Biological products for the control of *Pratylenchus brachyurus* in maize crops

Productos biológicos para el control de *Pratylenchus brachyurus* en cultivos de maíz

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**ABSTRACT**

This study aimed to evaluate the effect of seed treatment with biological nematicides on the control of *Pratylenchus brachyurus* population in maize and crop yield. For this, a greenhouse experiment and a field experiment on clay soil were conducted in Juranda, Paraná State, Brazil. In both experiments, seeds of the maize hybrid K9310 VIP3 were subjected to the following treatments: untreated control, abamectin (Avicta® 500 FS), *Bacillus subtilis* + *B. methylotrophicus* (Rizos® + Onix®), *B. amyloliquefaciens* + *Trichoderma harzianum* (NemaControl® + StimuControl®), and *Pochonia chlamydospora* (Rizotec®). In a greenhouse,
seeds were sown in pots containing 950 cm$^3$ of autoclaved substrate. Five days after emergence, each seedling was inoculated with 500 individuals of *P. brachyurus*. After 75 days, the plants were evaluated for nematode and vegetative variables. Prior to the field experiment, samples of soil and the crop preceding maize were collected for an initial characterization of nematode populations in the area. At 60 days after sowing, plants were evaluated for nematode reproduction. Crop yield was assessed at 150 days after sowing. All biological treatments reduced *P. brachyurus* in maize roots under greenhouse conditions, with the combination of *B. amyloliquefaciens* and *T. harzianum* providing the best results. Additionally, nematode variables were negatively correlated with plant height. Under field conditions, there was a negative correlation between *P. brachyurus* and crop yield, and biological treatments led to a mean increase of 6 bags ha$^{-1}$ in maize yield compared with the control.

**Keywords:** biological control, lesion nematode, seed treatment.

**RESUMO**
Este estudo teve como objetivo avaliar o efeito do tratamento de sementes com nematicidas biológicos no controle da população de *Pratylenchus brachyurus* no rendimento do milho e da cultura. Para isso, foram realizados um experimento em estufa e um de campo em solo argiloso em Juranda, Paraná. Em ambos os experimentos, as sementes do híbrido de milho K9310 VIP3 foram submetidas aos seguintes tratamentos: controle não tratado, abamectina (Avicta® 500 FS), *Bacillus subtilis* + *B. methylotrophicus* (Rizos® + Onix®), *B. amyloliquefaciens* + *Trichoderma harzianum* (NemaControl® + StimuControl®) e *Pochonia chlamydosporia* (Rizotec®). Em uma estufa, sementes foram semeadas em vasos contendo 950 cm$^3$ de substrato autoclavado. Cinco dias após a emergência, cada muda foi inoculada com 500 indivíduos de *P. brachyurus*. Após 75 dias, as plantas foram avaliadas quanto a variáveis nematóides e vegetativas. Antes do experimento de campo, amostras de solo e da cultura que precedia o milho foram coletadas para uma caracterização inicial das populações de nematóides na área. Aos 60 dias após a semeadura, as plantas foram avaliadas para reprodução de nematóides. O rendimento da cultura foi avaliado 150 dias após a sementeira. Todos os tratamentos biológicos reduziram o *P. brachyurus* em raízes de milho em condições de estufa, com a combinação de *B. amyloliquefaciens* e *T. harzianum* fornecendo os melhores resultados. Adicionalmente, as variáveis dos nematóides foram negativamente correlacionadas com a altura da planta. Em condições de campo, houve uma correlação negativa entre *P. brachyurus* e rendimento da cultura, e os tratamentos biológicos levaram a um aumento médio de 6 sacos ha$^{-1}$ no rendimento do milho em comparação com o controle.

