

QUANTIFICATION OF DAMAGE AND YIELD LOSSES AND MANAGEMENT OF ROOT-KNOT NEMATODES IN CONILON COFFEE¹

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ABSTRACT - Commercial exploitation of the coffee crop is one of the most important activities in the world's agricultural sector. One of the main phytosanitary problems affecting the crop is the presence of *Meloidogyne incognita*. Several measures have been tested for the management of this pathogen, albeit with low efficiency. The objective of this work was to quantify the damage and losses and to manage *M. incognita* race 1 with *Pochonia chlamydosporia* and *Trichoderma harzianum* biological nematicides, comparing them to a chemical nematicide. The experiment was carried out in a commercial area naturally infested by the nematode and cultivated with the conilon coffee variety 'Vitória INCAPER 8142', clone V02. The treatments were tested with Carbofuran nematicide and with biological nematicides composed of *P. chlamydosporia* Pc-10 and *T. harzianum* ESALQ 1306. The biological products were applied alone or in combination. The lowest NPF (final nematode population) occurred in plants treated with *P. chlamydosporia* and Carbofuran. *P. chlamydosporia* was the most effective biological agent in the management of *M. incognita*. There was a reduction in production with an increase in the nematode population. The highest application costs of management methods for *M. incognita* race 1 per hectare were for three and two applications of Carbofuran and three applications of *P. chlamydosporia* + *T. harzianum*. The treatments with lowest application costs were one application of *T. harzianum* and one application of *P. chlamydosporia*. It was concluded that all treatments were efficient for the management of *M. incognita* race 1, causing a decrease in the roots and soil population.

Keywords: Conilon coffee. Biological control. Damage. Loss.

QUANTIFICAÇÃO DE DANOS E PERDAS E MANEJO DOS NEMATOIDES DAS GALHAS EM CAFEIRO CONILON

RESUMO - A exploração comercial da cultura do café é uma das atividades mais importantes do setor agropecuário mundial. Um dos principais problemas fitossanitários da cultura é a presença de *Meloidogyne incognita*. Várias medidas têm sido testadas para o manejo desse patógeno, porém, com baixa eficiência. O objetivo deste trabalho foi quantificar os danos e as perdas e manejar *M. incognita* raça 1 com nematicidas biológicos à base de *Pochonia chlamydosporia* e *Trichoderma harzianum*. O experimento foi conduzido em uma área comercial naturalmente infestada pelo nematoide e cultivada com a variedade de café conilon 'Vitória INCAPER 81421', clone V02. Foram testados os tratamentos: nematicida Carbofurano; nematicidas biológicos à base de *P. chlamydosporia* Pc-10 e *T. harzianum* ESALQ 1306. Os produtos biológicos foram aplicados isoladamente ou associados. As menores populações finais de nematoides ocorreram em plantas tratadas com *P. chlamydosporia* e Carbofurano. *P. chlamydosporia* foi o agente biológico mais eficaz no manejo de *M. incognita*. Houve redução de produção com o aumento da população do nematoide. Os maiores custos de aplicação dos métodos de manejo para *M. incognita* raça 1 por hectare foram para três e duas aplicações de Carbofurano e três aplicações de *P. chlamydosporia* + *T. harzianum*. Os tratamentos com menores custos de aplicação foram uma aplicação de *T. harzianum* e uma aplicação de *P. chlamydosporia*. Conclui-se que todos os tratamentos foram eficientes para o manejo de *M. incognita* raça 1, acarretando diminuição da população do nematoide nas raízes e no solo.

Palavras-chave: Cafeeiro conilon. Controle biológico. Danos. Perdas.

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INTRODUCTION

Espírito Santo is the second most important Brazilian state in coffee production, and the first in the production of *Coffea canephora* Pierre ex A. Froehner (conilon coffee), in which it stands out as the most economically and socially important agricultural activity for the development of the state (CONAB, 2017).

Phytonematoids are among the main phytosanitary pests causing damage to coffee (CONTARATO et al., 2014). Worldwide, about fifteen species of *Meloidogyne* spp. have been described as parasites of this crop, with *M. exigua*, *M. paranaensis* and *M. incognita* the most harmful ones (OLIVEIRA et al., 2011).

To manage *Meloidogyne* spp. in the field, synthetic nematicides are generally used; however, due to their high toxicity, risk of environmental contamination and inconsistent field-level results (NASR, 2015), alternative management practices have been researched, such as the use of antagonist fungi (RODRIGUES et al., 2016). Among the fungi with potential use in agriculture for nematode management are *Pochonia chlamydosporia* Zare & Gams (syn. *Verticillium chlamydosporium* Goddard) (VIGGIANO; FREITAS; LOPES, 2014), and *Trichoderma harzianum* Rifai (MENDOZA et al., 2015).

In the field, to prevent damage to plants caused by *Meloidogyne* spp., the population of these pathogens should be kept below the economic threshold (ET), defined as the intensity of the disease at which the benefit of control equals its cost. For this, it is critical that damage and yield losses be quantified for this definition to be operational and for integrated management to be viable (ABDELGAWAD; ASKARY, 2015).

