REDIA, 103, 2020: 147-151 http://dx.doi.org/10.19263/REDIA-103.20.23

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HOST STATUS OF PLANT SPECIES WITH NEMATICIDAL ACTIVITY AGAINST *MELOIDOGYNE GRAMINICOLA* (GOLDEN & BIRCHFIELD) (1)

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Dallavalle E., Curto G., Santi R., Matteo R., Lazzeri L. - Host status of plant species with nematicidal activity against *Meloidogyne graminicola* (Golden & Birchfield).

The rice root-knot nematode, *Meloidogyne graminicola* (Golden & Birchfield), is a nematode first described in 1965 from grasses and oats in Louisiana (US), and currently spread in rice crops in Asia, parts of the Americas and Africa. It can be detected in a wide range of more than 98 host plant species. In 2016 *M. graminicola* was detected for the first time in some rice fields of Northern Italy.

In this paper, two greenhouse experiments (the first at 21-22 °C and the second one at 26-28 °C) are reported and discussed, in which three species containing bioactive compounds, *Lepidium campestre* (L.) R. Br., *Eruca sativa* Mill. cv. Nemat, and *Crotalaria juncea* L., were compared with *Cucumis sativus* L., a good host of *M. graminicola*, as control. Seedlings of each plant species were transplanted in pots containing mean 50 J2s 100 cm^{-3} soil. Three assessments were carried out about 30, 60, 90 days after transplantation, on both soil and roots. Temperature influenced *M. graminicola* life cycle, which was never completed at 21-22 °C; conversely, at 26-28 °C, both *E. sativa* and *C. juncea* reached a reproduction rate (R) = 0.01, confirming to be poor hosts, effective in decreasing the nematode infestation on the roots and in the soil, while *L. campestre* (R = 4.01) demonstrated to be a good host of *M. graminicola* more than the control *C. sativus* (R = 2.12), increasing considerably the nematode population after about 90 days.

KEY WORDS: *Meloidogyne graminicola*, *Lepidium campestre*, *Eruca sativa* cv. Nemat, *Crotalaria juncea*, Biofumigation, Glucosinolates, Isothyocianates, Alkaloids, Trichodesmine.

INTRODUCTION

The rice root-knot nematode, *Meloidogyne graminicola* (Golden & Birchfield) is a sedentary endoparasite nematode especially widespread in Central and South America, South Africa and Madagascar, South Asia (EPPO GLOBAL DATABASE, 2020). The main host of this nematode is rice (*Oryza sativa* L.), though more than 98 plant species have been also identified as additional hosts, including other cereals and weeds infesting rice fields as well as several crop species belonging to Asteraceae, Cucurbitaceae, Fabaceae, Solanaceae. In the last years, some outbreaks of *M. graminicola* have been reported also in the rice-growing areas of Piedmont and Lombardy, North Italy, as imposing the adoption of severe emergency measures.

Effective emerging solutions were suggested for *M. graminicola* control, from biological control (LIU *et al*., 2019) to resistant varieties selected for breeding (ZHAN *et al*., 2018). Among those solutions and despite of the great availability of resistant plants, the use of plants with nematicidal activity was overlooked.

In the recent past, the efficacy of the bioactive molecules released by Brassicaceae in the control of the root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitw., has been widely demonstrated (CURTO *et al*., 2008). In fact, Brassicaceae, and other plants from Brassicales order, contain the glucosinolate-myrosinase complex in their cells, which, after cell lesions and enzymatic hydrolysis, releases a number of biologically active compounds including isothiocyanates, nitriles, epithionitriles and thiocyanates (FAHEY *et al*., 2001). When this occurs, the nematode is poisoned by these volatile substances (LAZZERI *et al*., 2004) and either is not able to complete its life cycle into the roots of trap plants (CURTO *et al*., 2005), or is killed as second stage juvenile (J2) in the soil after a biofumigation with Brassicaceae (CURTO *et al*., 2004).

