

Full Length Research Paper

Reproduction of *Meloidogyne javanica* in crambe plant and influence on crop yield and oil content

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The crambe is an oilseed plant of the Brassicaceae family, which has the potential to grow in the winter, in succession to soybean crop, and whose seeds have been used for extraction of oil for biofuel production. However, in areas with nematode infestation, special care is needed in the selection of the species to be used in crop succession. Thus, the present study aimed to assess the susceptibility of crambe to plant-parasitic nematode, *Meloidogyne javanica* and its interference in vegetative growth parameters: crop yield and seed oil content. Therefore, crambe (*Crambe abyssinica* Hochst) seedlings were inoculated with a suspension containing 0, 1300, 2600 and 5200 eggs and second-stage juveniles (J2) of *M. javanica*, in experiment 1 (January to March, 2012), and 0, 1000, 2000 and 4000 eggs and J2, in experiment 2 (May to July, 2012). The tomato plant was inoculated to assess the viability of the inoculum. The assessments were done at two different times: 60 days after inoculation, assessment of the nematode reproduction factor, plant height and fresh and dry mass of the aerial part, was done with four repetitions. At the end of the crop cycle, 90 days after inoculation, seed yield and oil content were assessed in the remaining four replications. When grown in a period of higher temperatures, the crambe showed susceptibility to root-knot nematodes, with a negative impact on plant yield. However, this did not occur when the plant was grown in a more favorable season. In both experiments, seed oil content was not affected by the presence of the nematode.

Key words: *Crambe abyssinica*, oil, susceptibility, nematodes.

INTRODUCTION

The crambe is a cruciferous winter oilseed, belonging to the Brassicaceae family (Machado et al., 2008), originating in the Mediterranean and with short cycle,

which ranges from 90 to 100 days (Oplinger et al., 1991). The plant is 70-90 cm tall, blooms 35 days after seeding, and the seeds are rounded and covered with a gray

integument. Its main characteristic is the high concentration of oil, with levels ranging from 35 to 60% and used in biodiesel production (Oliveira et al., 2011).

Being a very robust plant, the crambe can grow in very adverse climate conditions, tolerating the frosts typical of the south of the country and temperatures as low as minus 6°C (Pilau et al., 2011), and show good yield in the dry season (Oplinger et al., 1991). Because it is resistant to drought and low temperature periods, crambe has been an alternative to sowing the winter crop in no-tillage system, with subsequent planting of soybeans (Pitol et al., 2010; Oliveira et al., 2011). However, there is little information on the management and potential of this plant, including its use as an alternative control importance of nematodes to soybeans, as *Meloidogyne javanica* (Treub) Chitwood and *Pratylenchus brachyurus* (Godfrey) Filipjev and Schuurmans Steckhoven.

The soybean, *Glycine max* (L.) Merrill is currently the most important crop in the world. According to data of USDA (2015), soybean yield in the 2015/16 harvest was 106.58 million of tons, in a total of 33.79 million hectares cultivated.

Despite the high yield, damage caused by phytonematodes are very common, and the losses caused by them have become a matter of growing concern among producers and researchers (Asmus, 2004; Kubo et al., 2004; Machado et al., 2006). The main nematodes associated with soybean crops include *Meloidogyne incognita* (Kofoid & White) Chitwood and *M. javanica* (Treub) Chitwood, that form root galls, *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans Stekh which cause root lesions, as well as the cyst nematode, *Heterodera glycines* Ichinohe, and the reniform nematode, *Rotylenchulus sreniformis* Linford & Oliveira (Dias-Arieira and Chiamolera, 2011).

The nematode galls are important plant pathogens that affect agricultural production worldwide (Al-Raddad, 1995). This is favored by the high reproductive capacity of the nematodes, which leads to the rapid growth of populations in the field (Ferraz, 1985).

Due to the limited number of varieties resistant to nematodes, adapted to the different farming regions and the lack of nematicides registered for the crop, alternative methods to control the pest are constantly sought. Therefore, the use of non-host plants in rotation or succession of cultures with soybean is one of the main management strategies. With this practice, the populations of nematodes can be kept below the economic damage threshold, without any risk to the environment (Carneiro et al., 2007). The main crops to be used in succession to soybean are corn, oats, wheat, and, to a lesser extent, sunflower (EMBRAPA, 2004).

