

HOST SUITABILITY OF WEEDS AND FORAGE SPECIES TO ROOT-KNOT NEMATODE *Meloidogyne graminicola* AS A FUNCTION OF IRRIGATION MANAGEMENT¹

*Hospedabilidade de Plantas Daninhas e Espécies Forrageiras ao Nematóide das Galhas *Meloidogyne graminicola* em Função do Manejo da Irrigação*

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ABSTRACT - The irrigated rice production can be limited by various phytopathogenic agents, including root-knot nematodes (*Meloidogyne* spp.). Thus, the aim of this research was to check the host suitability of plant species most often found off-season and during rice cultivation, to root-knot nematode *Meloidogyne graminicola*, under two irrigation managements. Two experiments were conducted in a completely randomized design. In the first experiment seven plant species that occur in an area of rice cultivation, in fallow, off-season were evaluated. For the second experiment nine weed species infesting the irrigated rice culture were tested in rainfed and flooding conditions. The sixteen species, kept individually in pots with sterilized substrate, were inoculated with 5,000 eggs and second stage juveniles (J₂) of nematode. BRS 410 IRGA rice plants inoculated with *M. graminicola* were used as control. Two months after inoculation, the root system of each plant was evaluated for number of galls and nematode reproduction factor. It was verified that the species of off-season of rice cultivation *Sida rhombifolia*, *Raphanus raphanistrum*, *Spergula arvensis*, *Lotus corniculatus* and *Trifolium repens*, and, during the cycle of rice cultivation, *Aeschynomene denticulata*, *Leersia hexandra*, are immune to nematode. The plant species off-season, *Avena strigosa* and *Lolium multiflorum* and of cultivation, *Alternanthera philoxeroides*, red rice, *Echinochloa crusgalli*, *Cyperus difformis*, *Cyperus esculentus*, *Cyperus iria* and *Fimbristylis miliacea* would behave as hosts of *M. graminicola*, mostly under rainfed conditions.

Keywords: hosts, reproduction, susceptibility, *Oryza sativa*.

RESUMO - A produção de arroz irrigado pode ser limitada por vários agentes fitopatogênicos, entre eles os nematóides das galhas (*Meloidogyne* spp.). Dessa forma, objetivou-se neste estudo avaliar a hospedabilidade de espécies de plantas, encontradas com maior frequência no entrecultivo e durante o período de cultivo do arroz irrigado, ao nematóide das galhas, *Meloidogyne graminicola*, sob dois manejos da irrigação. Para isso, foram realizados dois experimentos em delineamento completamente casualizado. No primeiro experimento, foram testadas sete espécies vegetais que ocorrem em área de arroz irrigado, em pousio, no período de entrecultivo. Para o segundo experimento, testaram-se, em condições de sequeiro e alagamento, nove espécies de plantas daninhas infestantes da cultura do arroz irrigado. As 16 espécies vegetais, mantidas individualmente em vaso com substrato esterilizado, foram inoculadas com 5.000 ovos e juvenis de segundo estágio (J₂) do nematóide. Plantas de arroz BRS IRGA 410 inoculadas com *M. graminicola* foram utilizadas como testemunhas. Dois meses após a inoculação, o sistema radical de cada planta foi avaliado quanto ao número de galhas e fator de reprodução do nematóide.

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Verificou-se que as espécies de entrecultivo da cultura do arroz irrigado *Sida rhombifolia*, *Raphanus raphanistrum*, *Spergula arvensis*, *Lotus corniculatus* e *Trifolium repens* e, durante o ciclo de cultivo do arroz irrigado, *Aeschynomene denticulata* e *Leersia hexandra* são imunes ao nematoide. As espécies vegetais de entrecultivo, *Avena strigosa* e *Lolium multiflorum*, e as de cultivo, *Alternanthera philoxeroides*, arroz-vermelho, *Echinochloa crusgalli*, *Cyperus difformis*, *Cyperus esculentus*, *Cyperus iria* e *Fimbristylis miliacea*, comportam-se como hospedeiras de *M. graminicola*, principalmente em condições de sequeiro.

Palavras-chave: hospedeiro, reprodução, suscetibilidade, *Oryza sativa*.

