

# Number of Nematicide Cycles per Year on Banana (*Musa* AAA cv VALERY) Root Nematode Control and Crop Yield

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**Abstract:** The effect of the number of nematicide cycles per year on banana (*Musa* AAA cv. Valery) root weight, root nematode control and crop yield were compared in a commercial banana plantation in Ecuador testing five treatments in a randomized complete block design with six replicates. Treatments consisted of one, two, three or four nematicide cycles per year plus the untreated control. Averaging the 24 root nematode samplings after treatments application, the nematicide applications reduced *R. similis* ( $P = 0.0114$ ) between 22 to 49 %, *Helicotylenchus* spp. ( $P = 0.0004$ ) between 23 to 40 % and total nematode ( $P = 0.0002$ ) populations between 25 to 45 %. At harvest, nematicide applications increased ratooning ( $P < 0.0001$ ;  $P < 0.0001$ ) at 12 and 24 months after treatment application, respectively. Even though, nematicide treatments increased yield between 66 and 471 (1.1-8.5 tm) boxes of 18.14 kg ha<sup>-1</sup> year<sup>-1</sup> at 12 months after treatments application, the difference was not large enough to reach significant difference ( $P = 0.2812$ ). At 24 months after treatment application, in plants treated with nematicides, the yield increased ( $P = 0.0061$ ) between 226 to 730 (4.0-13.2 tm) boxes of 18.14 kg ha<sup>-1</sup> year<sup>-1</sup>, which resulted in a net profit (deducted the treatment cost and the packing cost of the additional boxes) of US \$1, 050 to \$3, 432 ha<sup>-1</sup> year<sup>-1</sup>. The highest yield and net profit were obtained with the rotation of three nematicide cycles per year.

**Keywords:** crop yield, *Helicotylenchus* spp., nematode control, *Radopholus similis*, root weight, total nematodes

## 1. Introduction

Banana (*Musa* AAA) is the most important crop in Ecuador which generates 2, 5 million jobs and accounts for almost 10 % (Hidalgo, 2020) of the total exports. In 2019, 356, 8 million boxes of 18.14 kg (Bananotas, 2020) were exported, produced on an area of 200, 000 hectares (Salazar, 2019), which gave a total income of about US \$3, 100 million, which represents 2 % of the Gross Domestic Product and 35 % of the Agriculture Gross Domestic Product (El Telégrafo, 2019). A profitable banana production requires a rational use of inputs and labor to meet the requirements of certifications, supermarkets, and consumers, which are constantly looking for a final consumption product obtained with low use of inputs in harmony with the environment. This will entail flexible production models, where the strategies for pest control play a key role, since they threaten and reduce yield, and their control may represent close to 20 % of the production cost.

Within the root pests, nematodes are the main problem, being their presence common and in many cases in high quantities in Ecuadorian plantations (Aguirre et al. 2016a). In the five provinces; Cañar, El Oro, Guayas, Los Ríos and Santo Domingo where banana is cultivated, nematodes are common (Chávez and Araya, 2001; 2010, Aguirre et al., 2016a; 2016b) and usually only polyspecific communities occur, consisting of a mixture mainly of *Radopholus similis* and *Helicotylenchus* spp., with low frequency and numbers of *Pratylenchus* spp. and *Meloidogyne* spp. Here, banana growers started nematode control since 1970 (Triviño and Escobar, 2004) and recently, banana nematode population

studies, that included data since 1994 up to 2015 (Aguirre et al., 2016a; 2016b) confirm the high incidence of nematodes, where a large number of samples were found over the economic threshold, in all the provinces where the crop is grown. Banana nematodes live within the roots, where they weaken plant anchorage and restrict water and nutrients uptake, decreasing leaf photosynthesis, retard leaf emission, and reduce bunch weight, ratio, ratooning, plant longevity and yield.

To avoid or reduce nematode damage, the only alternative management strategy currently available, is the regular application of non-fumigant nematicides, of which growers know that is economically feasible. Nematicide application is recommended when the total phytoparasitic nematode population exceeds the economic threshold of 2, 500 individuals per 100 g of fresh roots (Instituto Nacional Autónomo de Investigaciones Agropecuarias-INIAP, 2018) collected in front of the follower sucker and extracted by the root maceration method (Araya, 2002) recovering the nematodes on the No 500 (0.025 mm) mesh. The nematicides registered for bananas are rotated according to their physico-chemical characteristics and weather condition to prevent their biodegradation. However, in Ecuadorian conditions, most banana growers stopped to applied nematicides which had resulted in high nematode population, root damage and severe yield reduction. Therefore, the objective of this study was to evaluate different nematicide cycles per year on banana root nematode control and crop yield and to determine the net profit of the chemical nematode control in the crop.

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## 2. Material and Methodology

### Experimental site and agro-ecological characteristics:

The field experiment was carried out in a 24 years old commercial banana (*Musa* AAA cv. Valery) farm infected by nematodes located in El Guabo county, province of El Oro, Ecuador. The soil was alluvial, taxonomically classified as an Inceptisol and it had a clay texture (20 % sand, 20 % silt and 60 % clay) with a pH of 6.7 and 1.58 % organic matter. The following concentrations of extractable bases were found, using Modified Olsen as the extractant: Ca 15.4, Mg 4.7, and K 0.85 cmol L<sup>-1</sup>, and P 33, Zn 17.2, Cu 4.8, Fe 61.0 and Mn 18.9 µg ml<sup>-1</sup>. The area where the experiment was established had an average production in 2015 of 2,600 boxes of 18.14 kg per hectare per year with a plant density of about 1450 plants by hectare.

Desuckering was carried out every 6-8 weeks, leaving the production unit with a bearing mother plant, a large daughter sucker (follower) and a small grand-daughter (pepper) when possible. Bunching plants were propped with double polypropylene twine to the bottom of two well-developed adjacent plants, reason why plant toppling was not considered as a variable in the experiment. The follower sucker of each production unit was fertilized every 28 days at the rate of 80 kg ha<sup>-1</sup> with a formula adapted to the soil and crop requirements, consisting of urea (46 % N).

Generally, during the rainy season, from January to May each year, water requirements was supplied by rainfall, where the annual precipitation was of 960, 1,020; and 1,060 mm per year, for 2016, 2017 and 2018, respectively. A complex system of primary, secondary, and tertiary drains was provided to disperse excess rainfall and prevent water logging during heavy rains. From June to December each year, water was supplied by sprinkling irrigation. Mean daily maximum/minimum temperatures were 29-31/25-22 °C, during the studied period.