**Palavras-chave:** controle biológico, nematoide de lesão, tratamento de sementes.

**RESUMEN**
Este estudio tuvo como objetivo evaluar el efecto del tratamiento de semillas con nematicidas biológicos en el control de la población de *Pratylenchus brachyurus* en el rendimiento del maíz y los cultivos. Para ello, se realizó un experimento de invernadero y un experimento de campo en suelo arcilloso en Juranda, Estado de Paraná, Brasil. En ambos experimentos, las semillas del híbrido de maíz K9310 VIP3 fueron sometidas a los siguientes tratamientos: control no tratado, abamectina (Avicta® 500 FS), *Bacillus subtilis* + *B. methylotrophicus* (Rizos® + Onix®), *B. amyloliquefaciens* + *Trichoderma harzianum* (NemaControl® + StimuControl®) y *Pochonia chlamydosporia* (Rizotec®). En un invernadero, las semillas se sembraron en macetas que
contenían 950 cm³ de sustrato en autoclave. Cinco días después de la aparición, cada plántula fue inoculada con 500 individuos de P. brachyurus. Después de 75 días, las plantas fueron evaluadas para variables nematodas y vegetativas. Antes del experimento de campo, se recolectaron muestras del suelo y del cultivo que precedió al maíz para una caracterización inicial de las poblaciones de nematodos en el área. A los 60 días después de la siembra, las plantas fueron evaluadas para la reproducción de nematodos. El rendimiento del cultivo se evaluó a los 150 días después de la siembra. Todos los tratamientos biológicos redujeron P. brachyurus en raíces de maíz bajo condiciones de invernadero, con la combinación de B. amyloliquifaciens y T. harzianum proporcionando los mejores resultados. Además, las variables de nematodos se correlacionaron negativamente con la altura de la planta. En condiciones de campo, hubo una correlación negativa entre P. brachyurus y el rendimiento del cultivo, y los tratamientos biológicos llevaron a un aumento medio de 6 bolsas ha⁻¹ en el rendimiento del maíz en comparación con el control.

**Palabras clave:** control biológico, nematodo de lesión, tratamiento de semillas.

### 1 INTRODUCTION

Maíz, a strategic crop for Brazilian agribusinesses, is affected by plant-parasitic nematodes that compromise both the quantity and quality of crop production (Contini et al., 2019; Oliveira; Inomoto, 2023). The lesion nematode *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven holds great economic importance for its wide geographical distribution, polyphagous nature, and high population densities, which can result in crop losses of up to 50% in early stages of cultivation (Oliveira; Inomoto, 2023).

Environmental conditions that are favorable to nematodes or unfavorable to the crop, such as soil compaction, low fertility, high sand content, and use of host crops (e.g., soybean–maize rotation systems), are known to be associated with increased nematode populations (Franchini et al., 2014; Favoreto et al., 2019). Given the complexity of managing *P. brachyurus*, it is recommended to integrate different control strategies for best results. However, the broad host range of the nematode, the scarcity of resistant cultivars, and the relatively short residual effect of chemical nematicides make it challenging to maintain nematode populations below the economic damage threshold (Santana-Gomes et al., 2014; Homiak et al., 2017; Favoreto et al., 2019; Oliveira et al., 2019; Dias-Arieira et al., 2022).

In this context, biological control emerges as a key component of integrated nematode management. This complementary approach has the potential to work in synergy with other
control methods or may produce additive effects that facilitate nematode control (Dias-Arieira et al., 2022). Microorganisms such as fungi and bacteria can be effective agents in reducing nematode populations. Important agents used in the control of *P. brachyurus* include bacteria of the genus *Bacillus*, fungi of the genus *Trichoderma*, and the fungal species *Purpureocillium lilacinum* (= *Paecilomyces lilacinus*) and *Pochonia chlamydospora* (Kath et al., 2017; Miamoto et al., 2017; Dias-Arieira et al., 2018; Oliveira et al., 2019; Pacheco et al., 2020; Oliveira et al., 2021).

Although biological control has received great attention in scientific research in recent years, the majority of studies have focused on managing nematodes in soybean crops. Efficient strategies are needed for the management of *P. brachyurus* not only in soybean but also in maize, benefiting the rotation system as a whole. Considering these observations, this study aimed to evaluate the efficiency of seed treatment with biological products in enhancing crop yield and controlling *P. brachyurus* in maize under field and greenhouse conditions.