Due to the serious problem that *M. incognita* race 1 represents for conilon coffee growing worldwide, this research aimed to quantify the damage and yield losses caused by *M. incognita* race 1 in conilon coffee and to test different strategies of conilon coffee management involving different numbers of applications of biological nematicides based on *P. chlamydosporia* and *T. harzianum*, applied alone and in combination, and the use of a chemical nematicide in plants grown in a commercial area.

MATERIAL AND METHODS

Characterization of the experimental area

The experiment was carried out in a commercial field during the months of September

2013 and March 2014, in the municipality of São Roque do Canaã, in the central highlands of Espírito Santo State, with longitude 40°39'17" W and latitude 19°45'17" S; average altitude of 225 m and rugged topography. The soil was characterized as eutrophic Red Yellow Latosol with good drainage.

According to the Köppen international classification, the climate of the region is of the "Cwa" type, i.e. humid warm tropical with cold and dry winter, with annual average temperature of 24.5 °C and annual average rainfall of 1,341 mm.

The harvest consisted of a 24-month Conilon coffee bush variety, 'Vitória INCAPER 8142' clone V02 (at the installation of the experiment), grown in rows, with spacing of 2.80 m x 1.20 m between rows and plants, respectively. We used five planting lines, chosen because it is a clone widely cultivated in Espírito Santo and very susceptible to *M. incognita*. Confirmation of the species used the methodology of Carneiro and Almeida, (2001). The characterization of race 1 was carried out using the method of Hartman and Sasser (1985).

Experimental design and conduct of the experiment

The experiment was arranged in the field in randomized blocks in a split plot scheme. The area was divided into four blocks, in which each one consisted of five management methods (four nematicides and control) and subplots, with different times of application, drawn within the plot.

The experimental unit consisted of five plants, but three plants were considered useful for analysis. The total number of plants in the experiment was 300 (5 management methods x 3 application numbers x 4 blocks x 5 plants).

The experimental plot consisted of 15 plants divided into 3 subplots, each with 5 plants.

The treatments, doses and times of application are listed in Table 1.

For treatments that received two applications, the second was performed 60 days after the first, and for three applications the third took place 60 days after the second.

The first application took place on September 15, 2013, the second on November 15, 2013 and the third on January 15, 2014.

The Carbofuran nematicide: 50 g/kg (5% w/w) was manually applied in holes opened in the surface of soil at a depth of 5.0 cm, in the projection of the coffee canopy, and then the biological nematicides were diluted in water and applied manually via backpack.

The management of fertilization, irrigation and other cultural or phytosanitary treatments was performed according to the needs of the crop.

Table 1. Dosages and methods of application of biological and chemical nematicides for the management of *M. incognita* race 1, in the conilon coffee variety 'Vitória INCAPER 8142' clone V02 in a commercial area naturally infested by the pathogen.

	Recommended dose (kg or L/ha)	Dose used per plant	Application means
Control	-----	-----	-----
Carbofurano: 50 g/kg 1*	89.34 kg/ha ¹	30 g	Solid/incorporated
Carbofurano: 50 g/kg 2	178.68 kg/ha	30 g	Solid/incorporated
Carbofurano: 50 g/kg 3	268.02 kg/ha	30 g	Solid/incorporated
<i>Trichoderma. harzianum</i> : 1	1.00 l/ha ²	200 mL syrup/plant: 595.6 l syrup/ha	Diluted/squirt
<i>T. harzianum</i> : 2	2.00 l/ha	200 mL syrup/plant: 595.6 l syrup/ha	Diluted/squirt
<i>T. harzianum</i> : 3	3.00 l/ha	200 mL syrup/plant: 595.6 l syrup/ha	Diluted/squirt
<i>Pochonia. chlamydosporia</i> : 1	2.00 kg/ha ³	200 mL syrup/plant: 595.6 l syrup/ha	Diluted/squirt
<i>P. chlamydosporia</i> : 2	4.00 kg/ha	200 mL syrup/plant: 595.6 l syrup/ha	Diluted/squirt
<i>P. chlamydosporia</i> : 3	6.00 kg/ha	200 mL syrup/plant: 595.6 l syrup/ha	Diluted/squirt
<i>P. chlamydosporia</i> + <i>T. harzianum</i> :1	2.00 kg/ha+1.00 l/ha	200 mL syrup/plant:5 95.6 l syrup/ha	Diluted/squirt
<i>P. chlamydosporia</i> + <i>T. harzianum</i> :2	4.00 kg/ha+2.00 l/ha	200 mL syrup/plant: 595.6 l syrup/ha	Diluted/squirt
<i>P. chlamydosporia</i> + <i>T. harzianum</i> :3	6.00 kg/ha+3.00 l/ha	200 mL syrup/plant: 595.6 l syrup/ha	Diluted/squirt

*Each of the treatments was applied once, twice or three times.

Population evaluation of *M. incognita* race 1

From each repetition, 100 g roots were collected from each of the three central plants to form a sample composed of 300 g, stored in plastic bags and packed in polystyrene boxes until the moment of the evaluations.

