Penetration of *Pratylenchus zeae* in antagonistic plants

Simone de Melo SANTANA-GOMES 1,∗, Claudia Regina DIAS-ARIEIRA 2, Júlio Cesar Antunes FERREIRA 3, Paula Juliana Grotto DÉBIA 2, Fabio BIELA 4 and Michelly Ragazzi CARDOSO 3

*Pratylenchus zeae* Graham is one of the most important nematode species found in sugarcane and has been reported in the main sugarcane-producing regions of Brazil, especially in areas of commercial production in the state of São Paulo (Novaretti et al., 1998, Dinardo-Miranda et al., 2003), in the northeast region (Moura et al., 2000) and in Paraná (Severino et al., 2010). Due to the scarcity of plants resistant to root-lesion nematode and limitations regarding the use of chemical control, crop rotation is becoming increasingly important. Thus, the use of leguminous plants as a green manure is crucial as it promotes, among other benefits, improvements in the physical and chemical conditions of the soil (McSorley & Gallaher, 1994).

An experiment was conducted in a glasshouse at Universidade Estadual de Maringá, campus Regional de Umuarama (State University of Maringa, Umuarama Regional Campus) in January 2014, with minimum and maximum temperatures of 20.2 and 32.4°C, respectively. The species assessed in this experiment were crotalaria (*Crotalaria spectabilis*), dwarf pigeon pea (*Cajanus cajan*), velvet bean (*Mucuna aterrima*), jack bean (*Canavalia ensiformis*) and stylosanthes (*Stylosanthes capitata + S. macrocephala*), with maize (*Zea mays*) cv. IPR 114 as control. The seedlings, produced in the same manner, were transplanted into 500 ml plastic containers of the same soil:sand mixture that had been previously autoclaved at 120°C for 2 h.

Two days after transplantation, the seedlings were inoculated with a suspension of 1000 *P. zeae*, obtained from a pure population maintained on maize in a glasshouse. For the extraction of nematodes from the roots, the methodology of Coolen & D’Herde (1972) was adopted. The suspensions obtained were examined with an optical microscope using a nematode counting chamber, and the sample was calibrated to a 4 ml solution. It was then applied to the soil in 3-4 cm deep holes. Five, 10 and 15 days after inoculation three seedlings from each species were removed. The roots were collected, carefully washed and weighed with a semi-analytical balance. Then, the nematodes in these roots were stained with acid fuchsin (Byrd et al., 1983) and the roots were left to de-stain in acidified glycerol. Subsequently, roots were mounted on temporary microscope slides and were assessed under a light microscope for the presence of nematodes and to determine the total number of individuals inside the root. The whole experiment was repeated in time and the combined data were subjected to analysis of variance and the means were compared by Duncan's test at 5% probability.

Five days after inoculation (DAI) there was no statistical difference between the values of penetration of *P. zeae* in maize roots and in the leguminous plants, and the values...
Table 1. Number of Pratylenchus zeae in maize, crotalaria, dwarf pigeon pea, velvet bean, jack bean and stylosanthes roots at 5, 10 and 15 days after inoculation (DAI) with 1000 nematodes.

<table>
<thead>
<tr>
<th>Plant</th>
<th>5 DAI</th>
<th>10 DAI</th>
<th>15 DAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>11.2 a</td>
<td>128.8 a</td>
<td>27.3 a</td>
</tr>
<tr>
<td>Crotalaria</td>
<td>0.0 b</td>
<td>0.5 b</td>
<td>1.9 b</td>
</tr>
<tr>
<td>Dwarf pigeon pea</td>
<td>0.3 b</td>
<td>0.1 b</td>
<td>1.6 b</td>
</tr>
<tr>
<td>Velvet bean</td>
<td>0.0 b</td>
<td>16.3 b</td>
<td>10.6 b</td>
</tr>
<tr>
<td>Jack bean</td>
<td>0.0 b</td>
<td>0.0 b</td>
<td>3.2 b</td>
</tr>
<tr>
<td>Stylosanthes</td>
<td>0.1 b</td>
<td>0.0 b</td>
<td>0.0 b</td>
</tr>
<tr>
<td>CV (%)</td>
<td>10.2</td>
<td>8.7</td>
<td>15.3</td>
</tr>
</tbody>
</table>

Means followed by the same letter in the columns are not statistically different (Duncan’s test at 5% probability). CV = coefficient of variation.

were very low, with maximum penetration of 11.2 nematodes (root system)$^{-1}$ in maize. Nevertheless, at 10 DAI, the penetration of $P$. zeae in maize was higher than that in the assessed antagonistic plants, with 128.8 nematodes (root system)$^{-1}$ of maize, followed by velvet bean (16.3), while the other plants had values lower than 1.0 (Table 1).

