Susceptibility of Crotalaria Species to Different Populations of Meloidogyne javanica

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Abstract
Crotalaria is one of the most widely used plants for the control of phytopathogenic nematodes. The plants act by inhibiting nematode multiplication, fixing atmospheric nitrogen, and improving soil quality. The pathogenicity of nematode populations can vary according to plant species. This study aimed to evaluate the response of different Crotalaria species to seven populations of Meloidogyne javanica. Crotalaria spectabilis, C. ochroleuca, C. juncea, and soybean (control) were inoculated with the nematode. Fresh root weight, root-knot index, total number of nematodes, nematode population density (number of nematodes per gram of fresh root), and reproductive factor (RF) were evaluated 60 days after inoculation. M. javanica populations varied in pathogenicity to all plants studied. Soybean was susceptible to all populations, and C. spectabilis and C. ochroleuca were resistant. C. juncea was susceptible to Mj-7 (RF=1.04). Mj-7 was the most pathogenic population.

Keywords: Antagonist plants; Pathogenic variability; Root-knot nematodes.

INTRODUCTION

Root-knot nematodes (Meloidogyne spp.) are responsible for major losses in soybean [1]. Monoculture is one of the factors that contribute to nematode population increase and reduce agricultural productivity [2]. The parasitism of Meloidogyne spp. is complex. The nematodes infect plant roots, from where they extract nutrients for their development and reproduction, and inject substances that cause damage to the roots, leading to cell hypertrophy and hyperplasia and the formation of galls. These alterations obstruct the flow of nutrients and water to the plant, reducing plant growth and development and increasing susceptibility to other plant pathogens [3].

Management of root-knot nematodes involves integrated control strategies, such as the use of resistant cultivars and chemical and biological nematicides [4]. Crop rotation is another important strategy. Some plant species used in crop rotation are able to inhibit nematode multiplication while enhancing soil quality by increasing nitrogen fixation and nutrient availability [5-7]. Crotalaria spp. are the most widely used plants for nematode control, particularly Crotalaria spectabilis Roth, Crotalaria ochroleuca G. Don and Crotalaria juncea L. They effectively reduce Meloidogyne spp. reproduction [8-11]. However, the efficiency of Crotalaria plants may vary depending on nematode population. Conflicting results were reported for C. juncea against Meloidogyne javanica (Treub) Chitwood and Meloidogyne incognita (Kofoid and White) Chitwood; the plant was reported to be resistant in some studies [10,11] but susceptible in others [12,13]. Similarly, C. ochroleuca has been reported as resistant [14] and susceptible [10,12] to M. javanica.

Genetic characteristics of the host, environmental conditions, and pathogenic variability among nematode populations can be responsible for differences in the susceptibility of Crotalaria species. For instance, climatic conditions and mutagenic events can generate more aggressive and virulent individuals able to adapt to new hosts and environments [15,16].

We hypothesize that the pathogenicity of M. javanica populations from different regions of Brazil varies between Crotalaria species, which could represent a risk to susceptible crops in rotation with...
Crotalaria. The aim of this study was to evaluate the response of different Crotalaria species to seven populations of M. javanica.

MATERIAL AND METHODS

Collection of nematode populations

Soil and symptomatic roots samples were collected between March and May 2017 and analyzed in the Laboratory of Nematology of the State University of Maringá, Brazil (23°24′17.39″S 51°56′27.00″W). Seven soil and root samples collected at different locations (Table 1) were maintained on tomatoes (Lycopersicon esculentum L. cv. Santa Clara) and okra [Abelmoschus esculentus (L.) Moench cv. Santa Cruz] in pots containing 3 L of infected soil under greenhouse conditions for nematode multiplication. Plants were irrigated as necessary to provide favorable conditions for nematode multiplication. The inoculum was maintained for 60 days, after which samples were subjected to isoenzymatic analysis and purification.