To quantify the nematode population in the root system (eggs + juveniles of second stage, J2), we used the methodology proposed by Hussey and Barker (1973), modified by Bonetti and Ferraz (1981). For the evaluation of the population of nematodes in the soil the extraction was done by the Jenkins technique (1964).

Damage Quantification

In March, 2014, coinciding with the last nematological evaluation, the following were evaluated: number of fruits per rosette (NFPR); total grain weight per rosette (TGWR) (g); weight of 100 processed grains (g) (WG100); processed (total sample processed coffee weight)/mature yield (1 kg) (PMY%).

To estimate NFPR and TGWR, the plagiotropic branches located at the average height of the plants were taken as the basis of calculation. Four branches were collected, one in each quadrant of the plant, and the fruits of the three productive rosettes were counted in the median position of the productive branch, being then weighed in an analytical balance with precision of 0.001g.

For the evaluation of WG100 and PMY%, samples of 10.0 kg of ripe fruits were taken from

each treatment and placed in plastic trays, and then taken into sunshine to dry in a plastic oven until reaching a moisture content of 12.0%, and then benefited in a Model DRC-1 peeler.

These processed grains were weighed on an analytical balance accurate to 0.001g to obtain the WG100, and the PMY% by the equation: (total sample processed coffee weight/10.0 kg) x 100.

Yield in bags per hectare (YBHa) calculations were prepared as proposed by Androcioli Filho and Androcioli, (2011), in which:

Mature coffee (kg/ha):

[No. plagiotropic branches x no. rosettes x grain weight per rosette x no. plants per ha];

60 kg bags of processed coffee/ha:

[Yield (%) x productivity of mature coffee)/60]

The value of US\$ 1.00 was considered equivalent to R\$ 4.00 for this search.

The production values (US\$/ha) were obtained by multiplying the productivity, in bags per hectare, by the average price of US\$ 56.50 per bag of 60 kg of conilon type 7/8 coffee, obtained through the series prices registered by the national supply company, CONAB, from 2003 to 2017 (CONAB, 2017).

Based on these results, the yield gains for each management method were obtained by subtracting the yield obtained in the area for each treatment from the yield obtained in plants that received no treatment (control). The yield value gain, where each management method was applied, was considered in US\$/ha, obtained by subtracting the yield obtained in each plot treated from the plant

production in the untreated plots.

Based on the production value obtained (US\$/ha) and the application costs of each management strategy (US\$/ha), the net production gain (US\$/ha) was calculated by decreasing the production value of each management method, by their respective application costs, without taking into account the production costs of the crop, which were not accounted for in this calculation.

Sieve classification analyses were performed from a 300g sample, according to the size of the sieves, and the sieves were numbered from 10 to 17 for flat and mocha grains, besides residue (BRASIL, 2003).

Yield loss quantification

Application costs were based on the recommended coffee dosages and the costs of each of the products tested. Dosage and cost calculations per hectare are explained below:

a) Carbofuran: 50 g/kg (5% w/w), Furadan® 50 GR commercial product (30.0 grams per plant; recommendation: 2978 plants/ha x 0.030 kg/plant: 89.34 kg/ha), average price per bag of 10 kg of nematicide in Espírito Santo: US\$ 24.00 (COOPEAVI, 2015);

b) *T. harzianum* biological nematicide, strain ESALQ 1306, recommendation 1.0 L/h, average price per package of 1 liter in Espírito Santo: US\$ 35.00 (KOPPERT, 2013);

c) Biological nematicide based on *P. chlamydosporia* isolate Pc-10, recommendation 2.0 kg/ha. Average price per package of 2 kg in Espírito Santo: US\$ 60.00 (RIZOFLORE, 2013).

In addition to the costs related to each product tested, the labor cost per application was added, which for the conditions tested was US\$ 12.50 per application per hectare.

Statistical analysis

The data obtained were analyzed using the statistical software "Program GENES", to adjust the statistical models for the mean test at 5% of significance using the Scott and Knott test. For the results that proceeded to the linear regression analysis, the software SIGMA PLOT® 11.0 was used, with the model chosen by the coefficient of determination (R^2) and significance of the regression coefficient (β_1).

RESULTS AND DISCUSSION

Population evaluation of *M. incognita* race 1

Biological nematicides applied alone and in combination caused a decrease in the egg + juvenile

of the second-stage (J2) population of *M. incognita* race 1 in plant roots (Table 2).

The largest populations of *M. incognita* race 1 were found in roots of untreated conilon coffee (control) (Table 2).

In March, carbofuran was the treatment that most reduced the nematode population with one and two applications, with means that did not differ (Table 2).

The nematode population in the experiment set up in September 2013, considering the average of the four blocks, was 3,000 eggs + J2/50 g of roots and 725 J2/100 cm³ of soil. The increase in nematode population from 3,000 to 52,666 eggs + J2/50g from September to November 2013 (Table 2) in control plants can be attributed to the life-cycle characteristics of *Meloidogyne* spp., in addition to the intense physiological activity of the coffee plant during this period. These caused an increase in the number of rootlets and the release of root exudates by plants, which favors nematode penetration and multiplication (SILVA et al., 2015).

