 and 97 days after inoculation (DAI), in Experiments 1 (Nema/g 1) and 2 (Nema/g 2) (N=7)[y].

Hybrids	Nema/g 1 76 DAI	Nema/g 2 76 DAI	Nema/g 1 97 DAI	Nema/g 2 97 DAI
DKB 390	120 aB	2,060 aA	1,195 aB	7,003 aA
2B688PW	71 aB	497 bA	239 bA	419 cA
DKB 177	84 aA	149 cA	131 bB	1,021 bA
FS 500	32 aA	160 cA	243 bA	191 cA
P30K75	96 aB	478 bA	183 bA	703 cA
P4285VYHR	102 aB	629 bA	171 bB	1,290 bA
SYN7205	115 aB	989 bA	225 bB	1,167 bA
TMG987VIP3	109 aA	211 cA	739 aA	374 cA

[y]Mean values from seven replicates.

[z]Means followed by the same lower case letter in the columns for the comparison between hybrids and capital letters in lines for the comparison between evaluation dates (inside each experiment separately) did not differ at the 5% significance level, according to Scott-Knott Test.

Filho, 2016; Silva *et al*., 2020). Besides the inherent difficulties in breeding for resistance to migratory nematodes, the absence of a standardized phenotyping processes for assessing genotype resistance leads to inconsistent and non-comparable results across experiments. The phenotyping process can be influenced by various factors, including initial population density, the plant´s age at the time of inoculation, and the timing of the evaluation post-inoculation (Machado, 2021; Pedro *et al*., 2024).

While some studies have aimed to standardize the phenotyping process for nematode resistance – such as those for root-knot nematodes in coffee (Silva *et al*., 2020) and for *P. brachyurus* in soybeans (Pedro *et al*., 2024) – similar studies for corn are lacking. One of the closest studies found in the literature is by Inomoto (2011), which primarily focused on the reaction of corn hybrids to *P. brachyurus* in rotation with soybean.

In addition to presenting the RF values for the evaluated corn hybrids, Inomoto (2011) conducted trials to evaluate the effect of two experimental periods (70 and 107 days after inoculation) on *P. brachyurus* reproduction. The author concluded that the longer period allowed for greater reproduction and recommended its adoption for assessing corn resistance to the nematode. However, this recommendation is often overlooked in practice, with Brazilian corn breeding companies adopting methodologies that do not allow for accurate classification of resistance or susceptibility of corn hybrids to *P. brachyurus*. This oversight can lead to significant losses for farmers if a hybrid is incorrectly used to manage *P. brachyurus*.

Although some studies on the reaction of corn hybrids to different nematodes in Brazil exist, the methodologies used for phenotyping vary or are incompletely described, preventing reproducibility across studies. Werle (2018) noted that corn hybrids with RF values ranging from 0.6 to 3.4 could be classified as resistant or having a low RF, but he did not specify the methodology used for these evaluations.

Carmo *et al*. (2015) evaluated corn hybrids at 40 and 90 days after planting in a field naturally infested with *P. brachyurus*. They observed that at 40 days, hybrids 20A55 PW, P3844 HX, and LG 6038 PRO 2 had RF values below 1.0, while at 90 days, all hybrids had RF values above 1.0. Similarly, Fonseca (2012) found that the Nema/g values on the corn hybrids were significantly influenced by the evaluation period, with lower values at 40 days and higher values at 90 days. However, the importance of this result for genotype evaluation was not discussed.

Soybean studies have shown similar *P. brachyurus* behavior. For instance, Santos *et al*. (2015) studied *P. brachyurus* reproduction at different evaluation times in soybean and found that when 600 eggs and juveniles were inoculated per plant, nematode reproduction peaked between 75 and 84 DAI. Evaluations conducted before these dates yielded incorrect results regarding cultivar resistance to root-lesion nematodes. Later, Pedro *et al*. (2024) suggested that in greenhouse conditions, evaluations of soybean genotypes inoculated with *P. brachyurus* after 70 days provide the best characterization of susceptibility and should be adopted as the optimal evaluation time.

The literature on soybean and corn confirms that to obtain a valid and reliable classification of genotypes regarding resistance or susceptibility to *P. brachyurus*, a longer interval between inoculation and evaluation is essential, as confirmed in the present study.

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