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# Reduction of banana (*Musa* AAA cv Grande Naine) leaf photosynthesis by *Radopholus similis*

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## ABSTRACT
*Objective:* to determine the effect of *Radopholus similis* on banana (*Musa* AAA cv. Grande Naine) leaf photosynthesis.
*Methodology and Results*: Four experiments were carried out under lathhouse conditions. *In vitro* plants were sown in pot of 1.8 L volume containing a soil (sterilized or unsterilized) from a commercial banana farm. Experiment 1: four treatments were evaluated. The treatments consisted of plants on unsterilized and sterilized banana soil without and with the inoculation of 500 (506 ± 18) *R. similis* per pot. During the three measuring times (7 am, 10 am, and 1 pm), at 45 days after inoculation, the highest photosynthesis rate was observed in the plants free of nematodes and the lowest in those plants inoculated with *R. similis*. In the evaluation at 10 am a reduction of 46% (P= 0.0307) in the photosynthesis rate was found on plants inoculated with *R. similis* that were grown in the sterilized banana soil. In experiment II: five treatments were evaluated on sterilized banana soil. One treatment was non-inoculated (control) and in the others, each plant was inoculated 15 days after sowing with 500 (509 ± 21), 1000 (1049 ± 34), 1500 (1526 ± 39) or 2000 (2056 ± 67) *R. similis*. After 75 days of the inoculation, from the six photosynthesis evaluation times (6-7, 8-9, and 10-11 am or 12-1, 1-2 and 2-3 pm) with exception of that at 2-3 pm, the highest photosynthesis rate was observed in the plants free of nematodes. Reductions in the photosynthesis rate with nematode inoculation varied between 12 and 36% at 6-7 am, between 13 and 57% at 8-9 am, between 32 and 57% at 10-11 am, and between 16 and 65% at 12-1 pm, and between 13 and 47% at 1-2 pm. The photosynthesis rate decreased linearly as the number of *R. similis* inoculated increased in the evaluations of 8-9 (P= 0.0070) and 10-11 am (P= 0.0049) or 12-1 pm (P= 0.0048) and 1-2 pm (P= 0.0255). In experiment III: two treatments were evaluated in sterilized banana soil in which the plants of one treatment were inoculated 19 days after sowing with 1500 (1564 ± 49) *R. similis* and the others were the control. A photosynthetic light response curve was determined at 75 days after inoculation showing that the area under curve of the potential assimilation rate of the plants inoculated with *R. similis* was reduced (P= 0.0153) by 70% compared to non-inoculated plants. In experiment IV: three treatments were evaluated where the plants of two treatments were sown in sterilized banana soil. One treatment was inoculated with 2000 (2078 ± 63) *R. similis* per pot, 21 days after sowing, and the other had no inoculation. The remaining treatment was set up in

unsterilized soil without nematode inoculation. The net assimilation rate curve before nematode inoculation differed (P= 0.0072) among treatments. A reduction of 33% in the accumulated net assimilation rate across the sequence of light points (0-2200 µmol $m^{-2}$ $s^{-1}$) was evidenced on the plants cultivated on banana soil without sterilization, which was infected with residual *R. similis* on the soil. The net assimilation rate curve at 4 (P< 0.0001), 11 (P= 0.0340) and 25 (P= 0.0127) days after nematode inoculation was higher on the plants free of nematodes.

*Conclusion and Application of results*: In the four experiments, the lowest photosynthetic rate was found in the plants infected by *R. similis*. This confirms that the infection or parasitism of banana roots by *R. similis*, independently of if there are obvious root and foliage symptoms, consistently it reduces their photosynthesis rate, which in a long term will reduce crop performance. Therefore, nematode population must be monitored during the crop cycle to apply control measures in time in order to prevent production losses.

**Keywords***:* banana, *Musa* AAA, nematodes, photosynthesis, *Radopholus similis*

## INTRODUCTION

The burrowing nematode, *Radopholus similis* (Cobb 1893, Thorne 1949, Sher 1968) is an obligate migratory endoparasite that causes serious yield losses (Gowen 1995, Salguero *et al*. 2016, Sikora *et al*. 2018, Jaramillo *et al*. 2019, Chávez *et al*. 2020), to bananas in the regions where the crop is grown around the world. In nematode infested areas of Africa, losses of over 75% have been reported (Sarah 1989) and yield responses in nematode treated areas in different countries varied greatly, between 38 and 376% (Gowen 1995, 1993, 1979). While high yield losses occur on susceptible crops with visible symptoms of damage with high nematode numbers, many times, yield losses up to 30% can occur without obvious symptoms (Niblack 1993). The damage is found in the roots and corm tissue. The nematode enters the root and periodically feed as they migrate through the root tissue. The intracellular migration and extended feeding cause large destruction of root tissue along the path of the migrating parasite. Nematode infection results in dark red lesions on the outer part of the root, which penetrates throughout the cortex and rarely to the stele (Araya and De Waele 2004). Adjacent lesions may coalesce and the cortical root tissue atrophies, turning black, killing the roots, which become withered. Nematode feeding and intra-root migration activities damages the epidermis, cortex and stellar elements that disrupts water and nutrient uptake and transport, and as a result, whole plant-water relations. Then, banana root damage by the nematode results in retard leaf emission, stunted and chlorotic plants, with a lengthening of the vegetative phase (Quénéhervé 2009, Sikora *et al*. 2018), that ends in bunch weight and total harvest reduction as well as plant lifespan reduction.

These observations have been made on what are obvious morphological symptoms. However, nematode parasitism is first related to physiological changes that affect the photosynthetic process (Hussey and Williamson, 1998, Wallace 1987). Labudda *et al*. (2018) found that *Heterodera schachtii* altered the plant physiological processes of *Arabidopsis thaliana*. Similarly, Goulart *et al*. (2019) working with coffee seedlings infected with *M. exigua* or *M. paranaensis* found alterations on plant physiology as a reduction in transpiration, stomatal conductance, $CO_2$ concentration and in the photosynthetic rate.

Information dealing with the effects of *R. similis* parasitism on the physiological processes, like photosynthesis, which determine the growth rate of a banana crop, may contribute to understand differences in crop yield. Therefore, the objective of this work was to study the effect of initial *R. similis* population densities on banana leaf photosynthesis. The photosynthesis was measured in infected and uninfected plants,

both before and after larval invasion by *R. similis* into banana (*Musa* AAA cv Grande Naine) plants grown under lathhouse conditions.

## MATERIALS AND METHODS:

**General procedure for the four experiments:** banana plantlets (*Musa* AAA cv. Grande Naine) were micropropagated through *in vitro* culture as described by Israeli *et al.* (1995) and Acuña (1993). After the laboratory and following 55-60 days of nursery stage, the plantlets were transplanted into plastic pots with five drainage holes of 1 cm-d in the bottom. In the four experiments, pots of 1.8 L volume were used. The soil used in the four experiments was from the same commercial banana farm infested with nematodes. Soil contained 2.05% organic matter, had a pH of 5.4 and a content of bases of Ca 4.1, Mg 1.37 and K 0.5 $cmol^{(+)} L^{-1}$ and of P 9, Fe 70, Cu 6, Zn 1.5, and Mn 8 mg $L^{-1}$. The soil was a sandy loam (sand 56%, clay 15% and loam 29%). When sterilized soil was used, the soil was sieved through a 2 mm sieve (No 10) placed in trays of 5 cm high, moistened and sterilized in an Autoclave (Tomin TM-322) for 3 hours at 120 °C. Plants were fertilized two times a week with the application of 100 ml of a water solution (1 L of tap water + 5 ml of Bayfolan Forte) to the soil, and one day before photosynthesis evaluation, irrigated with 100 ml of tap water.