<table>
<thead>
<tr>
<th>Populations</th>
<th>Locations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mj-1</td>
<td>Formosa do Rio Preto – BA</td>
</tr>
<tr>
<td>Mj-2</td>
<td>Formosa do Rio Preto – BA</td>
</tr>
<tr>
<td>Mj-3</td>
<td>Londrina – PR</td>
</tr>
<tr>
<td>Mj-4</td>
<td>Alegrete – RS</td>
</tr>
<tr>
<td>Mj-5</td>
<td>Luiz Eduardo Magalhães - BA</td>
</tr>
<tr>
<td>Mj-6</td>
<td>Formosa do Rio Preto – BA</td>
</tr>
<tr>
<td>Mj-7</td>
<td>Nova Maringá – MT</td>
</tr>
</tbody>
</table>

Isoenzymatic characterization and purification of populations

Isoenzymatic characterization of Meloidogyne spp. was carried out for each sample, and inocula were purified by removing the egg mass.

Twenty adult milky-white colored females were removed from each sample using fine-point forceps and a stereoscope microscope. The females were transferred to capillary tubes containing extraction buffer [17], macerated with a needle, mixed with bromophenol blue, and loaded onto a polyacrylamide gel. A sample of M. javanica was used as standard for phenotypic comparison. Vertical gel electrophoresis was carried out using a Bio-Rad PowerPac™ Basic (Singapore, Singapore) apparatus under refrigeration. Electrophoresis was initially carried out at 100 V until bromophenol blue reached the separating gel and then at 200 V. The gel was transferred to a glass flask containing a staining solution (50 mL of phosphate buffer, 50 mg of Fast Blue RR salt, and 1.5 mL of 1% α-naphthylacetate) for esterase detection. The gel was incubated in the dark at 37°C for 30 min until dark bands were revealed and then transferred to a fixative solution (50 mL of acetic acid, 50 mL of distilled water, and 10 mL of methyl alcohol solution) for 30 min. Finally, the gel was placed on wet cellophane paper and dried on acrylic frames. The relative mobility of the bands was measured in relation to the first band of M. javanica. The boundary between stacking and separating gels was used as a reference.

The egg mass of each M. javanica population was inoculated on a tomato plant. Tomatoes were transplanted to pots containing 2 L of autoclaved soil (120°C for 2 h) and kept under greenhouse conditions for five months for nematode multiplication.

Susceptibility of Crotalaria to M. javanica populations

Experiments were conducted in a greenhouse, under coordinates 23°24′17.39″S and 51°56′27.00″W, in two periods, from September to November 2018 (Experiment 1) and from December 2018 to February 2019 (Experiment 2). A completely randomized 4 × 7 factorial design with six replicates was used. C. ochroleuca, C. juncea, C. spectabilis, and soybean cv. M6210 IPRO (control) seeds were sown in plastic trays containing commercial substrate (Mecplan®, Telêmaco Borba – PR, Brazil). After 15 days, seedlings were transplanted to styrofoam pots containing 500 mL of substrate composed of clayey soil and sand at a 1:1 (v/v) ratio. Plants were inoculated with 1000 eggs and eventual second-stage (J2) juveniles of M. javanica at the time of transplant. Seven nematode populations were used. The inoculum of each population was extracted following the method proposed by Hussey and Barker [18] and modified by Boneti and Ferraz [19]. A 3 mL suspension containing 1000 eggs and J2 was obtained using a Peters chamber under a light microscope.

Plants were grown for 60 days under greenhouse conditions and irrigated daily. After this period, plants were removed, and the roots were carefully washed, dried on a paper towel to remove excess water, and weighed on a semi-analytical balance for determination of fresh root weight. The gall index (GI) was...
The number of total nematodes and the population density (number of nematodes per gram of fresh root) were determined for each root sample. The reproduction factor (RF) was calculated using the equation RF = final population/initial population. Plants with RF = 0 were considered immune; RF < 1, resistant; and RF ≥ 1, susceptible [21].

Data were submitted to analysis of variance, and means were compared by the Scott–Knott test and Tukey’s test for nematode populations and plant species, respectively, using SISVAR [22]. Statistical significance was set at p<0.05. To meet normality assumptions, data were transformed using \((x + 1)^{1/2}\).

