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Reaction of *Avena* spp. to different concentration levels of *Meloidogyne javanica* and *M. incognita* inoculum

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Abstract

The objective of this study was to evaluate the resistance reaction of *Avena* spp. after inoculation of different concentrations of eggs and juveniles of *M. javanica* and *M. incognita*. Ten oat genotypes (*Avena sativa*: UPFPS Farroupilha, UPFA Ouro, IPR Afrodite, AF1345 Ucraniana; *A. strigosa*: Agro Quaraí, Agro Esteio, Embrapa 139, Iapar 61 Ibiporã, UPFA 21 Moreninha, AF 12202) were evaluated in bioassays for resistance against the root-knot nematodes, *Meloidogyne javanica* and *M. incognita*. Susceptible (*Solanum lycopersicum* and *Glycine max*) and resistant (*Crotalaria spectabilis*) species were used as control treatments. Depending on the inoculum level, the oats showed a different response to nematodes. All genotypes of oats were resistant to *M. javanica* when a high inoculum level (5,000 eggs and J2 per plant) was used but under low inoculum level (1,500 eggs and J2 per plant), AF 12202, Iapar 61 Ibiporã, Agro Esteio and Agro Quaraí were susceptible. All genotypes of oats were resistant to the inoculation of 2,900 eggs and J2 of *M. incognita*, except for 'Iapar 61' that showed susceptibility in this concentration. At lower inoculum level (1,500 eggs and J2) Agro Quaraí, Agro Esteio and Iapar 61 Ibiporã were susceptible. The reaction of oats to *M. javanica* and *M. incognita* depends on the inoculum level. The resistance reaction at low inoculum density of *A. sativa* 'AF 1345 Ucraniana' and 'IPR Afrodite' allows its use in breeding programs in oats and suggests the possibility of management tool in areas infested with both root-knot nematode species.

Keywords: Oat; Genetic control; Root-knot nematodes; Resistance; Susceptibility.
Abbreviations: FP_final population density, HIC_higher inoculum concentration, J2_second-stage juvenile, LIC_lower inoculum concentration, R_resistant, RF_reproduction factor, S_susceptible.

Introduction

Oats (*Avena* spp., Poaceae) are cultivated as a winter crop in several regions around the world for different purposes. The main aptitude of 'white oat' (*A. sativa* L.) is for production of grains destined for human or animal consumption. On the other hand, 'black oat' or 'white oat' (*A. strigosa* Schreb.) is mainly used for pasture formation and soil cover. Both species can be used as an alternative for improvement of chemical, physical and biological properties of the soil. They can also aid in the management of diseases and pests such as root-knot nematodes [*Meloidogyne javanica* (Treub) Chitwood and *M. incognita* (Kofoid and White) Chitwood] that can cause severe damage to various agricultural species worldwide (Karuri et al., 2017).

The typical symptom of the attack of root-knot nematodes is hyperplasia commonly called 'gall', which is a thickening of the radical cortex cells that jeopardizes the absorption of water and nutrients. As a result, plant growth is affected and grain yield can be reduced. These nematodes have a high level of polyphagia and can easily adapt to diverse edaphoclimatic conditions, which makes the management very challenging (Cabrera et al., 2009).

It is known that the host plant can affect the dynamics of an established nematode population in a specific area (Oostenbrink, 1966). Therefore, the rotation or succession of crops with resistant and/or antagonistic plants may suppress the nematode population level and could be an alternative to the use of pesticides (Machado et al., 2015; Miamoto et al., 2016; Niro et al., 2016). Oats could be recommended for crop rotation targeting the management of nematodes as long as they do not allow the nematodes to multiply in their roots, which is type of resistance reaction. However, many authors have already reported that there is a great variability among *Avena* spp. regarding to the reaction towards *M. javanica* and *M. incognita* (Borges et al., 2009; Machado et al., 2015). Consequently, the evaluation of the resistance reaction of oat genotypes to nematodes should be considered for the selection of lines using bioassays. Moreover, the identification of the resistance reaction to root-knot nematodes could be used for seed companies as a commercialization strategy, which could add value to the product and provide greater safety to the growers regarding the use of these genotypes.