According to Escobar et al. (2015), each female of *M. incognita* can deposit in the region of the cortical parenchyma or on the root surface an egg mass containing on average 400 to 500 eggs. According to Moens, Perry and Starr (2009), the life cycle of *M. incognita* with temperatures around 29 °C may be only 21 days, and at this stage it is already possible to find egg masses. This indicates that the increase in the coffee root nematode population in the present research, including in plants that received the treatments, is mainly due to the availability of a susceptible host and favorable climatic conditions. The studied coffee crop is located in a region whose climate is characterized as tropical hot and humid, with a cool and dry winter, with an average annual temperature of 24.5° C, and rain beginning in October and ending in April. These conditions favored the multiplication of the nematode during September to March, the summer season.

The nematode population reductions promoted by the treatments generate knowledge of fundamental importance at a moment of transformation of coffee from Espírito Santo, due to the introduction of new genetic materials.

The fungus *T. harzianum*, applied alone, provided a decrease in NFP (final population) at all evaluated times, with the highest management effectiveness achieved in January with 2 and 3 applications in relation to the control (Table 2). *T. harzianum* also reduced soil final population compared to control in all periods and application quantities (Table 2). This proves that *T. harzianum* provides a long-term decrease in the soil nematode population, which is one of the advantages of biological nematode control. The fungus *T. harzianum* is an antagonist that inhibits and blocks

the development of phytopathogens in the soil, using some mechanisms such as antibiosis, parasitism and competition (MENDOZA et al., 2015). In addition to the direct effects on phytonematodes, *T. harzianum* also stimulates plant root development, releasing substances called metabolites, which act as phytohormones and resistance inducers, acting on

organic matter decomposition and nutrient release (KOPPERT, 2013). Several studies demonstrate that root colonization by *Trichoderma* spp. has elevated the level of enzymatic defense of plants, including peroxidase, chitinase, β -1,3-glucanases, lipoxygenase and phenylalanine ammonia lyase (HOWELL et al., 2000).

Table 2. Population of *Meloidogyne incognita* race 1 (eggs + second-stage juveniles, J2 / 50g root) in roots of coffee conilon clone V02 variety ‘Vitória INCAPER 8142’, and in soil (J2/100 cm³) in an area naturally infested by the pathogen, submitted to different management methods with evaluation at different times.

Treatments	Eggs + J2 / 50 g root			J2/ 100 cm ³ of soil		
	November (2013)	January (2014)	March (2014)	November (2013)	January (2014)	March (2014)
Control	52.666 cF ¹	54.333 aE	64.783 bH ¹	1.340 bF ¹	620 aF	1.823 cG
Carbofuran: 50 g/kg 1*	10.246 bB	11.778 bB	5.617 aA	350 cD	151 aA	248 bB
Carbofuran: 50 g/kg 2	8.800 bA	15.673 cC	5.866 aA	288 bC	250 bC	150 aA
Carbofuran: 50 g/kg 3	11.020 aB	14.365 bC	14.795 bC	312 bC	112 aA	350 bC
<i>T. harzianum</i> : 1	32.621 bE	22.030 aD	28.362 bG	418 aD	468 aE	710 bF
<i>T. harzianum</i> : 2	30.000 cE	14.483 aC	22.041 bE	302 aC	350 bD	300 aB
<i>T. harzianum</i> : 3	31.230 cE	15.255 aC	21.931 bE	358 bD	387 bD	298 aB
<i>P. chlamydosporia</i> : 1	14.700 aC	15.650 aC	23.681 bF	412 bD	122 aA	634 cE
<i>P. chlamydosporia</i> : 2	19.800 bD	8.700 aA	24.120 bF	200 aB	300 bD	750 cF
<i>P. chlamydosporia</i> : 3	16.780 bC	9.415 aA	10.857 aB	190 bB	189 bB	120 aA
<i>P. chlamydosporia</i> + <i>T. harzianum</i> : 1	20.579 bD	12.166 aB	35.320 cG	156.0 aA	456.0 bE	440.0 bD
<i>P. chlamydosporia</i> + <i>T. harzianum</i> : 2	22.130 bD	9.276 aA	20.744 bD	450.0 bE	250.0 aC	522.0 cE
<i>P. chlamydosporia</i> + <i>T. harzianum</i> : 3	21.200 bD	9.543 aA	19.153 bD	370.0 bD	275.0 aC	532.0 cE

¹Averages followed by the same lowercase letter in the rows and uppercase in the columns do not differ statistically by the Scott and Knott test (P > 0.05).

*Number and timing of treatment applications: (1 – September; 2 – September and November; 3 – September, November and January).