***Radopholus similis* isolation and identification**: The population of *R. similis* used for inoculation was obtained from the roots of 50 randomly chosen banana plants within a commercial banana farm. A hole of 15 cm wide, 15 cm length and 30 cm deep was dug at the follower sucker base. All roots were collected in a plastic bag and taken to the laboratory in insulated chests. In the laboratory, all the roots were washed with tap water and chopped into 2 to 3 cm pieces. Fractions of 100 g of roots were macerated (Taylor and Loegering 1953) in a kitchen blender (Sunbeam-Oster Household Products, model Osterizer with three speed levels) with 600 ml of tap water during 10 sec at low and 10 sec at high speed. The nematode suspension of each fraction was passed through three nested sieves with openings of 0.25mm (No 60), 0.106 mm (No 140) and 0.020 mm (No 635). Then, the nematode suspension from the last sieve No 635 was transferred to a Baermann-funnel and nematodes were recovered after 24h. Hereafter the nematode suspension was passed through a sieve with 0.020 mm openings, and the recovered nematodes were resuspended in 100 ml of tap water. At least 30 adult females of *R. similis* were individually handpicked under the stereo microscope with the nema-pick. After superficial sterilization with streptomycin sulphate, a single female was inoculated in each of 30 sterilized carrot disks for reproduction, following established procedures (Moens and Araya 2001, Speijer and De Waele 1997). Sixty days later, each *R. similis* population was extracted from the carrot disks (Speijer and De Waele 1997, Moens and Araya 2001,) and nematode identified under the light-microscope. Then, DNA was extracted from five composited samples of about 50 nematodes each, from each population, and was amplificated with specific PCR-primers (FsimFIRsimR) which confirmed the nematode identity as *R. similis*. Therefore, the *R. similis* from these populations were reared on carrot dish for the inoculations of the experiments.

***Radopholus similis* inoculation**: The initial *R. similis* population was estimated from five 2-ml aliquots. Before inoculation, soil surface in each individual pot was wetted with 50 ml of distillated water, and then five holes of 1 cm-d, 2-cm depth at 1.5 cm from the pseudostem base were made. The *R. similis* were pipetted as an aqueous suspension, then holes covered with the same soil and 50 ml of distillated

water applied again. During the inoculation, another 5 counts were done to confirm the inoculation numbers. **Lathhouse conditions and experimental design**: The four experiments were done in sequence, under lathhouse conditions, with 80-90% humidity and 24-26 °C at MONRERI facilities at Guápiles County, Costa Rica. In all experiments, for each treatment, six repetitions were use and pots were arranged in a completely randomized design.

**Photosynthesis evaluation:** In the first two experiments, the photosynthetic rates at specific times were measured and in the last two experiments, photosynthetic response to light curve were measured. The photosynthesis rate was measured with a LI-6800 Portable Photosynthesis System (LICOR Environmental Sciences). For the four experiments, the equipment was set with a 9-$cm^2$ leaf aperture, 410 mmol $CO_2$ $m^{-2}$ $s^{-1}$, 600-mmol $m^{-2}$ $s^{-1}$ of airflow, 30°C of leaf temperature, and relative humidity was kept between 60 to 70%. In all experiments, the apical portion of the second youngest leaf in every plant was used as an indicator of plant photosynthetic potential, being a young fully expanded and illuminated leaf for plant developed in pots under lathhouse conditions. Photosynthesis evaluations were done in a consistent manner, first repetition (one plant) of each treatment, then replicated two (second plant) of each treatment, and so on. For experiment III and IV an auto program with decreasing levels of photosynthetic active radiation (PAR), known as light response curves was used. Each plant was acclimatized with 800-µmol photons $m^{-2}$ $s^{-1}$ before the initiation of the light curve. Light points sequence was as following: 2200, 1900, 1600, 1300, 1000, 700, 400, 100, 75, 50, 25 and 0-mmol photons $m^{-2}$ $s^{-1}$. Light curves were measured from 7 to 10 am.

**Treatment description in each experiment**:

**Experiment I:** Four treatments were evaluated: Plants on unsterilized and sterilized banana soil with and without *R. similis* inoculation. The nematode inoculation was done 17 days after plant sowing with 500 (506 ± 18) *R. similis* per pot. Photosynthetic rates were evaluated at 45 days after *R. similis* inoculation at 7 am, 10 am, and 1 pm (2, 5 and 8 h after dawn). Measurements were taken using 150, 500 and 1,200-µmol $m^{-2}$ $s^{-1}$ light intensity, previously defined as being the light saturation under the lathhouse conditions.

**Experiment II:** Five treatments were evaluated on sterilized banana soil in which one treatment was non-inoculated (control) and in the other, each plant was inoculated with 500 (509 ± 21), 1000 (1049 ± 34), 1500 (1526 ± 39) or 2000 (2056 ± 67) *R. similis*. The nematode inoculation was done 15 days after plant sowing. Photosynthetic rates were evaluated at 75 days after *R. similis* inoculation at 6-7 am, 8-9 am, 10-11 am, 12-1 pm, 1-2 pm and 2-3 pm (1-2, 3-4, 5-6, 7-8, 8-9 and 9-10 h after dawn). Measurements were taken using 250, 500, 1000, 1200, 1500 and 1600 µmol $m^{-2}$ $s^{-1}$ light intensity, respectively, previously defined as being the light saturation under the lathhouse conditions.

**Experiment III:** Two treatments were evaluated where plants were sown in sterilized banana soil in which the plants of one treatment were inoculated with *R. similis* and the others were the control. The nematode inoculation was done 19 days after plant sowing with 0 (control) or 1500 (1564 ± 49) *R. similis* per pot. Photosynthetic light response curve was evaluated at 75 days after *R. similis* inoculation.

**Experiment IV:** Three treatments were evaluated where the plants of two treatments were sown in sterilized banana soil that were inoculated or not with *R. similis* while in the other treatment, plants were set up in unsterilized soil without nematode inoculation. The nematode inoculation was done 21 days after plant sowing with 2000 (2078 ± 63) *R. similis* per pot. The photosynthetic light response curve was evaluated the day before

nematode inoculation and then at 4, 11 and 25 days after inoculation at the same time.

**Growth variables evaluation**: After the photosynthesis evaluation at 45 days experiment I, 75 days Experiment II and III, and 25 days after the last photosynthesis evaluation experiment IV, plants were removed from the pots by pressing and losing the pots in the middle and applying gently water. Then, the soil was washed away from the roots and the roots were cut from the foliage (corm + stem + leaves) and weighted by separated on a scale (CAS Computing scale, model AD, precision 5 kg ± 1 g).

**Root nematode extraction**: Roots were chopped between one to two centimetres length, and after homogenizing, 25 g or the amount available, were taken for nematode extraction. Extraction was carried out by the maceration-sieving method (Taylor and Loegering 1953) following the modifications described by Araya (2002). Nematode were recovered on a 0.025 mm (No. 500) mesh and expressed as *R. similis* per 100 g of roots by plant.

**Data analysis:** Root and foliage weight data were subjected to ANOVA by Proc GLM of SAS and mean separation by LSD-test when required. The number of nematodes were analysed with a generalized linear model using the logarithmic transformation as a link function and assuming a negative binomial distribution of the errors using GemMod of SAS. To determine the effect of the inoculation in non-sterilized soil, the absolute control inoculated with *R. similis* was compared with the plants without inoculation and to determine the effect of sterilization, the plants inoculated in sterilized soil were compared with the un-inoculated plants growth on non-sterilized soil by contrasts. In experiment III, since two treatments on sterilized soil were compared in which one treatment was non-inoculated and the other inoculated with *R. similis*, the data were subjected to a non-parametric analysis of variance using Kruskal-Wallis in SAS. For the photosynthetic rate or light response curve evaluations, the gas exchange data were registered on an excel sheet and were analysed using Infostat v. 2018 software. In Experiment I and II, the photosynthetic rate of the treatments was compared at each time and means separated by T-test. For experiment II, in addition, a regression analysis of the photosynthetic rate on levels of *R. similis* inoculation at each time was done. For experiment III and IV, the area under the light response curve was calculated and compared by ANOVA or T-test. In addition, in each radiation level a T-test was done.

## RESULTS:

**Experiment I:** Root weight which varied between 43 and 55 g by plant was similar (P= 0.1442) among plants grown on unsterilized or sterilized banana soil that were inoculated or not with *R. similis* (Figure 1A). Foliage weight oscillated between 92 and 116 g by plant and was higher (P= 0.0270) for those inoculated with 500 (506 ± 18) *R. similis* grown in the sterilized soil (Figure 1B). The *R. similis* population after the photosynthesis evaluation confirmed that plants were infected with the nematode with differences in the number of *R. similis* (P< 0.0001) and total nematodes (P< 0.0001). As expected, in the plants grown in sterilized soil, without inoculation, no nematodes were detected (Figure 1C). In the plants grown in unsterilized soil that were inoculated with 500 (506 ± 18) *R. similis* the population of this nematode reached 19680 and total nematodes of 22280 per 100 g of roots, differing (P< 0.0005) with the population of 63280 *R. similis* and 63280 total nematodes found in plants grown in sterilized soil that were inoculated with *R. similis* (Figure 1C). In the three photosynthesis evaluation times (7 am, 10 am, and 1 pm or 2, 5 and 8 h after dawn) the highest photosynthesis rate was observed in the plants free of nematodes and

the lowest in those plants inoculated with *R. similis* (Figure 2). In the evaluation at 10 am, plants inoculated with *R. similis*, that were grown in the sterilized banana soil, a reduction of 46% (P= 0.0307) in the photosynthesis rate was found. Although, at 7 am and 1 pm, the plants inoculated with *R. similis*, that were cultivated on unsterilized or sterilized soil, also showed a reduction in the photosynthesis rate of 42 and 47%, and of 19 and 33%, respectively, the difference was not large enough to reach significant difference (P= 0.1249; P= 0.7032) due to the high variability.