INTRODUCTION

In Brazil, about 2.8 million hectares are cultivated with rice annually, of which over one million are located in the Brazilian states of Rio Grande do Sul (RS) and just over 150 thousand in Santa Catarina (SC). The country occupies a prominent position in the global paddy setting, with a total production of 11.2 million tonnes, standing out as the largest producer outside Asia (CONAB, 2012; FAO, 2012). However, the level of productivity considered ideal for the culture has not yet been reached due to weed interference and attack by pests and plant pathogens.

Among the pests affecting irrigated rice, plant parasitic nematodes are pathogens that infect both the aerial part and the root system of the parasitized plants, and may considerably reduce the culture yield (Karssen & Moens, 2006). *Meloidogyne graminicola* is considered one of the species with the greatest potential for damage to irrigated rice culture, reducing productivity between 11% and 80% (Padgham et al., 2004; De Waele & Elsen, 2007). In Brazil, only in the 1990s was reported the presence of *M. graminicola* in irrigated rice culture (Sperandio & Monteiro, 1991). Subsequently, the widespread occurrence of *M. graminicola* was related (Est. VS1) in irrigated rice fields of the central geological depression of Rio Grande do Sul (Steffen et al., 2007) and in Santa Catarina State (Gomes et al., 2009).

The *Meloidogyne* genus is considered the most important mainly due to its wide host range that includes more than 3,000 species of wild and cultivated plants (Hussey & Janssen, 2002). Among the *Meloidogyne* spp. Weed host, *Amaranthus spinosus*, *Bidens pilosa*,

Portulaca oleracea (Brito et al., 2008), *Cyperus rotundus*, *Amaranthus* spp., *Chenopodium album* and *Digitaria* sp. are often cited in the literature (Myers et al., 2004).

Due to its wide polyphagia, *M. graminicola* survives and reproduces in off-season on weeds and forage crops growing on fallow plants, contributing to the increase of inoculum in the soil, and then parasitizing rice cultivation in the next season (Pokharel et al., 2007). The barnyard grass (*Echinochloa* spp.), among other weeds (Siciliano et al., 1990; Sperandio & Amaral, 1994) is reported as good plant host of *M. graminicola*. However, little is known about the development and reproduction of this nematode in these species in different irrigation situations. In this context, the aim of this research was to check the host suitability of plant species, most often found off-season and during rice cultivation, to root-knot nematode, *Meloidogyne graminicola*, under two irrigation managements.

MATERIALS AND METHODS

The experiments were conducted at greenhouse (25 ± 5 °C) and laboratory conditions, where 16 species of weeds and forage crops were evaluated for resistance to *M. graminicola*. In both experiments, the design was completely randomized with six replications. BR-IRGA 410 rice plants were used as susceptible control (Steffen, 2007).

In the first experiment seven plant species that occur in an area of rice cultivation, in fallow, off-season were tested: *Avena strigosa* (black oat), *Lolium multiflorum* (ryegrass), *Sida rhombifolia* (arrowleaf sida), *Raphanus raphanistrum* (turnip), *Spergula arvensis* (corn

spurrey), *Lotus corniculatus* (bird's-foot trefoil) and *Trifolium repens* (white clover (also known as Dutch clover)).

For performing the second experiment, nine weed species that affects the irrigated rice culture were evaluated in rainfed and flooding conditions: *Alternanthera philoxeroides* (alligator weed), *Aeschynomene denticulata* (common names vary regionally), *Oryza sativa* (red rice or Asian rice – ARV), *E. crus-galli* (cockspur (or cockspur grass), barnyard millet, Japanese millet, water grass, common barnyard grass, or simply “barnyard grass”), *C. difformis* (known by several common names, including variable flatsedge and smallflower umbrella-sedge), *Cyperus esculentus* (chufa sedge, nut grass, yellow nutsedge, tiger nut sedge, or earth almond), *Cyperus iria* (rice flat sedge and rice flatsedge), *F. miliacea* (grasslike fimbry) and *Leersia hexandra* (southern cutgrass, clubhead cutgrass, and swamp rice grass).