**Cultural practices in the experimental site:** Leaf fungi, especially black Sigatoka (*Pseudocercospora fijiensis*), was managed by defoliation weekly to reduce the pressure of black Sigatoka inoculum and by aerial spraying of alternate fungicides which resulted in 28 sprayings each year at 11 to 13 days intervals. The sequencing of the fungicides applied were: 2-3 cycles with Thalonex® 720SC (chloralonyl-Crystal Chemical) 3 L ha<sup>-1</sup> in water, and then one cycle of Mancozin® 430SC (mancozeb-Crystal Chemical) 2.4 L ha<sup>-1</sup> in combination with Acord® 250EC (dificonazole-Crystal Chemical) 0.7 L ha<sup>-1</sup> in emulsion with miscible oil (Banole®-Total) and water, both cases in a spray solution of 23 L ha<sup>-1</sup>. Weeds were controlled spraying every 5-8 weeks a Glifonox® 480CS (glyphosate-Crystal Chemical) solution of 2 L in 200 L of water. Before the beginning of the experiment, nematodes were controlled every year by 1.5-1.8 nematicide cycles (Counter® 15GR-AMVAC, Rugby® 10GR-FMC, Vydate® 24SL-DuPont) per year, based on the nematode economic threshold.

**Treatments and experimental design:** Five treatments were evaluated: treatments 1, 2, 3 and 4 consisted of the rotation of 1, 2, 3 and 4 nematicide cycles per year, respectively, and 5. the untreated control (Table 1). The

applied nematicides were those available in Ecuador including Counter® 15GR (biodac-terbufos-AMVAC), Verango® 50SC (fluopyram-Bayer), Vydate® 24SL (oxamyl-DuPont), Mocap® 15GR (biodac-ethoproph-AMVAC) and Rugby® 10GR (cadusaphos-FMC), (Table 1).

The rectangular plots for each treatment consisted of 150-175 production units. Plots were arranged in a randomized complete block design with six replicates. The application was made by spreading the products in a banded arc with radius of approximately 0.40 meter around each follower sucker pseudostem, sprouting from the base of the sucker, using the Swissmex backpack equipment specific for Counter®, Rugby®, and Mocap® and the spotgun for Vydate®. The rates used per follower sucker were the recommended by the manufacturer in the product label of 3 g a. i. for Counter® and Mocap®, 2.4 g a. i. for Vydate®, 2 g a. i. for Rugby® and 0.3 g a. i. for Verango®. Verango® was applied in a water solution adding 1 L of the product to 150 L of water plus 200 g of blue coloring and 100 ml of this solution was spread onto the soil surface with a manual dosing snack pack. Plant debris was removed from the soil surface prior to distributing the nematicides onto moist soil as directed by the product label. During the development of the experiment, no rooting or organic matter was applied in the experimental area.

**Root sampling for nematode extraction:** One day before the nematicide application, and then every 30 days up to the 24 months that the experiment lasted, root samples were collected in each repetition. Each sample consisted of the roots of three follower suckers between 1.5-2.5 m height from recently flowered plants or prompt to bearing. In front of each follower sucker, a hole of 20 cm length, 20 cm wide and 30 cm depth (soil volume of 12 L) was dug at the plant base using a shovel. All the roots found were collected and placed in labelled plastic bags and delivered to NEMALAB laboratory in coolers.

In the laboratory, the root samples were registered and processed as soon as possible, and when it was necessary, stored in a refrigerator Indurama serie RS-10989-593 adjusted to 6-8 °C until being processed. The roots were rinsed free of soil, separated in living roots (white or cream-colored roots), dead roots by nematodes (with symptoms of nematode damage, with light necrosis, but without root decay) and dead roots by other causes (rotten roots by excess water, snapping), left to dry off the surface moisture and weighed (Fisher Scientific serie 10309201 scale precision 710 g ± 1 g). During the root separation process, in some roots, it was necessary to cut some damaged parts, which were classified accordingly. The total root weight corresponds to the sum of living roots, dead roots by nematodes and roots dead by other causes.

The three types of roots were cut into 1-2 cm long pieces separately and after homogenization, 25 g were randomly selected following the found proportion of each type of root. For example, in a sample of 132 g of total roots, with 80.1 g of living roots, 48.9 g of dead roots by nematodes, and 3 g of dead roots by other causes, there would be 60.7 % of living roots, 37 % of dead roots by nematodes, and 2.3 % of dead roots by other causes that multiplied by the used sample size

of 25 g, would have 15.2 g of living roots, 9.3 g of dead roots by nematodes and 0.5 g of dead roots by others causes in the 25 g sample. These roots were macerated (Araya 2002) in a kitchen blender (Osterizer; Sunbeam-Oster) for two periods of 10 seconds, at low and then at high speed, and nematode recovered in 0.025 mm (No 500) sieve. The nematodes were identified at the genus and species level when possible, based on the morphological characteristics under a light microscope, following the key of Siddiqi (2000). The population densities of all plant-parasitic root nematodes present were recorded, and the values were converted to numbers per 100 g of roots.

**Harvest and yield variables:** At the beginning of the experiment, and at 12 and 24 months after the first treatments application, 90 bunches of each treatment (15 per useful replicate), selected randomly, not including plants from plot edges, edge drains, cable edges, dumpings nor replanting plants or from double ratoon suckers, were evaluated. Bunches were harvested by calibration starting when bunches reached 10 weeks of age. When in the second hand, the central fruit of the outer whorl had a diameter of at least a grade of 45 (35.5 mm-diameter) the bunch was harvested. If in week 13, it did not reach the required minimum grade of 45, they were harvested with the grade they had. The harvest age, the date of harvest, the number of hands, the dehanging applied; the bunch weight (Tru-Test electronic scale XR3000 Kg  $\pm$  1g) and the calibration of the central fruit of the outer whorl of the second hand were registered. To calculate the ratio, which is the number of boxes of 18.14 kg given by each bunch, a reduction of 17 %

was considered, because is the average of the farm, which includes 11 % of bunch stalk and 6 % of non-marketable fruit. With the data of the number of bunches harvested in 2015 in the area where the experiment was located, and the number of plants per hectare, the initial ratoon was calculated and with the age of the bunches and harvested dates, the ratooning was estimated (number of bunches per stool per year) at 12 and 24 months.

### 3. Data Analysis

Root and nematode data were averaged by experimental plot across the 24 months excluding the first evaluation pre-treatment application. The composition of the nematode population was determined pre-treatment application and then for the average of the 24 root samplings. Data of roots weights pre-treatment application, and thereafter for the average of the 24 root samplings, were subjected to ANOVA by Proc GLM of SAS and mean separation by LSD-test. The number of nematodes was analyzed with generalized linear models, using the log transformation as link function and negative binomial distribution of the errors for the first nematode sampling alone, and thereafter for the average of the 24 nematode samplings together after the application. Bunch weight, number of hands per bunch, fruit calibration in the second hand, ratio, ratooning, and number of boxes of 18.14 kg per hectare per year (97 % bunch recovery, 1, 406 bunches \* ratio \* ratooning) were averaged for each repetition and harvest, and subjected to ANOVA and mean separation using LSD-test in PC-SAS® version 9.4.