**RESULTS**

*M. javanica* populations varied greatly in their reproductive ability on different plant species. Interaction effects between plant species and nematode populations were found for all parameters. Nematode reproduction was greater on soybean than on *Crotalaria* species. *Mj-7* had higher GI than other nematode populations on *C. ochroleuca* (0.83 in Experiment 1 and 0.33 in Experiment 2) (Table 2). For *C. juncea*, the highest GIs were observed for *Mj-5* in Experiment 1 (GI = 2) and *Mj-3* in Experiment 2 (GI = 0.50). *C. spectabilis* showed lower GI than other plants, with an GI different from zero only for *Mj-3* in Experiment 1. For soybean, the highest GIs were observed for *Mj-5* (4.50), *Mj-7* (4.00), *Mj-6* (3.67), *Mj-3* (2.83), and *Mj-1* (2.83) in Experiment 1 and for *Mj-7* (4.33), *Mj-4* (3.67), *Mj-3* (4.33), and *Mj-5* (3.17) in Experiment 2. In both experiments, *Mj-1*, *Mj-2*, *Mj-3*, *Mj-4*, and *Mj-6* did not differ in GI between *Crotalaria* species but had significantly greater values on soybean. In Experiment 1, the GI of the *Mj-5* population on *C. juncea* was greater than on other *Crotalaria* species but lower than on soybean. *Mj-7* had the highest GI on soybean, followed by *C. ochroleuca* and other *Crotalaria* species.

Table 2: Gall index (GI) of different populations of *Meloidogyne javanica* inoculated in *Crotalaria* and soybean species.

<table>
<thead>
<tr>
<th>Populations</th>
<th>Gall index (GI)</th>
<th>C. ochroleuca</th>
<th>C. juncea</th>
<th>C. spectabilis</th>
<th>Soybean</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mj-1</em></td>
<td>0.33 bB</td>
<td>0.00 bB</td>
<td>0.00 aB</td>
<td>2.83 cA</td>
<td></td>
</tr>
<tr>
<td><em>Mj-2</em></td>
<td>0.00 bB</td>
<td>0.00 bB</td>
<td>0.17 aB</td>
<td>2.17 da</td>
<td></td>
</tr>
<tr>
<td><em>Mj-3</em></td>
<td>0.50 aB</td>
<td>0.00 bB</td>
<td>0.17 aB</td>
<td>2.83 cA</td>
<td></td>
</tr>
<tr>
<td><em>Mj-4</em></td>
<td>0.17 bB</td>
<td>0.17 bB</td>
<td>0.00 aB</td>
<td>2.50 da</td>
<td></td>
</tr>
<tr>
<td><em>Mj-5</em></td>
<td>0.50 aC</td>
<td>2.00 aB</td>
<td>0.00 aC</td>
<td>4.50 aA</td>
<td></td>
</tr>
<tr>
<td><em>Mj-6</em></td>
<td>0.50 bB</td>
<td>0.17 bB</td>
<td>0.00 aB</td>
<td>3.17 cA</td>
<td></td>
</tr>
<tr>
<td><em>Mj-7</em></td>
<td>0.83 aB</td>
<td>0.00 bC</td>
<td>0.00 aC</td>
<td>4.00 bA</td>
<td></td>
</tr>
<tr>
<td><strong>C.V. (%)</strong></td>
<td>26.62</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Within each experiment, means followed by the same lowercase letter in the column and upper case in the line do not differ by the Scott-Knott and Tukey test, respectively, at 5% probability.

Soybean had the highest nematode population density in both experiments; *Mj-5* and *Mj-7* were found at higher concentrations than other populations in Experiment 1 and *Mj-1* in Experiment 2 (Table 3). There was no significant difference in nematode population density between *Crotalaria* species. On *C. ochroleuca* and *C. juncea*, *Mj-7* had higher population density in Experiment 1 and *Mj-2* in Experiment 2. On *C. spectabilis*, the *Mj-6* population was present at higher densities in both experiments.

Table 3: Number per root gram of different populations of *Meloidogyne javanica* inoculated in *Crotalaria* and soybean species.