The reaction of the host plant to the root-knot nematodes fundamentally depends on the gene expression of the

plant. However, in situations where the nematode population is high, host plants may express a "false" resistance reaction due to the high competition between these phytopathogens for infection sites. As a result, the roots are less infected, resulting in a reproduction factor less than 1.0 (Andreazi et al., 2015; Sera et al., 2009; Shigueoka et al., 2016), which indicates a resistance reaction. Therefore, the use of contrasting inoculum concentrations is indicated in bioassays to test the genotype reaction to root-knot nematodes. Thus, a genotype that confirms a resistance reaction at either high or low inoculum concentrations could be used as parent in oat breeding programs for resistance to root-knot nematodes. Hence, the objective of this study was to evaluate the resistance reaction of *Avena* spp. according to the inoculation of different concentrations of eggs and juveniles of *M. javanica* and *M. incognita*.

Results

Reaction of Avena spp. to M. javanica

The results obtained in both experiments with *M. javanica* (Table 1 and Table 2) showed a resistant reaction of all genotypes of *Avena* spp. at the higher inoculum concentration (HIC= 5,000 eggs and J2 plant^{-1}). The groups formed by the genotypes based on the RF corresponded to those that were established regarding to the absolute density of nematodes, which is expected since their RF is calculated based on this attribute. Seven resistant genotypes proved lower reproduction of *M. javanica* (RF < 0.30) compared to the three other cultivars (RF > 0.40). However, based on the values of the relative density of nematodes in the roots, there was greater discrimination of the genotypes, with the formation of three groups. For absolute density (Table 1), *A. sativa* 'IPR Afrodite' had the best performance showing the lowest number, but it did not differ significantly from all the other cultivars with exception of *A. strigosa* 'Agro Quaraí', 'Iapar 61 Ibiporã', and 'Embrapa 139' that had the highest absolute density (Table 1).

Under a lower inoculum concentration (LIC = 1,500 eggs and J2 plant^{-1}), some changes in the resistance reaction to nematode (Table 2) were observed. Four genotypes of *A. strigosa* showed a susceptible reaction (RF > 1.0). It is important to highlight that *A. sativa* 'IPR Afrodite' showed a high stability in the RF, which was between 0.02 to 0.03 for the high and low concentration tested. In addition, significant reduction of the nematode density in the roots was exhibited by *A. sativa* 'AF 1345 Ucraniana'. For *A. strigosa,* only two cultivars presented a consistent response in the resistance reaction: 'UPFA 21 Moreninha' and 'Embrapa 139', where both were characterized as resistant under the two inoculum concentrations.

Reaction of Avena spp. to M. incognita

The reaction of the oat genotypes to *M. incognita* varied according to the inoculum concentration. Under the higher concentration (Table 3), except for *A. strigosa* 'Iapar 61 Ibiporã' (RF > 1.0), all genotypes were characterized as resistant. Among the resistant genotypes, the lowest values of FR (< 0.04) were exhibited by *A. sativa* 'IPR Afrodite' and 'AF 1345 Ucraniana', as occurred in the *M. javanica* bioassays. For these genotypes, the lowest nematode densities were also observed in the roots (< 14 g^{-1}). Under the lower inoculum concentration, the *A. sativa* genotypes maintained their resistance reaction, but two cultivars of *A. strigosa* ('Agro Quaraí' and 'Agro Esteio') presented as susceptible. *Avena strigosa* 'Iapar 61 Ibiporã' maintained the susceptibility reaction already shown in the condition of higher inoculum concentration.