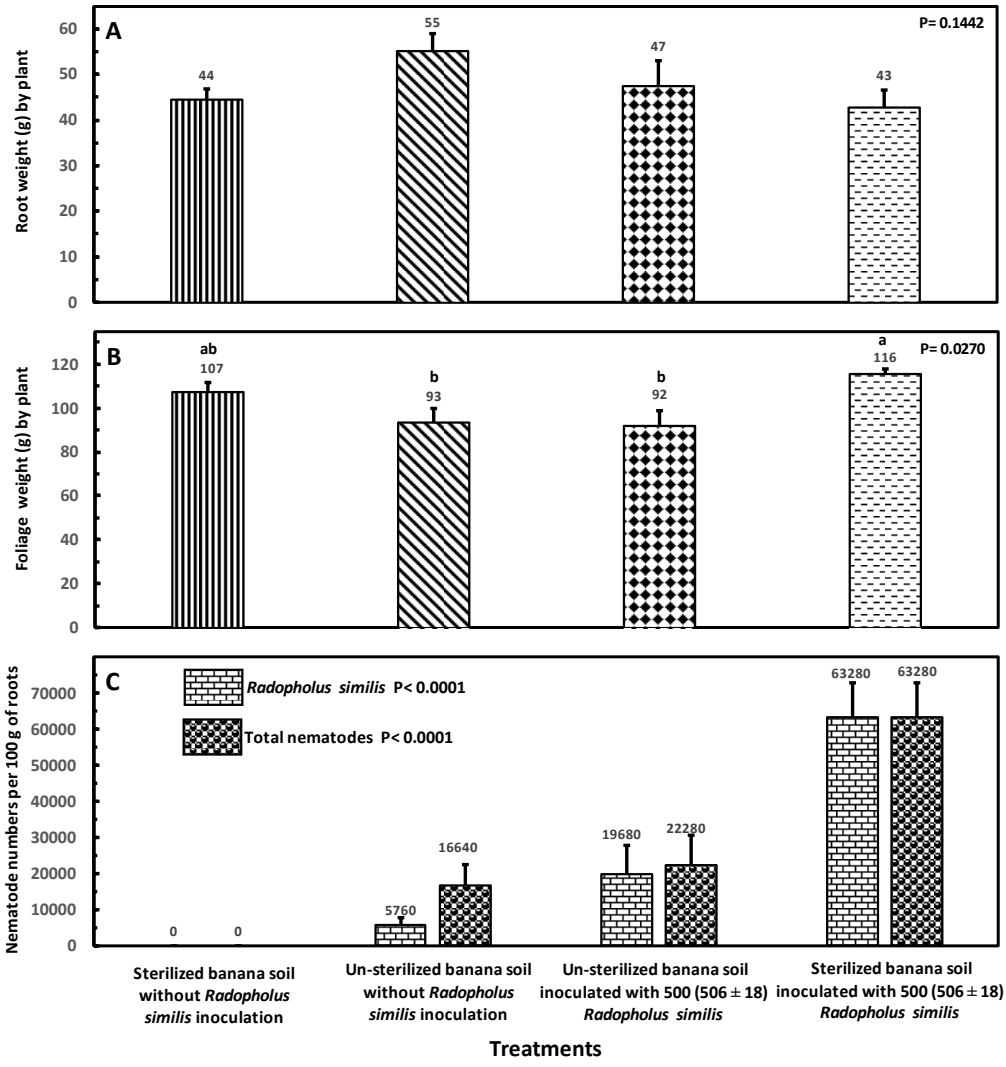


**Figure 1A-C:** Root and foliage weight (g) by plant and number of nematodes per 100 g of banana (*Musa* AAA cv. Grande Naine) roots by plant 45 days after plant inoculation or not with 500 (506 ± 18) *Radopholus similis*. Plants were cultivated on sterilized or un-sterilized banana soil. Each bar is the mean ± standard error of 6 repetitions. A) Root weight, B) Foliage weight, C) Nematode number by plant.

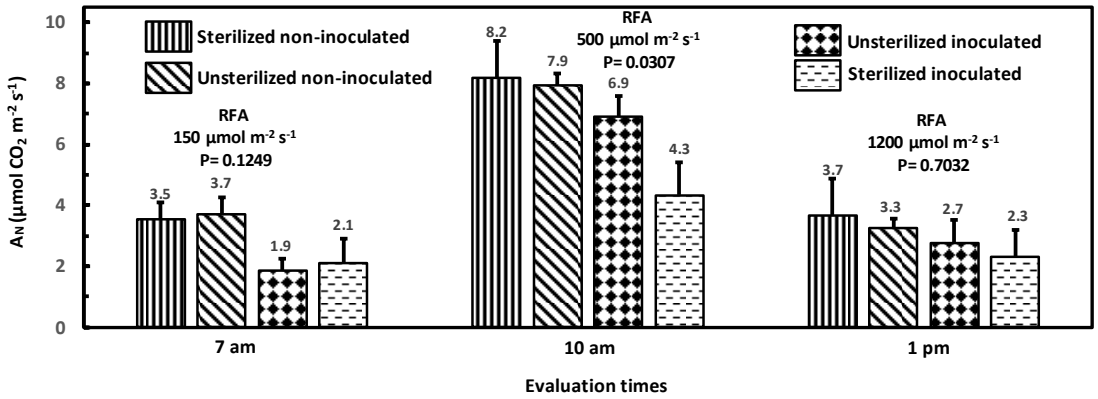


**Figure 2**: Net assimilation rate (µmol $CO_2$ $m^{-2}$ $s^{-1}$) of banana (*Musa* AAA cv Grande Naine) plants 45 days after inoculation with 500 (506 ± 18) *Radopholus similis* at 7 and 10 am or 1 pm (2, 5 and 8 h after dawn). Plants were cultivated on banana soil sterilized or un-sterilized under lathhouse conditions. Each bar is the mean ± standard error of six repetitions.

**Experiment II:** Root weight varied between 24 and 26 g by plant and was similar (P= 0.9623) among plants inoculated with different number of *R. similis* (Figure 3A). Foliage weight was also similar (P= 0.8091) among plants and fluctuated between 79 and 91 g by plant (Figure 3B). The *R. similis* population after the photosynthesis evaluation confirmed that plants were infected with the nematode (Figure 3C). In the non-inoculated plants, no nematodes were found, while in those inoculated, the highest (P< 0.0001) population of 28640 *R. similis* / 100 g of roots per plant, was found on the plants inoculated with 1000 (1049 ± 34) *R. similis*, without difference from those inoculated with 1500 (1526 ± 39) or 2000 (2056 ± 67) nematodes. From the six photosynthesis evaluation times (6-7, 8-9, and 10-11 am or 12-1, 1-2 and 2-3 pm (1-2, 3-4, 5-6, 7-8, 8-9 or 9-10 h after dawn), with exception of that at 2-3 pm (9-10 h after dawn), the highest photosynthesis rate was observed in the plants free of nematodes (Figure 4). Reductions in the photosynthesis rate with nematode inoculation varied between 12 and 36% at 6-7 am, between 13 and 57% at 8-9 am, between 32 and 57% at 10-11 am, and between 16 and 65% at 12-1 pm, and between 13 and 47% at 1-2 pm. At 2-3 pm, the photosynthesis rate was very similar in all the plants regardless of whether they were inoculated or not. Even though the photosynthesis rate decreased linearly as the number of *R. similis* inoculated increased, only in the evaluations of 8-9 (P= 0.0070) and 10-11 am (P= 0.0049) or 12-1 pm (P= 0.0048) and 1-2 pm (P= 0.0255) was significant. For each increase of 500 *R. similis* in the number inoculated, the net assimilation rate decreased by 0.3, 0.55, 0.55, 0.6 and 0.5 µmol $CO_2$ $m^{-2}$ $s^{-1}$ at 6-7 am, 8-9 am, 10-11 am, 12-1 pm and 1-2 pm, respectively.

