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Effect of different nematicide applications per year on banana (*Musa* AAA) root nematode control and crop yield

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ABSTRACT

Objective: The objective of this study was to evaluate the effect of different nematicide applications per year on control of banana root nematode, root weight and crop yield in Belize. The relationship between cost and benefit of the nematicide applications was also estimated.

Methodology and results: A field experiment was conducted in a 40 years old commercial banana (*Musa* AAA cv. Grande Naine) plantation from November 2011 to February 2013. Four treatments were evaluated: 1. Three nematicides (Nemacur®, Mocap®, Vydate®) applications per year with a 4-month interval, 2. Two nematicides (Nemacur®, Mocap®) applications per year with a 6-month interval, 3. Nematicides applied based on nematode threshold (100 nematodes per g of fresh root) which resulted in two applications; Nemacur® and Mocap® with a 7-month interval, and 4. An untreated control. Averaging the 12 root nematode samplings, the lowest *R. similis* ($P= 0.0008$), *Helicotylenchus* spp. ($P< 0.0001$) and total nematode ($P< 0.0001$) population were observed in the plots treated with the three nematicide applications per year. Compared with the untreated control, the three nematicide applications reduced *R. similis* in 53%, *Helicotylenchus* spp. in 48% and the total nematode population in 53%. Even though the three nematicide applications per year resulted in higher *R. similis* control efficacy with 42.4%, no difference ($P= 0.6372$) was detected with two 32% (at 6-month interval) applications per year, and two 33% (at 7-month interval) applications per year, based on nematode population threshold. For *Helicotylenchus* spp. ($P= 0.0047$) and total nematodes ($P= 0.0018$), three applications were better than two at 6-month interval or two applications per year based on nematode population threshold, with 65.3 and 58.5% of efficacy on nematode control, respectively. No difference in total root weight ($P= 0.9812$) and functional root weight ($P= 0.7742$) was observed among treatments, varying from 88 to 90 and 73 to 79 g / plant, respectively. At harvest, 12 months after the nematicide applications, bunch weight was increased ($P= 0.0013$) in 7.2 (41%), 4.8 (27%) and 4.7 (27%) kg per bunch resulting in an extra gain of \$2468, \$1660 and \$1427 ha^{-1} with three, two at 6-month interval and two applications per year based on nematode population density, respectively.

Conclusions and application of findings: The non-fumigant nematicide applications reduced banana root nematodes and improved crop yield. Rotation of the nematicides according to their physic-chemical properties and weather conditions is desirable in order to prevent their biodegradation.
Key words: Chemical control, *Musa* AAA, nematode control, nematicides.

INTRODUCTION

Banana (*Musa* AAA) is the most important crop in Belize accounting for almost 16% of the agricultural gross income. In 2014, 102118 tonnes were exported to the United Kingdom, produced on an area of 3000 ha, which gave a total income of US $45.5 million. Besides the constraints of banana market requirements and demands, there are other limiting factors. Considering the abiotic factors affecting yield, edaphic soil condition is a constraint to banana production mainly due to reduced soil depth, clay texture and poor structure. Among the biotic factors, banana-root nematodes are second after black Sigatoka caused by the fungi *Mycosphaerella fijiensis,* Morelet. Banana nematodes live within or around the roots, where they weaken plant anchorage and restrict water and nutrients uptake, retard leaf emission and reduce photosynthesis, bunch weight, ratio, ratooning, and plant longevity. In the 23 Belize banana plantations located in the Stann Creek and Toledo districts, nematodes are common (Ramclam and Araya, 2006) and usually only polyspecific communities occur, consisting of a mixture mainly of *Radopholus similis* (Cobb 1893, Thorne 1949, Sher 1968) and *Helicotylenchus* spp. To avoid or reduce nematode damage, the only alternative management strategy currently available, is the regular application of non-fumigant nematicides, which are economically feasible. Nematicide application is recommended when the total nematode population exceeds the economic threshold of 100 individuals per gram of fresh roots collected between the mother and its follower and extracted by the root maceration method (Taylor and Loegering, 1953, modified by Araya, 2002) recovering the nematodes on the No 500 (0.025 mm) mesh. The nematicides approved by the USA Environmental Protection Agency (EPA) and Codex Alimentarius Commission are rotated according to their physic-chemical characteristics and weather condition to prevent their biodegradation. However, in Belize most banana growers do not apply nematicide, which has resulted in high nematode population, root damage and severe yield reduction. The objective of this study was to evaluate the effect of different nematicide applications per year on banana root nematode control and crop yield.

