**Article**

# WEEDS HOSTS OF NEMATODES IN SUGARCANE CULTURE

*Plantas Daninhas Hospedeiras de Nematoides na Cultura da Cana-de-Açúcar*

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ABSTRACT - The presence of weeds and nematodes can affect of sugarcane yield. This research evaluated whether weeds that are very frequent in mechanized harvested sugarcane can be hosts for root-knot nematodes: *Meloidogyne incognita* or *Meloidogyne javanica*. Regarding nematode hospitability, ten weed species as well as a control sample (tomato) were evaluated; they were inoculated with *M. incognita* and *M. javanica*. The plants were inoculated with 2,000 eggs and second-stage juveniles ($J_2$), and there were three plants per experimental unit (6,000 eggs and $J_2$ per pot - repetition); 60 days after inoculation (DAI), the plants were removed and evaluated according to reproduction factor (RF), nematode final population (FP) and reproducibility index (RI). Regarding weed hospitability, it was found that *Luffa aegyptiaca* acted as a host for both nematodes, with RF > 1, which was higher than the control sample. *Digitaria horizontalis* was classified as a host for *M. incognita* and as a non-host for *M. javanica*. *Mucuna aterrima* and *Crotalaria spectabilis* presented the lowest RF and FP. *Ricinus communis* and *Ipomoea triloba* presented galls when inoculated with *M. incognita*, but were not considered host, since they presented RF < 1. None of the weed species was considered immune, i.e., with RF = 0. The lowest RF values of *M. incognita* (race 3) were related to *Crotalaria spectabilis* and *Euphorbia heterophylla* plants. This demonstrates the ability of crotalarias in decreasing nematode population in the field and justifies its use in several areas before planting main crops.

RESUMO - *A presença de plantas daninhas e de nematoides pode afetar a produtividade da cana-de-açúcar. Nesta pesquisa, foi avaliado se plantas daninhas com elevada frequência nos canaviais podem ser hospedeiras dos nematoides-das-galhas:* **Meloidogyne incognita** *ou* **Meloidogyne javanica**. *Foram avaliadas, quanto à hospedabilidade dos nematoides, dez espécies de plantas daninhas, além de uma testemunha (tomateiro), inoculadas com* **M. incognita** *ou* **M. javanica**. *As plantas foram inoculadas com 2.000 ovos e juvenis de segundo estádio ($J_2$), sendo que havia três plantas por unidade experimental (6.000 ovos e $J_2$ por vaso – repetição); 60 dias após a inoculação (DAI), as plantas foram retiradas e avaliadas segundo o fator de reprodução (FR), a população final de nematoides (PF) e o índice de reprodutividade (IR). Com relação à hospedabilidade, verificou-se que* **Luffa aegyptiaca** *comportou-se como hospedeira de ambos os nematoides, com FR > 1, sendo superior à testemunha.* **Digitaria horizontalis** *foi classificada como hospedeira de* **M. incognita** *e não hospedeira de* **M. javanica**. **Mucuna aterrima** *e* **Crotalaria spectabilis** *apresentaram os menores FR e PF.* **Ricinus communis** *e* **Ipomoea triloba** *apresentaram galhas quando inoculadas com* **M. incognita**, *mas não foram consideradas hospedeiras por apresentarem FR < 1. Nenhuma das*

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*espécies plantas daninhas foi considerada imune, ou seja, com FR = 0. Os menores valores do FR de* **M. incognita** *(raça 3) foram relacionados às plantas* **Crotalaria spectabilis** *e* **Euphorbia heterophylla**. *Isso demonstra a capacidade da crotalária em diminuir a população de nematoides e justifica seu uso em diversas áreas antes do plantio de culturas principais.*

**Palavras-chave:**   *Meloidogyne incognita, Meloidogyne javanica*, fitoparasitas, controle, *Saccharum officinarum.*

## INTRODUCTION

In worldwide terms, losses occasioned by nematodes to sugarcane cultures are estimated in 15.3%. *Pratylenchus zeae*, *Meloidogyne incognita* and *Meloidogyne javanica* are considered key-species for this culture, even if the ones causing damages can vary from one region to the other (Cadet and Spaul, 2005).

