Fusarium wilt (Foc Race 1) in relation to soil properties in smallholder's farms with 'gros Michel' banana (Musa AAA) in Costa Rica

Relación Suelo-Planta

FUSARIUM WILT (Foc RACE 1) IN RELATION TO SOIL PROPERTIES IN SMALLHOLDER'S FARMS WITH 'GROS MICHEL' BANANA (*Musa* AAA) IN COSTA RICA

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RESUMEN

La Marchitez por *Fusarium* (Foc Raza 1) y su relación con propiedades del suelo en fincas de pequeños productores de banano cv. 'Gros Michel' (*Musa* AAA) en Costa Rica. El objetivo del estudio fue comprender la relación entre propiedades del suelo y la Marchitez por *Fusarium* (Foc Raza 1) en el banano cv. 'Gros Michel'. La experimentación se basó en suelos de la región de Turrialba, Costa Rica. Un primer análisis se realizó en condiciones de invernadero con plantas inoculadas con Foc Raza 1 y sembradas en dos tipos de suelo representativos de dicha región. Se determinó la expresión de la enfermedad en relación con dos niveles de pH del suelo (menor de 5,0 y mayor de 6,0) y de tres dosis por semana de N (baja: 0,00 g planta^{-1}, media: 0,08 g planta^{-1} y alta: 0,28 planta^{-1}), aplicadas en una solución de nitrato de amonio en agua. El tipo de suelo, el pH y sus interacciones fueron significativas, donde plantas establecidas en un pH menor mostraron mayor presencia de la marchitez lo que causó una menor biomasa. Un segundo análisis consistió en un levantamiento de información a nivel de 20 fincas de toda la región donde se seleccionaron 49 áreas. Se determinó el estado de la Marchitez por *Fusarium*, mediante un índice de severidad (IS). También, se realizó un levantamiento de varias condiciones agroecológicas tales como altitud, precipitación anual, temperatura promedio y fertilidad del suelo. Además, se tomaron en cuenta variables de manejo como la densidad de población y el uso de coberturas del suelo. Un mayor IS de la enfermedad fue relacionado con un menor contenido de materia orgánica y, con una mayor presencia de acidez, calcio y magnesio en el suelo. El IS no fue significativo con respecto al resto de variables agroecológicas y de manejo evaluadas. Dado que las propiedades del suelo presentaron una alta correlación entre sí, es difícil atribuir diferencias en la presencia de la enfermedad a una única propiedad de suelo; cada caso debe verse como independiente. Sin embargo, los resultados generan nuevos conocimientos con respecto a la relación Suelo-Marchitez por *Fusarium* en el cultivo del banano. Prácticas de manejo del suelo como el encalado, la aplicación de fuentes de materia orgánica y un balanceado manejo de la nutrición tienen una aplicación importante para disminuir, en el mediano plazo, el impacto de la enfermedad en regiones bananeras con suelos infestados con Foc.

Palabras clave: calidad del suelo, encalado, Mal de Panamá, *Musa*, nutrición

ABSTRACT

The aim of the study was to understand the relationship between soil properties and *Fusarium* wilt (FW) by Foc Race 1 in banana cv. 'Gros Michel'. The experimentation was based on soils from the Turrialba region in Costa Rica. A first analysis took place under greenhouse conditions with Foc Race 1 inoculated plants and grown in two representative soil types from the region. The disease expression in relation with two soil pH levels (lower than 5.0 and higher than 6.0) and three weekly N doses (low: 0.00 g plant^{-1}, medium: 0.08 g plant^{-1} and high: 0.25 g plant^{-1}) applied with an ammonium nitrate solution in water. Soil type, pH and their interactions significantly affected the disease, where plants grown in the lower pH showed a higher FW expressed as a lower biomass. A second analysis used information at 20 farms in the region, where 49 fields were studied. FW expressed as a Severity Index (SI) was measured. Besides, agroecological variables such as altitude, slope, annual rainfall, average temperature, and soil fertility were determined. In addition, management variables as plant density and soil cover were gathered. A higher SI of FW was linked with a lower organic matter (SOM), and a higher acidity, calcium, and magnesium concentration in the soil. Other agroecological and management evaluated variables were not linked with the SI of FW. Given the fact that soil properties were highly correlated, it is difficult to attribute differences in SI of FW to individual soil properties. Each case should be considered as independent; however, results provide new insights into the relationship Soil-FW in banana. Practices of soil management such as liming, SOM founds application, and an adequate nutrient package are important options to alleviate the impact of the disease in the midterm in banana regions with Foc infested soils.

Key words: liming, *Musa*, Panama disease, plant nutrition, soil quality.