Discussion

In this study, we tested the hypothesis that the reaction of *Avena* spp. to root-knot nematodes (*M. incognita* and *M. javanica*) varies among the genotypes, whose expression of resistance or susceptibility varies according to the inoculum concentration and the nematode species. This has confirmed our results. The reaction of the genotypes to nematode was classified as resistance or susceptibile depending on the reproduction factor (RF) value, using tomato and soybean (susceptible species), and crotalaria (resistant species) as control species (Machado et al., 2015; Miamoto et al., 2016; Riede et al., 2015). At higher and lower inoculum concentration of both nematode species, there was a reaction to susceptibility to tomato and soybean, used in the experiments as a susceptible control regardless of inoculum concentration. Soybean and tomato are highly susceptible to both nematode species. The resistance reaction to crotalaria was also observed, proving the viability of the inoculum and experimental conditions.

Among the two tested oat species, *A. sativa* and *A. strigosa*, the first one stood out positively, since the white oats confirmed the resistance reaction independent of the nematode species and inoculum concentrations. In *A. strigosa*, a greater variability was observed since there was an alteration in the reaction of some genotypes to the variation of nematode species and/or inoculum concentration. This can partially be attributed to the lower number of genotypes tested in *A. sativa* (three cultivars and one line) than *A. strigosa* (six genotypes). Therefore, further studies should be performed to include more genotypes for *A. sativa*.

Based on the lower values of RF (< 0.03) and nematode density per gram of root (< 76 g^{-1}) (Tables 1 to Table 4), *A. sativa* genotypes 'IPR Afrodite' and 'AF 1345 Ucraniana' could be considered as highly resistant with great stability of the resistance reaction. A consistency was observed in the response of these two genotypes if we consider both RF and the relative density of nematodes, suggesting higher resistant level comparing to 'UPF Ouro' and 'UPFPS Farroupilha'. The values of the relative density of nematodes in the root system confirms the characterization of genotypes based on the RF as they show congruence between them (Shigueoka et al., 2016). The higher degree of resistance of 'IPR Afrodite' to both species of nematodes agrees to the results of research already done with this cultivar by other authors (Marini et al., 2016; Riede et al., 2015). Marini et al. (2016) found a reduction in the penetration of juvenile nematodes in the roots of this oat genotype, with lignified cells and intracellular spaces filled with phenols, which is an indicative of the plant defense response to nematode attack. This suggests that the chemical profile of oat genotypes is one of the determinant

Table 1. Absolute and relative final density of eggs and J2 of *Meloidogyne javanica*, reproduction factor (RF), and resistance reaction of oats (*Avena* spp.) under a high inoculum concentration (IC= 5,000 eggs and J2 per plant).

Genotypes	Absolute density (number per plant)	Relative density (number per gram of roots)	RF[1]	Reaction[2]
Avena sativa				
IPR Afrodite	115 b	17 c	0.02 b	R
UPFPS Farroupilha	1057 b	329 b	0.21 b	R
UPFA Ouro	1069 b	133 c	0.21 b	R
AF 1345 Ucraniana	1120 b	76 c	0.22 b	R
A. strigosa				
UPFA 21 Moreninha	1432 b	152 c	0.28 b	R
Agro Esteio	1462 b	171 c	0.29 b	R
AF 12202	1455 b	179 c	0.29 b	R
Embrapa 139	2033 a	284 b	0.41 a	R
Iapar 61 Ibiporã	2201 a	169 c	0.44 a	R
Agro Quaraí	2845 a	528 a	0.57 a	R

Means followed by the same letter in the column do not significantly differ from each other by Scott-Knott's test ($p > 0.05$). [1]Reproduction factor for the susceptible control (*Solanum lycopersicum:* 60.4; *Glycine max:* 8.0) and for the resistant control (*Crotalaria spectabilis:* 0.13). [2]Resistance reaction of the genotypes: resistant (R) and susceptible (S).

Table 2. Absolute and relative final population density of eggs and J2 of *Meloidogyne javanica*, reproduction factor (RF) and reaction of resistance (R) or susceptibility (S) of oats (*Avena* spp.) under low inoculum concentration (IC= 1,500 eggs and J2 per plant).