Among root-knot nematode species, *M. javanica* and *M. incognita* are considered more frequent within sugarcane in Brazil. The high population rate of these nematodes negatively affects sugarcane plantation productivity (Barros et al., 2005). In addition to the damage caused by using plant nutrients, nematodes from the *Meloidogyne* genus cause great harm to the root system. These parasites inject toxins, resulting into deformations called "galls"; they make plantations poorly developed and not very efficient, which may lead to reductions in culture productivity (Dinardo Miranda, 2005).

However, nematode attack is not only limited to cultures, that is, these phytoparasites may be hosted by weeds. This situation deserves to be highlighted because, in the absence of cultivated plants, weeds turn into a shelter for nematodes and they can freely multiply. Thus, control effectiveness over nematodes depends on a detailed survey about the main weed species that host these phytoparasites. This problem becomes extremely relevant, since studies on this area are scarce (Rizzardi et al., 2003) and, mostly, do not cover weeds that became recurrent in sugarcane cultures after the implementation of mechanized harvest system (Monquero et al., 2011a).

In this context, the goal of this work was to identify the species that may host *Meloidogyne javanica* and/or *M. incognita* nematodes (race 3), through a survey about weeds in sugarcane cultures.

## MATERIAL AND METHODS

The experiment was performed in a greenhouse and in laboratory. Weed tests included ten selected plant species, belonging to five botanical families, based on bibliographic survey, according to their importance in the sugarcane sector (Oliveira and Freitas, 2008; Monquero et al., 2011a). They are: Convolvulaceae: little bell (*Ipomoea triloba*) and *Merremia aegyptia*; Curcubitaceae: sponge gourd (*Luffa aegyptiaca*); Euphorbiaceae: Mexican fireplant (*Euphorbia heterophylla*) and castorbean plant (*Ricinus communis*); Fabaceae: showy rattlebox (*Crotalaria spectabilis*) and velvet bean (*Mucuna aterrima*); and Poaceae: windmill grass (*Chloris polydactyla*), crabgrass (*Digitaria horizontalis*) and signalgrass (*Urochloa decumbens*). *Solanum lycopersicum* tomato, Kada variety, was used to multiply *Meloidogyne javanica* and *M. incognita* race 3, in a greenhouse. Kada tomato plants were produced from seedlings that were planted in greenhouse trays; about 6 seedlings were transplanted into 5 L planters filled with sand and soil substrate (2:1, v:v), twice steam sterilized (20 min at 120 ºC).

To prepare the inoculum, 119 days of multiplication were necessary; tomato plants were transplanted when needed, due to the final cycle of the culture. Nematodes were extracted according to Hussey and Barker's methodology (1973), adapted by Bonetti and Ferraz (1981). Egg and $J_2$ quantification was performed on Peters slides, with the help of an optical microscope, counting three times and calculating the average in 1 mL. This value was multiplied by the total volume, thus determining the total number of eggs and $J_2$ for inoculation.

Weed seeding was performed on two different dates: on November 7th, 2014 the planting of weeds that one month later would have been inoculated with *M. incognita* (race 3) took place;

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after one week, on November 13th, the planting of weeds that were inoculated with *M. javanica* after one month occurred. Seeding was performed in 5 L planters filled with sand and soil substrate (2:1, v:v), twice steam sterilized (20 min., 120 °C), in order to kill any endogen nematode; samples were collected in order to make sure about the absence of any pathogen that may interfere. Fifteen days after seeding, thinning was performed, leaving three plants per planter.

At inoculation, weeds were between the second and fourth pair of real leaves (eudicots) or at the beginning of tillage (monocots); 6,000 $J_2$ and eggs of *M. javanica* and/or *M. incognita* (race 3) were used for each repetition (2,000 eggs and J2 per plant). The inocula were applied with an automatic pipette, in 1 mL suspension, into an open orifice near the roots and, after that, they were covered with substrate.

The experimental design was fully randomized, in factor sequence with six repetitions, summing up to 120 planters with weeds. In addition, the Kada variety of *Solanum lycopersicum* host plant, susceptible to *Meloidogyne* spp. phytonematode, was used as a comparison (12 planters).

Sixty days after inoculation, nematodes were extracted from the plant roots, as described by Hussey and Barker (1973), adapted by Bonetti and Ferraz (1981). After nematode quantification, the reproduction factor (RF) of the parasite was calculated in each treatment, as proposed by Oostenbrink (1966), being (RF = extracted egg n. and $J_2$/inoculated egg n. and $J_2$). Vegetal species with RF > 1 are considered hosts; species with 0 < RF < 1 are considered non-hosts; and those with RF equal to zero are considered immune.