Despite the aggressiveness of phytonematodes, which

attack crops of commercial interest, there is limited information on the reproduction of *M. javanica* and its interference in vegetative and productive parameters of the crambe. In view of the above considerations, the present study aimed at assessing the susceptibility of the crop to *M. javanica*, the interference of this nematode on the vegetative parameters of the crop and on the seeds and oil content.

MATERIALS AND METHODS

The experiments were conducted in greenhouse, at Universidade Estadual de Maringá, Campus Regional de Umuarama, Umuarama, Paraná, Brazil, in completely randomized designs, with four treatments (levels of initial inoculum) and eight replications. Period of the experiments: January to March 2012 (experiment 1) and repeated from May to July 2012 (Experiment 2).

The average temperatures recorded during the experimental periods were minimum of 21.2 and maximum of 31.9°C in experiment 1, and minimum of 12.9 and maximum of 21.7°C in experiment 2, according to data obtained from SIMEPAR (2013).

Initially, crambe seeds of the variety MS Brilhante were sown in trays containing the substrate Plantmax[®]. Seedlings were transplanted 15 days after germination to vases with capacity of 2 L, with two seedlings per vases. The substrate used was soil classified as Oxisol Dystrophic with a sandy texture (USDA, 1998), which was previously autoclaved for two hours at constant temperature of 120°C.

Two days after transplantation, the crambe was inoculated with suspension of *M. javanica*. In Experiment 1, the initial populations (Pi) were 0, 1300, 2600 and 5200 eggs and second-stage juveniles (J2), and in experiment 2, they were 0, 1000, 2000 and 4000 eggs and J2. The tomato plant of Santa Clara variety was inoculated with 5200 and 4000 eggs, in the respective experiments, to demonstrate the viability of the inoculum.

The inoculum was obtained from a pure population of nematodes, kept in the tomato plant, and extraction began in the roots, which were ground with a sodium hypochlorite solution in a blender, according to the method proposed by Hussey and Barker (1973) and adapted by Boneti and Ferraz (1981). The suspensions were calibrated using Peter's chamber, under optical microscope. Inoculation was performed by adding 4 mL of the respective suspensions to four equidistant holes, opened in the soil, around the plant.

After 60 days of cultivation, four plants of each treatment were randomly evaluated on vegetative parameters: plant height, dry mass of the aerial part, the latter obtained by drying in forced circulation at 65°C for three days. Regarding the nematological parameters, the number of galls and eggs per root was determined, which was considered the final population (Pf). The eggs were extracted according to the cited methodology and assessed in optical microscope in Peter's chamber.

The nematode reproduction factor (RF) in the crambe was calculated using the formula $RF = Pf/Pi$, as proposed by Oostenbrink (1966), in which plants are considered susceptible when $RF \geq 1$, resistant when $RF < 1$ and immune when $RF = 0$.

At the end of the crop cycle, the remaining plants were assessed for the number of seeds per plant, seed weight and oil content. The extraction of oil from the seeds was performed in laboratory using

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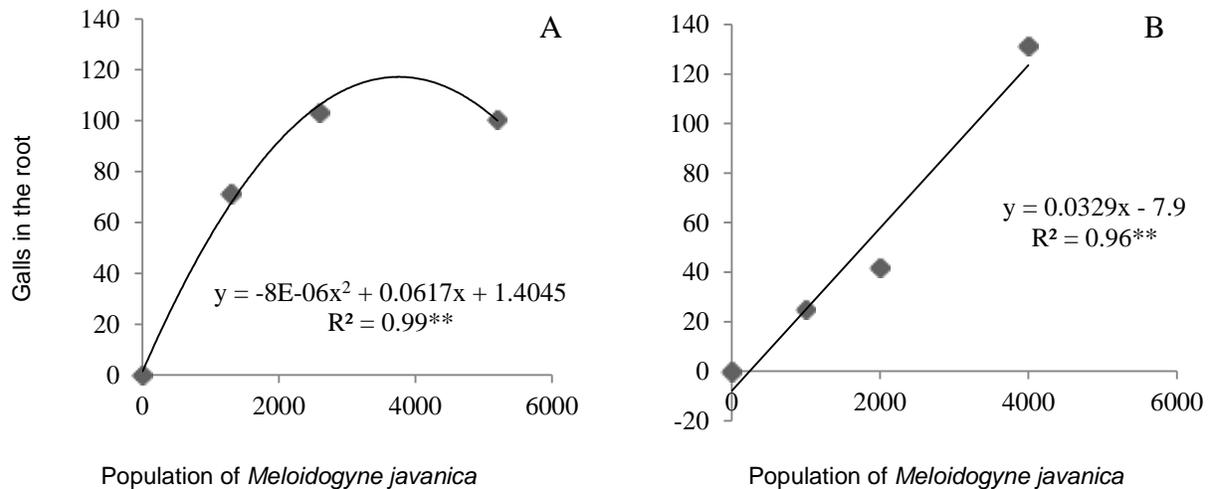


