

# RESEARCH/INVESTIGACIÓN

## ECOLIFE® AND MANGANESE PHOSPHITE IN THE CONTROL OF *MELOIDOGYNE JAVANICA* AND IN THE DEVELOPMENT OF SOYBEAN CULTIVARS SUSCEPTIBLE AND RESISTANT TO THE NEMATODE

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### ABSTRACT

Puerari, H.H., C.R. Dias-Arieira, C.A. Tavares-Silva, J.O. Arieira, F. Biela, and J.P. Poletine. 2013. Ecolife® and manganese phosphite in the control of *Meloidogyne javanica* and in the development of soybean cultivars susceptible and resistant to the nematode. *Nematropica* 43:105-112.

The objective of this study was to evaluate the activity of Ecolife® and manganese phosphite in the control of *Meloidogyne javanica* in soybean cultivars susceptible and resistant to the nematode. Seedlings of the susceptible (BRSMT-Pintado) and resistant (MG/BR 46 Conquista) soybean cultivars with 15 days of emergence were treated with Ecolife® (1.5 ml/L) or manganese phosphite (20 ml/L) at three different times: seven or one day prior to inoculation or seven days after inoculation with 2,000 eggs/plant. Untreated inoculated plants and untreated non-inoculated plants were used as controls. The number of galls, number of eggs/g root and vegetative parameters were evaluated 60 days after inoculation. The experiment was conducted in a greenhouse over two different experimental periods. The results demonstrated that the commercial product Ecolife® reduced the number of eggs/g root, mainly in the susceptible cultivar treated seven and one day prior to inoculation. Manganese phosphite reduced the number of nematode eggs/g root when the resistant cultivar was treated seven days prior to inoculation, but only in Experiment 1. Neither of the two products enhanced plant development.

*Key words:* induced resistance, management, root-knot nematode

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### RESUMO

Puerari, H.H., C.R. Dias-Arieira, C.A. Tavares-Silva, J.O. Arieira, F. Biela, and J.P. Poletine. 2013. Ecolife® e fosfito de manganês no controle de *Meloidogyne javanica* e no desenvolvimento de cultivares de soja suscetível e resistente ao nematoide. *Nematropica* 43:105-112.

O presente trabalho teve como objetivo avaliar a atividade de Ecolife® e fosfito de manganês, no controle de *Meloidogyne javanica*, em cultivares de soja suscetível e resistente ao nematoide. Plântulas de soja cv. BRSMT-Pintado (suscetível) e MG/BR 46 Conquista (resistente), com 15 dias de emergência, foram tratadas com Ecolife® (1,5 ml/L) ou fosfito de manganês (20 ml/L), em três épocas: sete ou um dia antes da inoculação ou sete dias após a inoculação, com 2000 ovos do nematoide/planta. Plantas não tratadas/inoculadas e não tratadas/não inoculadas foram utilizadas como testemunhas. Decorridos 60 dias da inoculação, avaliou-se o número de galhas, ovos/g de raiz e parâmetros vegetativos. Os resultados mostraram que o produto comercial Ecolife® proporcionou redução no número de ovos/g de raiz, principalmente para a cultivar suscetível tratada um e sete dias antes da inoculação. O fosfito de manganês reduziu o número de ovos do nematoide/g de raiz no tratamento com aplicação sete dias antes da inoculação em soja resistente, porém, apenas no experimento 1. Nenhum produto possibilitou melhoria no desenvolvimento da planta.

*Palabras clave:* indução de resistência, manejo, nematoide das galhas

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## INTRODUCTION

Root-knot nematodes are one of the main problems associated with global soybean production. In Brazil, *Meloidogyne javanica* (Treb) Chitwood is noted for its widespread distribution in soybean producing regions and for its aggressiveness (Embrapa, 1996). Parasitism by root-knot nematodes leads to poor root formation and obliteration of vascular vessels, resulting in alterations in the capacity of plant to translocate water and nutrients (Baldwin *et al.*, 1979; Carneiro *et al.*, 1999). As a result, plants infected with this nematode species present physiological and morphological abnormalities and alterations in a variety of biological processes, which lead to lower productivities (Asmus, 2001).

The control of these parasites is complex, and with the concern about the environment, a continuous search for nematode control is underway for cultural and other alternative methods such as crop rotation, use of antagonists plants, soil solarization, biological control and resistance induction using elicitor agents (Costa *et al.*, 2001; Bettiol and Ghini, 2003). These can lead to favorable results, mainly when used as part of an integrated management system.