The exploitation of this biochemical system is well-known as Biofumigation (LAZZERI *et al*., 2013a; MATTHIESSEN & KIRKEGAARD, 2006). This technique can be used in synergy with others sustainable solutions such as crop rotations and applications of natural products (OKA *et al*., 2000; CHITWOOD, 2002; PLOEG, 2002; D'ADDABBO *et al*., 2006), thus customizing and improving the cropping system on each single farm. Indeed, in these last years, the biofumigation technique was greatly improved, through plants selected for green manure application (LARKIN, 2013), catch-crops (CURTO *et al*., 2006), Brassica derived defatted seed meals (CURTO *et al*., 2016), liquid formulations for foliar (BENFATTO *et al*., 2015) and root treatments (DE NICOLA *et al*., 2013).

Besides Brassicaceae, also some Fabaceae plants such as *Crotalaria juncea* L. (Sunn Hemp) proved to be interesting for nematode control (WANG *et al*., 2002).

[1] Original scientific contribution presented and discussed at the XIII National Congress of the Italian Society of Nematology (SIN), Catania - Italy, October 2-4, 2019.

- Received 21 September 2020 Accepted 8 October 2020

Furthermore, *C. juncea* proved to be effective in the containment of pathogenic fungi such as *Ralstonia solanacearum* (S.) Y. (KAKUHENZIRE *et al*., 2013) and weeds (MOSJIDIS & WEHTJE, 2011; SKINNER *et al*., 2012). The biological activity of this plant is especially due to its pyrrolizidine alkaloids, mainly trichodesmine (ADAMS & GIANTURCO, 1956). *C. juncea* stimulates the biological complexity of edaphic biocoenosis, saprophytic nematodes above all, making it more competitive towards of phytoparasitic organisms (WANG *et al*., 2004a; WANG *et al*., 2004b).

Recently, it was demonstrated that *C. juncea* significantly decreased *M. incognita* population in field infested soils (CURTO *et al*., 2015), thus also confirming the adaptability of this plant to the Po valley summer conditions. Furthermore, the high nitrogen content of leaves, the great biomass production and the role in the soil cover during summer hottest days could play a substantial role in the prevention of organic matter mineralization, as avoiding the typical summer carbon depletion of Mediterranean soils (ROTAR & JOY, 1983; PARENTI *et al*., 2018).

The aim of the present study was to assess the host status to *M. graminicola* of two Brassicaceae [*Lepidium campestre* (L.) R. Br. and *Eruca sativa* Mill. cv. Nemat] and a Fabacea (*C. juncea*), as to verify their ability to interrupt the life cycle of this nematode and, consequently, their potential application for suppressive green manures in fields infested by *M. graminicola*.

MATERIALS AND METHODS

The experiments consisted in two trials repeated in 2018 and 2019, both carried out in a greenhouse located in Bologna, Italy (44°46′58″N; 11°33′79″E, 54 m a.s.l.).

Seeds of *L. campestre* and *E. sativa* cv. Nemat were provided by the *Brassicaceae* seed collection of CREA-CI (Bologna) (LAZZERI *et al*., 2013b), whereas *C. juncea* seeds were purchased from Nutrien Italia S.p.A. A schematic description of tested plants and their potential active compound release are reported in table 1. Seeds of *Cucumis sativus* L. cv. "Vert petit de Paris" were also provided as untreated control, due to the well-known susceptibility of this plant to *M. graminicola* (JAIN *et al*., 2012). The population of *M. graminicola*, used in both trials, was collected from a naturally infested soil of a paddy field located in Novara province (Piedmont, North-West Italy) and then reared on rice and cucumber in greenhouse, as to obtain the *inoculum* needed for the experiments.

Seeds of each species were sown in polystyrene plateau fulfilled with sterile soil. Seedlings at third/fourth true leaf stage were then transplanted in pots (12 cm diameter) containing 600 cm^3 of soil artificially infested (50 J2 100 cm^{-3} soil) with *M. graminicola*. In the first and second experiment plants were transplanted on December 6 2018 and April 5 2019, respectively.

