

Quantification of damage and yield losses caused by Root-knot nematode in lettuce in Brazil

Cuantificación de daños y pérdidas de rendimiento causadas por el nematodo agallador en un cultivo de lechuga en Brasil

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ABSTRACT

The objective of this work was to quantify damage and yield losses in lettuce parasitized by *Meloidogyne javanica*, as well as the growth characteristics of naturally cultivated field plants infested by this pathogen. The experimental area consisted of four seedbeds of 10.0x1.60 m cultivated with 160 plants in each, totaling 640 plants. The damages and yield loss were quantified in four evaluations (Ev1, Ev2, Ev3, and Ev4), with each evaluation corresponding to a cultivation cycle. The four plantings were made in the consecutive form. The fresh mass weight and dry mass weight (FMW and DMW, respectively), gall number (GN), and final population of the nematodes (FP) were evaluated. The population increase of *M. javanica* caused a reduction in the FMW and DMW in all evaluations. In Ev1 and Ev2, 100% of the plants were commercialized. In Ev3 and Ev4, 7% and 65% of the plants, respectively, could not be commercialized. The Ev4 was completely unviable since the produce had losses of US\$ 7.42; US\$ 23.19 and US\$ 1,484.10 for the cultivation of 640; 2,000 and 128,000 plants, respectively. The economic threshold in Ev4 was 10,151 nematodes/plant or 109 galls/plant.

Keywords: *Lactuca sativa*, *Meloidogyne javanica*, field.

RESUMEN

El objetivo de este trabajo fue cuantificar el daño agronómico en un cultivo de lechugas parasitadas por *Meloidogyne javanica* y caracterizar el crecimiento de plantas cultivadas en un campo infestado por este patógeno. El estudio se hizo en cuatro camas de 10.0 x 1.60 m cultivadas con 160 plantas en cada una, totalizando 640 plantas por cada ciclo de cultivo. Se hicieron cuatro tratamientos (Ev1, Ev2, Ev3 y Ev4), correspondientes a un ciclo de cultivo. Las cuatro plantaciones se realizaron de forma consecutiva y se seleccionaron 100 plantas al azar para cuantificar el efecto del nematodo en las lechugas. Los parámetros evaluados fueron peso fresco (PMF), peso seco (PMS), número de agallas (NG) y la población final de nematodos (PF). El aumento de la población de *M. javanica* provocó una reducción de PMF y PMS en todas las evaluaciones. En Ev1 y Ev2 se vendió el 100% de las plantas. En Ev3 y Ev4 hubo un 7% y un 65% de las plantas, respectivamente, que no pudieron venderse. El cuarto ciclo de cultivo (Ev4) resultó completamente inviable, ya que las pérdidas fueron de U \$ 7,42; U \$ 23,19 y U \$ 1.484,10 para el cultivo de 640, 2.000 y 128.000 plantas, respectivamente. El umbral económico en Ev4 fue de 10.151 nematodos / planta o 109 agallas / planta

Palabras clave: *Lactuca sativa*, *Meloidogyne javanica*, campo.

Introduction

Lettuce (*Lactuca sativa* L.) is among the ten most valued leafy vegetables for natural consumption in Brazil, cultivated in all regions of this country

(Ceasa-ES, 2018). Together with the tomato, it is the most used vegetable in the Brazilian kitchen.

Lettuce is a plant that is susceptible to root-knot nematodes, *Meloidogyne* spp. Goeldi 1887 (Rodrigues *et al.*, 2016), and depending on the cultivar, population

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density, virulence of the nematode, environmental conditions, the interaction between nematode and other pathogens, plant physiological conditions, and the soil organic material content, the plants may suffer damage quantitatively and qualitatively and yield losses caused by these pathogens in lettuce are estimated between 10 and 100% (Jesus Junior *et al.*, 2014; Rodrigues *et al.*, 2016).

According to Abd-Elgawad and Askary (2015), to avoid damage, the ideal is to maintain the nematode population below the economic threshold (ET), defined as the intensity of the disease at which the benefit of control is equal to its cost. However, for this definition to be operational and for integrated management to be viable, it is necessary to quantify the damage and yield losses caused by phytonematodes.

In Brazil, research on the effects of nematodes on lettuce cultivated in the field is rare. Some research has been done on lesion nematodes, *Pratylenchus* spp., Filipjev 1936 (Potter & Olthof, 1974); however, the majority has been done in other countries with the root-knot nematodes *Meloidogyne* spp., (Viaene & Abawi, 1998). This information emphasizes the importance of quantifying the damage and yield losses in commercially exploited crops parasitized by *Meloidogyne* spp., to guide the producers decision-making in managing these pathogens.