Genotypes	Absolute density (number per plant)	Relative density (number per gram of roots)	RF[1]	Reaction[2]
A. sativa				
AF 1345 Ucraniana	30 b	3 b	0.02 b	R
IPR Afrodite	43 b	8 b	0.03 b	R
UPFA Ouro	366 b	65 b	0.25 b	R
UPFPS Farroupilha	386 b	61 b	0.26 b	R
A. strigosa				
UPFA 21 Moreninha	873 b	138 b	0.58 b	R
Embrapa 139	1306 b	335 b	0.87 b	R
AF 12202	2006 a	354 b	1.34 a	S
Iapar 61 Ibiporã	2989 a	337 b	1.99 a	S
Agro Esteio	3083 a	776 a	2.06 a	S
Agro Quaraí	3496 a	787 a	2.33 a	S

Means followed by the same letter in the column do not significantly differ from each other by Scott-Knott's test ($p > 0.05$). [1]Reproduction factor for the susceptible control (*Solanum lycopersicum:* 34.5; *Glycine max:* 16.6) and for the resistant control (*Crotalaria spectabilis:* 0.04). [2]Resistance reaction of the genotypes: resistant (R) and susceptible (S).

Table 3. Absolute and relative final population density of eggs and J2 of *Meloidogyne incognita*, reproduction factor (RF) and reaction of resistance (R) or susceptibility (S) of oats (*Avena* spp.) under low inoculum concentration (IC= 2,900 eggs and J2 per plant).

Genotypes	Absolute density (number per plant)	Relative density (number per gram of roots)	RF[1]	Reaction[2]
Avena sativa				
IPR Afrodite	20 c	7 b	0.01 c	R
AF 1345 Ucraniana	97 c	13 b	0.03 c	R
UPFA Ouro	723 c	355 b	0.25 c	R
UPFPS Farroupilha	1326 c	958 a	0.45 c	R
A. strigosa				
Agro Quaraí	940 c	456 b	0.32 c	R
UPFA 21 Moreninha	1803 b	755 a	0.62 b	R
Agro Esteio	1863 b	838 a	0.64 b	R
AF 12202	2126 b	719 a	0.73 b	R
Embrapa 139	2136 b	1059 a	0.74 b	R
Iapar 61 Ibiporã	3634 a	406 b	1.25 a	S

Means followed by the same letter in the column do not significantly differ from each other by Scott-Knott's test ($p > 0.05$). [1]Reproduction factor for the susceptible control (*Solanum lycopersicum:* 34.7; *Glycine max:* 10.7) and for the resistant control (*Crotalaria spectabilis:* 0.04). [2]Resistance reaction of the genotypes: resistant (R) and susceptible (S).

Table 4. Absolute and relative final population density of eggs and J2 of *Meloidogyne incognita*, reproduction factor (RF) and reaction of resistance (R) or susceptibility (S) of oats (*Avena* spp.) under low inoculum concentration (IC= 1,500 eggs and J2 per plant).

Genotypes	Absolute density (number per plant)	Relative density (number per gram of roots)	RF[1]	Reaction[2]
Avena sativa				
AF 1345 Ucraniana	7 b	1 c	0.00 b	R
IPR Afrodite	17 b	4 c	0.01 b	R
UPFPS Farroupilha	116 b	25 c	0.08 b	R
UPFA Ouro	123 b	20 c	0.08 b	R
Avena strigosa				
UPFA 21 Moreninha	580 b	78 c	0.39 b	R
AF 12202	893 b	114 c	0.60 b	R
Embrapa 139	1406 a	234 b	0.94 a	R
Agro Quaraí	2000 a	433 a	1.33 a	S
Agro Esteio	2010 a	456 a	1.34 a	S
Iapar 61 Ibiporã	2200 a	232 b	1.47 a	S

Means followed by the same letter in the column do not significantly differ from each other by Scott-Knott's test ($p > 0.05$). [1]Reproduction factor for the susceptible control (*Solanum lycopersicum:* 26.3; *Glycine max:* 10.5) and for the resistant control (*Crotalaria spectabilis:* 0.04). [2]Resistance reaction of the genotypes: resistant (R) and susceptible (S).

factors of their reaction to root-knot nematodes, which indicates the value of this genotype in the search for genes conferring resistance to nematodes.