Nine replications were provided for each of the three species. During the first and second experiment, the greenhouse temperature was maintained at 22±1°C and 27±1°C, respectively.

In both experiments, soil nematode population and gall infestation on plant roots were checked on three pots of each species at around 30, 60 and 90 days after transplantation, i.e. on January 8, February 7 and March 5 2018 and May 17, June 27 and July 29 2019, respectively. Nematode J2s were extracted from soil of each pot by Baermann trays (BARKER, 1985; TACCONI & AMBROGIONI, 1995) and the number of J2s 100 cm^{-3} of soil was counted. Gall number and developmental stage of *M. graminicola* were also microscopically checked on each root.

At each sampling, analysis of variance (ANOVA) followed by Tukey test was performed on data of gall number per root and J2s 100 cm^{-3} soil from the three replicates of each treatment. The statistical analysis was performed by ARM7® software, Gylling Data Management, Inc. (Brookings, South Dakota, USA).

RESULTS

The obtained results demonstrated a different susceptibility of the tested plant species to *M. graminicola*.

In both experiments, as expected, *C. sativus* always behaved as good host of *M. graminicola*.

In Experiment n.1, the nematode population did not thrive in any different thesis, probably due to the adopted winter cycle. Nevertheless, some differences in gall number and *M. graminicola* reproduction in the soil were highlighted at lower temperatures too: *E. sativa* and *C. juncea* did never show galls on the roots, whereas only some J2 (0.33 J2 per pot) were found in soil with *C. juncea* and none in soil from pots with *E. sativa*. On the contrary, *L. campestre* demonstrated to be a host of *M. graminicola* even in this winter experiment, as some galls were found on its roots at the third assessment (2.33 galls per root) and a mean of 10 J2s were counted in soil, a number comparable to that found in soil cultivated with *C. sativus* (11 J2s per pot) (table 2).

Table 1 – Tested plant species and potential active compound released

Tested plant species	Family	Main active compound
Lepidium campestre	Brassicaceae	Tropaeolin (Benzyl ITC)
Eruca sativa cv. Nemat	Brassicaceae	Erucin (4-Methylthio-butyl ITC)
Crotalaria juncea	Fabaceae	Trichodesmine
Cucumis sativus cv. “Vert petit de Paris”	Cucurbitaceae	No compounds effective in nematode control

Table 2 – Experiment n.1 – The number of galls per root and J2s per pot are reported as mean of 3 repetitions in each assessment. Reproduction factor (R) stands for ratio of final (PF) to initial (PI) J2 population in the soil

Tested crop	2019-Jan-08		2019-Feb-07		2019-Mar-05		R (PF/PI)
	Galls per root (n)	J2 per pot (n)	Galls per root (n)	J2 per pot (n)	Galls per root (n)	J2 per pot (n)	
Lepidium campestre	1.67	1.33	3.00	4.33	2.33 ab	10.00 ab	0.20 ab
Eruca sativa	0.00	0.00	0.00	0.00	0.00 b	0.00 c	0.00 c
Crotalaria juncea	0.00	0.00	0.00	0.67	0.00 b	0.33 bc	0.01 bc
Cucumis sativus	1.00	0.00	2.00	1.67	5.67 a	11.00 a	0.22 a
F	NS	NS	NS	NS	**	*	*

Analysis of variance (ANOVA) on non-transformed data. Means followed by the same letters are not significantly different (P =0.05) according to Tukey test; * = statistically significant at $P \leq 0.05$; ** = statistically significant at $P \leq 0.01$

Table 3 – Experiment n.2 – The number of galls per root and J2s per pot are reported as mean of 3 repetitions in each assessment. Reproduction factor (R) stands for ratio of final (PF) to initial (PI) J2 population in the soil