At 15 DAI, penetration remained higher in maize (27.3 nematodes (root system)$^{-1}$), followed by velvet bean and jack bean (10.6 and 3.3, respectively).

It had been previously observed that cultivation using a sugarcane - C. spectabilis - rotation system in a soil naturally infested with a mixed population of Pratylenchus (P. zeae and P. brachyurus) reduced nematode populations by up to 48%, maintaining them at relatively lower levels and for a longer period, approximately 4 months, compared with the other assessed systems (Oliveira et al., 2008). Similarly to C. spectabilis, C. juncea showed positive results for the control of $P$. zeae in sugarcane when grown in succession with velvet bean, leading to a decrease in the total nematode population and an increase in productivity ranging from 10.8 to 22.1% compared with fallow (Moura et al., 2010). However, even when grown alone, C. juncea showed better control of P. zeae in sugarcane (Sundararaj & Mehta, 1990).

In our experiment, P. zeae penetrated the roots in small numbers, when compared to the control treatment. So the plant reacted as a non-host and this would have happened in different ways, such as producing repelling or unattractive substances, but further studies are needed to elucidate the mode of action.

The efficiency of pigeon pea in the control of P. zeae, demonstrated in the reduced penetration in the roots, corroborates the results obtained by Santana et al. (2012), who reported that, regardless of the type of soil (clay or sandy soil), growing pigeon pea for 90 and 110 days caused a reduction in the population of the parasite. The resistance of different strains of pigeon pea to P. zeae was demonstrated in other studies (Jones & Hillocks, 1995; Araújo Filho et al., 2010; Souto et al., 2011), but the antagonistic activity of pigeon pea has not been well defined because different genotypes respond differently to the presence of populations of P. zeae. However, it is hypothesised that the antagonistic effect depends on the amount of phenol produced by the plant (Thakar & Yadav, 1986).

Velvet bean was the leguminous crop with the highest penetration of nematodes in the assessment performed 10 and 15 days after inoculation. Some studies demonstrated that this crop might respond differently to nematode inoculation. Thus, cultivation of velvet bean for 3 months followed by cultivation of Crotalaria, and the reverse treatment, reduced the entire population of P. zeae (100%) in areas infested with the parasite in the state of Pernambuco (Moura & Oliveira, 2009). Rotations with maize and velvet bean reduced the population of P. zeae by 32% in maize and the low populations of the nematode reduced infection and reproduction (Marisa et al., 1996; Arim et al., 2006).

Penetration of P. zeae in jack bean in the roots occurred only 15 days after inoculation and in higher percentages than those obtained in the control crop. Jack bean crop had previously reduced the population of P. zeae in soils infested with this parasite, and, besides reducing reproduction, controlled the population in sugarcane grown in succession (Obici et al., 2011). Moreover, C. ensiformis reduced the population of P. zeae in maize (Arim et al., 2006), and was considered a non-host for P. neglectus (Al-Rehiyani & Hafez, 1998); results of the present study indicated it was a poor host.

In the cultivation of stylosanthes, the values 0.1 and 0.0 nematodes (root system)$^{-1}$ were observed at 5 and 15 DAI, which were lower than those observed in the control treatment. Likewise, the plant reduced the population of P. zeae after 90 and 120 days of cultivation in different soils, with prolonged effects on the subsequent crop (Obici et al., 2011). Similarly to other leguminous crops studies, this plant reacted as a poor host with low penetration of P. zeae.

Thus, all the leguminous crops had a lower invasion of P. zeae than that observed for maize. The findings showed that the plants assessed in this study reacted as poor hosts for P. zeae.