Figure 1. Number of galls in crambe roots depending on the population of *Meloidogyne javanica*. A = Experiment 1 and B = Experiment 2. ** = significant regression at 1% of probability.

methodology proposed by Silva et al. (2015).

The data were subjected to analysis of variance and the means to regression analysis, with a significance level of 5%, for the data concerning the inoculum levels used in the crambe. For the comparison of data with the susceptibility pattern (tomato plant), the means were compared by Tukey test at 5% probability, using the SISVAR 5.3 software (Ferreira, 2010).

RESULTS AND DISCUSSION

In Experiment 1, the crambe was susceptible to *M. javanica*, with reproduction factors equal to 3.3, 2.6 and 1.5, when inoculated with 1300, 2600 and 5000 eggs, respectively. In the second experiment, RF values were equal or close to 1, with means of 1.0, 0.7 and 0.8 for plants inoculated with 1000, 2000 and 4000 eggs, respectively. In both experiments, the reproduction values of the nematode in the crambe were significantly lower than those obtained in the tomato plant, used as control treatment, whose RF values were 14.9 and 53.8 in Experiments 1 and 2, respectively.

The difference in the reproductive activity of *M. javanica* in the two years can be explained by the experimental period. In Experiment 1, plant growth occurred from January to March, being affected by the high temperatures, resulting in physiological stress that made the plants susceptible to the establishment of the pathogen in the roots, while Experiment 2 was conducted in May and June, period of milder temperatures, which is more appropriate for the crambe crop (Knights, 2002; Pitol et al., 2010; Silva et al., 2013).

The climate factors have also directly impacted the reproductive activity of root-knot nematodes, because the genus *Meloidogyne* spp. usually has high activity in the soil and roots under high temperatures, and its ideal

temperature for reproduction is 25°C. However, species such as *M. incognita* and *M. javanica* cannot survive at temperatures lower than 10°C (Ritzinger et al., 2010; Campos et al., 2011). Campos et al. (2011) reported that the reproduction of *M. javanica* in soybean plants increased at a temperature of 28°C, both in resistant and susceptible varieties.

The number of galls and eggs was found to be directly proportional to the population levels of *M. javanica* (Figures 1 and 2). In experiment 1, the relationship between the number of galls and eggs in crambe roots and the population of phytonematodes was best fitted by the quadratic equation (Figures 1A and 2A). Both parameters showed decrease when the population of nematodes was higher than 4000 eggs (Figure 1A). This was possibly due to food restriction, as it has been observed in other pathosystems involving *Meloidogyne* spp., in which increase in nematode population reduced the number of galls, giving rise to the hypothesis that the parasite had difficulty establishing feeding sites, due to the competition generated by the penetration of a large number of J2 in the roots (Schochow et al., 2004; Fabry et al., 2009).

In Experiment 2 (Figures 3B and 4B), the number of galls and eggs showed linear increase in relation to the initial population of *M. javanica*. The data obtained corroborate the findings of Souza et al. (2011), that classified the crambe as susceptible to this nematode, and its reproduction was directly proportional to the increase in inoculum levels of the parasite.