**Table 1:** Description of the treatments evaluated with the sequence of the nematicides and month of application

Treatment	Nematicide and months of application-evaluation																									
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
1.1 c /year	Co												Mo													Ru
2.2 c / year	Co						Mo						Ru						Co							Vy
3.3 c / year	Ve				Co				Vy				Ru				Ve				Co					Mo
4.4 c / year	Ve			Co			Vy			Mo			Ru			Ve			Co			Vy				Mo
5. Untreated																										

Note: 0 = July 2016 when the experiment was established and 24 = July 2018 when the experiment ended, c / year = cycles per year, Co = Counter® 15GR terbufos-AMVAC 3 g a. i., Mo = Mocap® 15GR ethoprosfos-AMVAC 3 g a. i., Rugby® 10GR cadusaphos-FMC 2 g a. i., Vy = Vydate® 24SL oxamyl-DuPont 2.4 g a. i., Ve = Verango® fluopyram-Bayer 0.3 g a. i.

### 4. Results

**Effect of nematicide treatments on root content and nematode population:** In the sampling done before treatment application, no difference was found in the content of living roots ( $P = 0.0847$ ), dead roots by nematodes ( $P = 0.1969$ ), dead roots by other causes ( $P = 0.2525$ ), total roots ( $P = 0.0887$ ), and living root percentage ( $P = 0.4009$ ). Their contents varied between 17.6 to 29.3 g for living roots, the dead roots by nematodes ranged between 3.2 to 10.3 g, the dead roots by other causes oscillated between 1.2 to 4.6 g and total root weight between 24.2 to 40.7 g per follower sucker (Figure 1A-D). The percentages of living roots in the follower sucker ranged between 64.2 to 78.2 % (Figure 1E). Similarly, in this sampling, no difference was found among treatments in the populations of *R. similis* ( $P = 0.0788$ ), *Helicotylenchus* spp. ( $P = 0.9787$ ) and total nematodes ( $P = 0.1992$ ), that corresponds to the sum of all the phytoparasitic nematode species detected (Figure 2A-C). Nematode

populations among treatments varied in *R. similis* between 267 to 3, 562, in *Helicotylenchus* spp. between 3, 200 to 4, 200 and in total nematodes between 4, 000 to 8, 448 individuals per 100 g of roots. The composition of the nematode population before treatments application was: 23.5 % of *R. similis*, 64.1 % of *Helicotylenchus* spp., 9.6 % of *Meloidogyne* spp. with a negligible amount of 2.8 % of *Pratylenchus* spp. (data not shown).

Root content and nematode populations throughout the 25 samplings are presented in Figure 1A-E and Figure 2A-C. Across the different samplings, the roots content and nematode populations followed a similar trend in all the treatments. After treatments application, when comparing the average of the 24 samplings, differences were found among treatments in the contents of living roots ( $P = 0.0303$ ), dead roots by nematodes ( $P = 0.0232$ ), and total roots ( $P = 0.0103$ ), ranging between 57.8 to 63.8 g, between 10.3 to 12.6 g, and between 71.0 to 78.7 g per follower sucker, respectively (Figure 3A, B and D). In the other root



variables; dead roots by other causes ( $P = 0.5809$ ) that oscillated between 2.3 to 2.9 g, and percentage of living roots ( $P = 0.9805$ ) that fluctuated between 77.5 to 78, 0 % per follower sucker, no difference was found (Figure 3C, E).

The biggest nematode population per 100 g of roots of *R. similis* ( $P = 0.0114$ ), *Helicotylenchus* spp. ( $P = 0.0004$ ) and total nematodes ( $P = 0.0002$ ) was found in the untreated plants (Figure 2A-C and Figure 4A-C). Compared to the untreated plants, nematicide treatments reduced *R. similis* between 22 to 49 %, *Helicotylenchus* spp. between 23 to 40 % and the total nematode populations between 25 to 45 % (Figure 4A-C). Averaging the 24 samplings taken after treatments application, a change in the nematode population composition was observed, with *R. similis* increasing to 44.6 %, *Helicotylenchus* spp. was reduced to 42.1 %, *Meloidogyne* spp. had a similar participation with 10.3 %, and *Pratylenchus* spp. with 3.0 %, remain negligible (data not shown).

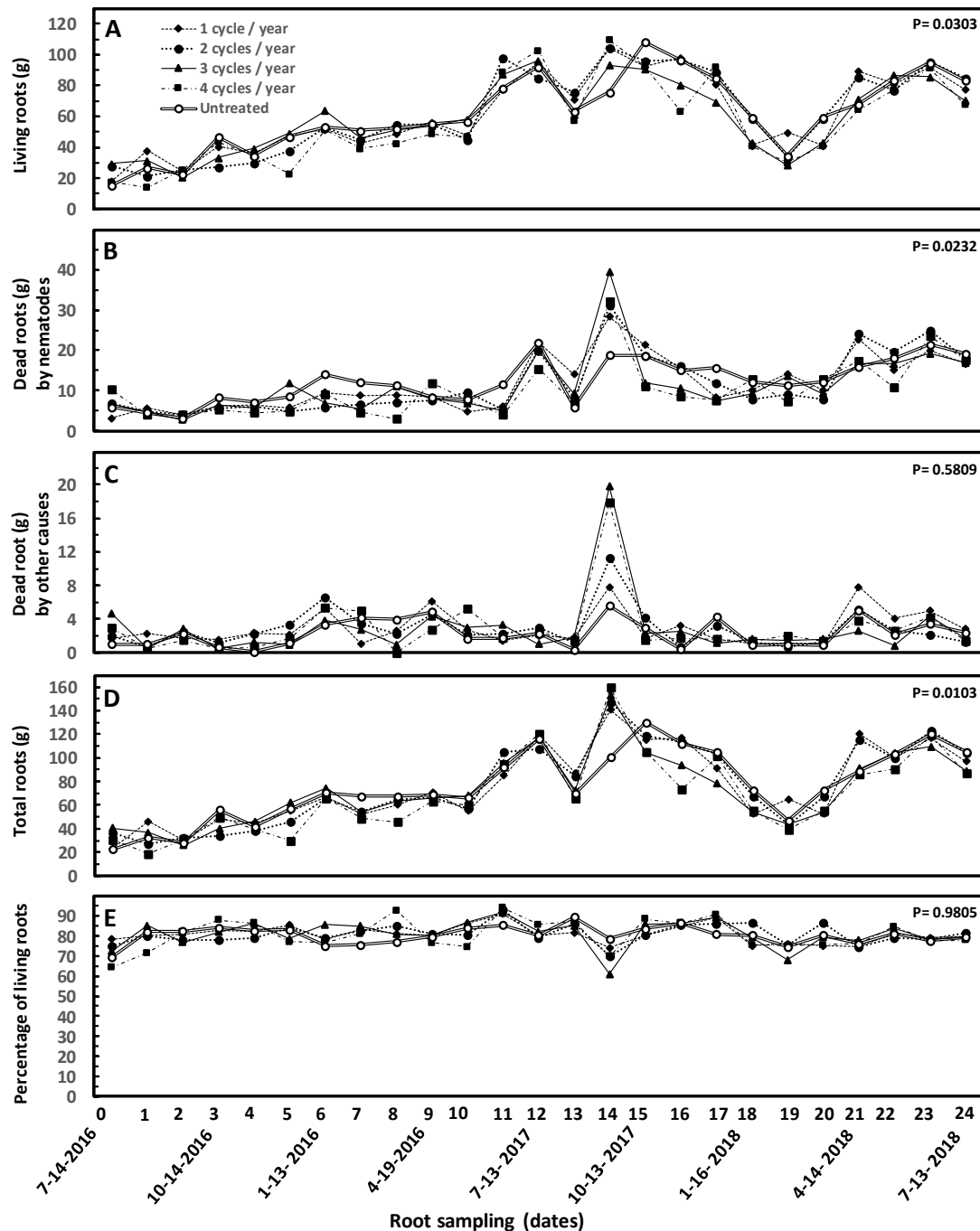
**Effect of nematicide treatments on yield variables:** The data of the three harvests carried out; at the beginning of the experiment, and at 12 and 24 months after the first treatments application, are presented in Table 2. The initial ratooning in the experimental area was 1.64 bunches harvested in each banana stool per year (Table 2) which is equivalent to an interval between harvests of 222.6 days. Bunch weight ( $P = 0.9622$ ) was similar among treatments in the initial harvest varying between 25.6 to 26.5 kg per bunch. In parallel, the number of hands ( $P = 0.1490$ ) that varied between 6.6 to 6.9 per bunch, and the ratio ( $P = 0.9693$ ), that fluctuated between 1.17 to 1.21 boxes per bunch, were also similar among treatments (Table 2). So, in congruence, the yield ( $P = 0.9630$ ), which ranged between 2, 698 to 2, 790 boxes of 18.14 kg per hectare per year was similar among treatments.