Reproduction factor (RF) and final population (FP) related data on nematodes from each species were submitted to analysis of variance, using F test. RF data were transformed into X = X+C, with C = 100. The analysis of variance was performed by ASSISTAT program, using the Scott-Knott test at p > 0.05 probability level.

*M. incognita* (race 3) and *M. javanica* reproducibility indices were determined considering *Solanum lycopersicum* var. Kada as a control sample of the inoculum feasibility (100%). Therefore, reproducibility indices were found through the division: nematode final population in that weed/nematode final population in tomato. Thus, the resistance of the tested plants was classified by reproducibility index according to the following reproduction criteria, established by Taylor and Sasser (1981): S - susceptible culture (normal reproduction), varying from 50% to 100% compared to tomato; SR - slightly resistant, from 25% to 50%; MoR - moderately resistant, from 10% to 25%; VR - very resistant, from 1% to 10%; HR - highly resistant, lower than 1%; and I - immune, where there was no reproduction.

## RESULTS AND DISCUSSION

The inoculum feasibility of both nematodes can be confirmed by the number of eggs and $J_2$ produced in the tomato plants, which had high multiplication level (Table 1). Among the weed species evaluated as possible hosts for *M. incognita* (race 3), 80% acted as bad hosts, whereas *L. aegyptiaca* and *D. horizontalis* were considered hosts because they presented RF > 1; the former was classified as susceptible, with reproducibility indices higher than the control sample, and the latter was classified as slightly resistant, presenting 26.72% RI (Table 2). However, only *L. aegyptiaca* differed from the control sample by Scott-Knott test, at p > 0.05 probability level.

As for *M. javanica,* it was observed that only *L. aegyptiaca* acted as a host, presenting higher RF than the standard plant, which is tomato (Table 1); it was classified as susceptible (Table 2), since it was the only one differing from the control sample. Damages caused by these nematodes in low crop yield areas are so severe that, many times, sugarcane does not complete its industrial cycle, thus contributing to the abandonment of infested areas or to the increase of environmental impact, because of nematicide use (Silva et al., 2012). Moreover, this information is important because the microclimate created by straw on the soil stimulates the germination of seeds and the development of some weed seedlings, such as *L. aegyptiaca*; therefore, in sugarcane areas with mechanized harvest, this plant has become important and is considered hard to control (Monquero et al., 2011b; Zera et al., 2012).

*D. horizontalis* species obtained a reproduction factor higher than 1 for *M. incognita* (race 3) (Table 1), acting as a host and being classified as slightly resistant (Table 2). Silva et al. (2013)

**Table 1** - Reproduction of *Meloidogyne incognita* (race 3) and *Meloidogyne javanica* in weeds and control sample (tomato), inoculated with 2,000 eggs and second-stage juveniles (J₂) by root system, 60 days afetr inoculation

| Specie | *M. incognita* (race 3) | | | *M. javanica* | | |
|---|---|---|---|---|---|---|
| | FP[1] | RF[2] | Reproduction[3] | FP[1] | RF[2] | Reproduction[3] |
| *I. triloba* | 3100 bA | 100.51 bA | NH | 3306 bA | 100.55 bA | NH |
| *M. aegyptia* | 1468 bA | 100.24 bA | NH | 1072 bA | 100.17 bA | NH |
| *L. aegyptiaca* | 187060 aA | 131.17 aA | H | 154013 aA | 125.66 aA | H |
| *R. communis* | 4370 bA | 100.72 bA | NH | 445 bA | 100.07 bA | NH |
| *E.heterophylla* | 276 bA | 100.04 bA | NH | 901 bA | 100.15 bA | NH |
| *C. spectabilis* | 205 bA | 100.03 bA | NH | 94 bA | 100.01 bA | NH |
| *M. aterrima* | 3363 bA | 100.56 bA | NH | 2954 bA | 100.49 bA | NH |
| *C. polydactyla* | 544 bA | 100.09 bA | NH | 1254 bA | 100.20 bA | NH |
| *D.horizontalis* | 7392 bA | 101.23 bA | H | 1909 bA | 100.31 bA | NH |
| *U. decumbens* | 698 bA | 100.11 bA | NH | 219 bA | 100.03 bA | NH |
| *S.lycopersicum* | 27661 bA | 104.61 bA | H | 18591 bA | 103.09 bA | H |
| RF Variable: $F_{(weeds)}$ = 29.4733** | | $F_{(nematodes)}$ = 0.6678 ns | | $F_{(weeds \times nematodes)}$ = 0.2841* | | |
| VC (%) (FR) = 5.22 | | | | | | |
| FP Variable: $F_{(weeds)}$ = 29.4653** | | $F_{(nematodes)}$ = 0.6884 ns | | $F_{(weeds \times nematodes)}$ = 0.2813* | | |
| VC (%) (PF) = 168.90 | | | | | | |