In *A. strigosa,* there was some changes in the resistance reaction in response to the inoculum concentration. *Avena strigosa* 'UPFA 21 Moreninha' and 'Embrapa 139' were both resistant to *M. javanica* and *M. incognita* under both inoculum concentrations, showed the best performance among the black oats. On the other hand, *A. strigosa* 'Iapar 61' showed the worst performance, since it was susceptible to the low concentration of *M. javanica* (Table 2) and the two tested concentrations of *M. incognita* (Tables 3 and Table 4). This genotype showed a resistance reaction only when treated with a high concentration of *M. javanica* (Table 1). Machado et al. (2015) identified a susceptibility reaction of this cultivar to *M. javanica* and *M. incognita* by inoculating 2,000 nematode eggs. However, Gardiano et al. (2011) and Moritz et al. (2003) verified a resistance reaction of the same cultivar to *M. incognita* when more than 5,000 eggs and J2 were inoculated. The variation in response to 'Iapar 61 Ibiporã' for the reaction to *M. javanica* and *M. incognita* can be explained by the genetic variability of this oat genotype in response to the virulence of different isolates of the same nematode species (Borges et al., 2009; Hagemann et al., 2010). Furthermore, due to the concentration of 5000 eggs and juveniles of root-knot nematodes per plant being very high, this can contribute to the expression of "false resistance" by the plant. Although *A. strigosa* 'Embrapa 139' was resistant when inoculated with a lower concentration of *M. javanica* and *M. incognita*, the RF for both nematode species in the roots was very close to 1 (Tables 2 and Table 4). Therefore, it implies that growing such plants could increase the population of the nematodes in areas, where a low nematode population is present. The interaction between the pathogen and the host plant can result in compatibility or incompatibility. When the pathogen can overcome all the defense barriers of the host plant and there is no recognition or the defense response is too slow, a compatible interaction takes place, which leads to disease development allowing the pathogen to spread in the host plant. However, when the host plant is resistant to the invading pathogen, there is a rapid response of induced defense, occurring the prevention of the development of the disease, characterized as an incompatible interaction (Garcia-Brugger, 2006).

The compatible or incompatible interaction between the pathogen and the host plant is evaluated when the plant is exposed to different inoculum concentrations of the pathogen. Under a higher concentration of the pathogen, a change in the reaction of some oat genotypes was observed, indicating a "false" resistance (Table 1 and Table 3). Susceptible plants may exhibit a resistant reaction, when exposed to high population densities of nematodes. The reaction of resistance of the plant in the highest inoculum concentration of the nematodes indicates that the phytopathogens did not multiply in the plant roots. In higher inoculum concentration of nematodes, the plants were resistant compared to the lower inoculum concentration, when the plants were susceptible. This can be explained due to the fact that in higher inoculum concentration, there is greater competition between the individuals by the infection sites and feeding in the roots of the host plant. Consequently, the roots are less infected and the reproduction factor (RF) is below 1.0, indicating a resistance reaction (Andreazi et al., 2015; Shigueoka et al., 2016).

The increase of the nematode density in the roots of the host plant results in reduction of food and space availability in the roots, hindering the development of nematodes. Thereby, the reduction rate of nematode reproduction occurs due to the decrease of the necessary resources for its reproduction and survival, avoiding the parasitism, in contrast to low inoculum concentrations (Greco and Di Vito, 2009).

When the roots of the plants are exposed to the lowest inoculum concentration of the nematodes, it indicates that the individuals do not need to compete for food and space in the roots of the host plant, favoring the infection. When there is availability of food and space in the roots of the host plant and both are sufficient for the development of the nematodes, there will be no competition among the individuals for these resources (Greco and Di Vito, 2009).