In the second harvest, carried out 12 months after the first treatments application, only in ratooning there was a difference ( $P < 0.0001$ ) among treatments, varying between 1.67 in the untreated plants to 1.83 on those plants treated 4 times a year (Table 2). Compared to the untreated plants, ratooning increased between 0.02 to 0.16 (1.2-9.6 %) units as the number of nematicide cycles per year increased, which means that the interval between harvests was reduced between 2.6 to 18.6 days, changing from 222.6 days at the beginning of the experiment to between 199.5 to 216.0 days. In bunch weight ( $P = 0.7181$ ) that varied between 32.5 to 34.3 kg, number of hands per bunch ( $P = 0.8293$ ), that fluctuated between 7.0 to 7.4, ratio ( $P = 0.7366$ ) that oscillated between 1.49 to 1.57, and yield ( $P = 0.2812$ ) that varied between 3, 569 to 4, 040 boxes per hectare per year, no difference was observed among treatments. Regarding to the untreated plots, yield was increased between 66 to 471 (2-13 %) boxes per hectare per year, as nematicide cycles per year increased. Comparing bunch weight of the second harvest, with the respective treatment at the first harvest (performed at the time of establishing the experiment), an increase between 6.4 (24 %) to 8.0 (31 %) kg was observed for all treatments, including the untreated plants. Similarly,

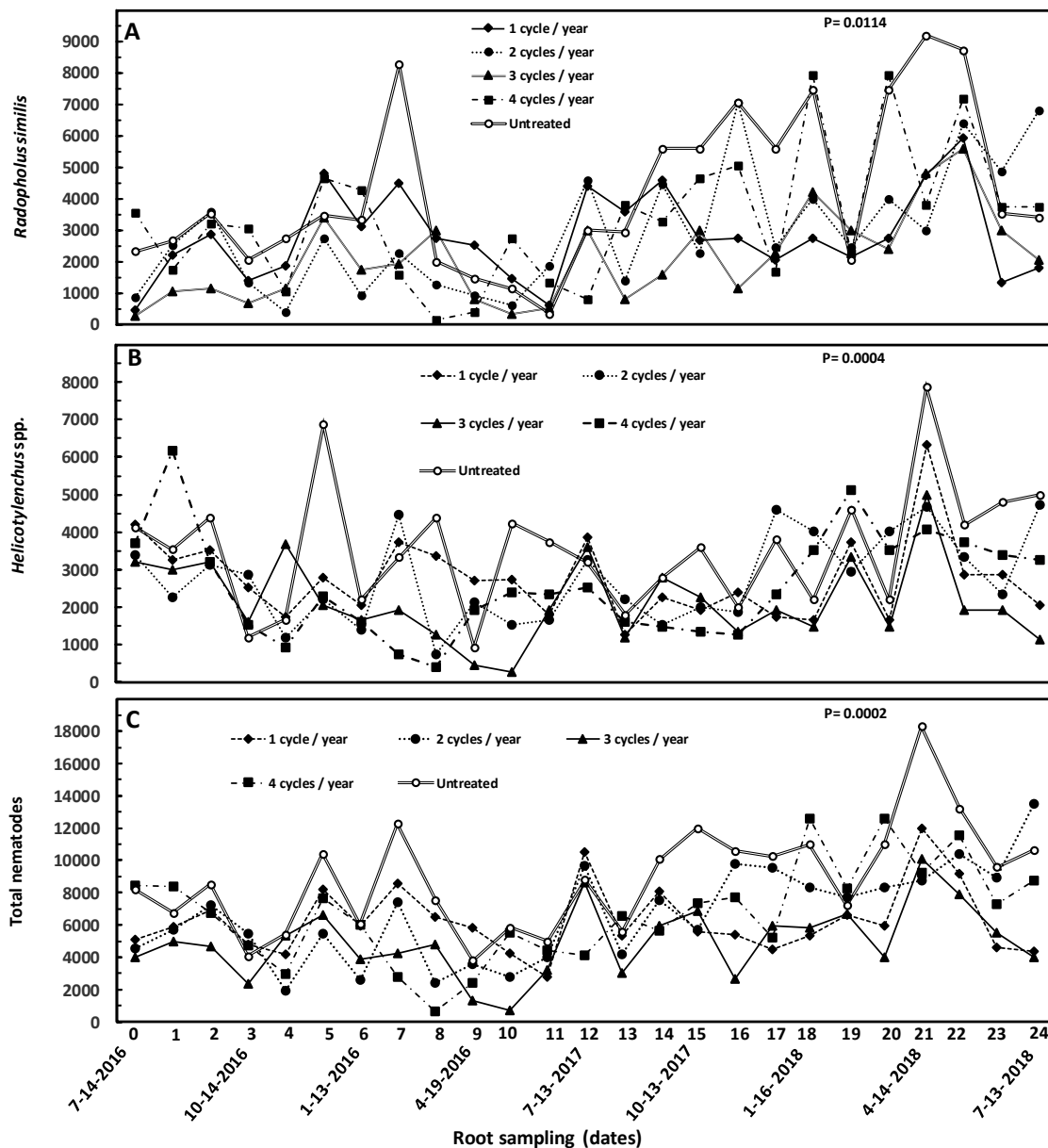
in all treatments increased the number of hands per bunch, between 0.3 to 0.7 (4-10 %), the ratio between 0.29 to 0.37 (25-31 %), and yield between 845 to 1, 273 (30-46 %) boxes per hectare per year.