MATERIALS AND METHODS

Crop management: The field experiment was carried out in a 40 years old commercial banana (*Musa* AAA cv. Grande Naine) farm located at Stann Creek district, Belize with a plant density of 2000 plants ha^{-1}. 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 soil was a Typic (Fluventic) Dystrudepts (silty loam: 12% sand, 61% silt and 27% clay) with 2.7% organic matter and 5.7 pH. The following concentrations of extractable bases were found, using Mehlich 3 (Mehlich, 1984) as the extractant: Ca 5.8, Mg 3.5 and K 0.9 $cmol^{(+)}$ kg^{-1}. Average rainfall in the area from 2011 to 2012 was 2041 mm distributed throughout the year. February and April were the driest months in 2012 with 94 and 14 mm, respectively. During the dry season, from February to May, supplementary sprinkle irrigation was used. Excess superficial water and water logging during heavy rains were prevented by a complex system of primary, secondary and tertiary drains. Mean daily maximum/minimum temperatures were 27-32/20-23 ^oC. The follower of each production unit was fertilized every 30 days with a mixture of nutrients adapted to the soil requirements, consisting of 23-0-30 (N-P_2O_5-K_2O) at 180 (90 g/follower) kg ha^{-1}. Black Sigatoka (*Mycosphaerella fijienses*) control was done with the rotation of chemical fungicides every 10-14 days with either mancozeb, chlorothalonil, pyrimethanil, difenoconazole, tridemorph, thiram, or epoxiconazole in emulsion with miscible oil and water, uniformly applied in a total volume of 22 L ha^{-1} and removing infected parts from the leaves. Weeds were controlled by regular applications of paraquat 1.5 L in 500 L of water ha^{-1} or glyphosate 1 L in 500 L ha^{-1} every 6-8 weeks.

Treatment description: The following treatments were evaluated: 1. Application of three nematicides (Nemacur® 15GR Biodac fenamiphos AMVAC, Mocap® 15GR Biodac ethoprophos AMVAC, Vydate® 24SL oxamyl Dupont) per year with a 4-month interval, 2. two nematicide (Nemacur® 15GR, Mocap® 15GR) applications per year with a 6-month interval, 3. Application of nematicide based on exceeding the nematode threshold (100 nematodes per g of root) which resulted in two applications Nemacur® 15GR and Mocap® 15GR with at 7-month interval, and 4. An untreated control (without application of nematicide). The rectangular plots for each treatment consisted of 125-150 production units. Plots were arranged in a randomized complete block design with six replicates. When the experiment started, all plots except those from the untreated control were applied with Nemacur® 15GR-Biodac on November 2011. The second nematicide application was done with Mocap® 15GR-Biodac in treatment 1 on March 27, 2012; treatment 2 on June 4, 2012; and treatment 3 on July 20, 2012. The third nematicide application in treatment 1 was with Vydate® on July 28, 2012. The nematicides were spread in a banded arc with radius of approximately 0.40 meter around each follower sprouting from the base of the sucker, using the Swissmex backpack equipment specific for Nemacur® and Mocap® and the spotgun for Vydate®. The rates used per follower were 3 g a.i. for Nemacur® and Mocap® and 2.4 g a.i. for Vydate®. Plant debris was removed from the soil surface prior to distributing the nematicides onto moist soil as directed by the product label.

Root sampling: One day before the nematicide application and every 30 days thereafter, root samples were collected up to 12 months after the first application. Each sample consisted of roots from three production units. Root samples were taken from a hole of 20 cm length, 20 cm wide and 30 cm depth (soil volume of 12 L) dug at the plant base between the mother plant and its follower using a shovel. All the roots from this 12 L soil volume were used for determining the weight (g) of total and functional roots. Functional roots were those that had no necrosis or root decay as opposed to non-functional roots. In some roots, it was necessary to cut some damaged parts, which were classified as non-functional roots. The remaining part was considered a functional root. The total root weight corresponds to the sum of the functional and non-functional roots.

Nematode extraction: Nematodes were extracted from 25 g of fresh functional roots, which were macerated in a kitchen blender (Taylor and Loegering, 1953, modified by Araya, 2002) for 10 sec at low and 10 sec at high speed, and the resulting mixture was washed from the blender through a series of nested sieves of (0.5/0.150/0.025 mm (No 60/140/500 sieves). The residue on the 0.5 and 0.150 mm mesh sieve was discarded and that on the 0.025 mm mesh washed off into a 250 ml beaker and the nematodes were counted from 2 ml aliquots. The number of *R. similis, Helicotylenchus spp.* and total nematodes (summary of both genera) were expressed per gram of root. The nematode population composition before nematicide application was determined for all plots, and then for the average of the 12 samplings after the application for treated and untreated plots, separately.