The infection becomes favorable to the nematodes when there is a satisfactory root volume in the plant, providing the nematode with space and nutrients that contribute to parasitism. So, the plant's reaction can only be confirmed

under different concentration levels of nematode inoculum compared to the root volume of the plant. When there is enough space, food in the roots of the host plant, the level of inoculum is low and there is no infection of the root tissue and reproduction of the nematodes, it can be stated that a plant is resistant to a certain nematode species. In this case, the interaction between the plant and the nematode species was not compatible because the root exudates were not attractive to the nematodes, preventing the parasitism (Licá et al., 2018). Thereby, the plant expresses its resistance or susceptibility reaction, indicating the importance of testing the genotypes under contrasting inoculum concentrations.

The classification of resistant or susceptible oat genotypes based on a single inoculum concentration may be unsatisfactory since the genotypes exhibit variability regarding to the reaction to root-knot nematodes under different levels of pathogen population.

The alteration of the genotype reaction at higher and lower concentrations of nematodes was verified in other crops such as coffee (*Coffea* spp.). Shigueoka et al. (2016) observed that the RF of 11 coffee genotypes were increased when the roots of the plants were in contact with the lower concentration of *M. paranaensis* (1,000 eggs), compared to the RF at the highest inoculum concentration (1,400 eggs), which altered the resistance reaction. On the other hand, Andreazi et al. (2015) did not verify a difference in the reaction of two coffee cultivars in concentrations between 500 and 8,000 eggs of *M. paranaensis*, although the resistance levels of the genotypes changed. Sera et al. (2009) observed that under concentrations of 500 to 2,000 eggs per plant of *M. paranaensis*, two coffee cultivars showed moderate resistance under 500 and 1,000 eggs of the nematode, while they were susceptible when inoculated with 1,500 and 2,000 eggs. Thus, the use of different concentrations of inoculum is important for the classification of the reaction of cultivars to nematodes (Andreazi et al., 2015).

Comparing the final absolute density of *M. javanica* and *M. incognita* in the roots of oat genotypes, the highest absolute density of *M. incognita* (Tables 3 and Table 4) was found under the highest inoculum concentration, in comparison with *M. javanica* (Tables 1 and Table 2). This response is a signal indicating that *M. incognita* has a higher reproductive ability in oats, mainly in black oats (Borges et al., 2009). *M. incognita* is also more aggressive to the roots of plants compared to *M. javanica*, which may lead to its greater capacity to infect the root tissue (Inomoto, 2016). In this study, when the genotypes were tested under lower inoculum concentrations, a higher absolute final population density of *M. javanica* was observed in comparison to *M. incognita* (Tables 1 to Table 4). This reiterates the need to elucidate the biochemical interaction between *Avena* spp. and *Meloidogyne* spp. as highlighted by Marini et al. (2016).

The reaction of the oats tested in this study demonstrates that there is a genotypic effect on the reaction of nematodes *M. javanica* and *M. incognita*. It is known that the hatching of juveniles of the phytonematodes species and, consequently, their penetration into the root tissue can be influenced by several factors such as the release of root exudates (Dias-Arieira et al., 2008). The molecular interaction between the plant and the nematode can be compatible (favorable host) or incompatible (unfavorable host), depending upon the exudation of organic compounds by the roots of the plant, which may or may not be attractive to the pathogen. When these substances are attractive to nematodes, they will look for these tissues to feed and host, which will not happen if the substances produced by the plant are not attractive to the nematodes. Non-host plants or antagonists do not release attractive exudates in the soil, but rather expels repellents, preventing the migration and establishment of nematode parasitism at the root (Licá et al., 2018).

In oats, the interaction between pathogen and host plant leads to the identification of a few chemical substances. Lectin is an example among these substances, which can bind to glycoproteins associated with the blockage of nematode chemoreceptors; thereby, preventing the detection of the host plant by the parasite (Lacerda et al., 2017; Molan et al., 2000). This substance is associated with the defense mechanism of the plants against predators and pathogens and is also related to the establishment of symbiotic interactions between the host plant and nitrogen-fixing microorganisms (De Hooff et al., 2009).