The objective of this work was to quantify the damage and yield losses in lettuce attacked by *M. javanica* (Treub 1885) Chitwood 1949, in field conditions.

Materials and Methods

The experiment was carried out in a field cultivated with lettuce cv. Vitória de Santo Antão naturally infested with *M. javanica* in Iúna Municipality, Espírito Santo State, Brazil. The area is at 649 meters altitude, 20° 23 '41' 'south latitude and 41° 32' 31 ' ' west longitude.

The region's climate is classified as Cwa-humid subtropical (Köppen climate classification), with average annual temperatures varying from 8 to 27° C.

The maximum and minimum air temperature and mean precipitation were monitored in the experiment region through automatic surface stations provided by the Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural of Espírito Santo - Incaper.

Table 1 shows the experimental periods, the air temperatures and the mean precipitation during the crop cycles.

The soil where the experiments were carried out was classified as medium texture, presenting 635.60 g/kg of sand; 53.62 g/kg silt; 310.19 g/kg clay and pH = 5.82.

The experimental area consisted of four 10.0m x 1.60m seedbeds, cultivated with 160 lettuce plants each (10 plants/m²), totaling 640 plants in each crop cycle.

The lettuce seedlings for conducting the experiments were produced *in situ* by the farmer, owner of the property, and taken to the field 20 days after sowing.

Fertilization was done in each cultivation cycle, according to recommendations for cultivation. Other cultural treatments, such as irrigation, were done according to the recommendations for the crop.

Identification of root-knot-nematode species

The identification of the species of *Meloidogyne* present in the naturally infested area was made by using the electrophoresis technique of esterase isoenzyme (Carneiro and Almeida, 2001). For that, before the experiment 30 plants with root galls were collected randomly and 10 females were taken from each plant for identification, resulting in a total of 300 analyzed females.

Table 1. Duration of four experiments with lettuce crop cv. Vitória Santo Antão in Iúna, ES, maximum and minimum temperatures of the air, and mean precipitation during the respective experimental periods.

Crop cycles	Experimental period		Duration of the experimental period (days)	Maximum and minimum T of the air (°C)	Average precipitation (mm)
	Beginning	End			
1 st	02/07/2009	17/08/2009	46	13 to 22	14
2 nd	04/09/2009	18/10/2009	44	15 to 24	53
3 rd	01/11/2009	18/12/2009	48	18 to 24	214
4 th	06/01/2010	20/02/2010	45	23 to 30	255

Determination of the initial population of *M. javanica* (Pi) in the experimental area

The Pi, expressed in number of second stage juveniles (J2) in 100 mL of soil, was determined by the Jenkins technique (1964). Therefore, 30 soil samples were randomly collected at a depth of 0-20 cm in each seedbed. These samples were mixed and homogenized, forming 4 composite samples.

The samples were packed in polyethylene bags, labeled, and immediately brought to the nematology laboratory for analysis.

Quantification of the damage

Four evaluations were done (Ev1, Ev2, Ev3 and Ev4). Each evaluation corresponded to a complete cycle of the lettuce crop. The four plantings were done consecutively. The first planting was only done after the land had been fallow for four months.

In each evaluation, a sample of 100 randomly selected plants from a total of 640 plants with different levels of nematode infestations (0 at the maximum level) was taken.

Immediately after collection, the weight of the fresh material of the leaves (FMW), the number of leaves (NL) and the weight of the stalk (WS) were determined. Leaves were dried in an incubator at 70 °C for 72 hours, to measure the weight of the dry material (DMW). The gall number (GN) and the final population of the nematodes (FP) in the infected plants were evaluated according to the method proposed by Bonetti & Ferraz (1981).

Reductions in FMW and DMW were compared to plants free of nematodes (level zero of the pathogen).

Data was statistically analyzed through two distinct procedures. When we considered each evaluation individually, the data were analyzed by linear regression, with the model chosen by the coefficient of determination (R^2) and significance of the regression coefficient ($B1$). The Kruskal-Wallis test was used for analyses involving all the evaluations. These analyses were done using the BioEstat 5.0 software at the 5% probability level.

Quantification of yield losses

The farmer, owner of the experimental area used in this study, sells 2,000 heads of lettuce each week. Thus, this amount was calculated as the potential weekly loss of the farmer, in addition

to the loss estimates of 640 heads of lettuce (total plants cultivated in the experimental area) and 128,000 heads (total plants cultivated in 1 ha). The cost of producing 2,000 heads of lettuce was provided by the farmer (Table 2), with the commercial value of these plants determined at a local market. The production values of 640 to 128,000 plants were estimated as a function of this information. Moreover, the calculation to estimate yield losses was based on commercialization data provided by the Central Market (Central de Abastecimento) of Espírito Santo State, Brazil (Ceasa-ES). Following the criteria of Ceasa-ES, lettuce plants weighing less than 300g are not well-accepted, but are sold when there is little supply of this vegetable in the market. Plants with a weight less than 200 g are not sold at all. In the present work only those plants weighing more than 200 g were considered to have commercial potential. The plants were divided into four categories based on the weight of fresh material (g): plants less than 100 g (category 1), plants between 100.01 and 200 g (category 2), 200.01 to 300 g (category 3) and plants weighing more than 300 g (category 4).