Research into resistance induction as an alternative method for nematode control has recently become more common. This method involves activating latent defense mechanisms in the plant using inducing agents (elicitors) (Smith, 1996), both biotic and abiotic (Bonaldo *et al.*, 2005; Nojosa *et al.*, 2005; Cavalcanti *et al.*, 2006). One of the characteristics of resistance induction is the absence of specificity, which allows a wide range of pathogens to be controlled through the use of different types of elicitors (Sticher *et al.*, 1997). Resistance induction occurs through the activation of genes that encode a series of pathogenesis-related proteins (PRs), enzymes involved in the synthesis of phytoalexins and lignin (Smith, 1996).

When compared with common nematicides, the cited advantages of resistance inducers in the control of nematodes include lower toxicity, improved biodegradation and the fact that they have multiple modes of action, reducing the probability of the parasite developing resistance (Barbosa *et al.*, 2010).

One of the most widely studied synthetic substances in the resistance induction process is salicylic acid, as well as its functional analogues, such as acibenzolar-S-methyl (ASM) and 2,6-dichloroisonicotinic acid (Boanova, 2008). Other substances that may act as inducers, but have still not been studied extensively, include the commercial product Ecolife<sup>®</sup> and manganese phosphite. According to information supplied by the manufacturer, Ecolife<sup>®</sup> is composed of citric biomass, has an aqueous and heterogeneous formulation and contains polyphenols, flavonoids, phytoalexins and diluted organic acids. This product also contains antioxidant substances, which cause alterations in plant metabolism and help in the prevention of disease,

in the regulation of vegetal growth and in reproductive processes, as well as improving the quality of crops in post-harvest (Motoyama *et al.*, 2003). However, the study of this compound is currently based on its use in the control of fungi (Cavalcanti *et al.*, 2006; Furtado *et al.*, 2010).

Phosphite is a precursor to phosphorous acid, which has a nitrogen atom on the position where the phosphates have an oxygen atom (Lovatt and Mikkelsen, 2006). The capacity of phosphite to stimulate defense mechanisms in plants, such as the production of phytoalexins, has been reported by Dercks and Creasy (1989). Resistance induction to nematodes using potassium phosphite has been proven in different pathosystems, such as for the resistance of wheat and oats to *Heterodera avenae* Wollenweber and *Meloidogyne marylandi* Jepson and Golden (Oka *et al.*, 2007) and for the resistance of corn to *Pratylenchus brachyurus* (Godfrey) and Filipjev and Schuurmans Steckhoven (Dias-Arieira *et al.*, 2012). However, there is a lack of information on the activity of manganese phosphite in the literature.

The objective of the present study was to evaluate the effect of the Ecolife<sup>®</sup> and manganese phosphite in the control of *M. javanica* in susceptible and resistant soybean cultivars.

## MATERIALS AND METHODS

The experiments were conducted in a greenhouse at the State University of Maringá, Regional Campus of Umuarama, Umuarama, Paraná, Brazil. Completely randomized experimental designs were used with six replicates and two different experimental periods: 10 October 2011 to 23 January 2012, with the temperature ranging from 19.5 to 30.5°C (Experiment 1) and 5 December 2011 to 5 March 2012, with the temperature ranging from 20.8 to 31.1°C (Experiment 2), to evaluate the effect of two products (Ecolife<sup>®</sup> and manganese phosphate), in two soybean cultivars.

Soybean seeds of BRSMT-Pintado cultivar, susceptible to *M. javanica*, and MG/BR 46 Conquista cultivar, resistant to *M. javanica*, were sown in polystyrene trays containing Plantmax<sup>®</sup> substrate. Fifteen days after sowing, when plants showed a pair of totally expanded trifoliate leaves, seedlings were transferred to vases containing 1.5 L of previously autoclaved soil (120°C for 2 h).

The plants were treated separately with Ecolife<sup>®</sup> (Quinabra), at the concentration recommended by the manufacturer (1.5 ml of the commercial product/L water), and with manganese phosphate, at a concentration of 20 ml of the commercial product/L. The applications were carried out at three time periods: 7 and 1 day prior to and 7 days after inoculation of the nematode. Untreated and inoculated plants were used as controls for the comparison of nematological parameters and untreated non-inoculated plants were used as controls for the analysis of vegetative

parameters. The applications were sprayed onto the shoots up to the point of runoff to prevent the soil from absorbing the product.