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INTRODUCTION

Fusarium wilt (also known as Panama disease) is recognized impacting disease in bananas production. It is caused by a soilborne fungus, *Fusarium oxysporum* f. sp. *cubense* or Foc (Stover 1962; Stover and Simmonds 1987). Race 1 of Foc (one of the strains of the fungus) devastated large-scale commercial production of 'Gros Michel' bananas in Latin America and the Caribbean (LAC) in the last century (Ploetz and Churchill 2011). In the large-scale production systems, the problem was resolved through the switch to the resistant Cavendish cultivar. However, Foc Race 1 is still a serious problem in smallholder production systems in several countries of LAC (Dita 2013). Although these systems in Costa Rica continue to suffer from Fusarium wilt (Foc Race 1), after shifting this cultivar by the Cavendish as the main for exportation cultivar, research in Fusarium wilt decreased. The awareness of the new threat of Foc TR4 (Ploetz 2011), re-initiated research into this disease in LAC, using the model 'Gros Michel'- Foc Race 1.

Production of banana cv. 'Gros Michel' (*Musa* AAA) in Costa Rica takes place due its specific and appreciated traits resulting in higher prices in local and regional markets. The production of this cultivar covers around 4,400 ha. Most production occurs in Talamanca followed by the Turrialba region. In general, the production occurs on small farms with areas between 1 and 2 ha in association with sugar cane and coffee (Tapia *et al*. 2009, Escobedo 2010, Chaves 2019). However, control of Foc Race 1 is still timely, as i) eradication from the soil is almost impossible due to the competitiveness of the fungus (Stover 1962), ii) almost all banana soils in Latin America are infected with the fungus, and iii) local markets still demand the 'Gros Michel' cultivar for its attractive traits. As a result, even though a resistant cultivar (Cavendish) is available in the country for the exportation, alternative options to deal with the disease in the cv. 'Gros Michel' are necessary.

Agroecological conditions have been shown to influence soilborne pests and diseases (Mazzola 2002, Ghorbani *et al*. 2008). For example, climatic conditions influence the occurrence of bacterial wilt in the East African highlands (Bouwmeester *et al*. 2016) and soil properties have been linked to nematode infestation in banana production (Pattison *et al*. 2008, Geense *et al*. 2015). It is also known that, for instance, soil pH and nitrogen concentrations influence Fusarium incidence in banana production (Rishbeth 1955, Segura *et al*. 2018). Soil conduciveness or suppressiveness for soilborne diseases is linked to specific biotic and abiotic soil conditions (Weller *et al*. 2002, Janvier *et al*. 2007, Orr and Nelson 2018). It has been shown that some soils suppress Fusarium wilt (Shen *et al*. 2015; Orr and Nelson 2018). Soil properties affect both the plant and the pathogen. For example, soil chemical, physical, and biological conditions directly influence the fungus population in the soil. At the same time, soil properties influence the plant nutritional status (Stoorvogel and Segura 2018) and, indirectly, this can modify the predisposition to Fusarium wilt. A better nurtured plant will be healthier and less predisposed to be affected by the fungus. Soil pH, moisture, and temperature were reported as factors associated with Fusarium wilt expression (Peng *et al*. 1999).

Disentangling the complex relationship between agroecology and crop disease incidence is a major challenge for scientists, the main being that there is a plethora of soil properties together with environmental conditions and crop management that determine the disease incidence. Different approaches can be followed, including greenhouse experiments (Gibson *et al*. 1999) and studies on farmer fields (Tripp and Woolley 1989). The advantage of greenhouse experiments is that we can keep many factors constant and only vary one or a limited number of factors to derive a specific relationship, but results from greenhouse experiments need in most of the cases subsequent validation under field conditions. On-farm trials or farm surveys are typically challenged by the variation that occurs under natural conditions. The advantage is that the experiment takes place under the actual farm conditions, but the disadvantage is the difficulty to attribute specific conditions to disease incidence. Field conditions can be extremely variable, requiring a larger number of observations so that one may carry out multivariate analyses to understand the complex relationships.

There is a lack in the known about the relationship between agroecological, soil conditions and Fusarium wilt in banana. However, insight into this relationship could provide opportunities to reduce Fusarium wilt through agricultural management. It is the generally accepted that soil properties influence the incidence of Fusarium wilt in bananas (Orr and Nelson 2018). Nonetheless, this relation and the subsequent impact of soil management on Fusarium wilt has not been quantified. Despite the advantages that management could offer to control Fusarium wilt, the disease control package rarely includes soil management. Therefore, this study aimed to obtain insight to what extent soil properties play a role in the incidence of Fusarium wilt by Foc Race 1 in banana cv. 'Gros Michel'.

MATERIALS AND METHODS

The study area

The study focused on the region of Turrialba in Costa Rica (09°47'14" N, 83°34'03" W), located within the central cordillera with considerable volcanic activity. The region is located in the tropical premontane and montane belt (Holdridge 1982). Environmental conditions in the mountainous region vary considerably with high volcanos and steep river valleys. Altitude ranges from 157 to 2,900 meters above sea level (m.a.s.l.), average annual temperatures vary between 10.8 and 25.5°C with minimal variability within the year, and average annual rainfall varies between 2,122 and 4,586 mm/year with a rainy season from May to November. Soil conditions vary in the area with Inceptisols, Ultisols, Andisols and Nitisols being present (Dijkshoorn *et al*. 2005). Most soils are suitable for the cultivation of bananas, although soil depth and acidity locally limit banana production (Kass *et al*. 1995). In a greenhouse experiment, the role of two soil properties as pH and nitrogen (N), commonly related to Foc incidence were tested. Besides, a gather of information at farm level looked the Foc-banana relationship at the farm level in different agroecological conditions including soil properties in the region.