We estimated the farmer's yield loss as a function of the parasitism of the nematodes on the plants. The economic threshold (ET) was estimated based on the equation generated by regression involving the FMW and/or GN and FP. The ET was calculated only for Ev4, since there was insufficient damage to cause yield losses in Ev1, Ev2 and Ev3.

To determine yield losses (Table 6), we calculated, for each evaluation, the income obtained from the commercially viable plants of categories 3 and 4 (US\$ 0.17/plant) and subtracted the cost of producing the entire crop.

Table 2. Cost estimates of producing lettuce cv. Vitória de Santo Antão, calculated for a) 2,000 heads of lettuce (total number of plants commercialized each week on the property), b) 640 heads of lettuce (total number of plants in the experiment), c) 128,000 plants (total number of plants cultivated in 1 ha).

Description	Value (US)
Total for production of 2,000 plants	US\$ 149.17
Total for production of 640 plants	US\$ 47.73
Total for production of 128,000 plants	US\$ 9.547,01

Results and discussion

Number of Leaves per Plant (NL)

The increase in the *M. javanica* population reduced NL in all evaluations. In Ev1, the extreme points are represented by plants with 55 leaves and pathogen level zero, in contrast to plants with only 31 leaves, in which 9,000 individuals were discovered in the FP of *M. javanica* and 17 galls. In Ev2, NL varied between 31 and 55, whereas PF increased, varying from 0 to 12,000 nematodes (Figure 1) with NG varying from 0 to 15. In Ev3, NL ranged from 21 to 47, with PF presenting a greater increase, varying from 0 to 25,000 nematodes and 0 to 38 galls. In Ev4, PF varied from 0 to 48,000, NG from 0 to 812 and NL from 18 to 28 (Figure 1). NL was reduced by 35%.

Stalk Weight (SW)

The increase of FP reduced SW in all evaluations. SW, in Ev1, was 102.34 g for plants free of nematodes (level zero of the pathogen), since those with PF 9,000 weighed only 41.24 g, as NG varied from 0 to 18. In Ev2 SW, varied from 41.54 to 104.56 g and the nematode level from 0 to 12,000. Plants presented an increase from 0 to 15 galls per plant. In Ev3 and Ev4, the minimum and maximum SW varied from 10.23 to 30.5 g and 3.67 to 29.32 g, while FP varied from 0 to 25,000 and 0 to 48,000, respectively, while GN increased from 38 in Ev3 to 812 in Ev4 (Figure 2). SW was reduced by 87%.

Plant fresh material weight (FMW)

The increase of the *M. javanica* population caused a reduction in FMW in all evaluations. In Ev1, the FMW varied from 728.49 g in plants with level zero of nematodes and 299.49 g in plants with a 9,000 PF. GN varied from 0 to 17. The FMW variation in Ev2 was 720.34 g for plants free of *M. javanica* and 310.34 g for plants with 12,000 individuals, with the GN varying from 0 to 15. The FMW of Ev3 ranged from 351.54 g to 175.21 for the populations with 0 and 25,000 nematodes, respectively, with an NG from 0 to 38. In Ev4, PF ranged from 0 to 48,000 for plants with FMW of 440.89 and 22.07 g, respectively, showing a 95% reduction of FMW. NG varied from 0 to 812 (Figure 3).

In Ev1, Ev2, Ev3 and Ev4 the maximum FP were 9,000, 12,000, 25,000 and 48,000, respectively,

and the maximum GN were 17, 15, 38 and 812, respectively (Figure 1). In these four evaluations reductions, were observed for all evaluated characteristics (Table 4).

Estimate of yield losses

In Table 5, note that in Ev1 and Ev2, 100% and 99% of the plants were commercialized. In Ev3 and Ev4, 7% and 65% of the plants were not commercialized.

We emphasize that throughout the entire experimental period, there was no incidence of other diseases or pests in the soil and shoots. The quantified damage was the result only of nematode parasitism on the plants.