[1] FP = final population; [2] RF (reproduction factor) = final population of eggs and J₂/initial population of eggs and J₂; [3] Reproduction: NH (non-host, 0<RF>1), H (host, RF>1); ns (non-significant); * (significant at 5% probability by F test); ** (significant at 1% probability by F test); VC (variation coefficient); averages followed by the same lowercase letters in the column and capital letters on the line do not differ among themselves by 5% probability Scoo-Knott test.

observed that *D. horizontalis* is resistant to *M. incognita* and *M. javanica*; this differs from what was discovered in this work. However, this weed did not differ from the control sample for any of the nematode species.

*C. spectabilis* was classified as highly resistant to two nematode species (Table 2). The main mechanism involved in nematode suppression by crotalarias is their ability to act as trap plants, allowing the penetration of juveniles into their roots but preventing their development to the adult phase. In addition to this mechanism, crotalarias produce some substances with nematicide potential, such as monocrotaline (Wang et al., 2002). The nematicide effect of *M. aterrima* and *C. spectabilis* is already known. This work proved that they are bad host plants for both nematodes; *C. spectabilis* is considered highly resistant and *M. aterrima* moderately resistant.

The use of crotalarias and mucunas as suppressor agents for nematodes from the *Meloidogyne* genus was verified in different works, highlighting the importance of crop rotation with legumes which, not only has the ability of reducing the population of these parasites, but also provides organic matter to the soil, contributing for physical-chemical improvements (Inomoto et al., 2006; Charchar et al., 2007; Inomoto et al., 2008).

**Table 2** - *M. incognita* (race 3) and *M. javanica* reproducibility indices in relation to *Solanum lycopersicum* var. Kada, used as control sample

| Specie | *M. incognita* | | *M. javanica* | |
|---|---|---|---|---|
| | [1]RI % | [2]R | [1]RI % | [2]R |
| *Solanum lycopersicum* | 100.00 | S | 100.00 | S |
| *Ipomoea triloba* | 11.20 | MoR | 17.78 | MoR |
| *Merremia aegyptia* | 5.30 | VR | 5.76 | VR |
| *Luffa aegyptiaca* | 676.25 | S | 828.42 | S |
| *Ricinus communis* | 15.79 | MoR | 2.39 | VR |
| *Euphorbia heterophylla* | 0.99 | HR | 4.84 | VR |
| *Crotalaria spectabilis* | 0.74 | HR | 0.50 | HR |
| *Mucuna aterrima* | 12.15 | MoR | 15.88 | MoR |
| *Chloris polydactyla* | 1.96 | MR | 6.74 | VR |
| *Digitaria horizontalis* | 26.72 | SR | 10.26 | MoR |
| *Urochloa decumbens* | 2.52 | VR | 1.17 | VR |

[1] Reproducibility index (IR%) = final nematode population in the species/final nematode population in tomato; [2] resistance; classification according Taylor and Sasser (1981): S - susceptible culture (normal reproduction), varying from 50% to 100% compared to tomato; SR - slightly resistant, from 25% to 50%; MoR - moderately resistant, from 10% to 25%; VR - very resistant, from 1% to 10%; HR - highly resistant, lower than 1%; and I - immune, where there was no reproduction.

Inomoto et al. (2006) analyzed six plants used for green manure in terms of *M. javanica* and *P. brachyurus* hospitability, and they concluded that only three of them can be used to control both nematodes, since they obtained a reproduction factor lower than 1. They are: *C. spectabilis*, *C. breviflora* and pigeon pea 'Iapar 43'. In this very same experiment, the authors could observe that *M. aterrima* may only help to control *M. javanica* and that velvet bean helped the multiplication of both nematodes. In a similar work, Charchar et al. (2007) also found reproduction factors lower than 1 for *C. juncea* (RF=0,30), *C. spectabilis* (RF=0,26) and *M. aterrima* (RF=0,26), when inoculated with *M. incognita* (race 1) and *M. javanica.* Inomoto et al. (2008) proved, in another experiment, that *C. juncea* (RF=0,21) ('IAC-KR-1') and *C. spectabilis* (RF=0,33) ('Common') are effective in reducing *M. javanica* population.