Inocula were obtained from a pure population of *M. javanica* maintained in tomato roots. To obtain the eggs, methodology of Hussey and Barker (1973) was followed. The suspension was calibrated to 500 eggs/ml, and 4 ml/plant was used for the inoculation process, equating to a total of 2,000 eggs and eventual second stage juveniles per plant. Inoculation was carried out by applying the suspension to three 3 - 5 cm-deep holes in the soil around the stem of the plant.

The plants were kept in a greenhouse for 60 days and irrigated daily. At the end of this period, plants were collected and shoots were separated from the root system. For the shoots, plant height was determined using a millimeter ruler and fresh and dry mass were determined using a semi analytical balance. To obtain the dry mass, shoots were placed into paper bags and maintained in a forced air circulation drying oven at 65°C until constant mass was achieved.

The root system was carefully washed and placed on absorbent paper to eliminate excess water. The fresh mass of the root was determined, and the number of galls was evaluated through direct counting. The number of eggs per root system was obtained after extraction using the methodology described above. A Peters chamber was used to determine population density and the total number of eggs per root system was divided by the fresh mass of the roots to determine the number of eggs/g root.

Data obtained for each product and each cultivar was submitted to analysis of variance and the averages were compared using the Tukey test with 5% of probability.

## RESULTS

The treatment of the cv. BRSMT-Pintado (susceptible) with Ecolife® did not reduce the number of galls in either Experiment 1 or Experiment 2, when compared to the control (Table 1). The same results were observed for both experiments using the soybean resistant cultivar MG/BR 46 Conquista, independent of the time period of application.

However, application of Ecolife® 7 and 1 day prior to inoculation with nematode significantly reduced the number of eggs/g root in both experiments in cv. BRSMT-Pintado compared with the control. The number of eggs/g root in cv. MG/BR 46 Conquista was lower than in the control for treatment one day prior to inoculation in Experiment 1, and for treatment seven days prior to and seven days after inoculation in Experiment 2.

Variable results were obtained for the vegetative parameters, as a reduction in height and fresh and dry mass of the shoots was observed for the application of Ecolife® one day prior to inoculation to cv. BRSMT-Pintado in Experiment 1, particularly when compared

to the non-inoculated control (Table 2). In Experiment 2, however, the inoculated control had lower values for plant height and fresh mass of the root than those observed for the other treatments, and the dry mass of the shoots and fresh mass of the root were lower than values observed for the treatment 7 days prior to inoculation. On analyzing Experiment 1 for cv. MG/BR 46 Conquista, it was observed that the treatment involving application one day prior to inoculation had lower values for plant height and fresh mass of the shoots than those observed for the treatment involving application seven days prior to inoculation, but was not observed symptom of phytotoxicity. However, these values did not differ from the control values (Table 2). The treatment seven days prior to inoculation presented additive effect, with higher values for dry mass of the shoots and fresh mass of the root compared to values observed for the controls. In Experiment 2, application of Ecolife® 7 days prior to inoculation had higher values for plant height compared to the value observed for the control. However, the values observed for the other parameters did not differ.

Manganese phosphite did not demonstrate any activity in the control of *M. javanica* in cv. BRSMT-Pintado in either of the experiments (Table 3). On the contrary, all of the treatments in Experiment 2 involving this product presented an increased number of galls compared to the control. For cv. MG/BR 46 Conquista, the number of galls in both experiments was not affected by the treatments. In Experiment 1, the number of eggs/g root was lower for the treatment involving application seven days prior to inoculation when compared to the control, however none of the treatments controlled the nematode in experiment 2.

Application of manganese phosphite to cv. BRSMT-Pintado did not affect the vegetative growth of the plants in Experiment 1 (Table 4). In Experiment 2, the non-inoculated control had higher values for plant height than the inoculated control. However, treatments involving application one and seven days prior to inoculation presented higher values compared to the inoculated control for fresh and dry mass of the shoots and fresh mass of the root. The treatment involving application seven days prior to inoculation also presented better values than the control for dry mass of the shoots.

In Experiment 1, cv. MG/BR 46 Conquista did not demonstrate a significant difference between the treatments in terms of fresh mass of the shoots and fresh mass of the root. However, for the dry mass of the shoots, the treatment involving application seven days prior to inoculation presented better values than the other treatments. In Experiment 2, the treatment involving application seven days prior to inoculation presented higher values for fresh and dry mass of the shoots compared to the inoculated control, but these results did not differ to those observed for the non-inoculated control.