Crop yield: At the beginning of the experiment and 12 months after the first nematicide application, 15 bunches from each experimental plot, selected randomly without considering edge plants, were weighed (Kg) and their number of hands recorded. Each bunch was trimmed to false + 2 hands 15 days after the flower emerged and covered with a plastic bag. First harvesting started in November 2011 and ended in January 2012 and second harvest was from November 2012 to January 2013.

Statistical analysis: Root and nematode data were averaged by experimental plot across the 12 months excluding the first evaluation. Data of total and functional root weight was subjected to ANOVA by Proc Glm of SAS for the first evaluation alone, before the nematicide application, and thereafter for the average of the 12 nematode samplings together, after the application with a factorial structure of 4 treatments times 12 evaluations, since nematode sampling were made in different plants. 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 12 nematode samplings together after the application, with the same factorial structure as before. The efficacy (Abott, 1925) on nematode reduction was calculated, using the data after the nematicide application, as percentage of reduction in number of nematodes, with respect to the untreated control, which were subjected to ANOVA and mean separation by LSD-test. Bunch weight and number of hands per bunch were subjected to ANOVA and mean separation using LSD-test in PC-SAS® version 9.4. The average of the root and nematode data of the 12 nematode samplings were linearly (Pearson coefficients) correlated with bunch weight and number of hands of the second harvest.

RESULTS

Nematode population composition: Pre-treatment nematode population was composed of 33% *R. similis* and 67% *Helicotylenchus* spp. After the nematicide applications, averaging the 12 evaluations, the proportion of *R. similis* and *Helicotylenchus* spp. was 33 and 67%, and 43 and 57% in the untreated and treated plots, respectively.

Nematode population and root content: In the root sampling done before the nematicide application, no difference among treatments was found for root weight, varying from 67-83 g / plant (P= 0.3159), functional root weight with 56-70 g / plant (P= 0.3257), *R. similis* population varied from 41-105 (P= 0.2970), *Helicotylenchus* spp. from 127-141 (P= 0.9924), and total nematode population from 176-237 (P= 0.7839) / g of root. The root weight (g) and nematode population per g of root through the 12 months after the nematicide application is depicted in Fig 1A-E. In all treatments, a slight increase in root weight was observed until May that thereafter decreased. With respect to the nematode population, a lower population was observed in those treated plots until July. Averaging the whole period of 12 months, no difference in total root (P= 0.9812) and functional root (P= 0.7742) weight was recorded varying from 88-90 and 73-79 g / plant, respectively (Fig 2A-B). All three treatments of nematicides reduced *R. similis* (P= 0.0008), *Helicotylenchus* spp. (P< 0.0001) and total nematodes (P< 0.0001) per gram of root (Fig 2C-E). The highest reduction was detected in the three nematicide applications per year with 47% for *R. similis*, 65% for *Helicotylenchus* spp. and 59% for total nematodes. In the treatments with two nematicide applications per year, every 6 months or every 7 months, corresponding to the nematode threshold of 100 g individuals per gram of root, the reduction in *R. similis* was 33 and 35%, in *Helicotylenchus* spp. was 36 and 52%, and in total nematodes was 35 and 46%, respectively.