In the third harvest, 24 months after the first treatments application, there was again a difference in ratooning ( $P < 0.0001$ ) varying between 1.65 in the untreated plants to 1.86 bunches per stool per year in those plants treated four times a year (Table 2). With respect to the untreated plants, ratooning increased between 0.05 to 0.21 (3-12 %) units as nematicide cycles per year increased, which means that the interval between harvests was reduced between 6.5 to 25 days, changing from 222.6 days at the beginning of the experiment to between 196.2 to 214.7 days. Similar trend followed the yield, which increased ( $P = 0.0061$ ) as nematicide cycles per year increased, varying between 4, 315 in the untreated plants to 5, 045 boxes in the plants treated three times a year. Regarding to the untreated plants, yield was increased between 226 to 730 (5-17 %) boxes per hectare per year as nematicide cycles per year increased. In bunch weight ( $P = 0.7723$ ) which varied between 40.7 to 42.7 kg, number of hands per bunch ( $P = 0.4518$ ) that fluctuated between 9.7 to 10.0, and ratio ( $P = 0.7711$ ) that varied between 1.86 to 1.95 boxes per bunch, no difference among treatments was found. Compared to the second harvest, all treatments increased yield between 746 to 1, 291 (21-34 %) boxes per hectare per year.

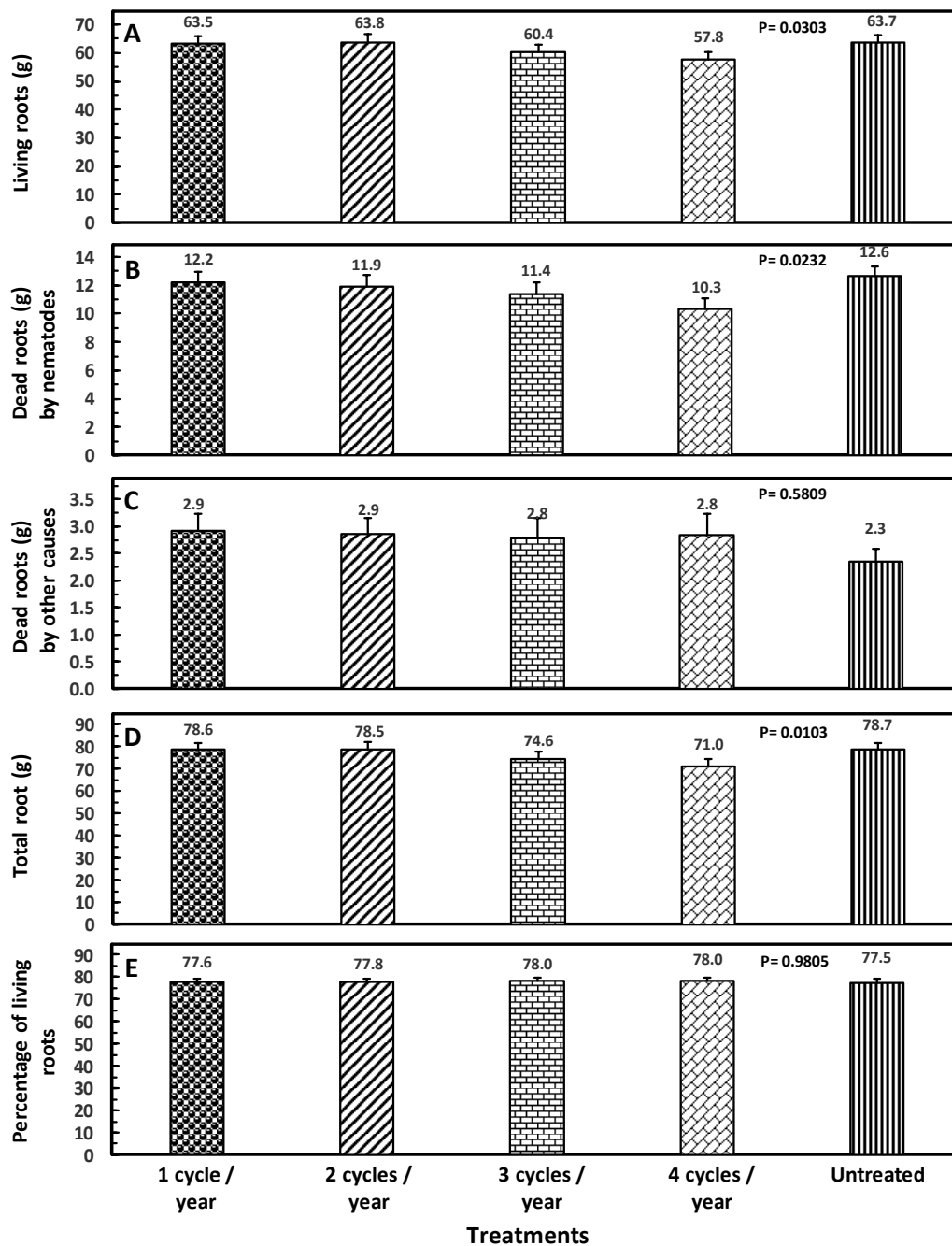
When checking the effect of ratooning in yield, it was found that with respect to the untreated plants at the first harvest (1.64 ratoon), treatments applied with nematicide increased the ratooning between 0.02 to 0.16 units, varying between 1.69 to 1.83 bunches per stool per year at 12 months, which means between 28 to 225 additional bunches per hectare per year, that multiplied by the ratio in each treatment resulted in an increase between 43 to 353 boxes of 18.14 kg per hectare per year. In the third harvest, at 24 months after the first treatments application, the increase in ratooning was between 0.05 to 0.21 units, from 1.65 in the untreated plants to 1.70-1.86 bunches per stool per year in plants treated with the different nematicide cycles per year, which means between 70 to 295 more bunches per hectare per year, that multiplied by the specific ratio of each treatment ended in an increase between 134 to 561 more boxes per hectare per year (Table 2). This means that the interval between harvests at 12 and 24 months was reduced between 2.6 to 18.6 and between 6.5 to 25.0 days in the nematicide treatments, while in the untreated plants, the interval was reduced in 4.0 days in the second harvest, and then extended in 2.6 days in the third harvest, changing from 222.6 days at the beginning of the experiment to 218.6 and 221.6 days between harvests at 12 and 24 months, respectively. The plant density was 1, 450 plants per hectare, of which 97 % of the bunches (1, 406) were processed. Then, when multiplied this number of plants harvested of 1, 406 bunches per hectare by the respective ratooning in the untreated plots, 2, 348 and 2, 320 bunches were harvested per hectare per year, at 12 and 24 months, respectively.