Table 1. Number of galls and eggs per gram of root (eggs/g root) in two soybean cultivars treated with Ecolife® 7 and 1 day prior and 7 days after inoculation with 2,000 *Meloidogyne javanica* eggs, in two experiments conducted at different time periods.

Treatments	Experiment 1		Experiment 2	
	Galls	Eggs/g root	Galls	Eggs/g root
<u>BRSMT-Pintado (Susceptible)</u>				
Ecolife® 7 days prior	42.5 <sup>ns</sup>	1624.3 a	64.8 <sup>ns</sup>	1102.8 a
Ecolife® 1 day prior	61.8	1682.2 a	29.4	921.5 a
Ecolife® 7 days after	71.5	1916.4 b	60.4	1912.7 ab
Untreated control	53.0	1918.4 b	35.8	3391.1 b
CV (%)	37.8	32.2	32.5	28.7
<u>MG/BR 46 Conquista (Resistant)</u>				
Ecolife® 7 days prior	13.5 <sup>ns</sup>	591.0 ab	59.8 <sup>ns</sup>	548.3 a
Ecolife® 1 day prior	16.0	319.7 a	82.5	878.2 ab
Ecolife® 7 days after	16.0	421.9 ab	58.5	660.3 a
Untreated control	17.6	786.8 b	34.6	1264.4 b
CV (%)	46.0	38.5	31.7	28.2

For each cultivar, averages followed by the same letter did not differ according to the Tukey test at 5% of probability.  
<sup>ns</sup> = not significant

Table 2. Mean values for plant height, fresh (FMS) and dry (DMS) mass of the shoots and fresh mass of the root (FMR) of two soybean cultivars treated with Ecolife® 7 and 1 day prior and 7 days after inoculation with 2,000 *Meloidogyne javanica* eggs, in two experiments conducted at different time periods.

Treatments	Experiment 1				Experiment 2			
	Height (cm)	FMS (g)	DMS (g)	FMR (g)	Height (cm)	FMS (g)	DMS (g)	FMR (g)
<u>BRSMT-Pintado (Susceptible)</u>								
Ecolife® 7 days prior	81.4 b	8.5 b	2.4 b	17.5 b	67.6 b	11.4 b	3.5 b	27.0 b
Ecolife® 1 day prior	49.8 a	3.3 a	0.9 a	8.5 a	67.6 b	9.2 b	3.0 b	18.6 ab
Ecolife® 7 days after	74.3 ab	7.7 b	2.4 b	12.2 ab	64.0 b	10.5 b	2.3 ab	16.1 ab
Inoc. control	65.3 ab	8.7 b	2.8 b	16.4 b	45.2 a	3.1 a	0.9 a	5.7 a
Non-inoc. control	80.8 b	7.3 b	2.1 b	5.1 a	65.4 b	6.2 ab	2.1 ab	10.6 a
CV (%)	30.0	43.5	38.8	38.6	18.0	32.2	37.1	45.3
<u>MG/BR 46 Conquista (Resistant)</u>								
Ecolife® 7 days prior	74.3 b	10.2 b	3.4 b	14.4 b	79.6 b	9.5 ab	3.0 ab	21.9 ab
Ecolife® 1 day prior	52.0 a	6.0 a	1.8 a	7.2 ab	70.2 ab	9.9 ab	3.5 ab	21.4 ab
Ecolife® 7 days after	74.6 b	8.9 ab	2.8 ab	12.4 b	78.4 b	12.8 b	3.8 b	27.4 b
Inoc. control	63.4 ab	6.4 a	1.8 a	5.0 a	61.2 a	8.9 a	2.6 a	20.5 ab
Non-inoc. control	62.0 ab	8.3 ab	2.2 a	5.9 a	65.2 a	11.4 b	4.0 b	14.8 a
CV (%)	25.7	35.3	42.7	34.4	18.3	31.6	28.3	32.4

For each cultivar, averages followed by the same letter did not differ according to the Tukey test at 5% of probability.  
<sup>ns</sup> = not significant

Inoc. control = Untreated/inoculated control

Non-inoc. control = Untreated/non-inoculated control

Table 3. Number of galls and eggs per gram of root (eggs/g root) in two soybean cultivars treated with manganese phosphite 7 and 1 day prior and 7 days after inoculation with 2,000 *Meloidogyne javanica* eggs, in two experiments conducted at different time periods.