The *P. chlamydosporia*-based biological nematicide, when applied alone, proved to be effective in the management of *M. incognita* race 1, as it provided a decrease in FP in coffee roots in the November and March evaluations, with 1 and 2 applications compared to the control. It also reduced soil FP in all evaluation periods with 3 applications (Table 2). This antagonist, when applied 2 or 3 times, in the January evaluation, proved to be more efficient than the chemical nematicide in reducing the NFP in the roots, proving the efficiency of this biological control agent, as well as its potential use in the management of *M. incognita* race 1 (Table 2).

Regarding the evaluation period, *P. chlamydosporia* was responsible for the largest

reduction in soil NFP in January with 1 application and in March with 3 applications (Table 2). Other authors have shown that *P. chlamydosporia* isolate Pc-10 is an efficient biocontrol agent of *Meloidogyne* spp. in other crops (DIAS-ARIEIRA et al., 2011; VIGGIANO; FREITAS; LOPES, 2014). One, two and three applications of *P. chlamydosporia* + *T. harzianum* reduced the FP in all evaluations when compared to the control (Table 2). In general, the associated use of the fungi *P. chlamydosporia* and *T. harzianum* showed results statistically equal to or close to those of their isolated uses in nematode population reduction.

The association between *P. chlamydosporia* + *T. harzianum* provided lower values of J2 in soil in

relation to the control, with the lowest value found with one application in November. In this case, this was the treatment that most reduced the soil NFP with one single application (Table 2).

Induction of soil suppressivity by the use of combinations of two or more antagonist organisms may provide better disease management compared to the use of individually applied organisms (MACHADO et al., 2012). Several organisms may enhance the efficiency of biological control due to their different mechanisms of action. Combinations can provide protection at different times or under different conditions and occupy different or complementary niches (SRIVASTAVA et al., 2010).

Based on the parasitism mechanism of eggs and females exerted by *P. chlamydosporia* and substance release antagonism by *T. harzianum*, the

application of the two biocontrol agents at different times of the phenological cycle could increase the management efficiency during the longest period of nutrient drainage by coffee. This is the post-flowering and grain-filling period, and is also the most favorable time for the nematode, due to the occurrence of higher soil moisture and temperature.

Damage Quantification

In the evaluations of the quantitative characteristics of the harvested grains, there was a difference between the averages obtained from plants treated with biological and chemical nematicides, and WG100 was the most influenced variable (Table 3).

Table 3. Number of fruits per rosette (NFPR), total grain weight per rosette (TGWR), weight of 100 processed grains (WG100), processed/mature yield (PMY%), yield in bags per hectare (YBH_a) and yield value (YVUS\$) of Conilon coffee clone V02, from the variety "Vitória" INCAPER 8142, submitted to different management methods of *Meloidogyne incognita* race 1 under evaluation in March 2014.

Treatments	Variables analyzed					
	NFPR	TGWR (g)	WG100(g)	PMY%	YBH _a	YVUS\$
Control	19.25 A ¹	13.60 A ¹	13.23 A	35.38 D ²	73.63 A ¹	4,178.405 A ²
Carbofuran: 50 g/kg 1*	32.00 C	21.00 B	16.29 C	35.50 D	155.56 D	8,828.17 D
Carbofuran: 50 g/kg 2	30.25 B	27.25 C	17.33 D	39.33 E	197.02 F	11,180.74 F
Carbofuran: 50 g/kg 3	29.25 B	29.17 D	16.60 C	32.58 B	170.47 E	9,673.935 D
<i>T. harzianum</i> : 1	27.50 B	28.22 D	15.49 B	31.58 D	156.77 D	8,894.555 D
<i>T. harzianum</i> : 2	25.75 B	22.15 B	18.28 F	38.38 E	120.07 B	6,814.17 B
<i>T. harzianum</i> : 3	26.00 B	23.95 C	18.59 F	38.88 E	157.48 D	8,934.585 D
<i>P. chlamydosporia</i> : 1	33.75 C	26.62 C	17.71 E	32.09 B	160.33 D	9,098.687 D
<i>P. chlamydosporia</i> : 2	29.25 B	28.95 D	17.34 D	31.86 B	115.16 B	6,535.05 B
<i>P. chlamydosporia</i> : 3	38.00 D	24.62 C	17.17 D	35.96 D	154.42 D	8,763.057 D
<i>P. chlamydosporia</i> + <i>T. harzianum</i> : 1	27.00 B	26.72 C	16.04 B	35.96 B	137.57 C	7,806.9 C
<i>P. chlamydosporia</i> + <i>T. harzianum</i> : 2	37.75 D	24.52 C	17.88 E	34.31 C	143.05 C	8,117.87 B
<i>P. chlamydosporia</i> + <i>T. harzianum</i> : 3	29.25 B	32.27 D	17.85 E	29.03 A	133.75 C	7,657.995 C

¹Averages followed by the same capital letter in the columns do not differ statistically by the Scott and Knott test (P> 0.05).

²Values obtained by dividing the amount of processed coffee obtained per kilogram of properly dried mature coffee with a moisture content of 12.00%.

*Number of applications.

The NFPR was influenced by the treatments, and the untreated plants (control) presented the lowest value. Thus, the quantitative damage caused by *M. incognita* race 1 is evident, as the lower weight per benefited grain indicates the use of a smaller sieve and the need for more grains to compose a 60 kg bag, which may compromise the profitability of the producer at sale time.