Asmus and Andrade (2001) also reported crambe susceptibility to *M. javanica*. However, the authors observed that the reproduction of the phytonematode was less intense than in other hosts, such as canola and quinoa. Despite the multiplication of *M. javanica* in the

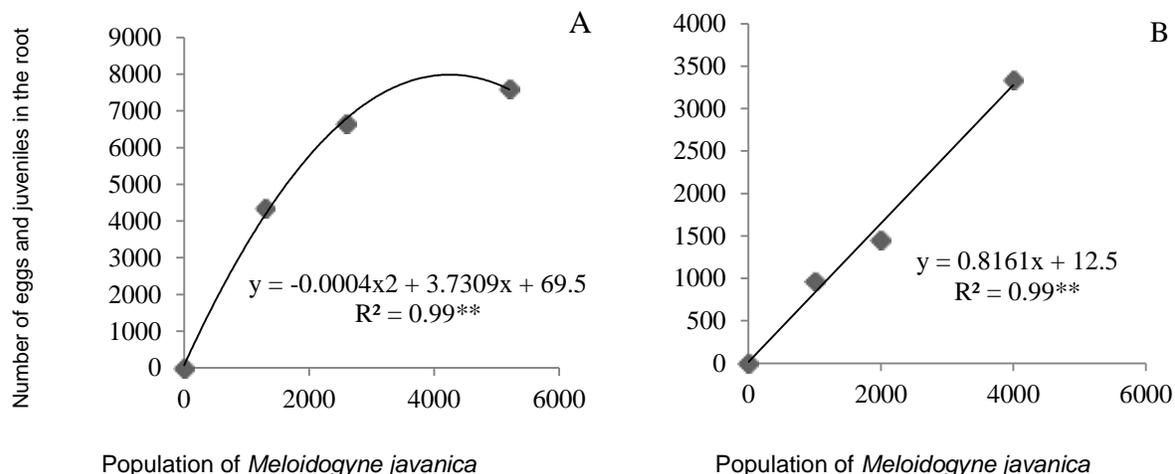


Figure 2. Regression of the number of eggs and juveniles in crambe roots depending on the population of *Meloidogyne javanica*. A = Experiment 1 and B = Experiment 2. ** = significant regression at 1% probability.

Table 1. Means related to the fresh mass of the aerial part (FMAP), dry mass of the aerial part (DMAP) and height of crambe plants depending on the different concentrations of *Meloidogyne javanica*.

Treatment	Experiment 1			Treatment	Experiment 2		
	Height cm	FMAP g	DMAP		Height cm	FMAP g	DMAP
0	46.8	1.9	0.8	0	59.2	3.8	1.6
1300	46.7	1.9	0.6	1000	56.7	6.7	2.5
2600	42.5	1.2	0.5	2000	51.7	3.3	1.6
5200	44.3	1.5	0.6	4000	58.5	4.6	1.8
CV(%)	17.3	26.7	30.3	CV(%)	19.2	35.2	32.0
L.R.	ns	ns	ns	L.R.	ns	ns	ns
Q.R.	ns	ns	ns	Q.R.	ns	ns	ns

L.R. = Linear regression; Q.R.= quadratic regression; ns = not significant at 5% probability.

crambe, RF indexes are still lower than those of other cultures commonly used in crop succession, such as sunflower, RF = 29.15 (Rosa et al., 2013) and black oat, RF=6.85 (Asmus et al., 2005).

The crambe is a commercially new culture, and, thus, further studies are needed on this species. Nevertheless, some authors have investigated the susceptibility of other brassicas to phytonematodes, and their results corroborate our experiment showing the susceptibility of *Brassica napus* Linnaeus (rapeseed) and *Brassica campestris* L. (turnip rape) to *Meloidogyne chitwoodi* Golden, O'Bannon, Santo and Finley 1 and 2, and *M. hapla* Chitwood (Mojtahedi et al., 1991), canola to *M. hapla* and *M. incognita* (Bernard and Montgomery-Dee, 1993), and *Sinapis alba* L. (mustard) to *M. enterolobii* Yang and Eisenback (*M. mayaguensis* Rammah and Hirschmann) (Brito et al., 2007), *Raphanus sativus* L. var. *oleiferus* Metzg. (forage turnip) to *M. javanica* (Rosa et

al., 2013) and *Brassica oleracea* to *M. javanicae* and *M. incognita* (Dias-Arieira et al., 2012). In turn, studies conducted by Charchar et al. (2007) indicated that black mustard (*Brassica nigra* (L.) Koch.) was considered a bad host for a mixed population of *M. incognita* and *M. javanica*.