The seeds of *L. corniculatus* and *T. repens* were acquired in the trade, and the species *A. philoxeroides* was reproduced from new seedling stems of the aerial part. For the other weeds, seeds were collected and placed in rice crops to germinate in commercial substrate (Germina Plant®) sterilized for two hours at 120 °C. The seeds of *S. rhombifolia* and *S. arvensis* were scarified, and the ones of *C. difformis*, *C. esculentus*, *C. iria*, *F. miliacea* and *L. hexandra*, were subjected to different conditions of light and photoperiod to overcome the dormancy. After the emergence, when the plants had between two and four leaves, the transplant was performed to plastic pots with sterilized substrate, keeping one plant per pot.

As inoculum of nematode was used a pure population of *M. graminicola* (Est VS1) kept in a greenhouse in BR IRGA 410 rice plants, with certification of purity of the species performed by electrophoresis with esterase isoenzyme (Carneiro & Almeida, 2001). The extraction of nematodes was performed by grinding the roots into a blender according to the technique described by Hussey & Barker (1973) and modified by Boneti & Ferraz (1981).

Five days after transplanting, each plant was inoculated with 5,000 eggs and second stage juveniles (J2) of *M. graminicola* (initial

population) and the inoculum was deposited into three holes approximately two centimeters deep around the plant. The plants from the experiments in the rainfed condition were irrigated when needed, while for the other water management condition, the pots containing the seedlings were filled with water (flooding condition) 48 hours later the inoculation, with water replacement when it was necessary.

Presence or absence of egg masses externally to the roots of the weeds was found at 60 days after inoculation, and the ones that presented were photographed under a microscope. Later, the root systems of the plants were washed and evaluated for number of galls. Next, each root system was processed according to the method of Hussey & Barker (1973), modified by Boneti & Ferraz (1981), for subsequent determination of the number of eggs and J_2 (final population) and calculation of the reproduction factor (RF) of the nematode/plant. The plant species with $0 < RF < 1$ were considered resistant; $RF \geq 1$, susceptible; and those with RF equal to zero, immune (Oostenbrink, 1966).

Data were analyzed for normality by the Shapiro-Wilk test; for homoscedasticity, by the Hartley test; and for independence of waste by graphical analysis. For the variables number of galls, reproductive factors, in both experiments, the transformation $\sqrt{(x + 0.5)}$ was necessary. Subsequently, the data were subjected to analysis of variance ($p \leq 0.05$). In case of statistical significance, the effects of plant species were compared by Tukey test ($p \leq 0.05$) and the effects of irrigation management by t-test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Assessing the response of plants occurring off-season of rice to *M. graminicola* (first experiment), it was found that only the forages *A. strigosa* and *L. multiflorum* were susceptible to the nematodes ($RF > 1$). For these two species, it was observed that *M. graminicola* developed typical galls on parasitized roots, although these have not shown statistical difference compared to control (Table 1). However, *A. strigosa* is considered a poor host of *M. graminicola* (Pokharel et al., 2007).



Table 1 - Number of galls, reproduction factor (RF) and reaction of weeds and forages that occurring in the off-season of irrigated rice and remaining under conditions of fallow in the crop, to *Meloidogyne graminicola*

Species	Number of galls	RF	Reaction ^{2/}
<i>Oryza sativa</i> (T)	98.3 a ^{1/}	74.7 a	S
<i>Avena strigosa</i>	111.2 a	15.4 b	S
<i>Lolium multiflorum</i>	65.5 a	3.8 c	S
<i>Sida rhombifolia</i>	0.0 b	0.0 d	I
<i>Raphanus raphanistrum</i>	0.0 b	0.0 d	I
<i>Spargula arvensis</i>	0.0 b	0.0 d	I
<i>Lotus corniculatus</i>	0.0 b	0.0 d	I
<i>Trifolium repens</i>	0.0 b	0.0 d	I

^{1/} Means followed by the same letter do not differ by the Tukey test ($p \leq 0.05$). T: control, rice plants cultivar BR-IRGA 410.

^{2/} S: susceptible (RF ≥ 1) and I: immune (RF = 0).