Greenhouse experiment

A factorial greenhouse experiment was established to evaluate the effect of soil properties on the incidence of Fusarium wilt by Foc Race 1 in plants of the cv. 'Gros Michel'. During a previous survey of banana soils in the Turrialba region, two representative soil types were selected (Klinkert 2014). These soils were described in detail and large topsoil (0-30cm) samples were taken. The soils were analyzed for pH, acidity, soil organic matter (SOM), Ca, Mg, K, P, Zn, Cu, Fe, Cu, and Mn following the methodologies described by Díaz-Romeu and Hunter (1978) and Mehlich (1984). The location of the samples and results of the analysis are presented in Table 1. Soil samples were used in a greenhouse experiment at the experimental station of CORBANA in La Rita (132 m.a.s.l., 10°15’54”N, 83°46’26” W, minimum temperature 17 °C and maximum temperature 35 °C, average temperature of 28 °C with an 85% relative humidity and 12 h daylight). The factorial design included 2 soil types x 2 levels of Foc Race 1 inoculation x 2 levels of soil pH x 3 levels of N fertilization x 3 replications resulting in 72 pots. Two contrasting soil pH levels were tested: 1) pH_{low} with a pH lower than 5.0, and 2) pH_{high} with a pH higher of 6.0. Soil pH was adjusted to these target levels by either applying a hydrochloride acid solution (10% HCl) to decrease pH or lime ($CaCO_3$) to increase soil pH. Soil pH was adapted before plant inoculation and planting. In each case, the acid or alkaline required units to achieve the lower and the higher pH were calculated before liming and/or acidifying. Soil pH was analyzed before each treatment and eight days after liming or acidification. The acidified soil samples reached pH values between 4.7 and 4.8, and limed soil samples reached pH values between 6.4 and 6.5. Typically, banana plants grow better in soils with pH above 5.5. Lower pH levels may result in poor banana development and production (Segura *et al.* 2015).

A total of 72 hardened 2-month-old tissue culture banana plants (*Musa* AAA, cultivar ‘Gros Michel’) were used in the experiment. These plants were grown in potting mix before the experiment. Two levels of Foc Race 1 inoculation by root dipping (Dita *et al*. 2010, García-Bastidas *et al*. 2014, Ordoñez *et al*. 2015) were tested: 1) $In_{0:}$ a control group, 30 minutes in clean water, and 2) In_1: an inoculated group, 30 minutes in a solution of water with 10^5 conidia mL^{-1} of Foc Race 1. The fungus strain was collected from Costa Rican soils and cultivated by CORBANA`s Center of Biological Control. Soil samples for the greenhouse experiment were collected from a banana region. As the soil samples were not sterilized in order to not disturb the plant response to the natural soil conditions, the plants in the control are exposed to a natural relatively low concentration of Foc Race 1 (but uniform for each of the soil types). Plants in In_0 can therefore still be infected but at a much lower level compared to In_1. Immediately after inoculation, plants were planted in 2 L pots (one plant per pot) filled with the treated soil samples. Three levels of N fertilization were established by varying the N dose: 1) N_{low} with no N addition, 2) N_{med} with 0.08 N g plant $week^{-1}$, and 3) N_{high} with 0.25 N g plant $week^{-1}$. N doses were respectively achieved through applications of 300 mL of differentiated solutions of water with ammonia nitrate (35-0-0) in concentrations of 0.00 g L^{-1} N, 0.14 g L^{-1} N and 0.43 g L^{-1} N, every 3-4 days. N_{med} emulated the average N requirement of plants during the first 10 weeks after planting in real field conditions. No other nutrients and agro-chemicals (e.g., fungicides and insecticides) were applied to the plants. Plants were followed for 8 weeks and were evaluated weekly on external symptoms. At the end, of the 8 weeks, total (above ground plus roots) fresh biomass (g $plant^{-1}$) of each plant was measured as the key indicator for disease incidence.