The low population of J2/100 mL of soil (16) found before the installation of the experiment was explained by the fact that the soil had been left to fallow for four months (Table 3). Rodrigues *et al.* (2016) also conducted an experiment in a cultivated area with lettuce in Iúna, Brazil, which remained fallow for six months, and found on average 6.3 J2 of *M. javanica*/100 mL of soil at the beginning of the experiment. Finding these pathogens in low populations after fallow periods is common, as they do not have the preferred host to feed on. Despite this, nematodes can survive in low populations due to dormancy and their ability to feed on other plants since they are polyphagous (Hand *et al.*, 2016).

It was observed that the population increase of *M. javanica* during the lettuce cultivation cycle caused all the growth characteristics analyzed to decrease. Taylor & Sasser (1978) affirmed that an increase in the phytonematode population is expected since nematodes multiply on a logarithmic scale, with females producing around 500 eggs with only about 5% surviving to complete the cycle. Over four generations, a single female can produce 25, 625, 15,625, and 390,625 adults. It is expected that with consecutive plantings of lettuce, the level of initial inoculation (Pi) in the soil is greater in the initial stage of each planting.

The mechanical damage caused by the nematodes penetrating and moving about in plant tissues leads to vascular tissue blockage at the feeding sites, limiting the translocation of water and nutrients, repressing plant growth and therefore the income from plant crops (Lu *et al.*, 2014). These types of physiological and mechanical damage lead to a reduction in plant growth (Rodrigues *et al.*,