Treatments	Experiment 1		Experiment 2	
	Galls	Eggs/g root	Galls	Eggs/g root
<u>BRSMT-Pintado (Susceptible)</u>				
Phosphite 7 days prior	106.3 <sup>ns</sup>	2483.9 <sup>ns</sup>	75.5 ab	2562.9 <sup>ns</sup>
Phosphite 1 day prior	55.5	1816.5	137.3 b	2291.6
Phosphite 7 days after	72.0	3230.3	119.8 b	1564.5
Untreated control	36.7	3189.7	39.8 a	1390.9
CV (%)	40.3	32.6	31.7	28.7
<u>MG/BR 46 Conquista (Resistant)</u>				
Phosphite 7 days prior	10.8 <sup>ns</sup>	275.1 a	29.0 <sup>ns</sup>	709.1 a
Phosphite 1 day prior	50.2	685.5 ab	29.8	1127.5 b
Phosphite 7 days after	61.0	1252.0 ab	28.2	849.1 ab
Untreated control	37.0	1264.4 b	17.6	710.9 a
CV (%)	32.3	28.2	40.1	32.1

For each cultivar, averages followed by the same letter did not differ according to the Tukey test at 5% of probability.  
<sup>ns</sup> = not significant

Table 4. Mean values for plant height, fresh (FMS) and dry (DMS) mass of the shoots and fresh mass of the root (FMR) of two soybean cultivars treated with manganese phosphite 7 and 1 day prior and 7 days after inoculation with 2,000 *Meloidogyne javanica* eggs, in two experiments conducted at different time periods.

Treatments	Experiment 1				Experiment 2			
	Height (cm)	FMS (g)	DMS (g)	FMR (g)	Height (cm)	FMS (g)	DMS (g)	FMR (g)
<u>BRSMT-Pintado (Susceptible)</u>								
Phosphite 7 days prior	44.8 <sup>ns</sup>	8.3 <sup>ns</sup>	2.2 <sup>ns</sup>	8.4 ab	48.8 ab	8.5 b	2.5 b	15.2 b
Phosphite 1 day prior	74.2	10.1	2.8	10.8 ab	49.4 ab	7.4 b	2.4 b	13.8 b
Phosphite 7 days after	59.8	9.2	2.6	12.3 ab	56.8 ab	6.2 ab	2.5 b	9.6 ab
Inoc. control	80.8	8.7	2.7	16.4 b	45.2 a	3.1 a	0.9 a	5.7 a
Non-inoc. control	65.3	7.3	2.1	5.1 a	65.4 b	6.2 ab	2.1 b	10.6 ab
CV (%)	31.1	32.7	35.8	35.3	21.7	37.9	39.0	33.7
<u>MG/BR 46 Conquista (Resistant)</u>								
Phosphite 7 days prior	38.7 a	7.0 <sup>ns</sup>	2.6 b	4.9 <sup>ns</sup>	74.6 b	13.2 b	3.8 b	21.8 b
Phosphite 1 day prior	41.0 a	6.7	1.8 a	5.7	56.0 a	11.2 ab	3.3 ab	19.4 ab
Phosphite 7 days after	46.0 ab	6.9	2.0 ab	8.0	55.4 a	9.9 ab	2.9 a	15.7 a
Inoc. control	63.4 b	6.4	1.7 a	5.0	61.2 ab	8.9 a	2.6 a	20.4 ab
Non-inoc. control	62.0 b	8.3	2.2 ab	5.9	65.2 ab	11.4 ab	4.0 b	14.8 a
CV (%)	31.8	29.0	52.5	40.2	28.3	30.5	28.1	38.9

For each cultivar, averages followed by the same letter did not differ according to the Tukey test at 5% of probability.  
<sup>ns</sup> = not significant

Inoc. control = Untreated/inoculated control

Non-inoc. control = Untreated/non-inoculated control

## DISCUSSION

The commercial product Ecolife® did not reduce the number of galls, but was efficient in reducing the number of eggs of *M. javanica* in the susceptible soybean cultivar when applied seven or one day prior to inoculation. Similar results have been reported by Guimarães (2007), when studying the use of inducers in the control of *Meloidogyne* spp. and *P. brachyurus* in sugarcane. This author observed that Ecolife 40® caused a reduction in the number of nematodes present in both the rhizosphere and roots themselves. One hypothesis is that the citric biomass causes an inducer effect, as previous studies have shown that citric extracts promote the production of the phytoalexin glyceollin in soybean cotyledons (Motoyama *et al.*, 2003).