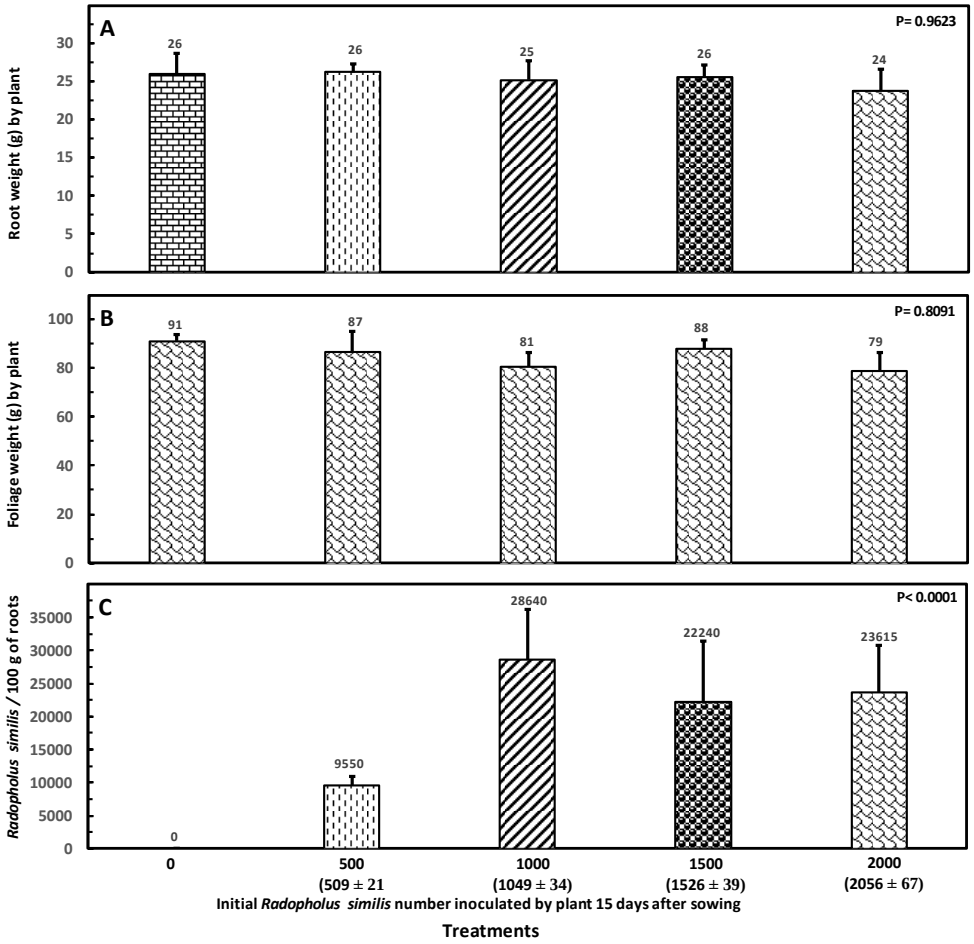


**Figure 3A-C.** Root and foliage weight (g) by plant and number of nematodes per 100 g of banana (*Musa* AAA cv. Grande Naine) roots by plant 45 days after plant inoculation with different number of *Radopholus similis*. Plants were cultivated on sterilized banana soil. Each bar is the mean ± standard error of 6 repetitions A) Root weight, B) Foliage weight, C) *Radopholus similis* by plant.

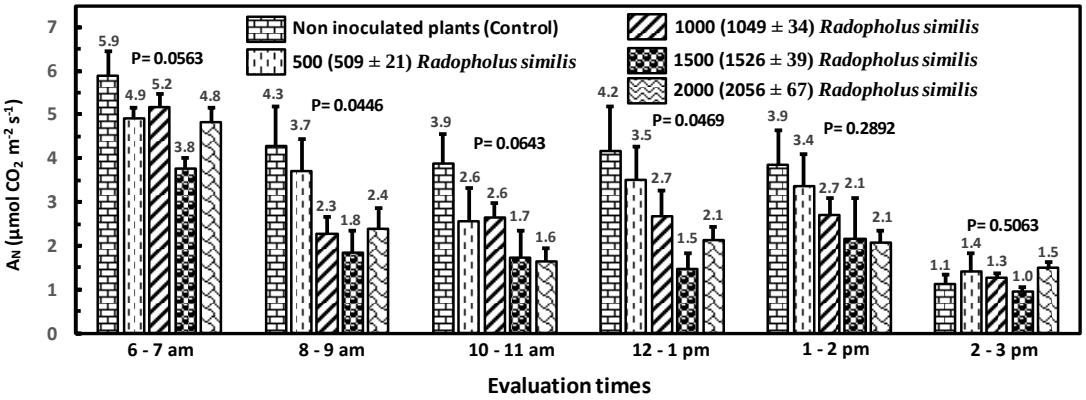


**Figure 4:** Net assimilation rate (µmol $CO_2$ $m^{-2}$ $s^{-1}$) of banana (*Musa* AAA cv Grande Naine) plants 45 days after inoculation with different *Radopholus similis* numbers at six evaluation times (6-7 am, 8-9 am, 10-11 am or 12-1 pm, 1-2 pm and 2-3 pm ((1-2, 3-4, 5-6, 7-8, 8-9 or 9-10 h after dawn). Each bar is the mean ± standard error of six observations. Plants were grown on sterilized banana soil.

**Experiment III:** Root (P= 0.3392) and foliage (P= 0.4627) weight was similar on inoculated (35 and 135 g) and non-inoculated (38 and 129 g) plants, respectively (Figure 5A-B). Again, the inoculation with *R. similis* was effective in infecting the banana plants (Figure 5C). The non-inoculated plants were free of nematodes, while in those inoculated with 1500 (1564 ± 49) *R. similis*, the population reached 35440 *R. similis* / 100 g of roots by plant (P= 0.0012). When the photosynthetic light response curve was measured, the area under the potential assimilation rate curve of the plants inoculated with *R. similis* was reduced (P= 0.0153) by 70% compared to non-inoculated plants (Figure 6). At a photosynthetic active radiation (PAR) of 0 and 25 µmol $m^{-2}$ $s^{-1}$ the net assimilation rate was very close (P> 0.1119) on non-inoculated or inoculated banana plants. After a photosynthetic active radiation (PAR) equal or above 50 µmol $m^{-2}$ $s^{-1}$ a higher (P< 0.0123) net assimilation rate was observed on plants free of nematodes and the difference in the net assimilation rate augmented as PAR increased up to 2200 µmol $m^{-2}$ $s^{-1}$. Then, the net assimilation rate on the inoculated plants was reduced between 30 to 88%.