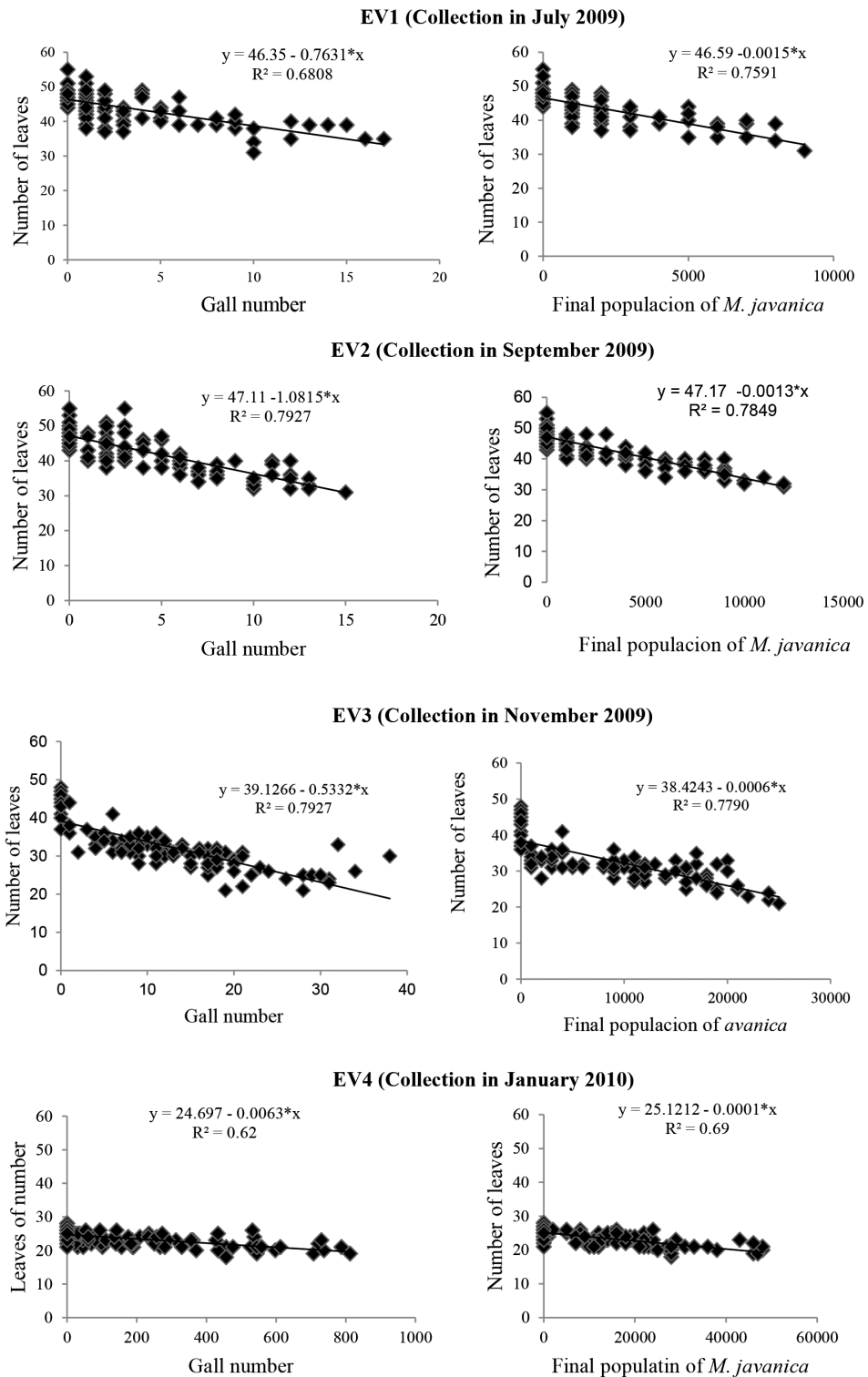


Figure 1. Number of leaves (NL) of lettuce cv. Vitória de Santo Antão as a function of the final population of *M. javanica* in the roots and number of galls in an area naturally infested by this pathogen in Iúna Municipality, Espírito Santo State, Brazil, in four evaluations (Ev1, Ev2, Ev3 and Ev4) done in the months of July, Sep and Nov 2009 and Jan 2010.

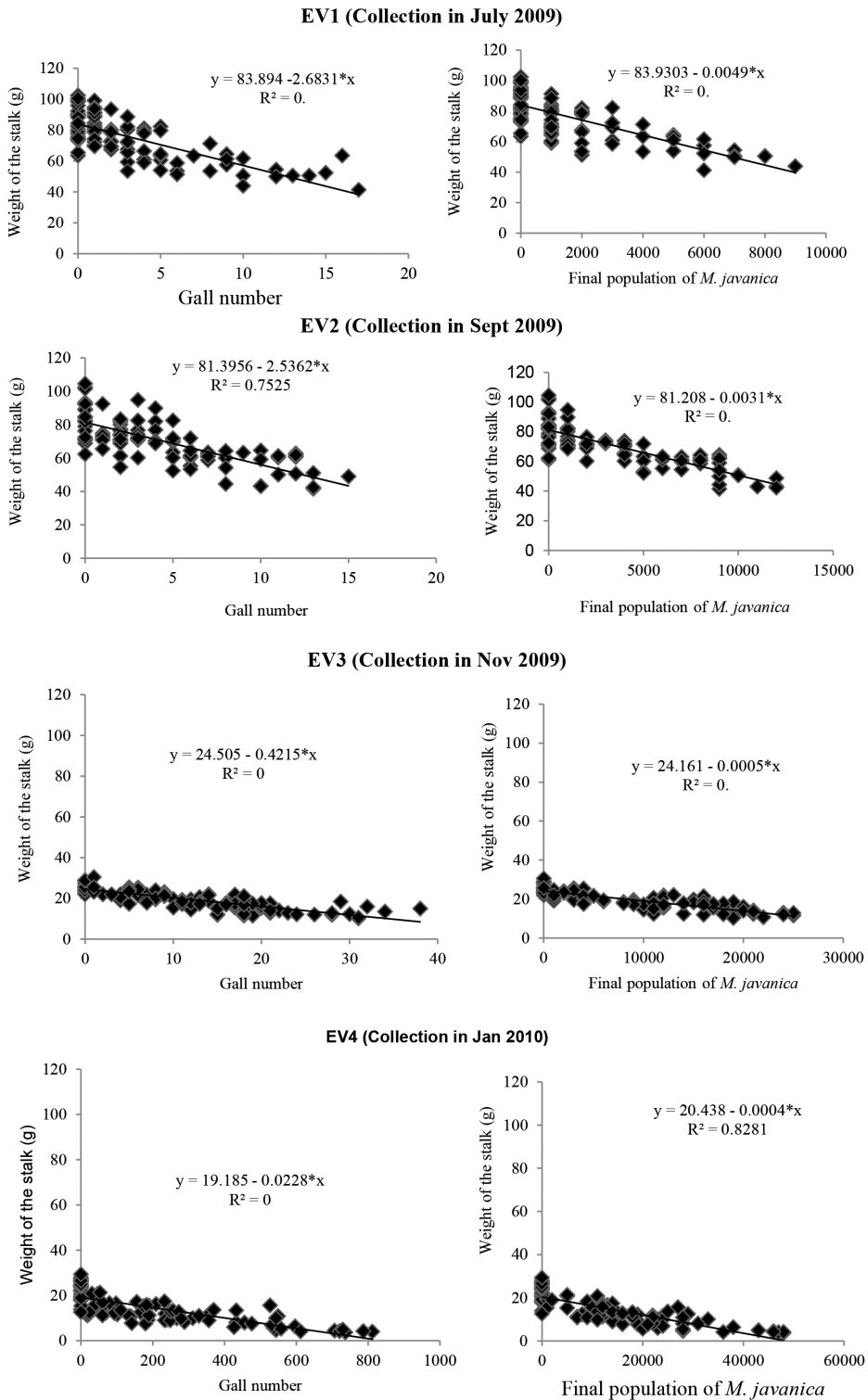


Figure 2. Stalk weight (SW) of lettuce cv. Vitória de Santo Antão as a function of the final population of *M. javanica* in the roots and number of galls, in an area naturally infested by this pathogen, in Iúna Municipality, Espírito Santo State, Brazil, in four evaluations (Ev1, Ev2, Ev3 and Ev4) done in the months of July, Sep and Nov 2009 and Jan 2010.