2 MATERIAL AND METHODS

Two experiments were conducted, one in a greenhouse (Experiment 1) and the other in the field (Experiment 2). In both experiments, seeds of the maize hybrid K9310 VIP3 were subjected to the following treatments: (i) untreated control, (ii) abamectin (Avicta® 500 FS, rate of 8 mL kg$^{-1}$ seed), (iii) *Bacillus subtilis* (Rizos®, rate of 4 mL kg$^{-1}$ seed) + *B. methylotrophicus* (Onyx®, rate of 3 mL kg$^{-1}$ seed), (iv) *B. amyloliquefaciens* (NemaControl®, rate of 3 mL kg$^{-1}$ seed) + *Trichoderma harzianum* (StimuControl®, rate of 4 mL kg$^{-1}$ seed), and (v) *P. chlamydospora* (Rizotec®, rate of 2.50 g kg$^{-1}$ seed).

Experiment 1 was conducted in a greenhouse at the State University of Maringá, Umuarama (23°47′55″S 53°18′48″W), Paraná State, Brazil, according to a completely randomized design with five treatments and eight replications. Each experimental unit consisted of a polystyrene pot containing 950 cm$^3$ of substrate (soil and sand mixed in a volumetric ratio of 2:1 and autoclaved for 2 h at 120 °C). Each pot was planted with one seed of maize. Five days after emergence, seedlings were inoculated with 500 specimens of *P. brachyurus*. The nematode suspension was deposited in a 2 cm deep hole made in the soil close to the base of the plant. The inoculum was obtained from a pure population of *P. brachyurus* maintained on soybean in a
Nematodes were extracted from the root system of soybean plants by the method proposed by Hussey and Barker (1973) and adapted by Boneti and Ferraz (1981).

At 75 days after inoculation, the plants were collected, and the root system was carefully separated from the aerial part. Roots were washed and placed on absorbent paper to remove excess water. Subsequently, roots were weighed to obtain the root fresh weight and subjected to the above-mentioned nematode extraction method. Then, the total number of nematodes was determined in a Peters chamber under an optical microscope. This parameter was divided by the root fresh weight to obtain the number of nematodes per gram of root (population density). Plant height was measured by using a millimeter ruler. Shoot fresh and dry weights were measured on a semi-analytical scale. For dry weight determination, shoots were dried to constant weight in a forced-air oven at 65 °C for 72 h.

Experiment 2 was carried out in a field in Juranda, Paraná, Brazil (24°23′43.7″ S and 52°51′26.7″ W, 507 m a.s.l). The area has a history of nematode infestation. The experimental period was from February to July 2022. Climate data for the period are shown in Table 1.

<table>
<thead>
<tr>
<th>Month</th>
<th>Cumulative rainfall (mm)</th>
<th>RH (%)</th>
<th>$T_{\text{min}}$ (°C)</th>
<th>$T_{\text{max}}$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>February</td>
<td>115.0</td>
<td>71</td>
<td>18.0</td>
<td>33.0</td>
</tr>
<tr>
<td>March</td>
<td>416.0</td>
<td>75</td>
<td>19.0</td>
<td>32.0</td>
</tr>
<tr>
<td>April</td>
<td>282.0</td>
<td>69</td>
<td>16.0</td>
<td>28.0</td>
</tr>
<tr>
<td>May</td>
<td>94.0</td>
<td>69</td>
<td>12.0</td>
<td>24.0</td>
</tr>
<tr>
<td>June</td>
<td>118.0</td>
<td>77</td>
<td>11.0</td>
<td>23.0</td>
</tr>
<tr>
<td>July</td>
<td>16.0</td>
<td>60</td>
<td>15.0</td>
<td>28.0</td>
</tr>
</tbody>
</table>

Source: Cooperativa Agroindustrial Coamo (COAMO, 2022).

The soil was classified as a dystroferric Red Latosol of clayey texture. The experiment was conducted according to a randomized block design with five replications per treatment. Each experimental unit consisted of a plot divided into eight 10 m long rows spaced 0.45 m apart, totaling 36 m$^2$ per plot. Plots were demarcated during soybean cultivation preceding maize planting. The initial population of *P. brachyurus* present in the area was surveyed by collecting root and soil samples from each plot. Additionally, soil samples were collected from the 0 to 0.20 m layer for physicochemical characterization. The results of soil analysis are described in Table 2.
Table 2. Soil chemical and physical properties at the experimental site in Juranda, Paraná, Brazil.