In *A. strigosa* and in rice the presence of egg masses was not observed externally to the root. As for *L. multiflorum*, some external egg masses were detected (Figure 1 and Table 1). These observations demonstrate that the expression of symptoms and reproduction of the nematode is dependent on the type of host (Roese & Oliveira, 2004). However, it should be emphasized that in irrigated rice, *M. graminicola* usually lays its eggs in a gelatinous mass inside the root (Prasad et al., 2013), a little studied fact to date.

In the other species, the reproduction factors were equal to zero and the plants

were immune to the nematode (Table 1). The immunity of *S. rhombifolia* to *M. graminicola* verified in this study corroborates the results observed by Siciliano et al. (1990), which found that plants of this species inoculated with *M. graminicola* neither exhibit galls in the roots nor allowed the nematode reproduction. *S. arvensis* and *T. repens* behaved in a immune way to *M. graminicola* (Table 1). However, some authors describe them as a host of the nematode (Windham & Pederson, 1992; Dabur et al., 2004). Thus, differences in adverse reactions to the same weed species can be credited to two hypotheses: intraspecific variability of weeds or physiological variation in populations of the nematode (Mônaco et al., 2008; Pokharel et al., 2010).

The immunity response of the species *S. rhombifolia*, *R. raphanistrum*, *S. arvensis*, *L. corniculatus* and *T. repens*, verified in this study (Table 1), represents an important information in the adoption of practices for the nematode management in irrigated rice because they reduce the populations of *M. graminicola*. Therefore, the use of pasture land, from the cultivation of turnips, clover and/or bird's-foot trefoil during autumn/winter, presents itself as a management alternative in areas highly infested with *M. graminicola*.

The species *L. multiflorum* and *A. strigosa* provided increase to the nematode population (Table 1). These plants, especially ryegrass,



Figure 1 - Egg masses and females of *Meloidogyne graminicola* observed outside *Lolium multiflorum* roots.

are considered the main cultivated or natural annual pastures used in succession to the rice crop. Generally, after the rice harvest, these plants occupy the areas, integrating the process of rotation of cereal with beef cattle or dairy, a widely used system in the lowland areas of the RS, where irrigated rice is grown. Although *L. multiflorum* and *A. strigosa* have shown lower susceptibility than the irrigated rice in the field, these host plants can ensure the survival of the nematode and contribute to rapid population increase in the soil, which in turn will constitute the source of primary inoculum for rice cultivation in the next cultivation season.

In the second experiment, there was an interaction between the treatment factors of plant species and managements of irrigation. It was observed in the rainfed system that *M. graminicola* developed a higher number of galls in the BR-IRGA 410 rice cultivar roots (control), followed by *A. philoxeroides*, *O. sativa*-ARV, *E. crusgalli*, *C. difformis*, *F. miliacea* and *C. iria*, while the species *A. denticulata*, *C. esculentus* and *L. hexandra* did not present these symptoms (Table 2). In the flooding condition, it was observed a higher number of galls in the roots of *E. crusgalli*, *C. difformis*, *O. sativa*-ARV, *A. philoxeroides*, in the control (BR-IRGA 410) and *C. iria*. The species *A. denticulata*, *C. esculentus*, *F. miliacea* and

L. hexandra did not shown galls in their respective roots in this irrigation system.

In comparing irrigation managements, differences occurred between rainfed and flooding both for variable numbers of galls and nematode reproduction. In the plants of *A. philoxeroides*, *O. sativa*-ARV, *E. crusgalli* and *F. miliacea*, a great number of galls was observed in the rainfed situation (Table 2). Probably in this situation a higher number of J2 of *M. graminicola* penetrated in the roots of these species, resulting in higher rates of reproduction.

Among the weeds assessed in the rainfed condition, *E. crusgalli* was the plant where the nematode had a higher reproduction rate, demonstrating greater susceptibility, followed by the control, *C. difformis*, *O. sativa*-ARV, *A. philoxeroides*, *C. iria*, *C. esculentus* and *F. miliacea* (Table 2). The greater susceptibility of *E. crusgalli* and of the other plants tested in rainfed, *M. graminicola* must be related to favorable conditions for penetration of juveniles of the root system, since in soil subjected to flooding juvenile they had only 48 hours to migrate and be introduced in the root system before the plants are subjected to saturation of the soil with water. The flooding of the soil with irrigation is the main barrier preventing the infection of rice roots by juveniles of *M. graminicola* (Bridge et al., 2005).