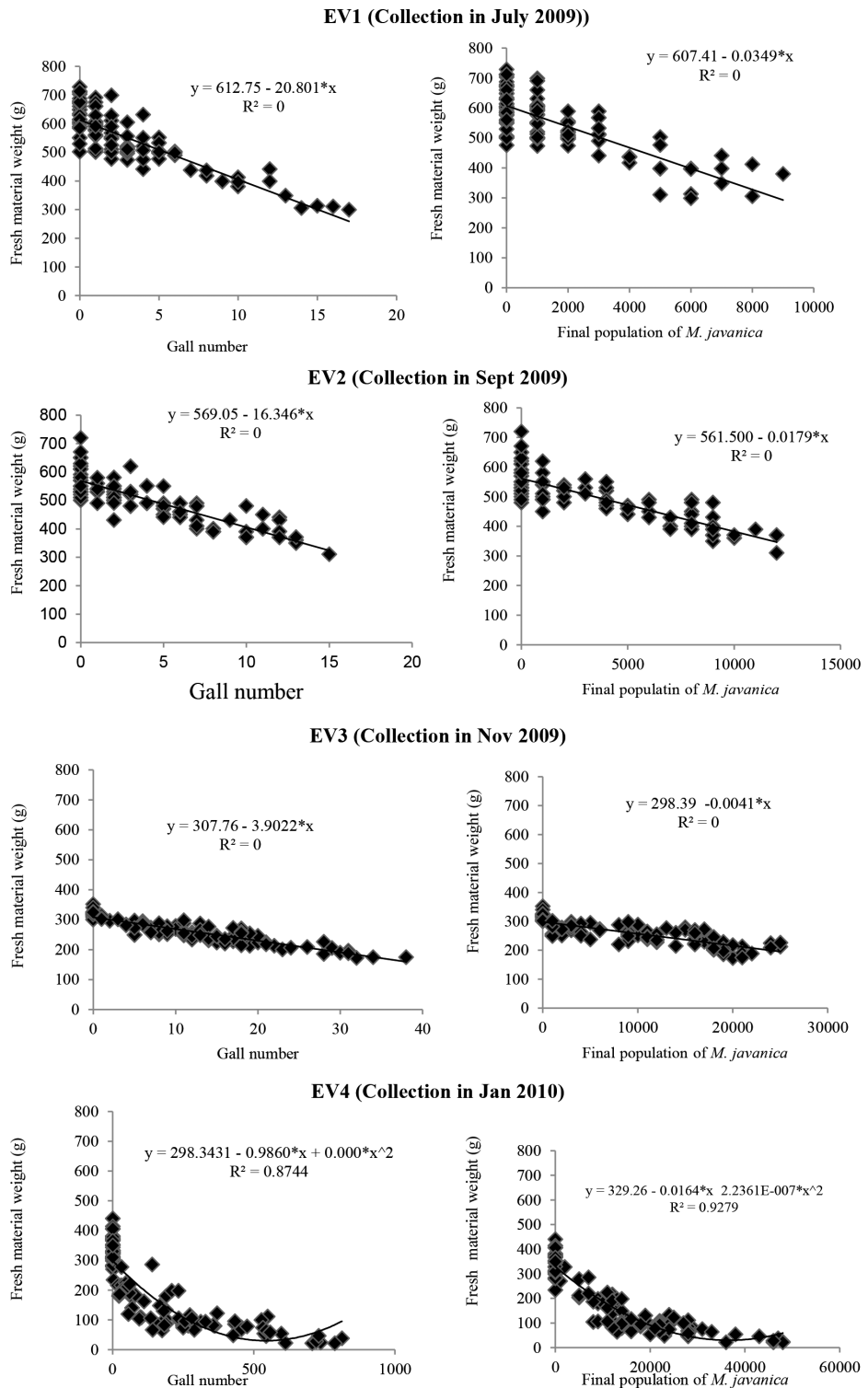


Figure 3. Fresh material weight (FMW) of lettuce cv. Vitória de Santo Antão as a function of the final population of *M. javanica* in the roots and number of galls, in an area naturally infested by this pathogen, in Iúna Municipality, Espírito Santo State, Brazil, in four evaluations (Ev1, Ev2, Ev3 and Ev4) done in the months of July, Sep and Nov 2009 and Jan 2010.