Farm Experiment

Previous studies in the region looked at the status of ‘Gros Michel’ banana production and Fusarium wilt by Foc Race 1 (Ramirez *et al*. 2010, Chavez *et al*. 2015). Those studies worked on 73 smallholders´ banana farms (1-2 ha) in the Turrialba region. The region was divided in sub-regions to cover the most representative predominant agroecological conditions such as altitude, slope, temperature, and annual precipitation. A total of 20 farms were selected based on the level of cooperation of the farmer and the adoption of disease management practices in the earlier studies (to minimize the variation within the survey). Besides, by covering the various sub-regions and ensuring the accessibility due the irregular landscape of the region. Each farm was surveyed concerning the current condition of the banana plants, the disease, age of the fields, and production. With this information a total of 49 fields (1, 2 or 3 per farm) were surveyed. Then, a strict protocol was followed for field observations and soil sampling. Field characteristics were registered including location and altitude with a GPS device, plant density and percentage cover crops. Banana production in the studied farms takes place in a narrow band between 617 and 1,065 m.a.s.l. The field observations aimed to get detailed insight into the agroecology, management (cover crop and plan density) and disease incidence. In general, in the production area mean annual temperature varies between 20 to 23°C, mean annual precipitation ranges from 2,666 to 3,155 mm/yr, and slopes vary between 3 and 33°. Plant density varied from a single plant to 2,000 plants ha^{1}. Cover crops varied from 0 to 100%. The observations were complemented with secondary data on total annual precipitation and average annual temperature (Fick and Hijmans, 2017) and topography (NASA JPL 2009). Fusarium wilt incidence was determined using a standard grading system (Poveda 2013). This system exists of five progressive classes of severity (i=1 to 5) with:

- Class 1: absence of symptoms,
- Class 2: minor symptoms in the leaves and pseudostem (small cracks),
- Class 3: moderate cracks in the pseudostem, shortening in petioles distance and new leave chlorosis, small size, and deformation,
- Class 4: severe cracks in the pseudostem, chlorosis and wilting in new and old leaves and leave deformation, and
- Class 5: all previous symptoms with plant wilting leading to plant death.

The percentage of plants with symptoms according to each class (CS_i in %) in each field area was measured. Finally, a severity index (SI) for the field was calculated as:

$$SI = \sum_{i=1}^{5} i \cdot CS_i$$

This resulted in a continuous value describing disease severity index (SI) on a scale from 100-500. As such, disease severity is composed by the proportion of plants affected and the expression of the disease in the plants (Peng *et al*. 1999). Composite topsoil (0-30 cm) samples were randomly taken from the fertilization band of 3-4 banana plants (in any status) throughout each field area. Those samples were analyzed for pH, SOM, acidity, N, Ca, Mg, K, P, Zn, Cu, Fe, Cu and Mn (following the methodologies by Díaz-Romeu and Hunter (1978), Mehlich (1984) and Elementar Analysensysteme GmbH (2011).

Statistical analysis

The greenhouse experiment aimed to reveal the effect of soil properties on the expression of Fusarium wilt. Data were analyzed using a factorial analysis of variance to evaluate the effect of four factors (soil type, inoculation, soil pH and N) and their interactions on the biomass. The differences in biomass according to the soil properties were evaluated through a Tukey's range test.

The focus of the statistical analysis of the farm gather of information was to find variables that influence the severity of Fusarium wilt in banana plantations. In the gather, many different agroecological conditions differ between the fields (in contrast to the controlled conditions in the greenhouse experiment). The relation of individual agroecological properties with disease severity was studied in three ways. First, the fields were split in two equal groups with low and high disease severity (using an admittedly arbitrary threshold of 350). Independent T-tests were performed to identify agroecological conditions that differed significantly between the two groups. Second, to analyze the system in a more integrated manner, the variation in soil properties was summarized in a K-means cluster analysis resulting in two cluster which can be interpreted as major soil types. In an independent T-test, the difference in disease severity was tested. Third, a principal component analysis was carried out to summarize the variation in soil conditions. The independent principal components were correlated with disease severity after which the soil properties underlying the correlated principal components were identified.

Table 1. Soil analysis for two representative soils types grown with 'Gros Michel' banana from the Turrialba region, Costa Rica.

Soil type	SOM	pH	Acidity	Ca	Mg	K	P	Fe	Cu	Zn	Mn
	%		cmol(+) L^{-1}				mg kg^{-1}				
Dystrudept	6.3	5.6	0.24	14	3.9	0.6	14	226	7	3	44
Haplohumult	12.3	6.1	0.05	5	0.9	0.6	3	78	4	1	13

RESULTS

Greenhouse experiment

Results of the experiment in terms of biomass per plant are summarized in Figure 1 and Figure 2. Without inoculation no external and internal symptoms of Foc were detected. The Dystrudept soil showed very limited effects of pH and N on biomass. However, the Haplohumult soil showed a significantly lower biomass under low pH ($P \leq 0.0001$), but no effect of N. Inoculation resulted in a considerable (63%) and significant ($P \leq 0.0001$) lower biomass. In the Dystrudept, inoculation resulted in an on-average lower biomass of 74% under pH_{low} versus 55% under pH_{high}. The strongest effect of inoculation in this soil was found under pH_{low} and N_{high} resulting in a 93% lower biomass. In the Haplohumult, plant inoculation also resulted in a considerable and significant ($P \leq 0.0001$) lower biomass. Although the remaining biomass in the inoculated plants did not differ with pH and N treatments, the reduction was higher under high pH (75%) compared to the low pH (64%). Overall, inoculation resulted in a significant lower biomass ($P \leq 0.0001$). In addition, the interaction between inoculation, soil type, and pH was found to be significant ($P = 0.0440$).