Analysis of vegetative parameters of the crambe (Table 1) showed that the presence of the nematodes did not affect plant growth. In comparison with data related to plant height found in the literature and cited by Oliveira et al. (2011), experiment 2 showed that, the development of the aerial part was nearly ideal for the culture, confirming that the period of the experiment was more favorable.

Despite the susceptibility of the crop, RF values close to 1 indicate that the crambe could be an option for use in crop succession systems, particularly because it ensures economic benefits to the producer. Also, since it is a species of Brassicaceae, release of glucosinolates may

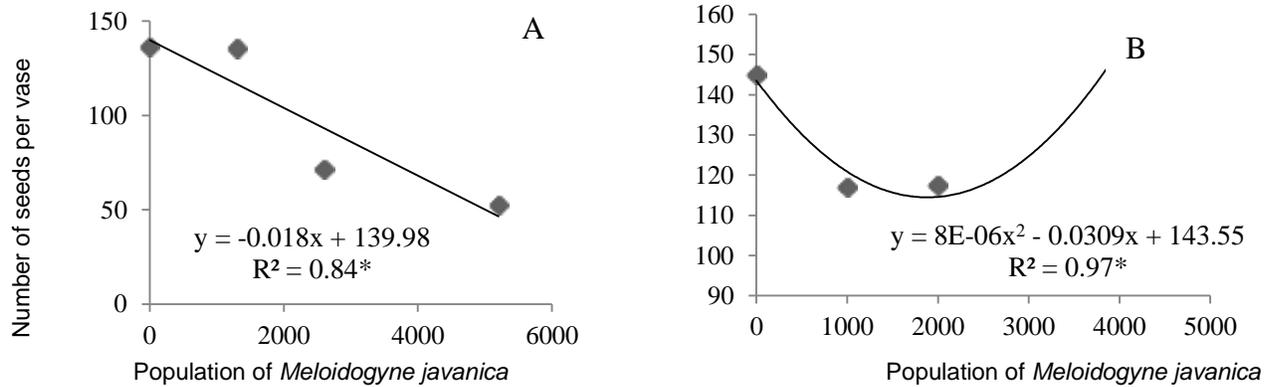


Figure 3. Regression to the number of crambe seeds per vase depending on the population of *Meloidogyne javanica*. A = Experiment 1 and B = Experiment 2. * = significant regression at 5% probability CV (%): A = 25.7 and B = 19.4.

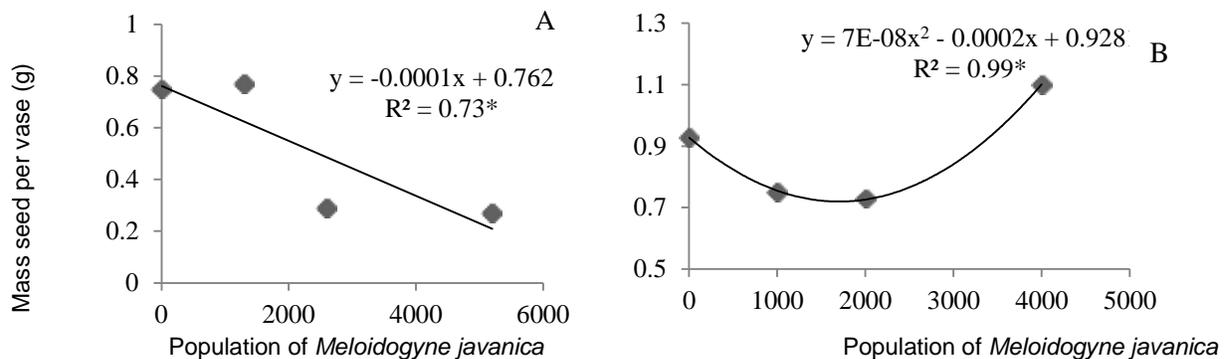


Figure 4. Regression of the mass of seeds (g) of crambe per vase, depending on the population of *Meloidogyne javanica*. A = Experiment 1 and B = Experiment 2 * = significant regression at 5% probability. CV(%): A = 29 and B = 22.1.

occur in the process of waste decomposition, as already observed for several species of the same family (Mojtahedi et al., 1991; Potter et al., 1999; Mazzola et al., 2001; McCully et al., 2008; Rizzarda et al., 2008; Reardon et al., 2013). However, Mojtahedi et al. (1991) observed that the suppressive effect of rapeseed on the nematodes was only possible when crop residues were incorporated to the soil.