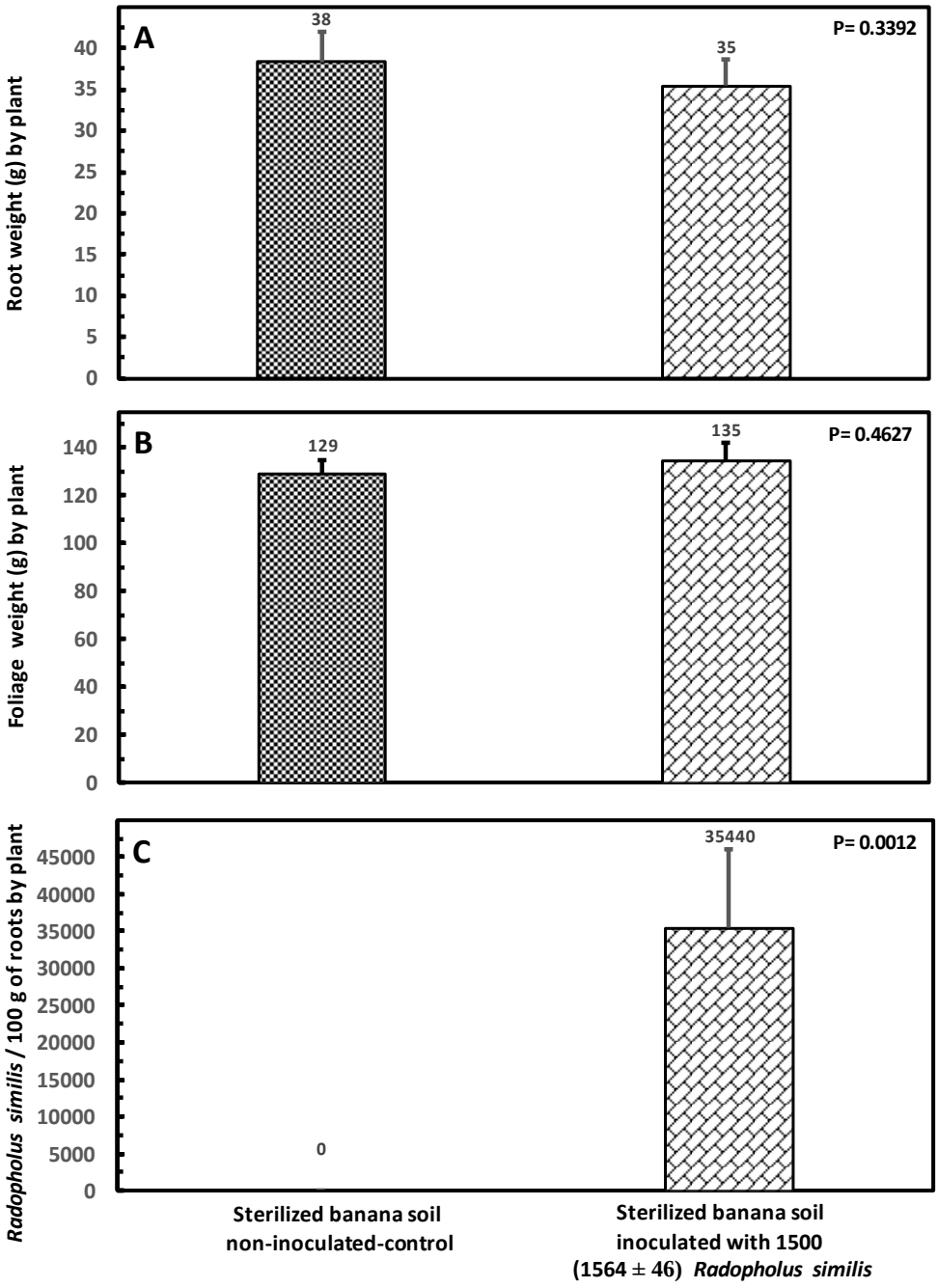


**Figure 5A-C:** Root and foliage weight (g) by plant and number of *Radopholus similis* per 100 g of banana (*Musa* AAA cv. Grande Naine) roots by plant 75 days after plant inoculation or not with 1500 (1564 ± 46) *Radopholus similis*. Each bar is the mean ± standard error of six repetitions. A) Root weight, B) Foliage weight, and C) *Radopholus similis* by plant.

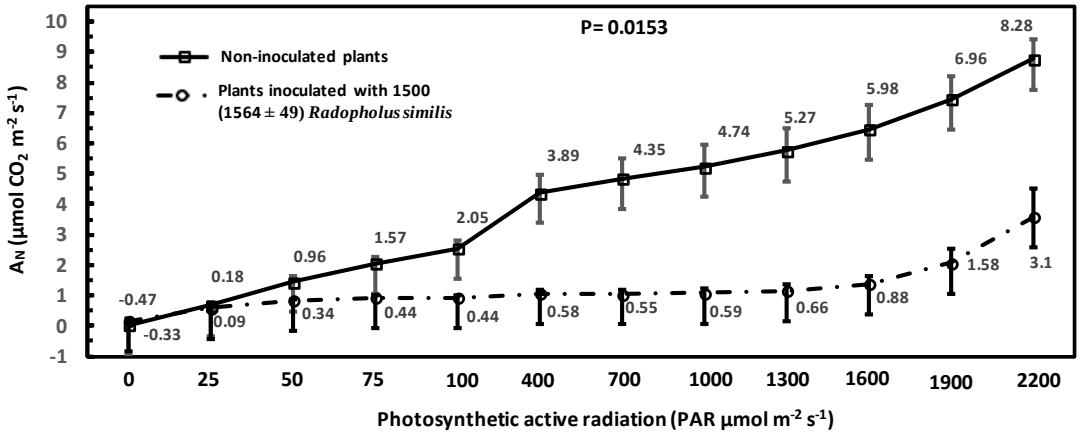


**Figure 6:** Net assimilation response curve to photosynthetic active radiation (PAR) of banana (*Musa* AAA cv Grande Naine) plants 75 days after inoculation or not with *Radopholus similis*. Each point is the mean ± standard error of six observations.

**Experiment IV:** A higher root weight (P< 0.0001) and foliage weight (P< 0.0001) was found on the plants cultivated on sterilized banana soil without *R. similis* inoculation with 48 and 154 g by plant, respectively (Figure 7A-B). The difference in root weight was induced by the lowest weight found on the plants grown on sterilized banana soil that were inoculated with 2000 (2078 ± 63) *R. similis* that showed 29 g per plant and in foliage weight, by the 106 g per plant, reported on the plants cultivated on the un-sterilized banana soil without inoculation. As new, the inoculation with *R. similis* was effective in infecting the banana plants (Figure 7C). The non-inoculated plants grown on sterilized banana soil were free of nematodes, while in those inoculated with 2000 (2078 ± 63) *R. similis*, the population reached 50758 *R. similis* / 100 g of roots by plant (P= 0.0007). The net assimilation rate curve before nematode inoculation differed (P= 0.0072) among treatments. A reduction of 33% in the accumulated net assimilation rate across the sequence of light points (0-2200 µmol $m^{-2}s^{-1}$) of the plants cultivated on banana soil without sterilization was found, compared to those plants on sterilized banana soil without inoculation (Figure 6). So, these plants were infected with residual *R. similis* on the soil as was confirmed at the end of the experiment where those plants reached 23733 individuals per 100 g of roots by plant. The net assimilation rate curve at 4 (P< 0.0001), 11 (P= 0.0340) and 25 (P= 0.0127) days after nematode inoculation was higher on the plants free of nematodes. At 4 days after *R. similis* inoculation, a reduction between 12 and 29% was observed on the plants cultivated on un-sterilized banana soil at photosynthetic active radiation between 100 and 2200 µmol $m^{-2}s^{-1}$. Plants cultivated on sterilized banana soil that were inoculated with 2000 (2078 ± 63) *R. similis*, showed a reduction of the net assimilation rate between 17 and 28% and between 15 and 29% at photosynthetic active radiation between 400 and 2200 µmol $m^{-2}$ $s^{-1}$ at 11 and 25 days after inoculation, respectively. On the plants cultivated on un-sterilized banana soil, the reduction at 11 and 25 days after inoculation was between 8 and 19% and between 5 and 30% at photosynthetic active radiation between 400 and 2200 µmol $m^{-2}$ $s^{-1}$, respectively.