Rosa et al. (2013) studied the reproduction of *M. javanica* in oleraceous and in plants used for green manure; for the *Mucuna* sp. they verified a lower than 1 reproduction factor, with gall index and egg mass index equal to 0; however, the reproduction factor for mucuna deeringiana was 5.25, classifying this species as susceptible to nematodes. As for crotalarias, a difference in reaction among the species was observed, too; for *C. spectabilis*, *C. juncea* and *C. breviflora* the reproduction factor was lower than 1, classifying them as resistant, with reduction ability in nematode final population. *C. mucronata* and *C. ochroleuca* species were susceptible to *M. javanica*, with reproduction factor 1.06 and 1.78, respectively. The results found by these authors reinforce the importance of knowing the species that will be used in crop rotation, since they do not have the same reaction to parasites and the wrong choice may increase population numbers, making control difficult.

In this work, *R. communis* was considered a bad host for both nematodes; however, during extraction, egg masses were observed in the plants inoculated with *M. incognita* (race 3); this would explain the numerically higher reproduction factor.

Dias-Arieira et al. (2009), while studying seven castorbean cultures, noted resistance to *M. paranaensis*, *M. javanica* and *M. incognita*, and obtaining reproduction factors that were very close to 0 and a lower number of galls and eggs, compared to the control sample (tomato). These results slightly differ from what was found in this work, where castorbean reproduction factors gets closer to 1 when it is inoculated with *M. incognita* (race 3).

In this work, *I. triloba* and *M. aegyptia* were not considered host plants for any nematode species; however, *I. triloba* presented galls in the treatments with *M. incognita* (race 3) and was classified as moderately resistant to these nematodes.

Some species of morning glory are important nematode hosts from the *Meloidogyne* genus. According to Mônaco et al. (2009), *Merremia cissoides*, *I. purpurea* and *I. nil* were susceptible to *M. incognita* (race 1) and *M. javanica*; for *M. incognita* (race 3), only *M. cissoides* was resistant. This shows the different reaction of species to different races, when talking about *M. incognita*.

In this work, *E. heterophylla* appeared to be non-host species for the studied nematodes. Mônaco et al. (2009) verified that *E. heterophylla* was resistant to *M. incognita* (races 1 and 3) and to *M. javanica*; this supports the results found in this work, where this species was considered a bad host for nematodes, since it presented a reproduction factor that was lower than 1. Silva et al. (2013) observed the opposite, finding reproduction factors of 7.9 for *M. incognita* and of 9.3 for *M. javanica*, thus considering it as a susceptible weed.

Cordeiro et al. (2014) studied the reproducibility and parasitism of ten weed species with respect to *M. incognita,* and also classified *E. heterophylla* as resistant, with gall index equal to 0, reproduction factor 0.180 and reproducibility index 8.7%, being classified as moderately resistant. These results agree with the ones presented here, except for the classification, which was "highly resistant" in this study.

In this work, *U. decumbens* acted as a bad host. Carneiro et al. (2006) showed in a study that signalgrass was resistant to *M. incognita* (races 1 and 3) and to *M. paranaenses* and it was considered immune to *M. javanica* (RF=0), 60 days after inoculation.

In a study about the reaction of 60 weed species to nematodes, Mônaco et al. (2009) observed that *U. decumbens* was resistant to *M. incognita* (race 1) and *M. javanica*, but immune to *M. incognita* (race 3); this partially agrees with the results displayed here, since this plant acted as a bad host for both nematodes, but did not appear immune in any case.

*L. aegyptiaca* species hosted *M. incognita* (race 3) and *M. javanica* nematodes, and *D. horizontalis* hosted *M. incognita* (race 3). These data highlight the importance of a proper control over these weeds, even when they are present in a level below economic damage.

Lower RF values for *M. incognita* (race 3) were related to *C. spectabilis* and *E. heterophylla*; this justifies the use of crotalaria in order to decrease damages provoked by these nematodes to main cultures, since it has the ability of reducing the initial population, providing control in the off-season.

## ACKNOWLEDGMENTS

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