<table>
<thead>
<tr>
<th>Populations</th>
<th>Nematode number per root gram</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. ochroleuca</td>
</tr>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
</tr>
<tr>
<td><em>Mj-1</em></td>
<td>10 aB</td>
</tr>
<tr>
<td><em>Mj-2</em></td>
<td>25 aB</td>
</tr>
<tr>
<td><em>Mj-3</em></td>
<td>36 aB</td>
</tr>
<tr>
<td><em>Mj-4</em></td>
<td>21 aB</td>
</tr>
<tr>
<td><em>Mj-5</em></td>
<td>25 aB</td>
</tr>
<tr>
<td><em>Mj-6</em></td>
<td>28 aB</td>
</tr>
<tr>
<td><em>Mj-7</em></td>
<td>55 aB</td>
</tr>
<tr>
<td><strong>C.V. (%)</strong></td>
<td>33.71</td>
</tr>
</tbody>
</table>

Within each experiment, means followed by the same lowercase letter in the column and upper case in the line do not differ by the Scott-Knott and Tukey test, respectively, at 5% probability.
Mj-7 (0.50) in Experiment 2. On between Crotalaria species in both experiments. However, the Meloidogyne javanica highest RF was observed for Mj-3 (0.34) in Experiment 1 and for Mj-1, Mj-2, Mj-4, Mj-5, and Mj-6 showed no differences in RF.

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**Table 4:** Reproduction Factor (RF) of different populations of Meloidogyne javanica inoculated in Crotalaria and soybean species.

<table>
<thead>
<tr>
<th>Populations</th>
<th>Reproduction Factor (RF)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. ochroleuca</td>
</tr>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
</tr>
<tr>
<td>Mj-1</td>
<td>0.11 aB</td>
</tr>
<tr>
<td>Mj-2</td>
<td>0.28 aB</td>
</tr>
<tr>
<td>Mj-3</td>
<td>0.34 aB</td>
</tr>
<tr>
<td>Mj-4</td>
<td>0.23 aB</td>
</tr>
<tr>
<td>Mj-5</td>
<td>0.24 aB</td>
</tr>
<tr>
<td>Mj-6</td>
<td>0.28 aB</td>
</tr>
<tr>
<td>Mj-7</td>
<td>0.30 aBC</td>
</tr>
<tr>
<td><strong>C.V. (%)</strong></td>
<td></td>
</tr>
</tbody>
</table>

**Experiment 2**

| Mj-1        | 0.05 aB       | 0.00 aB   | 0.18 aB       | 26.35 cA |
| Mj-2        | 0.38 aB       | 0.71 aB   | 0.22 aB       | 30.66 dA |
| Mj-3        | 0.28 aB       | 0.17 aB   | 0.17 aB       | 38.45 cA |
| Mj-4        | 0.39 aB       | 0.23 aB   | 0.28 aB       | 46.26 bA |
| Mj-5        | 0.06 aB       | 0.59 aB   | 0.24 aB       | 36.46 cA |

| Mj-6        | 0.25 aB       | 0.73 aB   | 0.27 aB       | 16.78 fA |
| Mj-7        | 0.50 aB       | 1.04 aB   | 0.23 aB       | 62.03 aA |

C.V. (%) 30.55

Within each experiment, means followed by the same lowercase letter in the column and upper case in the line do not differ by the Scott-Knott and Tukey test, respectively, at 5% probability.

Mj-1, Mj-2, Mj-4, Mj-5, and Mj-6 showed no differences in RF between Crotalaria species in both experiments. However, the RF of these nematode populations was significantly higher on soybean than on Crotalaria plants (Table 4). In Experiment 1, the RF of Mj-3 on C. ochroleuca was higher than on C. juncea but did not differ from that on C. spectabilis. On C. ochroleuca, the highest RF was observed for Mj-3 (0.34) in Experiment 1 and for Mj-7 (0.50) in Experiment 2. On C. juncea, Mj-7 showed higher RF than other populations in both.