The highest NFPR values were observed in plants that received three applications of

P. chlamydosporia and two applications of *P. chlamydosporia* + *T. harzianum* (Table 3).

The TGWR had its lowest value in the control. The highest averages were obtained with three applications of *P. chlamydosporia* + *T. harzianum*, two applications of *P. chlamydosporia*, one application of *T. harzianum* and three applications of Carbofuran (Table 2).

The highest averages of WG100 were obtained with two and three applications of

T. harzianum and three applications of Carbofuran (Table 3).

PMY% was lower in plants that received three associated applications of *P. chlamydosporia* + *T. harzianum* (29.03%), which indicates that for each 1000 g of mature coffee, 290.30 g of processed coffee is obtained (Table 3).

The highest YBHa were obtained with two and three applications of Carbofuran (Table 3). This is due to the fact that these two treatments were the ones that most reduced FP at harvest in March (Table 2). Another possible explanation for the higher productivity caused by the application of chemical nematicide is the prophylactic effects of carbofuran, which stimulate plant growth and consequently its resistance (SINGH; VARSHNEYA; SRIVASTAVAB, 2012).

The yield value (YVU \$) was higher than the control in all management strategies tested, with the highest values being obtained with one, two and three applications of Carbofuran, one and three applications of *P. chlamydosporia* and *T. harzianum* (Table 3).

Untreated (control) plants produced 73.63 sc/ha (Table 3), which is not a low yield for the cultivar 'Vitória INCAPER 8142' clone V02. This good yield may be explained by the fact that the crop in which the research was carried out followed the technical recommendations of nutrition, irrigation and pest and disease management. However, as *M. incognita* is a new challenge for many Espírito Santo coffee growers (BARROS et al., 2014), the non-use of efficient methods of crop management can cause a reduction in conilon coffee productivity due to plant parasitism by the nematode.

Young plants are the most damaged, although adult plants can also suffer serious defoliation, impairing the production of photoassimilates important for production (SALGADO et al., 2015). It is worth remembering that plants that receive good nutrition are more likely to resist nematode attack than nutrient-deficient plants (SANTANA-GOMES et al., 2013).

The results regarding damage caused by *M. incognita* race 1 found in this research corroborate those from the study of Oliveira et al. (2009), in a commercial crop of 'Vitória INCAPER 8142', clone V02 conilon coffee variety, infested with *M. incognita* in the municipality of Sooretama, Espírito Santo, Brazil. That study found root deformation, cracking, scaling and displacement of cortical root tissues in plants attacked by the pathogen. According to the authors, due to the damage caused by the nematodes, the plants started to exhibit a high number of fruits classified as black and dry. Other damage was also attributed to

parasitism, such as: reductions in plant height, number of leaves, productive and vegetative branches and rosettes per branch.

The quality of the benefited coffee regarding the grain size was influenced by the management method, as well as the number of applications of each treatment. For conilon coffee, the ideal is that the benefited grains have the highest percentage above sieve 13, as this ensures a higher yield and, consequently, market price.

Yield Loss Quantification

The highest application costs of management methods for *M. incognita* race 1 per hectare were: US\$ 680.46 and US\$ 453.50 for three and two applications of Carbofuran and US\$ 322.50 for three applications of *P. chlamydosporia* + *T. harzianum* (Table 4).

The treatments with lowest application costs were US\$ 47.50 for one application of *T. harzianum* and US\$ 72.50 for one application of *P. chlamydosporia* (Table 4).

Increasing the population of *M. incognita* race 1 at harvest led to reduced yield (Figure 1). Comparing the management costs (Table 4) with the NFP on the coffee roots in the March evaluation (Figure 1), it was possible to verify that *P. chlamydosporia* and *T. harzianum* fungi, applied alone, presented the best results for cost/benefit, because they are more economical and also proved to be efficient in the management of the nematode.

The use of Carbofuran, despite being efficient in the management of *M. incognita* race 1, presented a low cost/benefit, that is, it is a relatively expensive method for the producer and presents risks of environmental pollution (NASR, 2015).

These results of application costs, linked to the management of *M. incognita* race 1, are extremely important for coffee growing, because the management strategies tested, both biological and chemical, demonstrated management efficiency under the studied conditions. However, it is known that each of them has a cost to the producer, and their adoption should be based mainly on the pillars of phytosanitary management of plant diseases, which aims at environmental, economic and social sustainability, but must also have a holistic view of the agroecosystem.

The management methods that influenced the increase in the quality of the grains harvested in the experiment, which led to obtaining the highest percentages of coffee grains retained in sieves ≥ 13 , were one and two applications of *P. chlamydosporia* (Table 5).

Table 4. Recommended dose (DOS), unit price (UNP), price of the commercial quantity used (CQU) and application costs (APC) and total cost (TCO) of each treatment for management of *M. incognita* race 1 in conilon coffee clone V02 variety 'Vitória INCAPER 8142', in a field cultivated in the region of São Roque do Canaã, ES, naturally infested by the nematode.