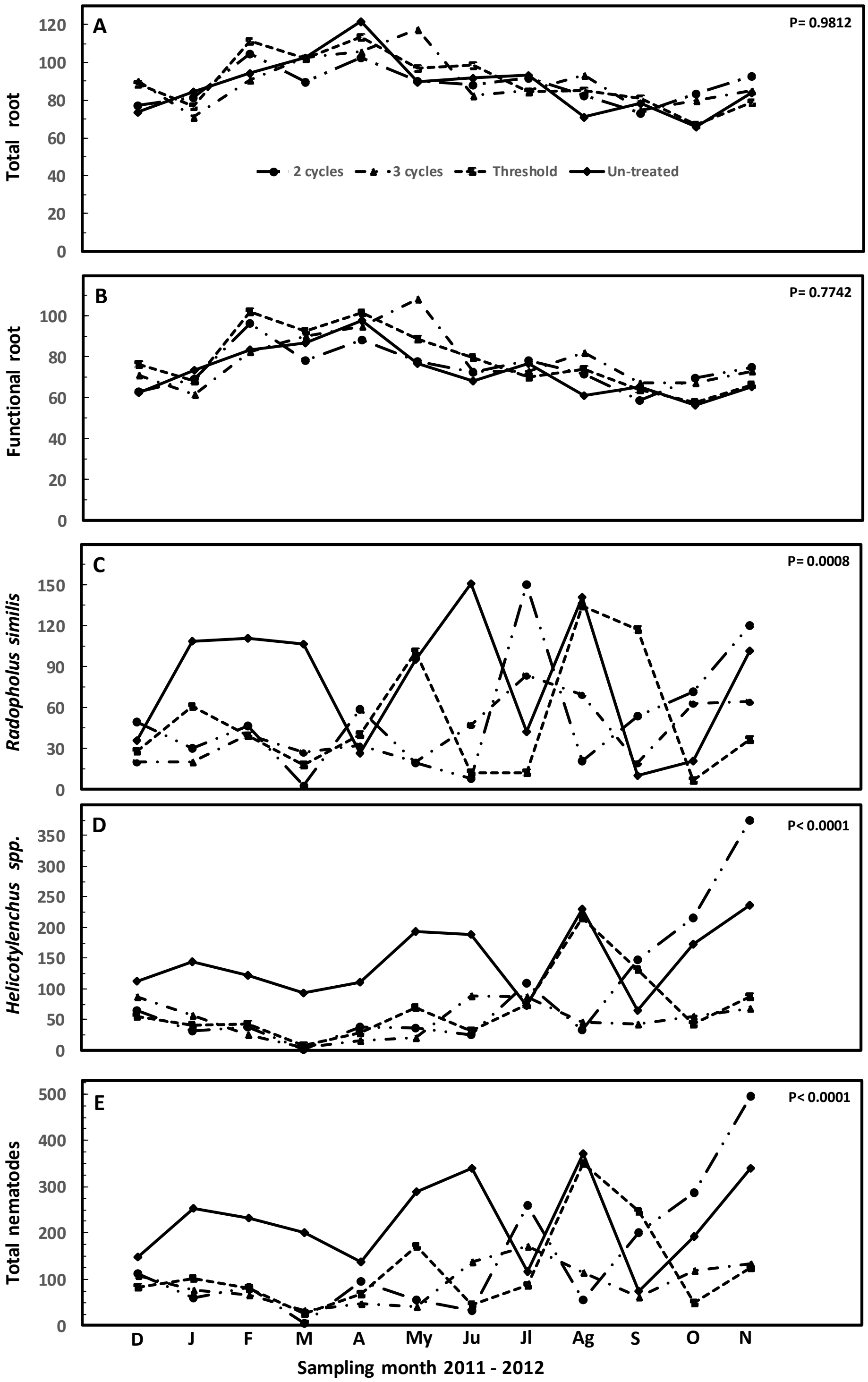

Figure 1A-E. Root weight (g) per plant and number of nematodes per g of root during the 12 samplings of the experiment after nematicide application for each treatment. Each point is the mean ± standard error of 6 repetitions and in each repetition, the root sample comes from three production units.