**DISCUSSION**

M. javanica populations differed in reproductive parameters between Crotalaria species. C. juncea was the only species of Crotalaria susceptible to Mj-7 in Experiment 2 (RF of 1.04). In general, C. juncea showed greater susceptibility to M. javanica populations than other Crotalaria species. In a previous study, Charchar et al. [23] found that C. juncea allowed the reproduction not only of M. javanica but also of M. incognita. Studies have also pointed this species as susceptible plant to the root lesion nematode Pratylenchus brachyurus (Godfrey) Filipjev & Schuurmans Stekhoven [24,25]. In contrast to C. juncea, C. spectabilis and C. ochroleuca were resistant (RF < 1) to all nematode populations in both experiments.

The positive results obtained with C. spectabilis in the control of root-knot nematodes are widely reported [11,14,26-28]. This plant antagonizes Meloidogyne via diverse mechanisms: it causes the nematode to form abnormally small nourishing cells, leads to nutritional deficiency, delays growth, and prevents the production of adult females [29,30]. Because it attracts and allows the penetration of juveniles but prevents their development and reproduction, C. spectabilis has potential as a trap crop [31,32].

C. ochroleuca is an efficient antagonist of Meloidogyne spp. [14], P. brachyurus [33], and Rotylenchulus reniformis Linford and Oliveira [34]. However, controversial results have been reported on its susceptibility to P. brachyurus [35], M. javanica [10], and different races of Heterodera glycines Ichinohe [35].

The antagonistic effects of Crotalaria species on nematodes have been attributed to the production of the pyrrolizidine alkaloid monocrotaline [36]. However, some studies concluded that, although this alkaloid reduces nematode mobility, it does not appear to be directly related to the antagonistic effects of Crotalaria, as several other plant species that produce monocrotaline are susceptible to nematodes [37,38].

In this study, there was wide variability in the pathogenicity of M. javanica populations. Some Crotalaria species differed in GI, population density, and RF, but marked differences were found when comparing Crotalaria species with soybean. The RF on soybean ranged from 3.67 to 22.25 (for Mj-1 and Mj-5, respectively) in Experiment 1 and from 16.78 to 62.03 (for Mj-6

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and Mj-7, respectively) in Experiment 2. In general, Mj-5 and Mj-7 were the most virulent populations to soybean, and Mj-1 and Mj-6 were the least.

Previous studies have observed variability in the virulence of *Meloidogyne* populations [16,39], which can be due to several factors. According to Woford et al. [40] and Davis et al. [41], the origin of the nematode population is an important factor for virulence, as plants may exhibit different resistances to nematode isolates of the same species but from different locations. Two populations of *M. paranaensis* Carneiro, Carneiro, Abrantes, Santos, & Almeida from different regions were found to differ in their aggressiveness to several hosts [39]. There is evidence of a positive correlation between geographic variables, such as high temperatures, may favor the development and reproduction of the nematode while negatively affecting plant responses [16]. Parthenogenetic species of the genus *Meloidogyne* are highly responsive to selection pressures arising from the environment and plant resistance genes [44,45].

Differences in pathogenicity between root-knot nematode populations may be a result of differences in aggressiveness and virulence. Aggressiveness, measured by the RF, determines the reproductive capacity of nematodes on a susceptible host, whereas virulence is the ability of the parasite to reproduce on a plant carrying resistance genes [43]. According to these definitions, Mj-7 was the most pathogenic population, as it had high RF on soybean and *C. juncea*.

In addition to nematode pathogenicity and plant genetics, climatic conditions also influence parasitism. Some climatic variables, such as high temperatures, may favor the development and reproduction of the nematode while negatively affecting plant responses [16]. Parthenogenetic species of the genus *Meloidogyne* are highly responsive to selection pressures arising from the environment and plant resistance genes [44,45].

**CONCLUSION**

*C. spectabilis* and *C. ochroleuca* were resistant to all studied populations of *M. javanica*. *C. juncea* was found susceptible to Mj-7 in one of the experiments. There were significant differences in the level of virulence of the nematode populations, with Mj-7 being the most aggressive. Soybean was susceptible to all *M. javanica* populations.

**ACKNOWLEDGEMENTS**

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