<table>
<thead>
<tr>
<th></th>
<th>P</th>
<th>K</th>
<th>C</th>
<th>Ca</th>
<th>Mg</th>
<th>H + Al</th>
<th>pH (CaCl₂)</th>
<th>Al</th>
<th>SB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15.64</td>
<td>0.48</td>
<td>23.07</td>
<td>5.94</td>
<td>2.05</td>
<td>5.55</td>
<td>5.12</td>
<td>0</td>
<td>8.47</td>
</tr>
<tr>
<td>Zn</td>
<td>5.3</td>
<td>23.4</td>
<td>130.9</td>
<td>7.6</td>
<td>60.41</td>
<td>14.02</td>
<td>13.2</td>
<td>13.7</td>
<td>73.1</td>
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<td>Fe</td>
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<td>CEC</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sand</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silt</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SB, sum of bases; BS, base saturation; CEC, cation-exchange capacity.
Units of measurement are as follows: P (Mehlich), Cu, Zn, Fe, and Mn, mg dm⁻³; K, Ca, Mg, H + Al, Al, SB, and CEC, cmol, dm⁻³; C, g dm⁻³; and BS, silt, sand, and clay, %.

Source: Author, 2024

During maize sowing, basal fertilization was carried out using NPK (15-15-15) fertilizer at 250 kg ha⁻¹. For the control of narrow- and broad-leaved weeds, glyphosate (Crucial®, rate of 2 L ha⁻¹) + atrazine (Primóleo®, rate of 6 L ha⁻¹) were applied when maize crops were at the V2 stage. Three insecticide applications were made for the control of bugs, as follows: (i) imidacloprid + bifenthrin (Galil®, rate of 350 mL ha⁻¹) at V2, (ii) acephate (Perito®, rate of 1 kg ha⁻¹) at V3, and (iii) acetamiprid + bifenthrin (Sperto®, rate of 150 g ha⁻¹) at V5. For the control of leafhoppers, plants were treated with a mixture of methomyl (Lannate®, rate of 1 L ha⁻¹) and acephate (Perito®, rate of 1 kg ha⁻¹) at the V4, V5, V6, V7, and V8 stages. Two fungicide applications were performed: azoxystrobin + tebuconazole (Azimut®, rate of 500 mL ha⁻¹) at V8 and azoxystrobin + epoxiconazole (Convicto®, rate of 600 mL ha⁻¹) at VT together with the insecticide imidacloprid + bifenthrin (Galil®, rate of 200 mL ha⁻¹) for aphid control.

At 60 days after maize sowing, roots and soil samples were collected from four plants in the second and penultimate rows of each plot. Samples were homogenized and stored in plastic bags (Silva; Machado, 2019). Then, the roots were sent to the Nematology Laboratory of the State University of Maringá, Umuarama Campus, where they were washed with water, placed on absorbent paper to remove excess water, and weighed for determination of the root fresh weight. Root samples were then subjected to nematode extraction, as described above. The total number of nematodes was evaluated using a Peters chamber under optical microscope. The total number of nematodes was divided by the root fresh weight to obtain the population density (nematodes g⁻¹ root).

At 150 days after maize sowing, 3 m long sections were harvested from four central rows per plot. Hundred grain weight and final yield per plot were determined. For yield determination, moisture content was corrected to 13%.
Experimental data were subjected to analysis of variance at the 5% significance level. When significant differences were detected, means were compared by Tukey's test at the 5% significance level using Sisvar software (Ferreira, 2014). Correlations between nematode and vegetative variables and yield were assessed by principal component analysis (PCA) using Statistica 10.0 software.

3 RESULTS AND DISCUSSION

In the greenhouse experiment, all bionematicides reduced the total number and population density of *P. brachyurus* in maize after 75 days of inoculation compared with the control. The most effective treatment was *B. amyloliquefaciens* + *T. harzianum*, which provided more than 85% reduction in both variables (Table 3). It is also noteworthy that all microbial agents were more efficient than chemical control in reducing *P. brachyurus*.