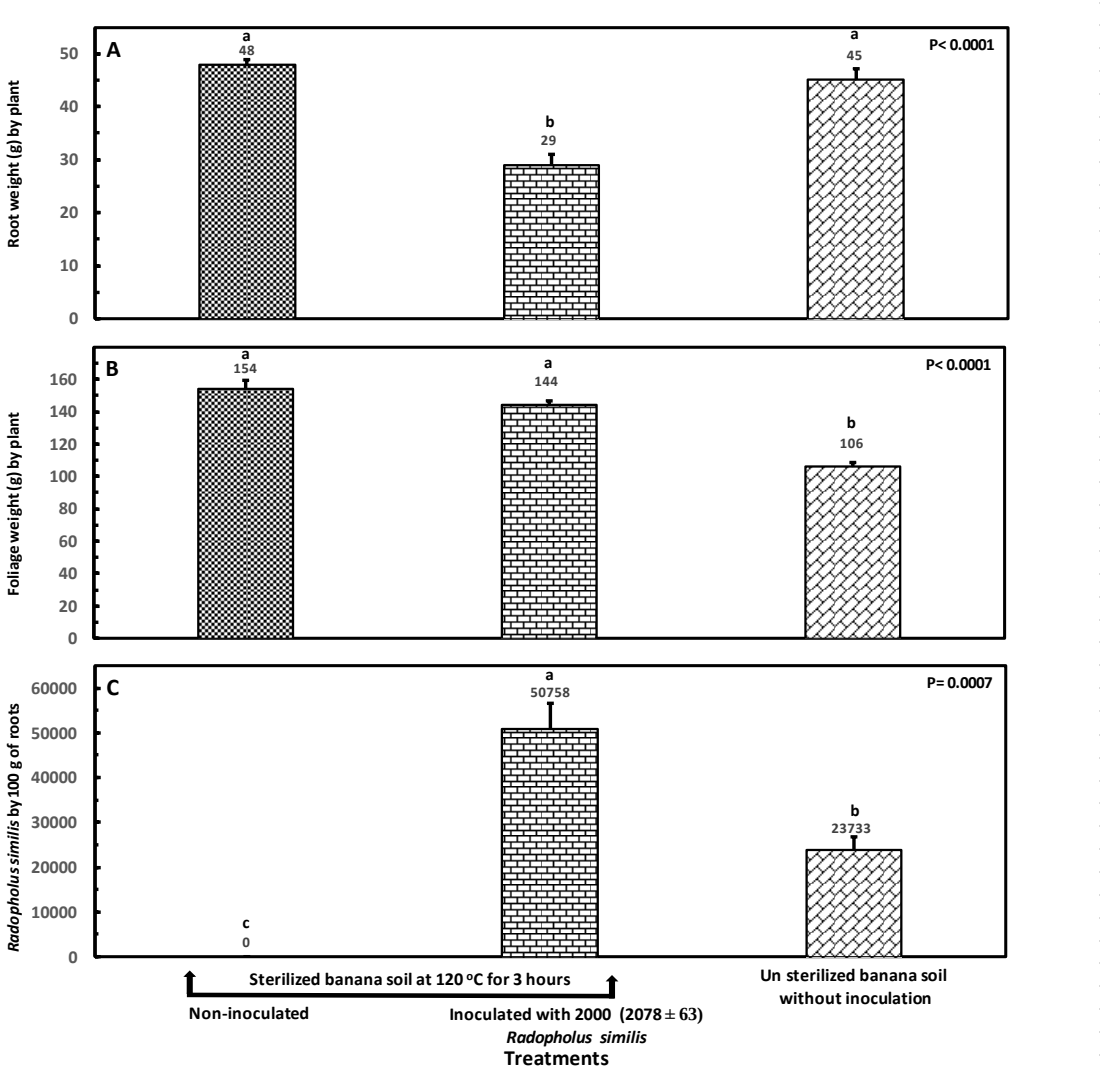


**Figure 7A-C.** Root and foliage weight (g) by plant and number of *Radopholus similis* per 100 g of banana (*Musa* AAA cv. Grande Naine) roots by plant 75 days after plant inoculation or not with 2000 (2078 ± 63) *Radopholus similis*. Each bar is the mean ± standard error of six repetitions.

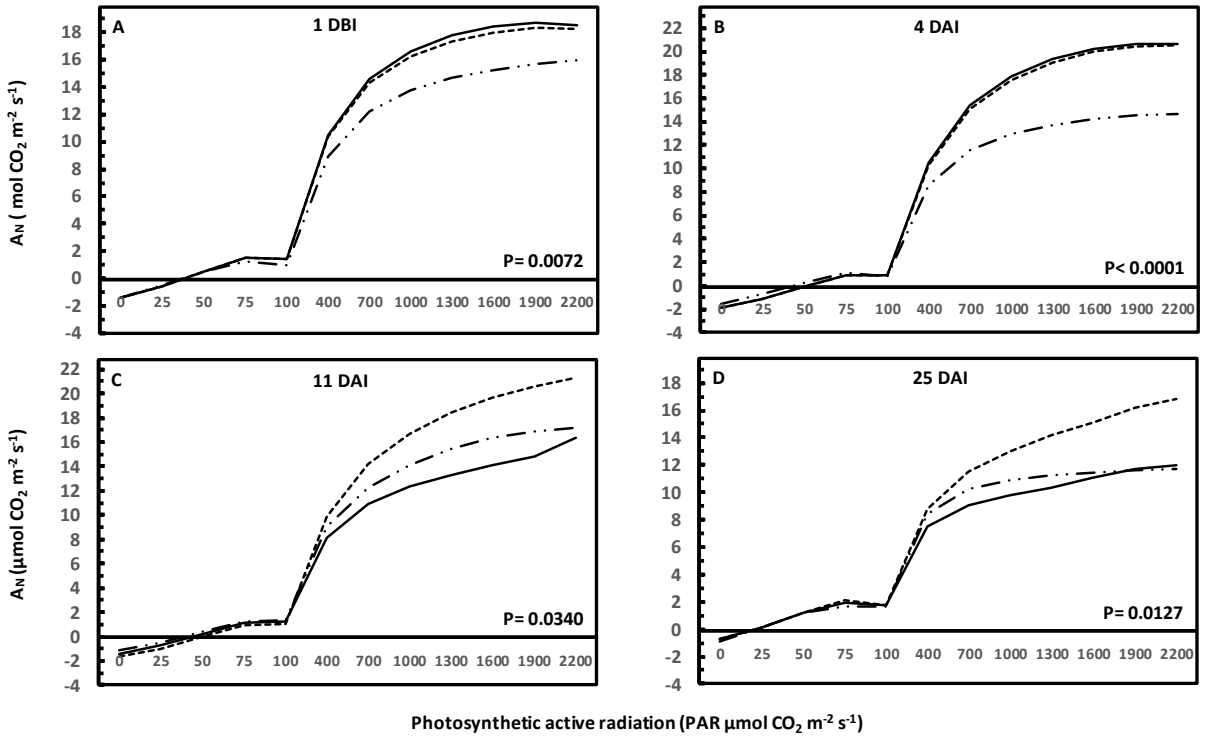


**Figure 8.** Net assimilation response curve to photosynthetic active radiation (PAR) of banana (*Musa* AAA cv Grande Naine) plants before and at 4, 11 and 25 days after inoculation or not with 2000 (2078 ± 63) *Radopholus similis*. Each point is the mean ± standard error of six observations. ▬ ▬ plants in sterilized banana soil without inoculation of *Radopholus similis*, ——— plants in sterilized banana soil inoculated with 2000 (2078 ± 63) *Radopholus similis* and —· · plants in non-sterilized banana soil without inoculation of *Radopholus similis*, 1DBI= one day before inoculation and 4, 11 and 25 DAI= 4, 11 and 25 days after inoculation.

## DISCUSSION

Soil sterilization was effective, given that the population of nematodes in the plants grown in such soil, without inoculation, no nematodes were found at the end of the experiments. Additionally, the sterilization allowed to determine the effectiveness of the inoculation with *R. similis*, being that those plants grown in the sterilized soil that were inoculated, a high population of *R. similis* per 100 g of roots by plant (Exp I: 63280, Exp II: 28640, Exp III: 35440, Exp IV: 50758) was reached at the end of the experiments. Similarly, in plants grown in unsterilized soil, which were inoculated, high populations of *R. similis* (Exp I: 19680 and Exp IV: 23733) by 100 g of roots by plant were found. When plants were grown on non-sterilized banana soil, beside the presence of *R. similis*, few nematodes of the genera *Helicotylenchus* spp. and *Meloidogyn*e spp. were found, which it is reasonable, and agrees with those reported in the country (Araya and Vargas 2018) and are also common parasites of banana roots worldwide (Gowen and Quénéhervé 1990, Gowen 1995, Gowen *et al*. 2005, Quénéhervé 2009, Daneel and De Waele 2017, Sikora *et al*. 2018). In the four experiments, the lowest photosynthetic rate was found in the plants infected by *R. similis*, independently if root and foliage weight were affected. This confirms that the infection or parasitism of banana roots by *R. similis* independently of if there are obvious root and foliage symptoms; consistently it reduces their photosynthesis rate, which in a long term will reduce crop performance. Depending on the time of the evaluation, the reduction in photosynthetic rate in the infected plants varies between 12 and 88% which are in parallel with