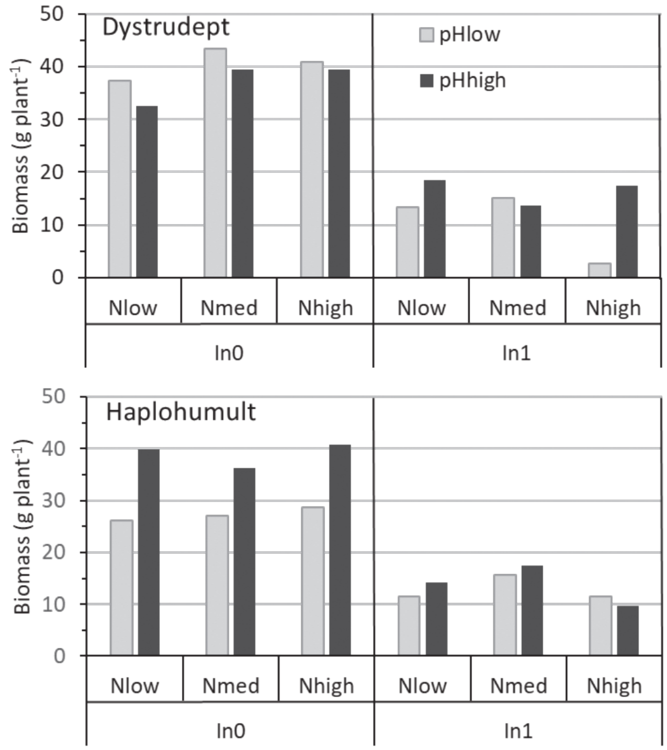

Fig 1. Plant biomass as an indicator for the effect of Fusarium wilt (Foc race 1) on 'Gros Michel' banana plants in a factorial greenhouse experiment testing two soil pH (lower than 5.0 and higher than 6.0) and three weekly N doses (low: 0.00 N g plant^{-1}, med: 0.08 N g plant^{-1}and high: 0.25 N g plant^{-1}) in two representative soils types from Turrialba, Costa Rica. In0 = not inoculated (control), In1= Inoculated.

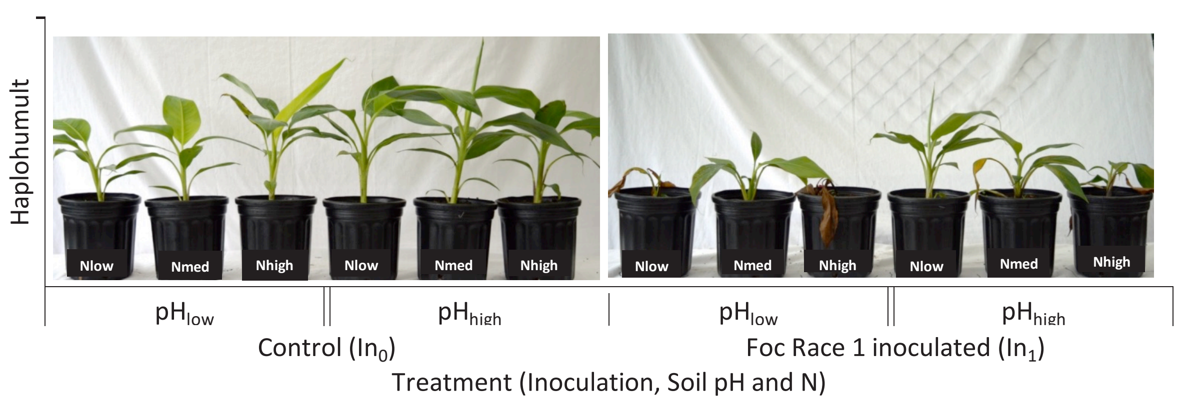

Fig 2. Example of inoculated (In_1) and not inoculated (In_0) 'Gros Michel' (*Musa* AAA) banana plants (greenhouse experiment) under two soil pH (lower than 5.0 and higher than 6.0) and three weekly N doses (low: 0.00 N g plant^{-1}, med: 0.08 N g plant^{-1}and high: 0.25 N g plant^{-1}) in one representative soil type from Turrialba, Costa Rica.

Farm Experiment

Fusarium wilt severity differed considerably between highly infested fields (SI= 500) whereas other fields did not present any symptoms of the disease (SI= 100). The average of SI was 340 with a standard deviation of 112. To better understand the differences in disease incidence, agroecological conditions in the 50% fields with the highest SI were compared to conditions in 50% field areas with the lowest SI. Results are presented in Table 2. No significant differences were found for the ecological and management variables. However, soil properties (SOM, acidity, Ca and Mg) were significantly different between the two groups. A higher concentration of SOM and a lower soil acidity were linked with the lower range of the severity index of Fusarium wilt. However, disease severity cannot be linked to individual soil properties it is not possible as soil properties are highly correlated to each other as shown in Table 3. This is partly illustrated by looking at the relation between acidity and disease severity (Figure 3).