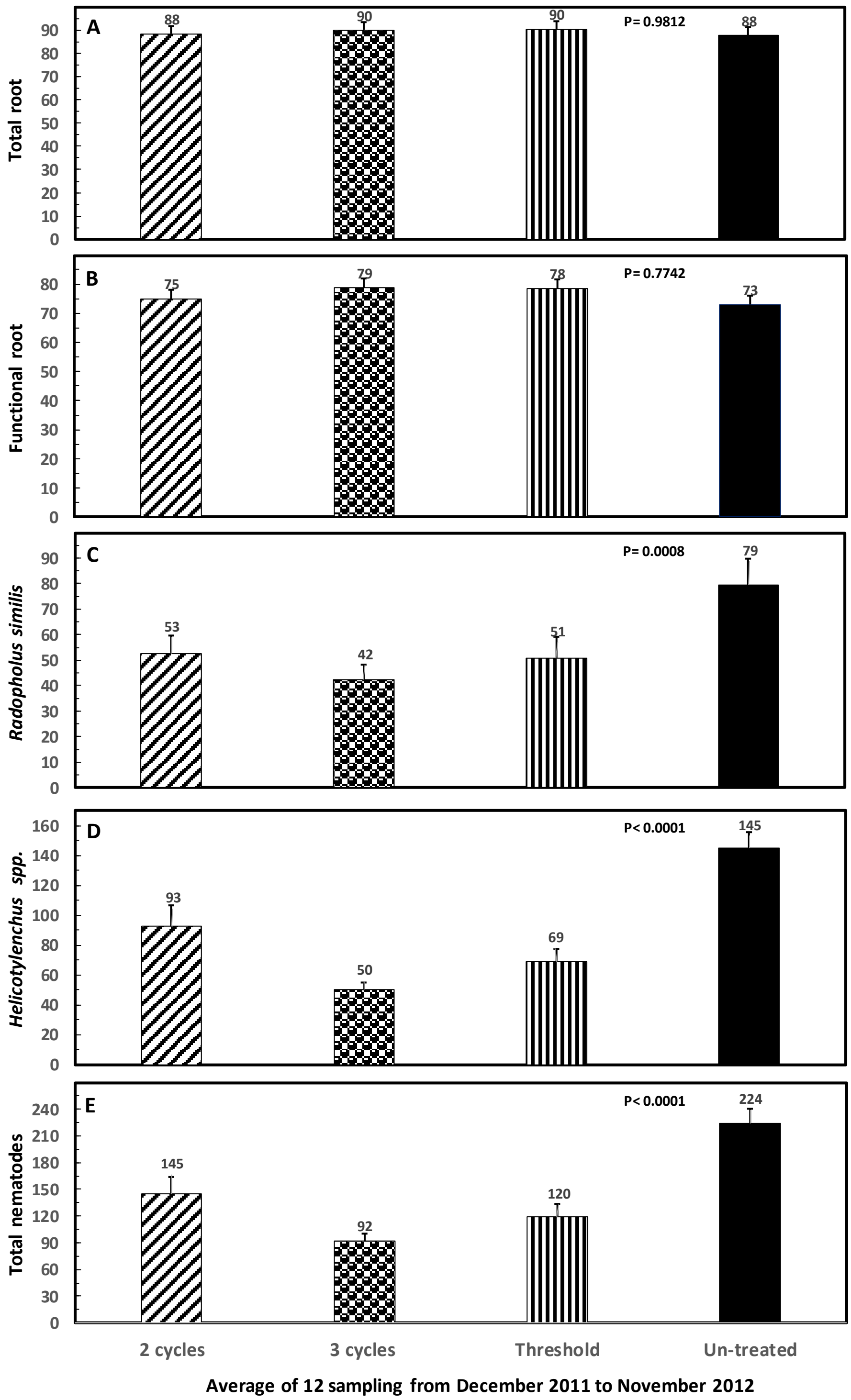

Figure 2A-E. Root weight (g) per plant and number of nematodes per g of root averaged of 12 samplings for each treatment. Each bar is the mean ± standard error of 72 observations (12 samplings x 6 repetitions, in each replicate the root sample comes from three production units.

Efficacy on nematode control: For *R. similis*, no difference (P= 0.6372) in efficacy was observed varying from 42% with three nematicide applications per year to 32 and 33% with two applications per year (Fig 3A). For *Helicotylenchus* spp. (Fig 3B) and total nematodes (Fig 3C) three applications per year gave the highest efficacy with a 65% reduction (P= 0.0047) and 59% reduction (P= 0.0018), respectively.