that found by Schans (1991) who reported a reduction of 38% on the photosynthesis rate of the potatoes cultivar Irene and of 43% on the cultivar Darwina infected by *Globodera pallida*. Swain and Prasad (1989) on rice inoculated with 100 infective juveniles of *Meloidogyne graminicola* per pot found a reduction in measured photosynthetic rates between 19 and 25%. On tomatoes plants, inoculated with *Meloidogyne ethiopica* a reduction of 70% in photosynthesis was reported (Strajnar *et al*. 2012). On coffee (*Coffea arabica* 'cv. Yellow Catuaí) the inoculation of 1500 juveniles of *Meloidogyne exigua* or *Meloidogyne paranaensis* caused 17 and 33%, respectively, of reduction in photosynthesis (Goulart *et al*. 2019). Other authors like Wallace (1974) and Loveys and Bird (1973) reported that parasitism of tomatoes roots by *Meloidogyne javanica* decreased its photosynthesis. Franco (1980) on potatoes plants parasitized with *Globodera rostochiensis*, Melakeberhan *et al*. (1984) on plant beans (*Phaseolus vulgaris*) infected by *Meloidogyne incognita*, and Melakeberhan and Ferris (1989) on *Vitis vinifera* plants infected with *M. incognita* also found lower leaf photosynthesis. Other crops where root parasitism by nematodes reduced leaf photosynthesis are; soybean (*Glycine max*) parasitized by *Heterodera glycines* race-3 (Asmus and Ferraz 2002), cotton (*Gossypium hirsutum*) parasitized by *Meloidogyne incognita* (Lu *et al*. 2014), *Arabidopsis thaliana* infected by *Heterodera schachtii* (Labudda *et al*. 2018). In the experiments reported here, a reduction on banana leaf photosynthesis was found with *R. similis* inoculation between 500 and 2000 infective juveniles by pot, but in other crops, a reduction on leaf photosynthesis was reported with the inoculation of only 100 infective juveniles of *Meloidogyne graminicola* on rice (Swain and Prasad 1989) or 250 larvae of *Meloidogyne javanica* on tomatoes (Wallace 1974). Therefore, more likely, the inoculation of lower *R. similis* numbers could also results in lower photosynthetic rates. The reduction on leaf photosynthesis with this low populations (500-2000) inoculated should be related with the nematode economic thresholds indicated for bananas and support that found by Guerout (1972) in Ivory Coast of 1000 *R. similis* per 100 g of roots and that indicated by INIAP (2018) of 2500 by 100 g of roots in Ecuador. Also is partially agreed with the number of 5000 per 100 g of roots suggested by Chávez *et al*. (2020) for bananas in Ecuador. The reduction on leaf photosynthesis with low *R. similis* population was confirmed in Exp IV where the drop was observed 4 days after inoculation with 2000 (2078 ± 63 *R. similis*), before nematode reproduction, since its life cycle takes 24 days (Loos 1962, Holdeman 1986, Haegeman *et al*. 2010, Guzmán 2011). This fast reduction on leaf photosynthesis after nematode invasion has also been reported on other crops. Wallace (1974) and Loveys and Bird (1973) on tomato plants, found that *Meloidogyne javanica* decreased leaf photosynthesis two days after root invasion. On potatoes plants, parasitized with *Globodera rostochiensis* (Franco 1980) or *Globodera pallida* (Schans 1991), a reduction on photosynthesis was found three days after nematode invasion. Melakeberhan *et al*. (1984) on *Phaseolus vulgaris*, cited reduction on leaf photosynthesis 3 days after *Meloidogyne incognita* invasion, and Labudda *et al*. (2018) indicated a reduced photosynthesis rate on *Arabidopsis thaliana* 7 days after invasion of *Heterodera schachtii.* This means that white and cream banana roots not always or necessarily are free of nematodes, and as indicated by Ayoub (1980), McKenry and Roberts (1985), Mai (1985), Niblack (1993), and Wang *et al*. (2003), extensive yield loss can occur when one or more nematode species may be feeding on a given plant, without showing obvious or specific plant symptoms.

In Exp IV, where the photosynthesis rate was evaluated on different times in the same plants,

the reduction in photosynthesis was observed at all evaluation times on the plants with nematodes. This agrees with observations of Wallace (1974), and Loveys and Bird (1973), who found decreased leaf photosynthesis throughout the crop growth. This means that in bananas infected with nematodes, leaf emission will be delayed. For ratoon banana plants to shooting or flowering, they must emit between 29 and 32 foliage (broad) leaves, excluding narrow leaves (Valle and Gonzáles 2009), or between 38 and 44 total leaves (broad + narrow leaves) as indicated by Stover (1979) and Gary (1977). Then, the period between bunch harvests in the same stool will be extended, reducing ratooning, as has been shown on experiments of banana nematode chemical control (Chávez *et al*. 2020, Jaramillo *et al*. 2019). The reduction on banana leaf photosynthesis, more likely comes from a disruption of the physiological processes efficiency throughout the whole plant induced by the nematode parasitism (Hussey and Williamson, 1998). It is known that nematode feeding and migration activities disrupt root tissue and alter root growth (Wilcox-Lee and Loria 1987) and consequently the host is deficient in water and nutrient uptake (Hurchanik *et al*., 2004, Strajnar *et al*., 2012). Labudda *et al*. (2018) found that *Heterodera schachtii* altered the plant physiological processes of *Arabidopsis thaliana*. Fatemy *et al*. (1985) suggested that reduced photosynthetic rate on potato plants infected by *Globodera rostochiensis* was due to stomatal closure induced by water stress. Similarly, Goulart *et al*. (2019) working with coffee seedlings infected with *M. exigua* or *M. paranaensis* found alterations on plant physiology as a reduction in transpiration, stomatal conductance, $CO_2$ concentration and in the rate of photosynthesis. Therefore, the rate of photosynthesis is a crucial parameter influencing crop yield and it consists of a sequence of events, which influence each other. In this sense, any disruption in its balance may change the physiological process. A reduction in photosynthesis, results in lower root and foliage growth rate, less vigour, lower leaf emission rate, lower bunch weight that in conjunction ends with lower productivity.

## CONCLUSION AND APPLICATION OF RESULTS

In the four experiments, the lowest photosynthetic rate was found in the plants infected by *R. similis*. This confirms that the infection or parasitism of banana roots by *R. similis*, independently of if there are obvious root and foliage symptoms; consistently it reduces their photosynthesis rate, which in a long term will reduce crop yield and its performance. Therefore, nematode population must be monitored during the crop cycle to apply control measures in time in order to prevent production losses.

## REFERENCES

Acuña PI. 1993. Micropropagación de banano a partir de ápices vegetativos. CORBANA 17(39):9-12.

Araya M., Vargas R. 2018. Frecuencia y densidades poblacionales de nematodos parásitos en plantaciones comerciales de banano (*Musa* AAA) muestreadas en el intermedio madre-hijo y al frente del hijo de sucesión. CORBANA 44(64):71-96.

Araya M, De Waele D. 2004. Spatial distribution of nematodes in three banana (*Musa* AAA) root parts considering two root thickness in three farm management systems. Acta Oecologica 26(2):137-148. DOI 10.1016/j.actao.2004.03.010.

Araya M. 2002. Metodología utilizada en el laboratorio de nematología de CORBANA S.A. para la extracción de

nematodos de las raíces de banano (*Musa* AAA) y plátano (*Musa* AAB). CORBANA 28(55): 97-110.

Asmus GL, Ferraz LCCB. 2002. Effect of population densities of *Heterodera glycines* race 3 on leaf área, photosynthesis and yield of soybean. Fitopatología Brasileira 27:273-278. DOI: 10.1590/S0100-41582002000300006

Ayoub SM. 1980. Plant nematology an agricultural training AID. NemaAid Publication. Sacramento, California. 195p.

Chávez C, Rubio D, Tobar M, Salas E, Araya M. 2020. Number of nematicide cycles per year on banana (*Musa* AAA cv Valery) root nematode control and crop yield. International Journal of Science and Research, 9(7):1651-1663. DOI: 10.21275/SR20722023421

Daneel MS, De Waele D. 2017. Nematode pests of banana. In: Fourie H, Spaull VW, Jones RK, Daneel MS, De Waele D. Eds. Nematology in South Africa: A view from the 21st Century. Pp: 359-371. Springer International Publishing Switzerland.

Fatemy F, Trinder PKE, Wingfield JN, Evans K. 1985. Effects of *Globodera rostochiensis* and water stress and exogenous abscisic acid on stomatal function and water use of Cara and Pentland Dell potato plants. Revue de Nematologie 8(3):249-255.

Franco J. 1980. Effect of potato cyst-nematode, *Globodera rostochiensis*, on photosynthesis of potato plants. Fitopatologia 15:1-6.

Gary J. 1973. Étude du développement du système foliare du bananier en fonction de la temperature. Fruits. 28:499-516.

Goulart RR, Terra WC, Salgado SML, Alves JD, Campos VP, Fatobene BJR, Marchiori PER, De Souza SR, Oliveira RDAL. 2019. *Meloidogyne paranaensis* and *M. exigua* alter coffee physiology. Nematology 21:459-467. http://dx.doi.org/10.1163/15685411-00003226

Gowen SR, Quénéhervé P, Fogain R. 2005. Nematode parasites of bananas and plantains. In: Luc M, Sikora RA, Bridge J. Eds. Plant parasitic nematodes in subtropical and tropical agriculture. Pp: 611-643, CABI Publishing, CAB International.

Gowen S. 1995. Pests. Pp: 382-402. In: Gowen S. Eds. Bananas and plantains. Chapman & Hall.