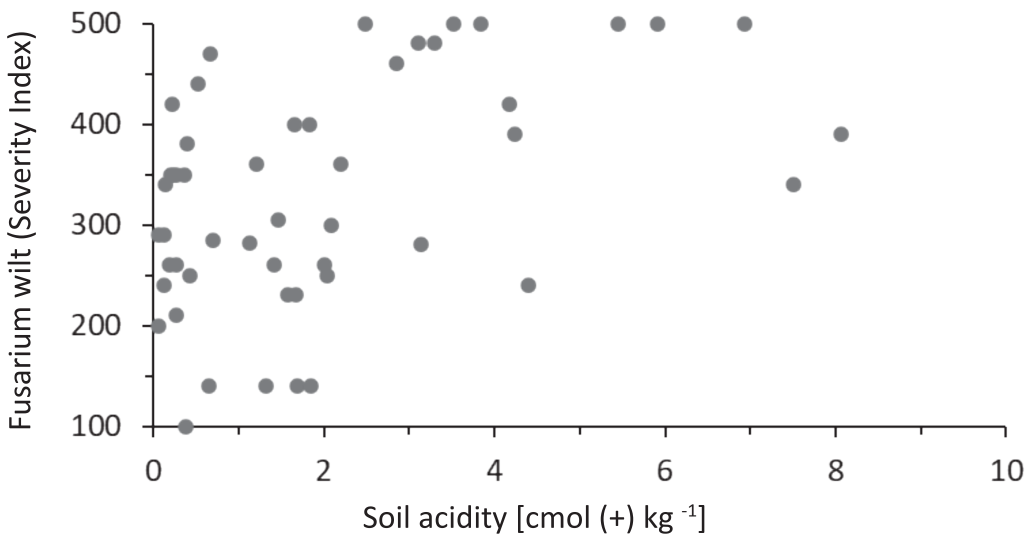

Fig 3. Scatter plot of soil acidity vs. Fusarium wilt index of severity by Foc Race 1 from 'Gros Michel' banana fields in Turrialba, Costa Rica.

An alternative way to look at the variation in disease severity, while considering the interactions between soil properties, is through a cluster analysis. In a cluster analysis the entire set of soil properties is considered, and the information is summarized by identifying clusters that can be considered to represent specific soil types. Subsequently, the differences in disease severity for the clusters was tested. The results are presented in Table 4. The clusters were significant differences in disease severity. SOM, acidity Ca and Mg were again linked with the SI of FW. The rest of soil properties were not linked with the disease. The variation in soil conditions can also be summarized through a principle component analysis as presented in Table 5. Five principle components were identified of which the second one, explaining 23% of the total soil variation, was significantly (at the 0.010 level) correlated to disease severity. The second principle component described particularly the variation in SOM and N.

Table 2. Agroecological conditions in 'Gros Michel' banana fields according the severity index of Fusarium wilt (Foc race 1) in Turrialba Costa Rica. Differences are tested for significance by an independent t-test (standard deviation between parentheses)

Agroecological variables	Severity index Fusarium wilt		
	<350	≥350	Overall
Ecological conditions			
Altitude (m.a.s.l.)	880 (135)	857 (152)	870 (142)
Slope (˚)	14.5 (8.8)	14 (8.1)	14.3 (8.4)
Temperature (˚C)	21.6 (0.6)	21.7 (0.91)	21.6 (0.8)
Precipitation (mm/yr)	2841 (154)	2826 (120)	2834 (138)
Soil conditions			
pH (-)	5.4 (0.4)	5.3 (0.4)	5.3 (0.4)
SOM (%)	7.2 (3.1)*	5.7 (1.5)	6.5 (2.6)
N (%)	0.35 (0.11)	0.33 (0.07)	0.34 (0.1)
Acidity (cmol (+) kg^{-1})	1.41 (1.63)*	2.65 (2.28)	2 (2.05)
Ca (cmol (+) kg^{-1})	7.3 (5.8)*	11.0 (6.1)	9.1 (6.2)
Mg (cmol (+) kg^{-1})	1.87 (1.38)**	2.9 (1.08)	2.34 (1.34)
K (cmol (+) kg^{-1})	0.38 (0.26)	0.40 (0.32)	0.39 (0.28)
P (mg kg^{-1})	11 (17)	10 (13)	11 (15)
Fe (mg kg^{-1})	151 (48)	173 (45)	161 (47)
Cu (mg kg^{-1})	4.5 (2.1)	4.9 (2.1)	4.7 (2.1)
Zn (mg kg^{-1})	3.1 (6.2)	2.4 (2.5)	2.8 (4.7)
Mn (mg kg^{-1})	58 (56)	70 (52)	64 (53)
Crop management			
Plant density (plants/ha)	570 (290)	390 (410)	480 (360)
Cover crop (%)	31.5 (33.5)	46.7 (40.4)	38.7 (37.3)

*P<0.050, **P< 0.010, ***P< 0.001

Table 3. Correlations between soil properties in 'Gros Michel' banana fields in Turrialba, Costa Rica.