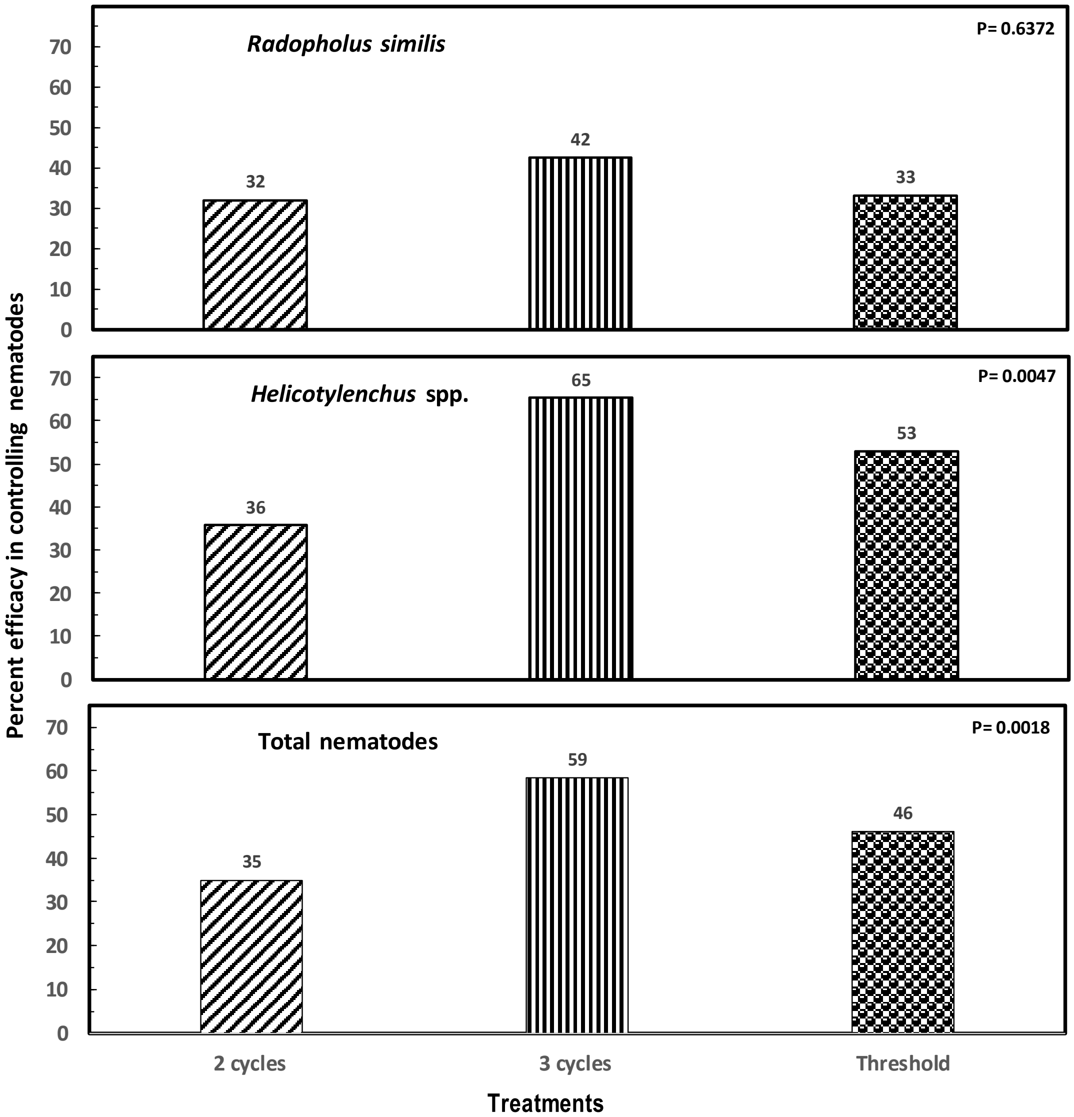

Figure 3A-C. Percentage efficacy on banana (*Musa* AAA) root recovery and nematode control. Each bar is the mean ± standard error of 72 observations (12 samplings x 6 repetitions).

Crop yield: At the beginning of the experiment no difference in the number of hands (P= 0.8680) per bunch which varied from 5.4 to 5.5 or bunch weight (P= 0.5621) varying from 13.8 to 14.8 kg was detected among treatments (Fig 4A-B). A year later, the number of hands improved slightly, 0.2 hands per bunch, however this difference was not significant (P= 0.1813). The use of nematicide clearly augmented (P= 0.0013) bunch weight. Compared to the untreated control, three nematicide applications per year increased bunch weight by 7.2 kg (41%) and two nematicide applications by 4.8 (27%) and 4.7 kg (27%) when applied at either a 6 or 7 (threshold) months interval. A significant, negative linear correlation was found between bunch weight and the number of *R. similis* (r= -0.70, P= 0.0001), *Helicotylenchus* spp. (r= -0.66, P = 0.0004) and total nematode (r= -0.75, P< 0.0001) population. No correlation (P> 0.8547) was detected between total and functional root weight and bunch weight.