Treatments	Variables analyzed				
	DOS (kg or L/ha)	UNP (US\$)	CQU	APC (US\$/ha) ⁴	TCO (US\$/ha)
Control	0	0	0	0	0
Carbofuran: 50 g/kg 1*	89.34 kg/ha ¹	24.00	2.23	12.50	226.82
Carbofuran: 50 g/kg 2	178.68 kg/ha	24.00	4.46	25.00	453.50
Carbofuran: 50 g/kg 3	268.02 kg/ha	24.00	6.69	37.50	680.46
<i>T. harzianum</i> : 1	1.00 l/ha ²	35.00	0.25	12.50	47.50
<i>T. harzianum</i> : 2	2.00 l/ha	35.00	0.5	25.00	95.00
<i>T. harzianum</i> : 3	3.00 l/ha	35.00	0.75	37.50	142.50
<i>P. chlamydosporia</i> : 1	2.00 kg/ha ³	60.00	0.25	12.50	72.50
<i>P. chlamydosporia</i> : 2	4.00 kg/ha	60.00	0.5	25.00	145.00
<i>P. chlamydosporia</i> : 3	6.00 kg/ha	60.00	0.75	37.50	217.50
<i>P. chlamydosporia</i> + <i>T. harzianum</i> : 1	2.00 kg/ha + 1.00 l/ha	60.00 + 35.00	0.25 + 0.25	12.50	107.50
<i>P. chlamydosporia</i> + <i>T. harzianum</i> : 2	4.00 kg/ha + 2.00 l/ha	60.00 + 35.00	0.5 + 0.5	25.00	215.00
<i>P. chlamydosporia</i> + <i>T. harzianum</i> : 3	6.00 kg/ha + 3.00 l/ha	60.00 + 35.00	0.75 + 0.75	37.50	322.50

¹Recommendations for coffee: source: (AGROFIT, 2013), 30.00 g per plant (recommendation: 2978 plant/ha x 0.030kg plant: 89.34 kg/ha). Furadan[®] 50 GR, average price per bag of 10 pounds in Espírito Santo: US\$ 24.00. ²Recommendations for coffee: source: (KOPPERT, 2013), 1.00 l/ha. Trichodermil[®] SC, strain ESALQ 1306, average price per pack of 1 liter in Espírito Santo: US\$ 35.00. ³Recommendations for coffee: source: (RIZOFLORA, 2013), 2.00 kg / ha. Rizotec[®] Insulated Pc-10, average price per pack of 2 kg in Espírito Santo: US\$ 60.00. ⁴Application cost based on the monetary value of US\$ 12.50 for 1 work-day of 1 rural worker in the studied region, establishing that the worker spends 1 day on each application in 1 ha. *Number of applications.

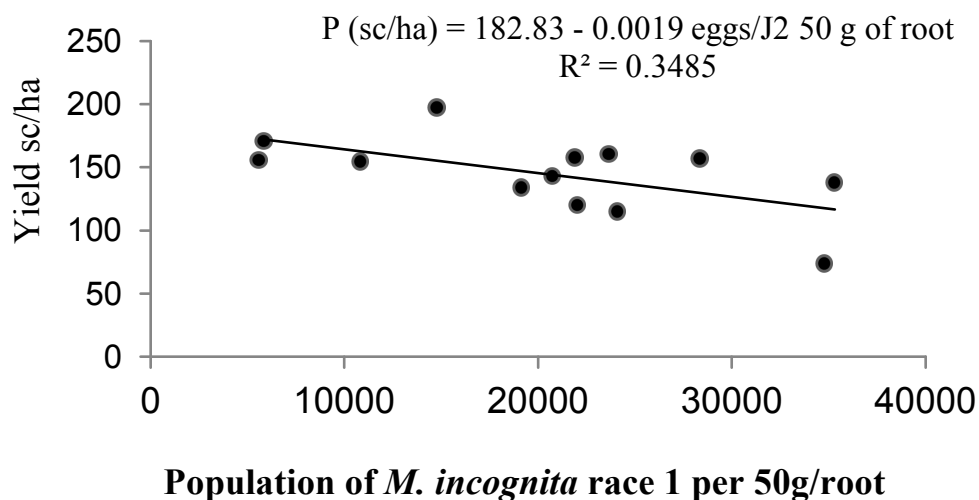


Figure 1. Relationship between yield of conilon coffee variety 'Vitória INCAPER 8142' clone V02 and different populations of *Meloidogyne incognita* race 1 found in field treatments (evaluation in March 2014).

Table 5. Percentage of grains benefited removed in sieves ≥ 13 , residue (sieve ≤ 13), yield value (YVA), total production custody (TCP) and liquid yield (JNI) of clone V02, variety 'Vitória INCAPER 8142', subject to different management methods for *Meloidogyne incognita* race 1.