Tested crop	2019-May-17		2019-Jun-27		2019-Jul-29		R (PF/PI)
	Galls per root (n)	J2 per pot (n)	Galls per root (n)	J2 per pot (n)	Galls per root (n)	J2 per pot (n)	
Lepidium campestre	1.50 a	1.00	4.33 a	2.33 a	16.04 a	200.33 a	4.01 a
Eruca sativa	0.00 b	0.33	0.00 b	0.00 b	0.00 b	0.67 c	0.01 b
Crotalaria juncea	0.00 b	0.00	0.00 b	0.00 b	0.26 b	0.67 c	0.01 b
Cucumis sativus	0.00 b	0.33	5.67 a	1.00 ab	3.38 ab	106.00 ab	2.12 ab
F	**	NS	**	**	** log	*	*

Analysis of variance (ANOVA) on either non-transformed data or log-transformed data in "galls per root" of third assessment. Means followed by the same letters are not significantly different (P = 0.05) according to Tukey test;* = statistically significant at $P \leq 0.05$; ** = statistically significant at $P \leq 0.01$

In Experiment 2, population of *M. graminicola* considerably increased throughout the trial and differences among nematicidal plants and host control (*C. sativus*) became more evident. At the third assessment, 3.38 galls per root and 106 J2s per pot were counted in soil with *C. sativus*. A mean of 0.67 J2 per pot and no root galls were observed for *E. sativa*, whereas *C. juncea* recorded 0.67 J2 per pot soil and 0.26 gall per root. In this experiment, *L. campestre* acted as host specie even more than the susceptible control. In fact, in the last assessment, *L. campestre* counted 16 galls per root, five times more than *C. sativus*, and 200 J2s per pot soil, almost double compared to *C. sativus*. Consequently, the reproduction rate (R) of nematode population, represented by the ratio between final and initial J2 population (PF/PI), reached R= 4.01 in *L. campestre* and R = 2.12 in *C. sativus* (table 3) while, in the winter test, R values were always lower than 0 in all tested species (table 2).

Regarding the completion of *M. graminicola* life cycle, neither females nor egg-masses of the rice root-knot nematode were ever observed on *E. sativa* and *C. juncea* in both experiments. Only few females were observed, starting from the second assessment, on *L. campestre* and *C. sativus* in the winter experiment, whereas the completion of the nematode life cycle, with the formation of egg-masses in all root galls, was observed since the second assessment in the spring-summer test.

DISCUSSION

Previous experiments have showed how *E. sativa* cv. Nemat (CURTO *et al*., 2016) and *C. juncea* (CURTO *et al*., 2015) can play a role in containing *M. incognita* populations, also in highly infested soils. In both experiments, absence or poor appearance of root galls, as well as minimal values of R rate on both *E. sativa* and C. *juncea* up to 90 days after transplantation, indicated the interesting potential of these two plant species in containing also the infestations of *M. graminicola*, as acting as poor hosts and releasing compounds that can severely limit the life cycle of the rice root-knot nematode. Therefore, the use of both these plants as green manure, though needing a further validation in field, could represent a useful tool for a sustainable control of *M. graminicola*.

Adversely, *L. campestre* showed to be a good host of *M. graminicola* which reproduced very rapidly on the roots of this plant and completed more than one generation in 90 days. Therefore, *L. campestre* is not suitable for green manuring and its use should be highly discouraged.

However, the evaluation of these experimental data should take into account also the different climate conditions of the two experiments, as *M. graminicola* showed an extremely high number of galls and was able to complete its life cycle on host species (*C. sativus* and *L. campestre*) roots only in the spring-summer trial, during which average 26-28 °C temperatures were recorded in the greenhouse. Adversely, nematode reproduction rate was always lower than 0 in the winter test, during which 21-22 °C mean temperatures were recorded.

More generally, the results of these experiments indicated that, aiming to limit *M. graminicola* infestation in rice fields, a strong attention should be given to novel plant selections developed for nematode control as well as to improve rotation with non-host crops.

AKNOWLEDGEMENTS

This study was supported from Emilia-Romagna Region. The authors acknowledge the colleagues of Plant Protection Service of Turin (Piedmont) and Minoprio Foundation of Como (Lombardy) for providing soil and rice plants infested by *M. graminicola*.

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