Table 3. Initial population of *Meloidogyne javanica* (Pi) expressed in number of second stage juveniles (J2) in 100 mL of soil in the experimental area before beginning the experiment.

	Seedbed 1*	Seedbed 2	Seedbed 3	Seedbed 4	Average
	20	28	28	28	26
	21	25	27	29	26
	16	32	28	32	27
	24	27	20	24	24
	28	19	19	25	23
	16	24	32	31	26
Average	21	26	26	28	25

* Thirty soil samples were collected at a depth 0-20 cm randomly in each of four seedbed, mixed and homogenized, forming 4 composite samples from the 30 single samples per seedbed.

Table 4. Reductions in fresh material weight (FMW) and dry (DMW) in lettuce cv. Vitória de Santo Antão parasitized by *M. javanica* in four evaluations (Ev1, Ev2, Ev3 and Ev4) done in the months of July, Sep and Nov of 2009 and Jan 2010 compared to plants free of nematodes (level zero of the pathogen).

Characteristics evaluated	Reductions observed (%)			
	EV1	EV2	EV3	EV4
FMW	59	57	50	95
DMW	55	51	57	83

2016), agreeing with the results in this study, in which in the fourth consecutive planting (Ev4), only 35% of the cultivated lettuce plants were above the acceptable weight for market consumption. According to Abd-Elgawad & Askary (2015), the root-knot nematodes may reduce production by up to 86%, depending on the intensity of the infestation and the cultivated plants.

The succession of lettuce plantings contributed to the increase in the number of plants with levels of damage that resulted in plants below the commercially accepted weight. With only two consecutive plantings, significant damage was not observed; in other words, the pathogen did not reach populations capable of causing significant damage.

In three sequential plantings (Ev1, Ev2, and Ev3), the crops presented perceptible damages, albeit at a low level (7% of plants below the commercially acceptable weight in Ev3), which do not prevent cultivation nor significantly reduce income, meaning the nematode has not yet been characterized as a phytopathological hindrance to the commercial cultivation of the lettuce.

Table 5. Percentage of lettuce plants cv. Vitória de Santo Antão capable of being commercialized when cultivated in an area naturally infested by *M. javanica* in the Lúna Municipality in Espírito Santo State, Brazil in four evaluations (Ev1, Ev2, Ev3 and Ev4) done in the months of July, Sep and Nov 2009 and Jan 2010. Plants with a fresh material weight (FMW) higher than 200 g were considered able to be commercialized.

Evaluation	FMW (%)			
	≥ 300	200.01 a 300	100.01 a 200	≤100
Ev1	100	0	0	0
Ev2	99	1	0	0
Ev3	18	75	7	0
Ev4	24	11	30	35

The ET concept is specific for each crop and is based on the disease intensity at which the benefits of control are equal to the cost. Costs above this level are characterized as yield losses. In the present study, the ET in Ev4 was 10,151 nematodes/plant or 109 galls/plant. In the study by Potter & Olthof (1974), the ETs for beet, spinach, and lettuce crops were 17%, 13%, and 10%, respectively. In the present study, the reduction of lettuce FMW reached 93% in plants parasitized by 48,000 individuals of *M. javanica*, in agreement with the results of Potter & Olthof (1974), who observed a reduction of 81% in plants parasitized by *M. hapla* Chitwood, 1949.

In the study by Chen *et al.* (1999), *M. hapla* caused a 178 to 103 g/plant reduction in lettuce, corresponding to 21 and 17% in 1995 and 1996, respectively. In Ev4 in the present study, DMW oscillated between 18.97 g and 3.21 g for an FP that

Table 6. Estimate of yield losses (US\$) in area of commercial planting of lettuce cv. Vitória de Santo Antão in Iúna Municipality, Espírito Santo State, Brazil, cultivated in the presence of *M. javanica* in four evaluations (Ev1, Ev2, Ev3 and Ev4) done in the months of July, Sep and Nov 2009 and Jan 2010. Only plants weighing more than 200 g were considered suitable for commercialization.