Gowen SR. 1993. Yield losses caused by nematodes to different banana varieties and some management techniques appropriate for farmers in Africa. Pp: 199-208. In: Gold CS, Gemmill B. Eds. Biological and Integrated Control of Highland Banana and Plantain Pest and Diseases. Proceedings of a Research Coordination Meeting, Cotonow, Bénin.

Gowen S, Quénéhervé P. 1990. Nematode parasites of bananas, plantains, and abaca. Pp: 431-460. In: Luc M, Sikora RA, Bridge J. Eds. Plant parasitic nematodes in subtropical and tropical agriculture. CAB International, Wallingford, UK.

Gowen SR. 1979. Some considerations of problems associated with the nematode pests of bananas. Nematropica 9(1):79-91.

Guerout R. 1972. Relation entre les populations de *Radopholus similis* COBB et la croissance du bananier. Fruits 27(5): 331-337.

Guzmán POA. 2011. En nematodo barrenador (*Radopholus similis* (Cobb) Thorne) del banano y plátano. Revista Luna Azul 33:137-153. http://www.redalyc.org/articulo.oa?id=321727235012

Haegeman A, Elsen A, De Waele D, Gheysen G. 2010. Emerging molecular knowledge on *Radopholus similis*, an important nematode pest of banana. Mol. Plant Pathol. 11(3), 315-323. DOI:10.1111/j.1364-3703.2010.00614.x

Holdeman QL. 1986. The burrowing nematode, *Radopholus similis*, sensu lato. Division of Plant Industry, California Department of Food and Agriculture and the Agricultural Commissioners of California. California. 52p.

Hurchanik D, Schmitt DP, Hue NV, Sipes BS. 2004. Plant nutrient partitioning in coffee infected with *Meloidogyne konaensis*. *Journal of Nematology* 36, 76-84.

Hussey RS, Williamson VM. 1998. Physiological and molecular aspects of nematode parasitism. pp. 87- 108. In: Barker KR, Pederson GA, Windham GL Eds. Plant and nematode interactions. Madison, Wisconsin. American Society of Agronomy.

INIAP (Instituto Nacional Autónomo de Investigaciones Agropecuarias), 2018. Resultado nematológico. 1p.

Israeli Y, Lahav E, Reuveni O. 1995. *In vitro* culture of bananas. Pp. 147-178. In: Gowen S. Eds. Bananas and plantains. Chapman & Hall, London, U.K.

Jaramillo J, Vintimilla M, Rubio D, Soto G, Tobar M, Salas E, Araya M. 2019. Effect of nematicide rotation on banana (*Musa* AAA cv. Williams) root nematode control and crop yield. Agronomía Colombiana 37(2):153-165. DOI: 10.15446/agron.colomb.v37n2.79099

Labudda M, Rózanska E, Czarnocka W, Sobczak M, Dzik JM. 2018. Systemic changes in photosynthesis and reactive oxygen species homeostasis in shoots of *Arabidopsis thaliana* infected with the beet cyst nematode *Heterodera schachtii*. Molecular Plant Pathology 19(7):1690-1704. DOI:10.1111/mpp.12652

Loos CA. 1962. Studies on the life-history and habits of the burrowing nematode, *Radopholus similis*, the cause of black-head disease of banana. Proceedings of the Helminthological Society 29(1): 43-52.

Loveys BR, Bird AF. 1973. The influence of nematodes on photosynthesis in tomato plants. Physiological Plant Pathology, 3:525-529.

Lu P, Davis RF, Kemerait RC, Van Iersel MW, Scherm H. 2014. Physiological effects of *Meloidogyne incognita* infection on cotton genotypes with different levels of resistance in the greenhouse. Journal of Nematology 46(4):352-359.

Mai WF. 1985. Plant-parasitic nematodes: their threat to agriculture. In: Sasser JN, Carter CC. (Ends). An advanced treatise on *Meloidogyne*: Vol I Biology and control. Raleigh, NC: North Carolina State University Press. Pp: 12-17.

McKenry MV, Roberts PA. 1985. Phytonematology study guide. Division of Agriculture and Natural Resources University of California. 56p.

Melakeberhan H, Ferris H. 1989. Impact of *Meloidogyne incognita* on physiological efficiency of *Vitis vinífera*. Journal of Nematology 21(1):74-80.

Melakeberhan H, Webster JM, Brooke RC. 1984. Improved techniques for measuring the $CO_2$ exchange rate of *Meloidogyne* infected bean plants. *Nematologica*, 30:213-221.

Moens T, Araya M. 2001. Metodología para evaluar la susceptibilidad de cultivares de *Musa* a *Radopholus similis*. CORBANA 27(54):119-132.

Niblack, T.L. (ed.) 1993. Protect your soybean profits: Manage soybean cyst nematode. University Missouri Printing Services, Columbia, MO. 20p.

Orton WKJ, Siddiqi MR. 1973. *Radopholus similis*. C.I.H. Description of Plant Parasitic Nematodes Set 2 No 27. 4p. Commonwealth Agricultural Bureaux.

Quénéhervé P. 2009. Integrated management of banana nematodes. In: Ciancio A, Mukerji KG. (Eds). Integrated Management of Plant Pest and Diseases. Integrated Management of Fruit Crops and Forest Nematodes. Springer. Pp: 3-61.

Salguero D, Rudon G, Blanco R, Moya C, Ramclam W, Medina L, Azofeifa D, Araya M. 2016. Effect of different nematicide applications per year on banana (*Musa* AAA) root nematode control and crop yield. Journal of Applied Biosciences 101:9598-9609. http://dx.doi.org/10.4314/jab.v101i1.4

Sarah JL. 1989. Nematological review: Banana nematodes and their control in Africa. Nematropica 19(2):199-216.

Schans J. 1991. Reduction of leaf photosynthesis and transpiration rates of potato plants by second-stage juveniles of *Globodera pallida*. Plant Cell and Environment 14:707-712.

Sikora R, Coyne D, Quénéhervé P. 2018. Nematode parasites of bananas and plantains. Pp: 617-657. In: Sikora RA, Coyne D, Hallmann J, Timper P. Eds. Plant parasitic nematodes in subtropical and tropical agriculture. 3rd Edition. CABI.

Speijer PR, De Waele D. 1997. Screening of *Musa* germplasm for resistance and tolerance to nematodes. INIBAP Technical Guidelines, INIBAP-IPGRI. 47p.

Stover, H. 1979. Pseudostem growth, leaf production and flower initiation in the Grand Nain Banana. Bulletin No 8. Tropical Agriculture Research Services, La Lima, Honduras.

Strajnar P, Širca S, Urek G, Šircelj H, Železnik P, Vodnik D. 2012. Effect of *Meloidogyne ethiopica* parasitism on water management and physiological stress in tomato. *European Journal of Plant Pathology* 132, 49-57. DOI: 10.1007/s10658-011-9847-6

Swain B, Prasad JS. 1989. Photosynthetic rate in rice as influenced by the root-knot nematode, *Meloidogyne graminícola*, infection. Revue Nématol. 12(4):431-432.

Taylor AL, Loegering WQ. 1953. Nematodes associated with root lesions in Abaca. Turrialba 3:8-13.

Valle H, González M. 2009. Determinación de la diferenciación floral en plantas de banano (*Musa* AAA) de cuarta generación en los cvs. Gran Naine, Valery y Williams. En: Sandoval J. Eds. III Congreso Científico Técnico Bananero Nacional. Corporación Bananera Nacional, Guápiles, Costa Rica. p. 51.

Wallace HR. 1987. Effects of nematode parasites on photosynthesis. In: Veech JA, Dickson DW. Eds. Vistas on Nematology. Pp: 253-259. Society of Nematologists.

Wallace HR. 1974. The influence of root knot nematode, *Meloidogyne javanica*, on photosynthesis and on nutrient demand by roots of tomato plants. Nematologica 20:27-33.

Wang J, Niblack TL, Tremain JA, Wiebold WJ, Tylka GL, Marett CC, Noel GR, Myers O, Schmidt ME. 2003. Soybean cyst nematode reduces soybean yield without causing obvious aboveground symptoms. Plant Dis. 87:623-628.

http://lib.dr.iastate.edu/plantpath_pubs/126?utm_source=lib.dr.iastate.edu%2Fplantpath_pubs%2F126&utm_medium=PDF&utm_campaign=PDFCoverPages

Wilcox-Lee D, Loria R. 1987. Effects of nematode parasitism on plant-water relations. In: Veech JA, Dickson DW. Eds. Vistas on Nematology. Pp: 260-266. Society of Nematologists.