Treatments	Variables analyzed				
	Sieve ≥ 13 (%)	residue (%)	(YVA) US/ha	(CTP) (US/ha)	GLP (US/ha)
Control	70.10 C	29.90 C	4.178.40 A ¹	0	4.178.40A ¹
Carbofuran: 50 g/kg 1*	81.10 B	18.90 B	8.828.17 D	453.56	8.601.35 D
Carbofuran: 50 g/kg 2	80.00 B	20.00 B	11.180.50 F	680.46	10.727.10 E
Carbofuran: 50 g/kg 3	81.45 B	18.55 B	9.673.93 E	226.75	8.993.47 D
<i>Trichoderma harzianum</i> : 1	68.77 D	31.23 D	8.896.80 D	47.50	8.849.30 D
<i>T. harzianum</i> : 2	82.20 B	17.80 B	6.814.17 B	95.00	6.719.17 B
<i>T. harzianum</i> : 3	76.55 B	23.45 B	8.934.58 D	142.50	8.292.08 D
<i>Pochonia chlamydosporia</i> : 1	85.60 A	14.40 A	9.098.68 D	72.50	9.026.18 D
<i>P. chlamydosporia</i> : 2	86.00 A	14.00 A	6.535.05 B	145.00	6.390.05 B
<i>P. chlamydosporia</i> : 3	78.20 B	21.80 B	8.763.05 D	217.50	8.545.55 D
<i>P. chlamydosporia</i> + <i>T. harzianum</i> : 1	68.90 D	31.10 D	7.806.90 C	107.50	7.699.40 C
<i>P. chlamydosporia</i> + <i>T. harzianum</i> : 2	83.54 B	16.46 B	8.117.96 C	215.00	7.902.87 C
<i>P. chlamydosporia</i> + <i>T. harzianum</i> : 3	80.00 B	20.00 B	7.657.99 C	322.50	7.335.49 C

¹Averages followed by the same capital letter in the columns do not differ statistically by the Scott and Knott test ($P > 0.05$).
*Number of applications.

The treatments with the lowest percentage of coffee grains retained in sieves ≥ 13 were: control; one application of *P. chlamydosporia* + *T. harzianum* and one application of *T. harzianum* (Table 4). This may be related to the fact that the association of *P. chlamydosporia* + *T. harzianum* in a single application, as well as one application of *T. harzianum*, did not exert management effectiveness, as observed in the other treatments, which led to an increase in productive and qualitative damage to grains from plants that received these treatments (Tables 2, 3 and 4).

The percentage of coffee grains retained in sieves ≥ 13 obtained in the unmanaged area of *M. incognita* race 1 is related to the interference caused by nematodes in plant development. This interference is related to nutrient spoliation during nematode feeding (FORTI et al., 2015); change in absorption and translocation of water and nutrients; modification or destruction and decrease of the roots, resulting in symptoms of the shoot, such as yellowing (CARVALHO et al., 2015); and reduction of the size and smaller proportion between shoot weight and root system.

The production value was calculated as a function of productivity in bags benefited per hectare obtained in each treatment, with the producer's gross revenue being influenced by the tested management method. The sale of the production obtained in the control would result in a gross production value of US\$ 4,178.40, which is low compared to the

US\$ 11,180.74 obtained with two nematicide applications (Table 4).

All treatments increased the average yield of the crop because the soil addition of antagonists to *M. incognita* race 1 or nematicide caused a decrease in the root and soil pathogen population, resulting in gains in average coffee yield (SRIVASTAVA et al., 2010; MACHADO et al., 2012).

The TCO was higher in all treatments than in the control, and the highest values were obtained with one, two and three applications of carbofuran, one and three applications of *P. chlamydosporia* and two and three applications of *P. chlamydosporia* + *T. harzianum* (Table 4).

The initial population of *M. incognita* race 1 already established in the field at the beginning of the the experiment was 3,000 eggs + J2 / 50 g of roots. The plants were 24 months old with a fully developing root system and plenty of food available for the nematode, which contributed to a significant increase in its population over the following months (TAYLOR; SASSER, 1978) (Table 2).

In the present research, the lowest yields, total grain weight per rosette, weight of 100 processed grains and percentage of processed coffee grains retained in sieves ≥ 13 were caused by the largest populations of *M. incognita* race 1 in the roots and soil (Tables 3 and 4). Plants that were not submitted to any management method (control) during the productive cycle (September-March) presented the greatest damage caused by the nematode.

The severity of damage caused by parasitism of *Meloidogyne spp.* in coffee is directly related to the number of root-eating individuals, which influences the physiological processes of the plant and culminates in dwarfism, nutritional deficiencies or toxicity, wilting, and reduced yields (ROSELI et al., 2019). All these types of damage were observed in our research, highlighting the decrease in the number of fruits in the rosettes and the fall of leaves.

CONCLUSIONS

We conclude that all treatments are efficient for the management of *M. incognita* race 1 in brown coffee, causing a reduction in root population in isolation. However, there is no management measure implemented, or performance and damage not large or sufficient nematode parasitism. Finally, the fungi *P. chlamydosporia* var. *chlamydosporia* and *T. harzianum*, tested in isolation and in combination, were efficient biological methods I do not administer to breed *M. incognita* 1.

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