Kaplan *et al.* (1980) observed that the production and accumulation of glyceollin in soybean cultivars resulted in resistance to *M. incognita* (Kofoid and White) Chitwood and *M. javanica*. In another study, research about the *in vitro* activity of this phytoalexin on root-knot nematodes demonstrated that *M. incognita* was more sensitive, while *M. javanica* could withstand higher concentrations of the product (Huang, 1985).

The fact that there was no reduction in the number of galls indicates that the inducer did not interfere in the penetration of the nematode and establishment of feeding sites, but caused a reduction either in the reproductive potential of the nematode or its survival after gall formation. Studies have shown that a rapid hypersensitivity reaction can occur after activation of induction, causing the degeneration of the feeding sites. This can lead to death or to a reduction in the availability of nutrition, thus affecting the reproductive potential of the nematode (Bakker *et al.*, 2006).

Similar results have been observed for other inducers, which despite causing a reduction in nematode reproduction, do not affect the production of galls. These include acibenzolar-S-methyl (Chinnasri *et al.*, 2003; Molinari and Baser, 2010; Puerari *et al.*, 2013) and DL- $\beta$ -amino-n-butyric acid (Oka and Cohen, 2001). The results from these studies confirmed the time necessary for the induction of resistance mechanisms of plants, as studies have shown that acibenzolar-S-methyl does not affect hatching, survival or penetration of nematodes in the roots of its host (Chinnasri *et al.*, 2003; Salgado *et al.*, 2007; Molinari e Baser, 2010), but instead reduces its development and reproductive potential (Chinnasri *et al.*, 2003; Molinari and Baser, 2010).

Application of manganese phosphite did not cause reduction in the number of galls for either cultivar, independent of the treatment. Furthermore, all of the treatments in Experiment 2 involving the susceptible cultivar (BRSMT-Pintado) presented higher numbers of galls than the control.

In relation to the number of eggs/g root, only the treatment involving application of manganese

phosphite seven days prior to inoculation to the cv. MG/BR 46 Conquista caused a reduction in this variable. Studies on the use of phosphites for resistance induction to nematodes mainly focus on the use of potassium phosphite, and many of these demonstrate the efficiency of this product. In a study conducted by Dias-Arieira *et al.* (2012), it was observed that potassium phosphite applied on leaves was efficient in reducing populations of *P. brachyurus* in corn. Similarly, Oka *et al.* (2007) observed that potassium phosphite was efficient in controlling *H. avenae* and *M. marylandi* when applied to shoots of wheat and oat crops. This result can be attributed to the capacity of phosphite to stimulate the production of phytoalexins (Dercks and Creasy, 1989).

The fact that manganese phosphite did not present similar results for the controls in both experiments does not mean that it is not an efficient product for the purposes of this study, since the control has been observed in previous studies. Some authors have drawn attention to factors that may cause variations in the results of resistance induction to nematodes, including dosage, method and time of application and the level of genetic resistance in the host (Owen *et al.*, 2002; Molinari and Baser, 2010; Puerari *et al.*, 2013). Furthermore, inducers generally need to be applied prior to inoculation at times ranging from 3 to 15 days (Owen *et al.*, 2002).

Dosage is a crucial point that deserves further investigation so that the best concentration of this product for resistance induction can be determined. The efficiency of acibenzolar-S-methyl, currently the most widely studied inducer, has been shown to be proportional to increases in the concentration applied (Chinnasri *et al.*, 2003; Molinari and Baser, 2010). The reduction of *Rotylenchulus reniformis* Lindford and Oliveira in the root system of cowpea, for example, was proportional when the concentration was increased from 50 to 100 mg/L water (Chinnasri *et al.*, 2003).

For the vegetative variables, there was no similarity in the results from both of the experiments involving the application of Ecolife®, independent of the cultivar studied. When evaluating the application of citric biomass for the control of disease in soybean, Kuhn *et al.* (2009) observed no significant differences between plants pulverized with water or with the product for the number of pods/plant, number of grains/pod, mass of 100 grains and productivity. Similar results were observed by Mesquini *et al.* (2011), who did not verify any significant differences in productivity or 1000 grain weight when using Ecolife® to control Asian soybean rust.

The fact that manganese phosphite did not present positive results for the vegetative parameters may have been explained by Marschner (1997), who challenge the use of phosphite as a nutritional source of phosphorous, emphasizing that there is no concrete evidence that plants use it as a direct source of this mineral. Conversely, Nojosa *et al.* (2005) explain that

phosphite promotes an improvement in the nutritional state of plants, principally during stages of higher metabolic activity, as the plant absorbs phosphorous at a higher rate than other phosphate-based products.