Oats are endowed with preformed structural toxicants, which constitute a physical and chemical defense barrier, restricting infection by pathogens. An example of such compounds is avenacin A-1, lignin, and suberin. The plants synthesize these compounds in larger quantity when attacked by phytopathogens (Mert-Türk, 2006; Soriano et al., 2004). The methyl-jasmonate hormone, derived from jasmonic acid, is responsible for plant regulation and plant defense. It was identified in white oat. This hormone is an inducer of flavone synthesis in response to nematode attack and may be indicative of plant resistance to the pathogen (Soriano et al., 2004). The scopoletin (6-methoxy-7-hydroxycoumarin), belonging to the class of coumarins and ferulic, coumaric, siringeic, vanillic and p-hydroxybenzoic acids, which are phenolic acids, have been identified as allelopathic, inhibiting seed germination and the growth of seedlings (Fay and Duke, 1977; Lupini et al., 2014).

The chemical traits of *Avena* spp. suggest a possible association with the reaction of oats to the parasitism of *M. javanica* and *M. incognita*, necessitating future studies to understand these mechanisms. The effectiveness of genotype selection for cultivation and/or breeding programs depends on the genetic variability and heritability of the parent material associated with the interaction of the genotypes with the different levels of nematode infection (Machado et al., 2015; Rocha et al., 2009). However, there is no accessible information on genetic parameters of resistance of oats towards root-knot nematodes. Therefore, developing cultivars with this characteristic is something that should be explored (Machado et al., 2015). Also, the mechanisms of interaction between the nematode population and the compounds produced by resistant plants to the pathogen need better characterization in case of *Avena* spp. (Marini et al., 2016).

Plants that synthesize biocidal substances such as oats can be used as an alternative to pesticides thereby serving as possible sources of new compounds (Niro et al., 2016). It is also essential to have knowledge of the mechanisms involved in the resistance or antagonism of plants with the virulence of nematodes of the genus *Meloidogyne* (Marini et al., 2016). Studies of this type aim to subsidize the

control of alternative practices of the root-knot nematodes through cultural control, because the control or restriction of nematodes by the treatment of seeds with chemical nematicides create a short period of protection (Cabrera et al., 2009; Gardiano et al., 2009, 2011). In this way, the management of the root-knot nematodes can be performed through oat cultivation that can reduce the nematode population preventing their penetration and multiplication in the roots (Borges et al., 2009). Thus, the use of resistant cultivars has been considered as the most practical, efficient and economical measure for the management of nematodes (Machado and Araújo Filho, 2016; Mukhtar et al., 2017).

Although many oat genotypes, classified as resistant, may reduce the population of root-knot nematodes, their reaction to nematodes mainly depends on: (i) the genetic variability of the genotypes, (ii) the variability of the nematode populations, and (iii) their population in cultivated areas. Therefore, it is necessary that each genotype is tested for the reaction to nematodes before cultivation (Borges et al., 2009; Gomez-Rodríguez et al., 2017) targeting the management of nematodes.

Materials and Methods

Plant material and experimental design

The oat genotypes used in this study corresponded to three cultivars (UPFPS Farroupilha, UPFA Ouro, IPR Afrodite) and a line (AF1345 Ucraniana) of *A. sativa*, and five cultivars (Agro Quaraí, Agro Esteio, Embrapa 139, Iapar 61 Ibiporã, UPFA 21 Moreninha) and a line (AF 12202) of *A. strigosa*. Crotalaria (*Crotalaria spectabilis* Roth.), which was included as resistant control, whereas soybean [*Glycine max* (L.) Merrill 'Nidera 6909 IPRO'] and tomato (*Solanum lycopersicum* L. 'Santa Clara') were included as susceptible control. The control species were included (i) to verify if the environmental effect of the experimental site is adequate, and (ii) to determine the viability of the inoculum.