Number of plants	Ev1 (100% of plants commercializable)			Ev2 (99% of plants commercializable)		
	Sales/production (US\$)	Costs/production (US\$)	Profit/producer (US\$)	Sales/production (US\$)	Costs/production (US\$)	Profit/producer (US\$)
640*	115.17	47.73	67.44	114.99	47.73	67.26
2,000**	359.92	149.17	210.75	359.91	149.17	210.57
128,000***	23,035.39	9,054.00	13,488.90	23,035.37	9,546.49	13,488.72
	Ev3 (93% of plants commercialized)			Ev4 (35% of plants commercialized)		
	Sales/production (US\$)(US\$)	Costs/production (US\$)	Profit/producer (US\$)	Sales/production (US\$)	Costs/production (US\$)	Profit/producer (US\$)
640	107.11	47.73	59.38	40.31	47.73	-7.42
2,000	334.73	149.17	185.76	125.97	149.17	-23.19
128,000	21,422.92	9,546.49	11,876.42	8,062.38	9,546.49	-1,484.10

*Total number of plants in the experimental area.

** Total number of plants commercialized per week by the farmer.

*** Total number of plants cultivated in 1 ha.

varied from 0 to 48,000; in other words, DMW and FMW were reduced by 83.07 and 95%, respectively.

In the fourth planting (Ev4), the FP was already high in the area, to the point of causing considerable damage to the lettuce crop, rendering 65% of the plants unviable for sale. This demonstrates the risk of carrying out four sequential lettuce plantings in the same area since, in the last planting, the cost of production will exceed the commercial value of the plants. Thus, it is clear that in the most studied area, lettuce cv. Vitória de Santo Antão can be grown for three consecutive cycles, after that different management strategies should be considered, such as leaving land fallow, rotating the crop, implanting a crop that is not susceptible to *M. javanica*, or even cultivating lettuce resistant to the pathogen. Production of lettuce cv. Vitória de Santo Antão may be resumed when the initial nematode soil population is reduced.

It is important to emphasize that plants weighing less than 200 g are not commercially valuable, and the estimated values of loss are valid for the period in which the experiment was developed.

The first step was to calculate the costs of producing lettuce plants to make this estimate. To do this, we considered all of the raw materials, labor, work, and equipment costs in preparing seedlings, the plantation, irrigation, fertilization,

and commercialization, as well as the depreciation of the machinery.

Until the third planting, the farmer will profit from commercially exploiting crops of lettuce cv. Vitória de Santo Antão on his property in Iúna, because in the first two plantings (Ev1 and Ev2), there were no yield losses due to the occurrence of the nematodes, and in the third crop (Ev3), 93% of the plants were sold, justifying the cultivation of the lettuce.

In Ev3, the net gain was reduced compared to Ev1 and Ev2 (only US\$ 1.612,47). Nevertheless, it was worth cultivating the plants because the producer made profits of US\$ 59.38, US\$ 185.56 and US\$ 11,876.42, considering the crop sizes of 640, 2,000, and 128,000 plants, respectively.

The lettuce's fourth cycle (Ev4) was shown to be completely unviable since the produce had losses of US\$ 7.42; US\$ 23.19, and US\$ 1,484.10 for cultivating 640, 2,000, and 128,000 plants, respectively.

Higher air temperatures and precipitation were observed in the last two cycles of lettuce (EV3 and EV4), which contributed to the significant population increase of *M. javanica* compared to EV1 and EV2, when air temperatures and precipitation did not exceed 24° C and 53 mm, respectively. Thus in EV1 and EV2, the cycle of *M. javanica* was longer since, at lower temperatures, the cycle of

the nematode can reach 60-70 days (Bird, 1974). According to the author, this variation is due to the reduction of the speed of the enzymatic reactions in the various events of the development of the nematode. Furthermore, under optimum temperature conditions, *Meloidogyne* spp. complete their cycle in 28 days (Bird, 1959).

It is important to emphasize that at higher temperatures (EV3 and EV4), there is a greater penetration of *M. javanica* juveniles into lettuce roots and significant J2 outbreaks (Khan *et al.*, 2014). Morris *et al.* (2011) observed that the percentage of J2 of *Meloidogyne* spp. was significantly lower at 15 °C than at 20 and 25 °C, with the fastest hatching occurring at 23 °C. The mean temperatures recorded in our study in EV3 and EV4 were 21 and 26.5 °C, respectively.

Nematodes require minimal oxygen content in the soil so they can develop their cycle normally. Therefore, prolonged saturation conditions reduce of some nematode species by excess water, limiting movement and hatching, and favoring the production of toxins by anaerobic organisms (Kitazume *et al.*, 2018). Although pluviometric indexes were higher in EV3 and EV4 (214 and 255 mm, respectively), there was no prolonged period of precipitation, and the higher soil moisture in these last two lettuce cycles favored the population increase of *M. javanica*.

Many studies have been done to quantify the damage to lettuce parasitized by *Meloidogyne* spp. (Viaene & Abawi, 1998; Chen *et al.*, 1999). Many of these works mention that these pathogens cause yield losses; however, they do not systematically quantify the yield losses, as carried out in the present work.

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