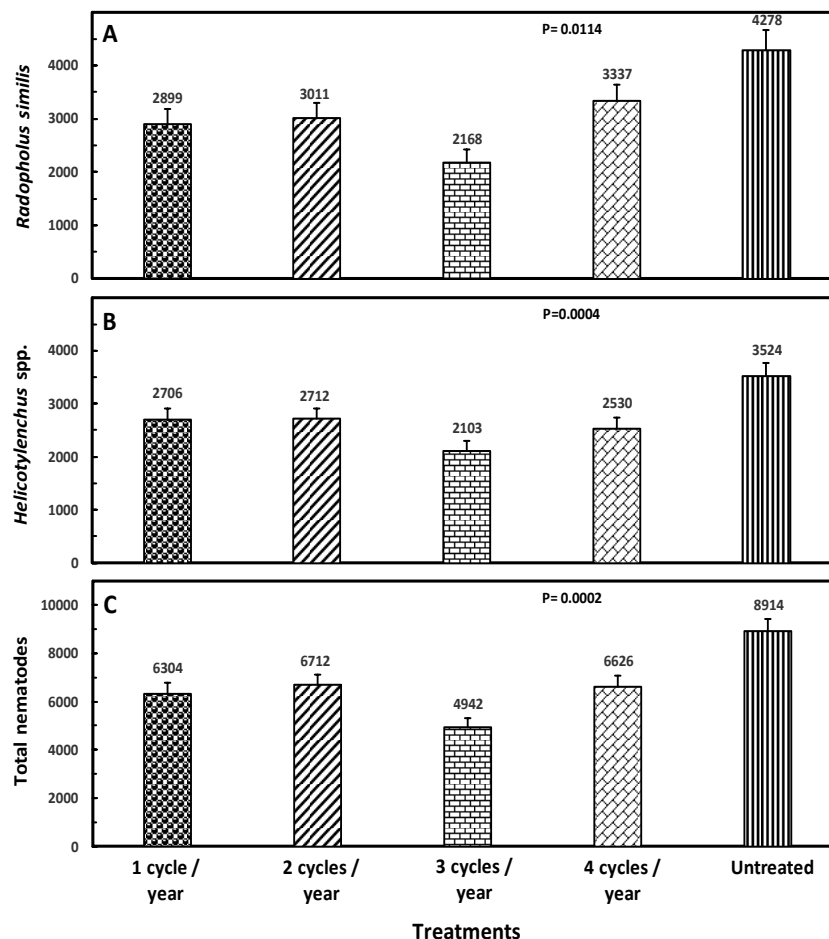
**Figure 1A-E:** Root content (g) by follower sucker and percentage of living roots per sucker in banana plants (*Musa* AAA cv Valery) treated with different number of nematocide cycles per year. Each point is the average of six repetitions. In each repetition, three follower suckers from 1.5-2.5 m height were excavated at its base and in front of it, making a hole of 20 cm long by 20 cm wide and 30 cm depth from where all the roots were collected.



**Figure 2A-C:** Number of nematodes per 100 g of banana (*Musa* AAA cv. Valery) roots treated with different nematocides cycles per year. Each point is the average of six repetitions. In each repetition, three follower suckers of 1.5-2.5 m height were dug in their base and in front, making a hole of 20 cm long by 20 cm wide and 30 cm depth from where all roots were collected.



**Figure 3A-E:** Average root content (g) per follower sucker and average percentage of living roots per follower sucker in banana plants (*Musa* AAA cv Valery) treated with different nematocidal cycles per year. Each bar is the average of 144 observations (24 samplings x six repetitions) and in each repetition the value is the average of three follower suckers. In each follower sucker, a hole 20 cm long by 20 cm wide and 30 cm depth was excavated at the base, and all roots were collected.



**Figure 4A-C.** Number of nematodes per 100 g of banana roots (*Musa* AAA cv. Valery) treated with different nematicide cycles per year. Each bar is the mean  $\pm$  standard error of 144 observations (24 samplings \* six repetitions) and in each repetition the value is the average of three follower suckers of 1.5-2.5 m high. A hole of 20 cm long x 20 cm wide and 30 cm depth was dug in front of each follower sucker and all roots were collected.

**Table 2:** Banana (*Musa* AAA cv. Valery) yield parameters as affected by nematicide treatments.

Treatment	Bunch weight Kg	Number of hands / bunch	Ratio	Ratoon	boxes ha <sup>-1</sup> year <sup>-1</sup>	Difference in boxes with untreated	Additional income US \$	Treatment cost US \$	Additional packing cost US \$0.75	Net income US \$	Net profit by dollar
First harvest at experiment set up, dehanging falls + 3											
1 cycle / year	26.5	6.7	1.21	1.64	2,790						
2 cycles / year	26.1	6.8	1.19	1.64	2,744						
3 cycles / year	26.1	6.6	1.19	1.64	2,744						
4 cycles / year	26.3	6.9	1.20	1.64	2,767						
Untreated	25.6	6.6	1.17	1.64	2,698						
Probability	P = 0.9622	P = 0, 1490	P = 0.9693		P = 0.9630						
Second harvest at 12 months after the first treatment application, dehanging falls + 1											
1 cycle / year	33.5	7.3	1.53	1.69	3,635	66	406	170	50	186	1.1
2 cycles / year	32.5	7.0	1.49	1.76	3,687	118	726	320	88	318	1.0
3 cycles / year	32.7	7.2	1.50	1.78	3,754	185	1,138	510	139	489	1.0
4 cycles / year	34.3	7.4	1.57	1.83	4,040	471	2,897	680	353	1,864	2.7
Untreated	33.2	7.3	1.52	1.67	3,569						
Probability	P = 0.7181	P = 0.8293	P = 0.7366	P<0.0001	P = 0.2812						
Third harvest after 24 months of the first treatment application, dehanging falls + 1											
1 cycle / year	41.5	9, 9	1.90	1.70	4,541	226	1,390	170	170	1,050	6.2
2 cycles / year	41.6	9, 7	1.90	1.78	4,755	440	2,706	320	330	2,056	6.4
3 cycles / year	42.7	10, 0	1.95	1.84	5,045	730	4,489	510	547	3,432	6.7
4 cycles / year	41.5	9, 9	1.90	1.86	4,969	654	4,022	680	490	2,852	4.2
Untreated	40.7	9, 7	1.86	1.65	4,315						
Probability	P = 0.7723	P = 0, 4518	P = 0.7711	P<0.0001	P = 0.0061						

Ratio = number of boxes of 18.14 kg per bunch (83 % of the bunch weight was packed (17 % rejection that includes 11 % bunch stalk and 6 % rejected bananas) per 18.14 kg by box. 1,450 plants per hectare from which 97 % of the bunches were processed (1,406 bunches), ratoon = number of bunches harvested by each banana stool by year, boxes per hectare per year = (1,406 bunches \* ratio \* ratoon). Each value is the mean of six replicates and in each replicate 15 bunches were harvested. Sale prices of each banana box was US \$6.15.