Four bioassays were established, two for *M. javanica* and two for *M. incognita*, using two concentrations of inoculum for each nematode species. For *M. javanica*, concentrations of 5,000 (Experiment 1) and 1,500 (Experiment 2) eggs and juveniles of second stage (J2) were used, whereas for *M. incognita* concentrations of 2,900 (Experiment 3) and 1,500 (Experiment 4) eggs and J2 were used. The experiments were carried out in a completely randomized design, where six replicates of the experiments were treated with the highest inoculum concentration, while the other three replicates were subjected to the lowest inoculum concentration. The experimental units were plastic pots distributed in a growth chamber at 23 ± 2 ºC with a photoperiod of 12 h. The experiments were carried out from October 2016 to September 2017 at the University of Passo Fundo (28º 15´S, 52º 24´W), in the north of the southern Brazilian state of Rio Grande do Sul.

Collection, quantification, and inoculation of M. javanica and M. incognita

The *M. javanica* isolate was obtained from infected soybean roots, while the *M. incognita* isolate was obtained from infected tomato roots. Identification of the nematode species was carried out based on the perineal pattern of the females (Taylor and Sasser, 1978). The isolates were multiplied in tomato plants that were kept in a growth chamber at 27 ± 3 ºC with a photoperiod of 12 h. The inoculum was extracted from roots by grinding in a blender containing 0.5% sodium hypochlorite solution (Bonetti and Ferraz, 1981). The number of eggs and J2 present in the suspension was quantified with the aid of a Peters chamber under an optical microscope. Ten seeds of each oat genotype and control species were seeded in the pots with 1 L of the substrate composed of sand: soil, 2:1, autoclaved for 2 h at 120 ºC. Ten days after emergence, thinning was performed leaving only two seedlings per pot. Two days after thinning, 1 mL of the nematode suspension was pipetted out at 4 cm deep in the substrate and 1 cm away from seedlings. After inoculation, the plants were maintained in a growth chamber at 23 ± 2 ºC with the photoperiod of 12 h. Plants were watered daily during the whole period of the experiment.

Evaluation method

Sixty days after inoculation, the plants were removed from the pots and the substrate was washed off. The roots were separated from the aerial part, cut into pieces of approximately 1 cm and placed to dry on absorbent paper. The total fresh mass of the root system was determined. Then, the extraction and counting of eggs and J2 were carried out in the same way as mentioned above for preparation of the inoculum.

The final population density (FP), which is the number of eggs and J2 found in the root system of each plant was determined directly. On the other hand, the relative population density of nematodes was calculated indirectly, corresponding to the number of eggs and J2 per gram of fresh root. From the FP value, the nematode reproduction factor (RF) was calculated according to equation cited to Oostenbrink (1966):

$$Reproduction\ factor\ (RF) = \frac{final\ population\ density}{initial\ population\ density}$$

The number of eggs and J2 present in the suspension at the time of inoculation was considered as the initial density. The nematode counts were performed for the two plants of each pot separately. From that, the mean was calculated for each experimental unit. Based on the nematode reproduction factor in the roots of the plants, the oat genotypes were classified as resistant (RF < 1) or susceptible (RF ≥ 1) (Oostenbrink, 1966). In order to verify the stability of the resistance reaction, the RF of *M. javanica* and *M. incognita* in the oat roots were compared for the two inoculum concentrations. Oat genotypes that did not alter the resistance reaction at both inoculum concentrations were considered stable.

Statistical analysis

The data were submitted to analysis of variance by the F test ($p \leq 0.05$), with a comparison of means by the Scott-Knott test ($p \leq 0.05$).

Conclusion

The reaction of *Avena* spp. to *M. javanica* and *M. incognita* depends on the level of nematode infestation, indicating the need for tests under different inoculum concentrations. The stability of the resistance reaction and the lower relative density of nematodes in *A. sativa* 'AF 1345 Ucraniana' and 'IPR Afrodite' indicate their potential for use as parents in breeding programs as well as for utilization in areas infested with both nematode species. Besides, the inoculum concentration that allowed a greater discrimination of the resistance reaction among the genotypes against the two species of nematodes was 1,500. Thus, his concentration could be a reference to future studies regarding to the response to oats to *M. javanica* and *M. incognita.*

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