Therefore, it can be concluded that the commercial product Ecolife® caused a reduction in the number of eggs of *M. javanica*/g root, especially when applied seven or one day prior to inoculation to a susceptible soybean genotype. Manganese phosphite reduced the number of eggs/g root in the treatment with application seven days prior to inoculation in resistant soybean, but only in Experiment 1. In soybean crop it is impossible to apply the resistance inductor prior to nematode inoculation, since the process occur naturally in areas, even that, the treatment is important because it may reduce feeding sites formed from the second parasite life cycle.

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#### LITERATURE CITED

- Asmus, G. L. 2001. Danos causados a cultura da soja por nematoides do gênero *Meloidogyne*. Pp. 39-62 in J. F. V. Silva, ed. Relações parasito hospedeiro nas meloidoginose da soja. Londrina, PR: Sociedade Brasileira de Nematologia.
- Bakker, E., R. Dees, J. Bakker, and A. Goverse. 2006. Mechanisms involved in plant resistance to nematodes. Pp. 314-334 in S. Tuzun, and E. Bent, eds. Multigenic and induced systemic resistance in plants. New York: Springer Science.
- Baldwin, J. G., K. R. Barked, and L. A. Nelson. 1979. Effects of *Meloidogyne incognita* on nitrogen fixation in soybean. Journal of Nematology 11:156-161.
- Barbosa, L. F., E. P. R. Amorim, V. K. S. Costa, R. C. P. Trindade, G. S. Peixinho, and S. J. S. Cruz. 2010. Efeito de resíduos vegetais sobre *Scutellonema bradys*, agente causal da casca preta do inhame (*Dioscorea* sp.). Revista Raízes e Amidos Tropicais 6:271-279.
- Bettiol, W., and R. Ghini. 2003. Controle físico de doenças e de plantas invasoras. Pp. 165-190 in C. Campanhola, and W. Bettiol, eds. Métodos alternativos de controle fitossanitário. Jaguariúna: Embrapa Meio Ambiente.
- Boanova, L. P. 2008. Ação de indutores bióticos e abióticos no controle da ferrugem do eucalipto, atividades enzimáticas e expressão gênica durante o processo de infecção. Tese de Doutorado. Universidade Estadual Paulista, Botucatu, SP.
- Bonaldo, S. M., S. F. Pascholati, and R. Romeiro. 2005. Indução de resistência: noções básicas e perspectivas. Pp. 11-28 in L. S. Cavalcanti, R. M. Di Piero, P. Cia, S. F. Pascholati, M. L. V. Resende, and R. S. Romeiro, eds. Indução de resistência em plantas a patógenos e insetos. Piracicaba, SP: FEALQ.
- Carneiro, R. G., P. Mazzafera, and L. C. C. B. Ferraz. 1999. Carbon partitioning in soybean infected with *Meloidogyne incognita* and *M. javanica*. Journal of Nematology 31:348-355.
- Cavalcanti, F. R., M. L. V. Resende, A. B. Zacaroni, P. M. Ribeiro Jr., J. C. B. Costa, and R. M. Souza. 2006. Acibenzolar-S-metil e Ecolife® na indução de respostas de defesa do tomateiro contra a mancha bacteriana (*Xanthomonas vesicatoria*). Fitopatologia Brasileira 31:372-380.
- Chinnasri, B., B. S. Sipes, and D. P. Schmitt. 2003. Effects of acibenzolar-S-methyl application to *Rotylenchulus reniformis* and *Meloidogyne javanica*. Journal of Nematology 35:110-114.
- Costa, M. J. M., V. P. Campos, D. F. Oliveira, and L. M. Pfenning. 2001. Toxidade de extratos vegetais e de esterco a *Meloidogyne incognita*. Fitopatologia Brasileira 27:245-250.
- Dercks, W., and L. L. Creasy. 1989. Influence of fosetyl-Al on phytoalexin accumulation in the *Plasmopara viticola* grapevine interaction. Physiology and Molecular Plant 34:203-213.
- Dias-Arieira, C. R., P. M. Marini, L. F. Fontana, M. Roldi, and T. R. B. Silva. 2012. Effect of *Azospirillum brasilense*, Stimulate® and potassium phosphite to control *Pratylenchus brachyurus* in soybean and maize. Nematropica 42:170-175.
- Embrapa. 1996. Recomendações técnicas para a cultura da soja na região central do Brasil. Londrina, PR: CNPSo, Documentos, 96.
- Furtado, L. M., A. A. C., Rodrigues, V. S. Araújo, L. L. S. Silva, and A. M. Catarino. 2010. Utilização de Ecolife® e acibenzolar-s-metil (ASM) no controle da antracnose da banana em pós-colheita. Summa Phytopathologica 36:237-239.
- Guimarães, L. M. P. 2007. Eficiência de indutores no manejo integrado de *Meloidogyne* spp. e *Pratylenchus zaei* em cana-de-açúcar. Universidade Federal de Pernambuco, Recife, PE. Tese de Doutorado em Fitopatologia.
- Huang, J. S. 1985. Mechanisms of resistance to root-knot nematodes. Pp. 11-17 in J. N. Sasser, and C. C. Carter, eds. An advanced treatise on *Meloidogyne*: Biology and control. Raleigh: North Carolina State University Graphics.
- Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reporter 57:1025-1028.
- Kaplan, D. T., N. T. Keen, and I. J. Thomason. 1980. Studies on the mode of action of glyceollin in soybean incompatibility to the root-knot nematode *Meloidogyne incognita*. Physiology Plant Pathology 16:319-325.