Soil property	pH	Acidity	Ca	Mg	K	P	Fe	Cu	Zn	Mn	SOM	Ca	N
pH	-												
Acidity	**-0.67**	-											
Ca	**0.63**	-0.13	-										
Mg	**0.53**	-0.06	**0.80**	-									
K	0.43	-0.13	0.42	0.33	-								
P	**0.50**	-0.23	**0.56**	0.37	**0.61**	-							
Fe	0.29	-0.18	**0.64**	**0.53**	**0.52**	**0.51**	-						
Cu	0.11	-0.28	0.11	-0.03	0.12	0.00	0.43	-					
Zn	0.35	-0.17	0.12	0.06	0.35	0.18	0.02	0.29	-				
Mn	0.13	-0.12	0.03	0.31	-0.04	-0.19	0.03	-0.33	-0.12	-			
SOM	-0.05	-0.25	-0.40	**-0.51**	-0.11	-0.09	-0.32	0.39	0.02	-0.41	-		
Ca	0.17	-0.27	-0.26	-0.36	0.62	0.25	-0.06	0.15	0.37	-0.21	0.46	-	
N	-0.01	-0.16	-0.29	-0.39	-0.04	0.04	-0.29	0.31	0.02	-0.38	**0.92**	0.06	-

Table 4. K-means cluster analysis on soil properties in 'Gros Michel' plantations from Turrialba, Costa Rica including a test for differences between the clusters for soil properties and Fusarium wilt (Foc Race 1) SI in 'Gros Michel' bananas fields (standard deviation between parentheses).

Soil property/FW IS	Cluster 1	Cluster 2	Overall	T-Test (P≤f)
pH (-)	5.4 (0.4)	5.3 (0.4)	5.3 (0.4)	n.s
SOM (%)	7.4 (3.3)	5.7 (1.4)	6.5 (2.6)	0.001
N (%)	0.36 (0.12)	0.33 (0.06)	0.34 (0.10)	n.s
Acidity (cmol (+) kg^{-1})	1.19 (1.1)	2.69 (2.4)	2.0 (2.1)	0.010
Ca (cmol (+) kg^{-1})	7.10 (6.0)	10.7 (5.9)	9.1 (6.2)	0.050
Mg (cmol (+) kg^{-1})	1.78 (1.4)	2.86 (1.1)	2.4 (1.3)	0.001
K (cmol (+) kg^{-1})	0.37 (0.25)	0.41 (0.32)	0.39 (0.28)	n.s
P (mg kg^{-1})	10 (15)	11 (14)	11 (15)	n.s
Fe (mg kg^{-1})	150 (49)	171 (44)	161 (47)	n.s
Cu (mg kg^{-1})	3 (2.2)	5 (2.0)	4.7 (2.1)	n.s
Zn (mg kg^{-1})	3 (6.6)	3 (2.4)	3 (4.7)	n.s
Mn (mg kg^{-1})	58 (57)	68 (50)	64 (53)	n.s
Fusarium wilt (SI)	229 (59)	416 (64)	330 (112)	0.001

Table 5. Principal component analysis on soil properties in 'Gros Michel' banana plantations from Turrialba, Costa Rica including the correlation of the principal components with the Fusarium wilt (Foc Race 1) severity index.

Soil property	Principal Components				
	1	2	3	4	5
Variance explained (%)	31.2	22.8	12.3	8.0	7.5
Correlation with FW SI	0.019	0.496**	0.274	0.063	-0.149
SOM	-0.197	0.922	0.103	0.119	0.100
N	-0.134	0.852	0.184	0.158	0.231
pH	0.823	0.116	-0.302	-0.170	0.207
Al	-0.591	-0.498	0.569	0.023	0.093
Acid	-0.560	-0.503	0.603	0.027	0.073
Ca	0.788	-0.350	0.199	0.087	-0.009
Mg	0.667	-0.520	0.000	0.096	-0.033
K	0.651	-0.028	0.352	-0.194	0.244
P	0.719	0.030	0.344	0.096	0.430
Fe	0.729	-0.191	0.300	0.255	-0.415
Cu	0.291	0.522	0.281	-0.079	-0.710
Zn	0.324	0.185	0.106	-0.820	0.001
Mn	0.104	-0.417	-0.646	0.208	0.012

*P<0.050, **P< 0.010, ***P< 0.001

DISCUSSION

Banana plants in young stages from tissue culture, as used in this experiment are known to be more sensitive to Foc infestation (Brake *et al*. 1995, Smith *et al*. 2008). This ensured plant response to the disease according to the soil tested treatments. Found results indicate that soil pH indeed influenced the effect of inoculation expressed in the plant biomass but that the effect differs per soil type. Overall, soil type, pH, N, and inoculation explained 68% of the variation in the biomass. The lower biomass in the inoculated plants was caused by growth reduction as well as wilting of the leaves at the end of the greenhouse experiment.