Table 3. Total number and population density of Pratylenchus brachyurus in maize crops under greenhouse conditions at 75 days after inoculation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total nematode number</th>
<th>Relative reduction (%)</th>
<th>Population density (nematodes g⁻¹ root)</th>
<th>Relative reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>4285 a</td>
<td>-</td>
<td>175 a</td>
<td>-</td>
</tr>
<tr>
<td>Abamectin</td>
<td>1760 b</td>
<td>58.92</td>
<td>72 b</td>
<td>58.85</td>
</tr>
<tr>
<td><em>Bs + Bm</em></td>
<td>685 cd</td>
<td>84.01</td>
<td>30 bc</td>
<td>82.85</td>
</tr>
<tr>
<td><em>Ba + Th</em></td>
<td>635 d</td>
<td>85.18</td>
<td>20 c</td>
<td>88.57</td>
</tr>
<tr>
<td><em>Pochonia chlamydosporia</em></td>
<td>1420 bc</td>
<td>66.86</td>
<td>40 bc</td>
<td>77.14</td>
</tr>
<tr>
<td>CV (%)</td>
<td>25.26</td>
<td>44.28</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means within columns followed by the same letter are not significantly different from each other by Tukey's test (*p* < 0.05). Relative reduction compared to the untreated control. CV, coefficient of variation; *Bs*, *Bacillus subtilis*; *Bm*, *Bacillus methylotrophicus*; *Ba*, *Bacillus amyloliquefaciens*; *Th*, *Trichoderma harzianum*.

The literature contains numerous reports of successful nematode control with abamectin, particularly for *P. brachyurus* (Homiak et al., 2017; Oliveira et al., 2019; Rosa et al., 2021; Gardiano-Link et al., 2022). Abamectin affects the nervous and muscular systems of nematodes by inhibiting acetylcholinesterase activity and acting on allosteric modulators of glutamate-gated chloride channels (IRAC-BR, 2022). Additional deleterious effects include disruption of ATPase activity, leading to neurotoxicity and respiratory failure (Ebone et al., 2019). Abamectin-based nematicides, although efficient in reducing nematode penetration and reproduction in the early stages of crop development, have a relatively short residual effect, providing only temporary
protection (Cabrera et al., 2009; Ribeiro et al., 2014; Silva et al., 2016; Homiak et al., 2017; Oliveira et al., 2019).

An alternative with long-term efficiency is biological control agents. Here, *B. amyloliquefaciens* + *T. harzianum* afforded greater pathogen reductions than the chemical control. The high efficiency of these microorganisms is in agreement with previous research (Miamoto et al., 2017). Bacteria of the genus *Bacillus* are noteworthy among biocontrol agents, with proven efficiency for nematode management. In general terms, Bacillus act as growth promoters and inducers of systemic resistance in plants and also colonize the rhizosphere, forming associations with plant roots, stimulated by root exudates (Abbasi et al., 2014; Saraf et al., 2014; Xing et al., 2020). As a result, a physicochemical barrier consisting of bacterial cells and metabolites with nematicidal or nematostatic properties (Engelbrecht et al., 2018) is formed. Rhizosphere colonization also triggers changes in root exudate composition, influencing the recognition of host plants by nematodes and thereby reducing pathogen penetration (Saraf et al., 2014; Lee; Kim, 2016; Sikder; Vertergard, 2020; Dias-Arieira et al., 2022).

Filamentous fungi of the genus *Trichoderma* are saprophytic microorganisms that inhabit the soil and can form symbiotic associations with various cultivated plants. *Trichoderma* contributes to pathogen control through the parasitism of eggs and juveniles, antibiosis, and competition, as well as promotion of plant growth and resistance induction (Martínez-Medina et al., 2017; Poveda et al., 2020; Sood et al., 2020). *T. harzianum* has been shown to effectively control migrating nematodes, such as *P. brachyurus*, by more than 40% (Dias-Arieira et al., 2018; Pacheco et al., 2020). Furthermore, the fungus reduced penetration levels and increased the secretion of enzymes associated with plant defense mechanisms; *in vitro*, it increased pathogen mortality (Kath et al., 2017) by up to 65% (Oliveira et al., 2021).