- Kuhn, R. A., R. L. Portz, and J. R. Stangarlin. 2009. Uso da biomassa cítrica no controle de doenças da soja. *Scientia Agraria Paranaensis* 8:85-98.
- Lovatt, C. J., and R. L. Mikkelsen. 2006. Phosphite fertilizers: What are they? Can you use them? What can they do? *Better Crops* 90:11-13.
- Marschner, H. 1997. Mineral nutrition of higher plants. London: Academic Press.
- Mesquini, R. M., K. R. F. Schwan-Estrada, R. A. Vieira, and J. F. Nascimento. 2011. Controle e progresso temporal da ferrugem asiática da soja sob controle alternativo em campo. *Summa Phytopathologica* 37:24-29.
- Molinari, S., and N. Baser. 2010. Induction of resistance to root-knot nematodes by SAR elicitors in tomato. *Crop Protection* 29:1354-1362.
- Motoyama, M. M., K. R. F. Schwan, J. R. Stangarlin, A. C. G. Fiori-Tutida, and C. A. Scapim. 2003. Indução de fitoalexinas em soja e em sorgo e efeito fungitóxico de extratos cítricos sobre *Colletotrichum lagenarium* e *Fusarium semitectum*. *Acta Scientiarum Agronomy* 25:491-496.
- Nojosa, G. B. A., M. L. V. Resende, and A. V. Resende. 2005. Uso de fosfitos e silicatos na indução de resistência. Pp. 139-153 in L. Cavalcanti, R. M. Di Piero, P. Cia, S. F. Pascholati, M. L. V. Resende, and R. S. Romeiro, eds. *Indução de resistência em plantas a patógenos e insetos*. Piracicaba, SP: FEALQ.
- Oka, Y., and Y. Cohen. 2001. Induced resistance to cyst and root-knot nematodes in cereals by DL-amino-butyric acid. *European Journal of Plant Pathology* 107:219-227.
- Oka, Y., N. Tkachi, and M. Mor. 2007. Phosphite inhibits development of the nematode *Heterodera avenae* and *Meloidogyne marylandi* in cereals. *Nematology* 97:396-404.
- Owen, K. J., C. D. Green, and B. J. Deverall. 2002. Benzothiadiazole applied to foliage reduces development and egg deposition by *Meloidogyne* spp. in glasshouse-grown grapevine roots. *Australasian Plant Pathology Society* 31:47-53.
- Puerari, H. H., C. R. Dias-Arieira, T. S. Dadazio, D. Mattei, T. R.B. Silva, and R. C. F. Ribeiro. 2013. Evaluation of acibenzolar-S-methyl for the control of *Meloidogyne javanica* and effects on the development of susceptible and resistant soybean. *Tropical Plant Pathology* 38:44-48.
- Salgado, S. M. L., M. L. V. Resende, and V. P. Campos. 2007. Efeito de indutores de resistência sobre *Meloidogyne exigua* do cafeeiro. *Ciência e Agrotecnologia* 31:1007-1013.
- Smith, C. J. 1996. Accumulation of phytoalexins: defense mechanisms and stimulus response systems. *New Phytologist* 132:1-45.
- Sticher, L., B. Mauchi-Mani, and J. P. Metraux. 1997. Systematic acquired resistance. *Annual Review of Phytopathology* 35:235-270.

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