Table 2 - Number of galls, reproduction factor (RF) and reaction of species of weeds, that occur in the period of rice cultivation, *Meloidogyne graminicola*, in irrigation management, rainfed and flooding

Species	Irrigation management					
	Rainfed	Flooding	Rainfed	Flooding	Rainfed	Flooding
	Number of galls		RF		Reaction ^{2/}	
<i>Oryza sativa</i> (T)	138.5 a ^{1/} *	28.8 a	88.4 b *	7.7 a	S	S
<i>Alternanthera philoxeroides</i>	104.0 ab *	30.8 a	22.4 d *	3.1 bcd	S	S
<i>Aeschynomene denticulata</i>	0.0 e ^{ns}	0.0 c	0.0 e ^{ns}	0.0 f	I	I
<i>Oryza sativa</i> (red rice)	100.3 ab *	38.0 a	38.0 c *	3.7 bcd	S	S
<i>Echinochloa crusgalli</i>	88.7 b *	45.0 a	110.2 a *	5.4 ab	S	S
<i>Cyperus difformis</i>	51.2 c ^{ns}	38.3 a	51.8 c *	3.9 bc	S	S
<i>Cyperus esculentus</i>	0.0 e ^{ns}	0.0 c	14.0 d *	0.1 ef	S	R
<i>Cyperus iria</i>	12.3 d ^{ns}	11.2 b	20.4 d *	1.9 cd	S	S
<i>Fimbristylis miliacea</i>	24.7 d *	0.0 c	13.8 d *	1.5 de	S	S
<i>Leersia hexandra</i>	0.0 e ^{ns}	0.0 c	0.0 e ^{ns}	0.0 f	I	I

^{1/} Means followed by the same letter in the column analyze species within irrigation management and do not differ by Tukey test ($p \leq 0.05$); ^{ns} and * non significant nor meaningful difference, respectively, by t test ($p \leq 0.05$), for each species between the managements. T: control, rice plants cultivar BR-IRGA 410. ^{2/} S: susceptible (RF ≥ 1); I: immune (RF = 0) and R: resistant ($0 < \text{RF} < 1$).



However, after penetration, the nematode can develop normally and have several cycles inside the roots, even at flooding condition.

In the flooding irrigation system, the nematode showed lower RF, *comparing to the rainfed system*, in the control (irrigated rice), followed by the species *E. crusgalli*, *C. difformis*, *O. sativa*-ARV, *A. philoxeroides*, *C. iria* and *F. miliacea* (Table 2). As for *C. esculentus*, it has shown to be resistant in the flooding condition and susceptible in the rainfed management; and the species *A. denticulata* and *L. hexandra* were immune to the nematode in both situations. The lowest nematode reproduction at flooding condition is probably due to the reduction hatching and infectivity of the J2 in the roots of rice plants subjected to this system of irrigation (Bridge et al., 2005). These observations corroborate the occurrence record of *M. graminicola* in rice crops in RS (Sperandio & Amaral, 1994; Steffen et al., 2007), whose symptom expression has been detected primarily in portions of areas with smallest water surfaces or no water accumulation points.

The off-season species of the irrigated rice crop, *S. rhombifolia*, *R. raphanistrum*, *S. arvensis*, *L. corniculatus* e *T. repens*, and, during the irrigated rice cultivation cycle, *A. denticulata* and *L. hexandra* are immune to *M. graminicola*. The weeds and forage plants that occur off-season, *A. strigosa* and *L. multiflorum*, and during the irrigated rice cultivation cycle, *A. philoxeroides*, *O. sativa*-ARV, *E. crusgalli*, *C. difformis*, *C. esculentus*, *C. iria* and *F. miliacea*, behave as hosts of *M. graminicola*, specially in rainfed situations. Besides, the irrigation water reduces the reproduction of the nematode and the number of galls in *A. philoxeroides*, *O. sativa*-ARV, *E. crusgalli*, *C. difformis*, *C. iria* and *F. miliacea*.

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