In the greenhouse experiment, treatments had a significant effect on root fresh weight only. *P. chlamydosporia* treatment afforded the highest root weight (Table 4).
Table 4. Height, shoot fresh and dry weights, and root fresh weight of maize under different biological treatments at 75 days after inoculation with Pratylenchus brachyurus.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant height (cm)</th>
<th>Shoot fresh weight (g)</th>
<th>Shoot dry weight (g)</th>
<th>Root fresh weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>139.33**</td>
<td>133.40**</td>
<td>26.14**</td>
<td>25.71b</td>
</tr>
<tr>
<td>Abamectin</td>
<td>177.00</td>
<td>126.46</td>
<td>25.46</td>
<td>25.07b</td>
</tr>
<tr>
<td>Bs + Bm</td>
<td>172.66</td>
<td>140.24</td>
<td>27.53</td>
<td>22.96b</td>
</tr>
<tr>
<td>Ba + Th</td>
<td>184.16</td>
<td>155.23</td>
<td>30.96</td>
<td>32.71ab</td>
</tr>
<tr>
<td>Pochonia chlamydosporia</td>
<td>172.50</td>
<td>153.07</td>
<td>28.27</td>
<td>36.70a</td>
</tr>
<tr>
<td>CV (%)</td>
<td>17.74</td>
<td>17.93</td>
<td>13.12</td>
<td>21.14</td>
</tr>
</tbody>
</table>

Means followed by the same letter are not significantly different from each other by Tukey’s test (p < 0.05). CV, coefficient of variation; ns, not significant; Bs, Bacillus subtilis; Bm, Bacillus methylotrophicus; Ba, Bacillus amyloliquefaciens; Th, Trichoderma harzianum.

Source: Author, 2024

Classified as a chitinolytic fungus, *P. chlamydosporia* uses several mechanisms of action against nematodes, including egg parasitism, colonization of females, and induction of systemic resistance and growth in several plant species (Dallemole-Giaretta et al., 2015; Larriba et al., 2015; Zavala-Gonzalez et al., 2017; Monteiro et al., 2018; Pacheco et al., 2020; Coutinho et al., 2021). Although *P. chlamydosporia* is most effective against sedentary nematodes, which deposit eggs in egg masses, the fungus also acts against migrating nematodes, such as *P. brachyurus* (Dias-Arieira et al., 2018; Pacheco et al., 2020; Rosa et al., 2021). Moreover, the fungus can colonize roots endophytically, exerting positive effects on plants and negative effects on nematodes (Dias-Arieira et al., 2023).

In addition to minimizing nematode reproduction, *P. chlamydosporia* can increase the activity of antioxidant enzymes associated with resistance induction (Medeiros et al., 2015) and solubilize phosphorus, significantly enhancing root development and maize yield (Rosa et al., 2021). Of note, soils with high levels of organic matter offer more favorable conditions for *P. chlamydosporia* development. As observed by Podestá et al. (2016), the period between mycelial establishment and contact with nematodes plays a crucial role in the effectiveness of biological control. Thus, it is important that fungi acquire a competitive advantage over nematodes by colonizing the rhizosphere or rhizoplane before the arrival of pathogens (Dias-Arieira et al., 2023).

PCA of the vegetative parameters of maize (Fig. 1) revealed two principal components, which together explained 92.60% of the variance in the dataset. The first component explained 69.81% and the second component, 22.79%. The PCA biplot indicated a strong negative correlation of total nematode number and population density with plant height. Shoot fresh and
dry weights and root fresh weight were positively and strongly correlated, as were total nematode number and population density.

These correlations can be explained by the direct impact of *P. brachyurus* on root development, interfering with water and nutrient absorption. The nematode causes lesions that necrotize roots and can compromise the full development capacity of plants. Thus, infected plants may exhibit typical secondary symptoms in shoots, such as atrophy and reduced size (Oliveira; Inomoto, 2023).