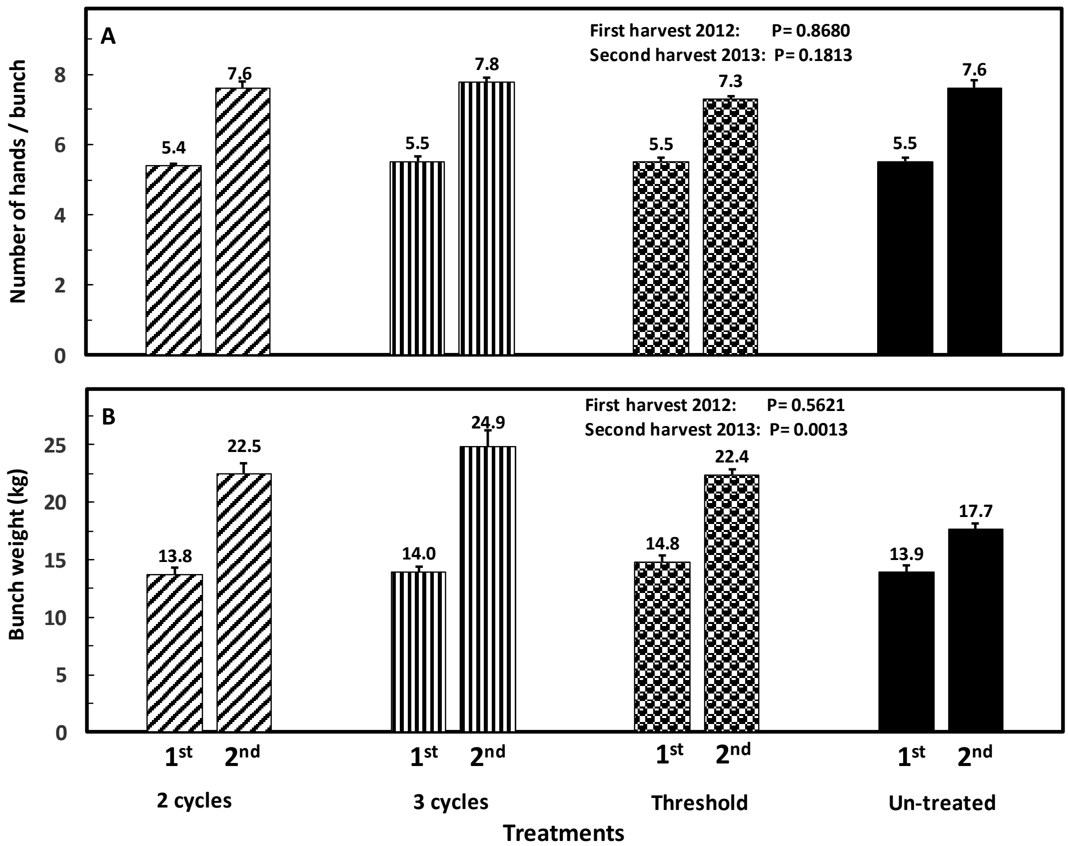

Figure 4A-B. Effect of the nematicide applications per year on number of hands per bunch and bunch weight in banana (*Musa* AAA cv. Grande Naine) before treatment effect (first harvest 2012) and after treatment effect (second harvest 2013). Each bar is the mean of 90 observations (6 repetitions and in each repetition 15 bunches were recorded).