## 5. Discussion

No differences among treatments were found in root contents and nematode populations in the sampling done before treatments application. In the production variables evaluated at the time of establishing this experiment, also no differences were found. This means that any difference that was found after applying the treatments should be attributed to its effect. The four nematode genera detected are well known pathogens in banana roots (Gowen et al., 2005; Quénéhervé, 2008; Dubois and Coyne, 2011; Volcy, 2011; Guzmán-Piedrahita 2011a, 2011b, Sikora et al., 2018), and agreed with those found in Ecuador (Chávez et al., 2010, Aguirre et al., 2016a, 2016b, Jaramillo et al., 2019).

At the beginning of the experiment, the nematode population consisted mainly of *Helicotylenchus* spp. (64.1 %) and *R. similis* (23.5 %), reducing the proportion of *Helicotylenchus* spp. to 42.1 % at the end of the experiment, while *R. similis* increased to 44.6 % of the phytoparasitic nematode community, while *Meloidogyne* spp. and *Pratylenchus* spp. remain similar to the initial proportion with 10.3 % and 3 %, respectively. In Cavendish banana plantations, where the four nematode genera found here are presented, greater proportion of *Helicotylenchus* has been observed in conditions of insufficient nematode control, as reported earlier in Ecuador by Jaramillo et al. (2019). A similar behavior has been found in Costa Rica (Araya and Moens, 2005), and Belize (Salguero et al., 2016), where higher proportion of *Helicotylenchus* spp. was found in areas with insufficient nematode control. *Helicotylenchus* spp. is an ecto-endoparasite (Blake, 1966; Orion and Bar-Eyal, 1995; Guzmán-Piedrahita, 2011b, Sikora et al., 2018) that induces necrotic lesions on the surface of the roots. In contrast, *R. similis* is a migratory endoparasite that causes necrotic lesions along the entire root; in the epidermis, cortical parenchyma and vascular cylinder (Blake, 1966; Orton and Siddiqi, 1973; Jackson et al., 2003, Volcy 2011, Guzmán-Piedrahita 2011a, Sikora et al., 2018). The high population of *Helicotylenchus* spp. and *R. similis* was favored, because even though banana is an annual crop, its production is in perennial monoculture.

The reduction found in nematode population with the application of nematicide between 22 to 49 % for *R. similis*, 23 to 40 % for *Helicotylenchus* spp. and 25 to 45 % for total nematodes agreed with results of Jaramillo et al. (2019) in Ecuador who reported reductions between 20 and 49 % for *R. similis*, 31 to 50 % for *Helicotylenchus* spp. and 29 to 49 % for total nematodes. These percentage decreases in nematode population were also in parallel with Araya and Cheves (1997a, 1997b) in Costa Rica, who found reductions of 22-63 % for *R. similis* and 25-89 % for *Helicotylenchus* spp. and Moens et al. (2004), also in Costa Rica, who recorded drops between 18-59 % for the total phytoparasitic nematodes. Quénéhervé et al. (1991a; 1991b; 1991c) in Ivory Coast, indicated reductions of *R. similis* between 22.7 to 90.7 % and 32.5 to 100 % for *Helicotylenchus* spp., and Castillo et al. (2010) in Colombia found drops of 24 % for *R. similis*, between 38-60 % for *Helicotylenchus* spp., and between 25-33 % for total nematodes. In Belize, Salguero et al. (2016), found decreases between 33-47 % for *R. similis*,

36-65 % for *Helicotylenchus* spp. and between 35-59 % for total nematodes.

In parallel with the significant reduction of nematodes in treatments with nematicide, a significant lower content of dead roots by nematodes was registered in these treatments. The differences found in living roots and total roots was induced by a lower content in plants treated with four nematicide cycles a year. This probably means that when a plant had a healthy root system lower root mass is required. The classification of living roots, dead roots by nematodes and dead roots by other causes is subjective (visual) and depends on root symptoms. Roots infected by *R. similis* show reddish-brown lesions on the outer part of the roots penetrating throughout the cortex and then turns necrotic and *Helicotylenchus* spp. feeds on the outer cells of the root cortex, it produces a small-dashes reddish-brown to necrotic lesions. When roots are snapping either by excess soil humidity or by the presence of number of pathogens (fungi-bacteria) in the nematode-induced lesions, this probably hastens the destructions of roots. However, if the banana roots are still white and cream, it does not mean that they are free of nematodes. As indicated by Ayoub (1980), Mai (1985), McKenry and Roberts (1985) extensive loss of yield can occur when one or more nematode species may be feeding on a given plant, without showing obvious or specific plant symptoms. Here, maybe the nematode population, lower of 5, 000 per 100 g of roots, in many samplings, was not enough to develop root symptoms but it reduced ratooning and yield. This partially confirms the economic threshold suggested by INIAP (2018) of 2500 nematodes by 100 g of roots. It is known that in white-cream roots infected with nematodes histological and physiological cell alterations occurs (Blake 1966, Wyss 2002, Grunewald et al., 2009; Haegeman et al., 2010, Jones et al., 2016) which restrict water and nutrients uptake (Agrios 2005, Haegeman et al., 2010, Sikora et al., 2018).

Compared to the first harvest, when the experiment was set up, all yield variables (bunch weight, number of hands, ratio, boxes per hectare per year) were improved 12 months after the first application in all treatments, including the untreated plants, but without difference among treatments. This improvement resulted mainly from the changed in bunch dehanding, that was modified from falls + 3 in the first harvest to falls + 1 when the experiment started, which means that all bunches were harvested with more hands in the second harvest. The grower shifted the buyer and started to sell to a company that has a specialty market with specifications for large, medium, and small fruit.