Figure 1. Principal component analysis of total *P. brachyurus* number (total Pb) and population density (Pb/g), plant height, shoot fresh weight (SFW), shoot dry weight (SDW), and root fresh weight (RFW) of maize grown under greenhouse conditions.

In the field experiment, *P. brachyurus* was found to be uniformly distributed across areas planted with the predecessor crop (soybean) (Table 5).

**Table 5. Initial population of Pratylenchus brachyurus in soybean roots (predecessor crop) and maize roots (60 days after sowing) under different biological treatments, Juranda, Paraná State, Brazil.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nematode population (nematodes 10 g⁻¹ root)</th>
<th>Relative reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soybean roots</td>
<td>Maize roots</td>
</tr>
<tr>
<td>Control</td>
<td>280ns</td>
<td>350ns</td>
</tr>
<tr>
<td>Abamectin</td>
<td>180</td>
<td>240</td>
</tr>
<tr>
<td><em>Bs</em> + <em>Bm</em></td>
<td>280</td>
<td>320</td>
</tr>
<tr>
<td><em>Ba</em> + <em>Th</em></td>
<td>140</td>
<td>250</td>
</tr>
<tr>
<td><em>Pochonia chlamydosporia</em></td>
<td>150</td>
<td>330</td>
</tr>
</tbody>
</table>
Root lesion nematodes are a recurrent pathogen in Brazilian crops, being considered the most damaging nematodes to maize. Although *P. brachyurus* is native to the country, its population levels have significantly increased in recent years, increasing damage to crops. According to a 2022 survey, *Pratylenchus* spp. were detected in about 73.6% of samples from maize fields in Brazil; in the southern region, the frequency of lesion nematodes reached 67.7% (Syngenta, 2022). Several factors might be related to such an increase in nematode populations, including intercropping with host plants (e.g., soybean–maize), soil compaction, lack of crop rotation, low fertility, and sandy soils (Dias-Arieira et al., 2021).

There were no differences in *P. brachyurus* number between the control and treatments at 60 days after sowing (DAS). However, varied effects were observed in the relative reduction percentage (Table 5). Abamectin and *B. amyloliquefaciens* + *T. harzianum* afforded the largest reductions, namely of 31.43% and 28.57%, respectively. A non-uniform nematode distribution in naturally infested areas or neighboring plants makes it difficult to study management strategies in the field. It is easier to analyze the effects of treatments on plant development. Under these conditions, it is important that nematode control be carried out for initial crop protection, such as via seed treatment or in-furrow application. With this, the root system has a better chance to develop adequately.

In the case of biological control, it is necessary for control agents to acquire a competitive advantage over nematodes, being present in the rhizosphere or rhizoplane before pathogen arrival (Dias-Arieira et al., 2023). Furthermore, the period needed for complete plant protection may vary depending on the ecology and biology of soil. A wide range of biotic and abiotic factors may influence the survival and development of the biocontrol agent, such as soil texture, organic matter content, moisture, temperature, and competition between microorganisms (Abd-Elgawad; Askary, 2020). Competition between introduced and native microorganisms in soil is believed to be directly related to soil quality, being more intense in poorer soils. In such soils, native microorganisms are highly adapted to local edaphoclimatic conditions. Thus, bionematicides
added to soil tend to be gradually eliminated because of the intense competition for available resources (Dias-Arieira et al., 2023).

At 150 DAS, there were no differences between treatments in hundred grain weight or final yield. However, there was a mean increment of 6 bags ha\(^{-1}\) with biological treatment compared with the control. The highest increase (6.8 bags ha\(^{-1}\)) was observed in maize treated with \(B.\) \textit{subtilis} + \(B.\) \textit{methylotrophicus} (Table 6).