DISCUSSION

There were no differences among treatments for root weight, nematode populations and crop yield before the nematicide treatment, that means that any difference detected later should be attributed to treatment effect. The nematode population consisted mainly of *Helicotylenchus* spp. and *R. similis*. This agrees with the proportion of nematodes observed in nematode infested Cavendish banana plantations without an effective nematode control, where the predominant nematode was *Helicotylenchus* spp. (Araya and Moens, 2005). *Helicotylenchus* spp. is and ecto-endoparasite (Blake, 1966; Orion and Bar-Eyal, 1995; Gowen 2000) and induces necrotic lesions on root surface. *Radopholus similis* is a migratory root endoparasite causing necrotic lesions through intra- and intercellular migration in the cortex (Blake, 1966; Orton and Siddqi, 1973; Jackson *et al*. 2003). The high population densities found for *Helicotylenchus* spp. and *R. similis* are fostered by the lengthy banana monoculture, absence of effective control measures and neglect of quarantine measures. The application of either two or three nematicides per year on soil surface reduced *R. similis*, *H. multicinctus*, and total nematode populations. However, the highest nematode reduction was observed with three nematicide applications per year varying from 47 to 65%. This reduction in nematode population agrees with results obtained by Quénéhervé *et al*. (1991a, 1991b, 1991c), Araya and Cheves (1997a, 1997b), Moens *et al*. (2004), Araya and Lakhi (2004), Castillo *et al*. (2010) who had tested different nematicides for banana root nematode control applying them on the soil surface. Even though a significant nematode reduction was observed, the increase in total and functional root weight was not significant. Most likely that was due to the narrow magnitude of the difference among treatments in root content after July. The precipitation varied from 195.4 to 315.5 mm per month from August to December 2013 resulting in soil anoxia in the conditions where the experiment was established which is known to affect root growth and development (Valverde, 1998). Percentage efficacy in nematode control varied from 23 to 42% for *R. similis*, 36 to 65% for *Helicotylenchus* spp. and 35 to 59% for total nematodes, with the best control seen with three nematicide applications per year. Those percentages are within the rates reported by Araya and Cheves (1997a, 1997b) and Calvo and Araya (2005) controlling nematodes on Cavendish banana roots with granular non-fumigant nematicides applied on soil surface. For three nematicide applications per year, the percentage control found for *Helicotylenchus* spp. and total nematodes is within the range of 50-90% cited by Van Gundy and McKenry (1977) and Schmitt (1985). The nematicide application improved crop yield, which is in agreement with results from Quénéhervé *et al*. (1991a, 1991b, 1991c), Pattison *et al*. (1999), Stanton and Pattison (2000), Araya and Lakhi (2004), and Castillo *et al*. (2010). The higher yield is probably due to the effect of the nematicide applications, which resulted in a significant nematode reduction, the non-significant increase in root content, which improved the root system health favoring water and nutrient uptake by the plant resulting in better bunch growth and development. Plants with a damaged root system have a lower capacity to absorb water and nutrients and have a compromised photosynthetic capacity. The high *Helicotylenchus* spp. population means that its parasitism most likely resulted in a significant bunch weight reduction, in agreement with observations of McSorley and Parrado (1986), Gowen and Quénéhervé (1990), Chau *et al*., (1997), Barekye *et al*. (1998, 2000), Gowen (2000) who reported that *H. multicinctus* and *H. dihystera* can damage the banana root system, and reduced yield between 19% (Speijer and Fogain, 1999) and 34% (Reddy, 1994). *Radopholus similis* is well known to cause damage to banana roots and thus yield (Gowen and Quénéhervé, 1990; Gowen, 1993, 1995). Therefore, development of nematode management tactics requires consideration of the damage caused by the total phytonematode population as has been suggested by Araya (2004), Ramclam and Araya (2006), Chávez and Araya (2010). It is known that induction and maintenance of *Helicotylenchus* spp. feeding sites causes physiological changes in cellular structure (Sijmons *et al*., 1994). *Radopholus similis* damages the cells of the epidermis and the central cylinder of banana roots, which explains the necrosis observed in the roots. The presence of different parasitic habits of the migratory endoparasitic (*Radopholus*) and ecto-endoparasitic (*Helicotylenchus*) nematodes are likely to exacerbate root damage, because lesions can develop at feeding sites throughout the root tissue. Moreover, plants often activate post-infection resistance mechanisms, even in cases where there is an increase in nematode population over time and the nematode-plant interaction is considered compatible. Therefore, together these processes probably represent a high expenditure of energy for the plants, and they may interfere with bunch filling and development. Additionally, the experiment was run on a low fertility soil, estimated by the sum of cations (Ca + Mg + K) plus extractable acidity in 10.9 and Braide and Wilson (1980) reported higher nematode damage in soils of poor fertility. The highest yield increase was found with the three nematicide applications per year in agreement with that found by Araya (2003) who recorded higher yield as the nematicide applications per year increased in banana plantations infested with nematodes. The significant, negative linear correlation found between bunch weight and populations of *R. similis*, *Helicotylenchus* spp. and total nematodes partially agrees with results obtained by Guerout (1972), Charles *et al*., (1985), Quénéhervé *et al*. (1991a, 1991b) who reported significant correlations between *R. similis* populations and banana yield parameters. The application of nematicide increased the bunch weight in 7.2 (41%), 4.8 (27%) and 4.7 (27%) kg with three, two at 6-month interval, and two at 7-month interval, applications per year, respectively. This corresponds to an improvement of 15.1 (812 boxes of 18.65 kg), 10.2 (548 boxes) and 9.9 (533 boxes) mt in yield, assuming 1800 harvested plants ha^{-1}, a ratooning of 1.3 which is the mean value of the farm, and a ratio of 0.9 considering the bunch weight with two nematicide applications of 22.4 kg with 10% of rachis and 15% of banana fruit rejection. During the study period, the market price for one nematicide application averaging the cost of the three products used was about US$241 per hectare including the labor. The cost of fertilizer, weed and black Sigatoka control, desuckering, propping, bagging, harvesting, transport of bunches to the packing house, and others were the same for treated and untreated plots, because the increase recorded was in bunch weight and not in number of bunches. The additional income for the 15.1, 10.2 and 9.9 mt ($468 each mt) would be $6905, $4658 and $4533 minus $1868 ($127 mt^{-1}), $1260 and $1227 for banana fruit packing, respectively for three, two (6-month interval) and two (7-month interval) nematicide applications per year. Therefore, the end result subtracting banana fruit packing and nematicide cost was an extra gain of $4314, $2916 and $2825 ha^{-1} with three, two and two nematicide applications per year, respectively. The use of nematicides increased the bunch weight by an average of 31.6% (5.6 kg). This average lies between the percentages found by Araya and Cheves (1997a, 1997b) of 22.1% and 40.8% but is lower than the 44% found by Stanton and Pattison (2000), the 45% (9.8 kg) recorded by Moens *et al.* (2004) and the 48.8% (9 kg) reported by Quénéhervé *et al.* (1991a) and the overall yield improvement cited by Vilardebo and Guerout (1976), Gowen (1993, 1995), and Pattison *et al.* (1999). In the long term, it is expected that an even greater yield difference between the treatments and untreated control will be visible mainly due to the deterioration of the root system in the untreated plants. Especially if the schedule and choice of application of the different nematicides can be further optimized. This implies that information like the soil and climatic conditions, physical and chemical characteristics of each product and previous product applications must be taken into consideration before selection of the nematicide to prevent its biodegradation. Yield improvement shown in this study was based only on bunch weight, but other variables such as fruit length and diameter might also be improved, which could result in a lower percentage banana rejection increasing the ratio resulting in an even better yield. Furthermore, when a nematicide is applied, the follower growth is larger, reducing the period from harvest to harvest resulting in higher ratooning as reported by Quénéhervé *et al.* (1991a, 1991b) and Araya and Lakhi (2004).

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