The improvement in ratooning found in the second (0.02 to 0.16 units) and third harvest (0.05 to 0.21 units) with the nematicide cycles means that the interval between harvest was reduced between 2.6 to 18.6 and between 6.5 to 25 days, respectively, in agreement with Quénéhervé et al. (1991b), who found a cumulative reduction in time to harvest according to the cycle of 28 days in the first, 57 days in the second and 128 days in the third harvested cycle in plants treated with nematicide. Similarly, Quénéhervé et al. (1991a) and Gowen (1995) reported an increase in the harvest period from 13 to 32 and from 22 to 40 days, respectively, in plants infected with nematodes that were not

treated compared with those applied with nematicide. In congruence with this extension in the period to harvest, Roderick et al. (2012) reported an increase of 13.6 more days to harvest in Mbwasirume banana plants to which they added nematodes compared to plants without the addition of nematodes.

The highest number of boxes per hectare per year was due to the application of nematicide that resulted in a significant reduction of nematodes, which led to an increase in the percentage of living roots that favored water and nutrients up take, allowing a better growth of the crop, which led to a higher ratooning. In the second and third harvest, nematicide treatments improved yield between 66 to 471 (1.1 to 8.5 tm) and between 226 to 730 (4 to 13.2 tm) more boxes per hectare per year than plants of the untreated plots, at 12 and 24 months, respectively, of the applied treatments. The lower increase in yield in the second harvest was due to the nematode control done in the farm before the experiment was established of 1.5 to 1.8 nematicide cycles per year. Since in a commercial banana plantation different phenological stages (peppers, suckers in different vegetative growth, flowering and fruiting plants) are present at the same time, which allows fruit harvest all year around, in the second harvest most of the harvested plants still had the nematode control effect in the untreated plots, while in the third harvest, all harvested plants were free of nematode control. Additionally, in treated plots, those with three and four nematicide cycles a year showed higher yield indicating that the 1.5 to 1.8 cycles used by the farm was insufficient to prevent nematode damage in an area with 2, 700 boxes of 18.14 kg per hectare per year. These results confirm that banana nematodes are serious threat to banana production in Ecuador as was found by Jaramillo et al. (2019) and agreed with Dita et al., (2013) thoughts, that nematodes continues to be a serious threat to banana production in Latin America and the Caribbean.

The percentages of yield increase varied between 2-13 % and 5-17 % at 12 and 24 months, respectively, which were agreed with some of the percentages compiled by Gowen and Quénéhervé (1990), who mentioned increases from 14-263 % and Gowen (1995), who cited increases from 5 to 275 % and were lower than that reported by Stanton and Pattison (2000) of 46 %. The increased in production found were in line with that reported by Quénéhervé et al. (1991b), who indicated increases in production between 523 to 1, 157 boxes (9.5-21 tm), with Pattison et al. (1999) who reported increases between 655 to 953 boxes of 13 kg (8.5-12.3 tm), with Salguero et al. (2016), who found increases between 545 to 832 boxes of 18.14 kg (9.9-15.1 tm), and was lower than that reported by Araya and Lakhi (2004), who cited increases of 1, 245 boxes of 18.14 kg (22, 6 tm) per hectare per year, controlling nematodes through the application of nematicides.

The highest yield (number of boxes per hectare per year) was observed in plants treated with three nematicide cycles per year in parallel with that reported by Jaramillo et al. (2019) in Ecuador and Araya (2003) in Costa Rica, who registered higher yields as the number of nematicide cycles per year increased in banana plantations infected with nematodes. These increased in production as a result of

nematodes control were in parallel with Guerout (1972), Charles et al. (1985), Quénéhervé et al. (1991a, 1991b), and Salguero et al. (2016), who cited negative and significant linear correlations between the populations of *R. similis*, *Helicotylenchus* spp. and total nematodes with bunch weight in bananas.

The high population of *Helicotylenchus* spp. and the increased achieved in production with the application of nematicide indicated that their parasitism reduces growth, development and production in accordance with observations by McSorley and Parrado (1986), Gowen and Quénéhervé (1990), Chau et al., (1997), Barekye et al. (1998, 2000), Gowen (2000), Ssango et al. 2004, Guzmán-Piedrahita (2011b), Coyne et al. (2013), Salguero et al. (2016) who reported that *H. multicinctus* and *H. dihystra* damaged the banana root system and reduced yield between 19 % (Speijer and Fogain, 1999) and 34 % (Reddy 1994). Additionally, Sijmons et al. (1994) indicated that the induction and maintenance of feeding sites of *Helicotylenchus* spp. causes physiological changes in the structure of cells. In the case of *R. similis* it was well supported that it reduced the yield in banana (Gowen and Quénéhervé 1990, Gowen 1993, 1995, Araya 2004, Roderick et al., 2012, Coyne et al. 2013).

The presence of nematodes with different parasitic habits; *R. similis* migratory endoparasite and *Helicotylenchus* spp. an ecto-endoparasite most likely exacerbates root damage since lesions can develop at feeding sites and through root tissue. In addition, plants often activate post-infection resistance mechanisms, even in cases where the population of nematodes increases over time, and the nematode-plant interaction is compatible. Therefore, together these processes can represent a high energy expenditure for plants that can interfere with the filling and development of the bunch. Given that both nematode genera cause damage to the crop, for the implementation of options for their management, the population of all the phytoparasitic nematodes present should be considered, as has been suggested by Araya (2004), Ramclam and Araya (2006), Salguero et al. (2016), and Aguirre et al. (2016a; 2016b). During the development of the experiment, the market price of a box of 18.14 kg of bananas was US \$6.15 and of a nematicide application cycle including the application cost was Counter® 15FC \$150, Verango® \$200, Vydate® 24SL \$150, Rugby® 10GR \$160, Mocap® 15GR \$170 per hectare. The cost of the fertilizer, control of black Sigatoka and weeds, and other tasks was the same for the control plots and those treated with nematicide, since the increase recorded was for ratooning. The additional net income from the increase in yield, deducted the cost of labor of \$0.75 of packing for each additional box and the cost of the product and its application was from US \$186 to \$1, 864 at 12 months and from \$1, 050 to \$3, 422 per hectare per year 24 months after the treatments were applied. This net gain agrees with that indicated by Jaramillo et al. (2019) who found amounts between \$2, 550 to \$5, 759 and Pattison et al. (1999) who reported amounts between \$2, 494 to \$5, 910 per hectare per year. This means, that for every dollar invested in nematode control, at 12 months, the net profit ranged from US \$1.0 to \$2.7 and at 24 months from \$4.2 to \$6.7. In the second harvest, the highest production was in

the plants that received four nematicide cycles per year, while in the third harvest, the highest yield was found with three nematicide cycles per year. This suggest that after lower the nematode population, three cycles a year should be enough to prevent the nematode damage at this condition.

## 6. Conclusion

Nematicide treatments reduced nematode population and improved crop yield, ending with a net profit between \$1050 to \$3452 more by hectare by year, which confirmed that nematodes are a serious pest to banana production.

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