Table 6. Hundred grain weight, grain yield, and grain yield relative to the control of maize subjected to different biological treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hundred grain weight (g)</th>
<th>Yield (bags ha(^{-1}))</th>
<th>Relative yield (bags ha(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27.78(^{ns})</td>
<td>121.9(^{ns})</td>
<td>-</td>
</tr>
<tr>
<td>Abamectin</td>
<td>27.64</td>
<td>126.2</td>
<td>4.3</td>
</tr>
<tr>
<td>(Bs) + (Bm)</td>
<td>29.79</td>
<td>128.7</td>
<td>6.8</td>
</tr>
<tr>
<td>(Ba) + (Th)</td>
<td>28.21</td>
<td>127.7</td>
<td>5.8</td>
</tr>
<tr>
<td>\textit{Pochonia chlamydospora}</td>
<td>28.56</td>
<td>127.4</td>
<td>5.5</td>
</tr>
</tbody>
</table>

\(^{ns}\), not significant at the 5% significance level by Tukey's test; CV, coefficient of variation; \(Bs\), \textit{Bacillus subtilis}; \(Bm\), \textit{Bacillus methylotrophicus}; \(Ba\), \textit{Bacillus amyloliquefaciens}; \(Th\), \textit{Trichoderma harzianum}.

Source: Author, 2024

Steffen et al. (2020) found that seed treatment with \textit{T. harzianum} led to an increase of 40 bags ha\(^{-1}\) in maize yield compared with the untreated control. According to the authors, these results were probably due to the increased root volume and stem diameter, which contributed to enhancing the capacity of plants to absorb and translocate water and nutrients. \textit{Trichoderma} not only acts in pathogen control but also synthesizes indoleacetic acid, an important inducer of plant growth (Kribel et al., 2019; Bader et al., 2020). The genus was reported to stimulate rooting, directly contributing to plant nutrition (Steffen et al., 2020).

Bacteria of the genus \textit{Bacillus} are also known to increase maize yield (Chagas et al., 2017). In a field study with \textit{B. subtilis} and \textit{B. megaterium}, Paiva et al. (2020) observed yield gains of up to 50%. Several species belonging to this genus have been identified as potential promoters of plant growth because of their multifunctional characteristics. Such characteristics include the ability to solubilize phosphate (Bahadir et al., 2018), produce indoleacetic acid and other phytohormones (Mohite, 2013), and synthesize siderophores, which are specific chelators of iron ions (Bjelić et al., 2018).

PCA of \textit{P. brachyurus} initial population, population at 60 DAS, and maize yield (Fig. 2) revealed two principal components, which together explained 90.07% of the total variance in the
dataset. Principal component 1 explained 48.77% and principal component 2, 41.30%. The biplot shows a strong positive correlation between initial nematode population and population at 60 DAS. These variables, however, correlated negatively with yield, demonstrating that nematodes directly affected maize yield.

There are several mechanisms by which *P. brachyurus* negatively impacts crop yield, including direct damage to roots, which compromises water and nutrient absorption. Such an effect can further result in water and/or nutritional stress, reduced photosynthetic activity, and increased susceptibility to diseases and opportunistic pathogens (McDonald et al., 2017).

As discussed, biological control agents are advantageous for long-term nematode management. These agents have multiple mechanisms of action, are compatible with chemical nematicides, can complement cultural management by conferring protection to plants, and are registered specifically for the target pest rather than the crop (Dias-Arieira et al., 2023). Furthermore, the combined use of fungi and bacteria may have a synergistic and additive effect on *P. brachyurus* control (Mamutoto et al., 2017).

The results of this study demonstrated the efficiency of biological nematicides in controlling *P. brachyurus* in maize. Of note, a single control strategy may not be sufficient to reduce the damage caused by nematodes, particularly when dealing with high population...
numbers. Thus, integrated management, such as the combined use of chemical and biological products and rotation with antagonistic plants or bad hosts, is essential for successful management.

4 CONCLUSIONS

All biological products applied via seed treatment were efficient in reducing *P. brachyurus* populations in maize grown under greenhouse conditions, particularly *B. amyloliquefaciens* + *T. harzianum*. Under greenhouse conditions, there was a negative correlation between *P. brachyurus* and plant height. Under field conditions, the pathogen correlated negatively with crop yield. In the field, biological treatment afforded a mean yield increment of 6 bags ha$^{-1}$ compared with the control.
REFERENCES