In experiment 1, the increase in the population of *M. javanica* promoted the proportional decrease in seed production (Figure 3A). The same was observed in experiment 2 (Figure 3B). However, this decrease occurred in populations of up to 2000 eggs. The increase in population levels led to increase in the production of crambe seeds. Similar results were observed for the parameter mass of seeds per vases (Figure 4).

Although increase in inoculum levels of the nematode affected the number of seeds and mass of seeds, a difference was observed in the fitting of the regression equation in experiment 1, the reductions were linear and in Experiment 2, there was reduction up to the level of

2000 eggs per plant.

Some factors can explain these results, and the temperature during the experimental period is possibly the most important. According to Falasca et al. (2010), the ideal temperatures for the vegetative period of the crambe are between 15 and 25°C. But in experiment 1, the experiment was conducted from January to March, during which temperatures were above the ideal for the crop, ranging from 21.2 to 31.9°C. Thus, the decrease in seed production cannot be associated only with increase in nematode population, but also to adverse climate conditions, as observed in previous studies that reported that the crambe had lower growth rates under higher temperatures, resulting in lower grain production (Silva et al., 2013).

On the other hand, in experiment 2 carried out in the months of May and July, the climate conditions were more favorable to plant development, with temperatures between 12.9 and 21.7°C, which are indicated for crambe crop (Knights, 2002; Pitol et al., 2010; Silva et al., 2013). Therefore, the decrease in seed production, and,

Table 2. Means related to oil content in seeds depending on the population of *Meloidogyne javanica*.

Experiment 1		Experiment 2	
Treatment	Oil content (%)	Treatment	Oil content (%)
0	30.4	0	30.0
1300	30.2	1000	30.2
2600	30.6	2000	30.4
5200	30.7	4000	30.5
CV (%)	1.7	CV (%)	1.2
L.R.	ns	L.R.	ns
Q.R.	ns	Q.R.	Ns

L.R.= Linear regression; Q.R.= quadratic regression; ns = not significant.

consequently, in seed mass, can be directly associated with the increase in the population of *M. javanica* up to the level of 2.000 eggs per plant, in which the plant production recovered. The increase in seed production may have occurred because of the competition between nematodes for feeding sites, as already discussed.

Despite the decrease in seed production (Table 2), oil content was not significant, in the two experiments, showing that the population levels of *M. javanica* did not influence oil content in crambe seeds. The average oil production of the analyzed plants was 30.5 and 30.3% in experiments 1 and 2, respectively. According to Laghetti et al. (1995), the crambe has an oil content of approximately 38% in the mass, higher than the one found in the present study, which was about 30%.

Being a rustic species, the crambe did not show changes in oil content in the seeds, even under unfavorable climate and soil conditions, as those found in experiment 1. Silva et al. (2013) also observed the oil content in crambe seeds was not affected when the plants were grown in different climate conditions. Thus, it can be inferred that even in conditions of higher population levels of *M. javanica* in the soil, oil content in the seeds will not be affected.

The reproduction of *M. javanica* was lower at the season most favorable for crambe vegetative development, indicating that winter cultivation can be recommended, since the pathogen will have decreased reproductive activity. Also, the crop will not be affected, which demonstrates that crambe is a cost-effective crop. However, further research in this field is needed to confirm this hypothesis.

Conclusion

The crambe was susceptible to root-knot nematodes. However, parasitism did not affect the vegetative growth of the crop. Regarding the production of crambe seeds, it was affected by the nematode population, in association with climate factors, such as high temperatures. The population of nematodes in the plants did not affect the seed oil content.

Conflict of Interests

The authors have not declared any conflict of interests.

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