Literature identifies soil pH as an important indicator for soil health for banana systems (Pattison *et al*. 2008, Segura *et al*. 2015, Geense *et al*. 2015), and its influence on soil suppressiveness for Foc (Orr and Nelson 2018). The role of pH is confirmed by the greenhouse experiment, although its effect differs between the two soil types. As a result, general validity referring management recommendations (in terms of e.g., liming and the avoidance of acidification) should be warranted. This is also confirmed by previous reports indicating that a multitude of chemical and physical soil properties would be linked to soil conduciveness of Fusarium wilt (Scher 1980, Domínguez *et al*. 2001, Orr and Nelson

2018). Confirming the role of the soil type and pH in FW expression, as in current results, is essential to find adequate strategies to at least alleviate the effect of Fusarium wilt in banana.

The farm survey showed the importance of SOM, Ca, Mg and acidity in the expression of the disease. SOM plays an important role in banana soil quality (Rivero *et al*. 2004; Riveros *et al*. 2006). Although Ca and Mg are two main nutrients in banana (Stoorvogel and Segura 2018), both were significantly higher in the range of high disease severity. A higher Ca concentration for instance is linked with a lower severity of fungal diseases in crops (Heyman *et al*. 2007, Sugimoto *et al*. 2007). The results in this experiment desagree with those previous results. At the same time, the graph of the soil acidity and the disease allows to illustrate that the correlation between soil properties (2 or more) can play a role in the expression of the disease. It is expected after the significance of the acidity according the disease level a higher SI of Fusarium wilt in the field areas with the higher acidity. However, a low acidity appears not to tell much about disease severity. In that case, likely other properties and their correlation also influence the disease. Besides, the possible implementation of managing soil properties implies considering the effect of changing one single soil property in other properties and their interaction in the disease incidence.

The cluster analysis confirmed the role of SOM, Ca, Mg and acidity in the disease. Probably those soil properties that were not correlated to disease severity played an important role in the definition of the clusters. SOM (and N) are considered to be important soil properties for the soil quality (Pattison et al., 2008). An optimal condition of SOM and N in the soil are crucial in the banana plant status. Besides, SOM is reported in the suppression of soil borne fungal diseases (Bananomi *et al*. 2007; Janvier 2007), as is the case of Fusarium wilt. Soil N concentration can be related with the plant response to the disease. Soil N was correlated with SOM and it also can be playing a role in the disease expression. Probably their role in the disease severity is also linked with other soil properties, but this was not evidenced in this research. Soil texture and particle size, for instance, are linked with the incidence of Fusarium wilt in banana (Dominguez *et al.* 2001).

Soil properties were found to have significant relationship with Fusarium wilt expression in both experiments. The interaction between inoculation and soil pH was significant in one of the evaluated soils with biomass as the key element of the disease expression at the greenhouse experiment. In the farm experiment, it also became apparent that many different soil properties are interacting, which hampers the quantification of the effect of individual soil properties. In general, the field survey confirmed the effect of soil properties such as SOM, Ca, Mg and acidity on the disease. Furthermore, correlations between soil properties were found. This shows the complexity of the systems and how more soil properties, even the soil type, can be playing a role in the disease.

The call for alternative control packages for diseases like Fusarium wilt is highly relevant as currently the very aggressive strain, the Foc Tropical Race 4 (Foc TR4) is spreading rapidly through many banana productions systems in Asia and Oceania (Ploetz and Churchil, 2011, García-Bastidas *et al*. 2014, Ploetz *et al*. 2015). In a worst scenario where Foc TR4 is already reported in LAC (Garcia-Bastidas *et al*. 2019) and the fact that resistant varieties will become available after year , there is an urgent need to develop control packages that allow farmers to manage the disease and slow down its dissemination.

The long-term goal of the research is to identify management strategies that may result in a reduction in the risk of Foc infestation. Results suggest that an integral soil management, including for example, SOM founds as organic amendments, liming to rise pH, and a balanced N-fertilization, may be considered as part of the strategy for alleviating the disease in smallholder farms of the cv. 'Gros Michel' and with other susceptible cultivars. These practices are being tested in current field experiments. Results also show that soil properties and their interactions play a role in the disease severity, contrary to the more general statements found in handbooks (e.g., Marschner 2012) and studies from other regions (Alvarez *et al*. 1981, Zhang *et al*. 2013, Sun *et al*. 2018). Soil management as a key factor of the integrated crop of banana, can potentially play an important role in disease management.

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REFERENCES

Alvarez, CE; Garcia, V; Robles, J; Diaz, A. 1981. Influence des caractéristiques du sol sur l'incidence de la Maladie de Panama. Fruits 36, 71–81.

Bananomi, G; Antignani, V; Pane, C; Scala, F. 2007. Suppression of soilborne fungal diseases with organic amendments. J. Plant Pathol. 89: 311–324.

Bouwmeester, H; Heuvelink, GH; Stoorvogel, JJ. 2016. Mapping crop diseases using survey data: the case of bacterial wilt in bananas in the East African highlands. Europ. J. Agronomy 74: 173-184.

Brake, VM; Pegg, KG; Irwin, JAG; Chaseling, J. 1995. The influence of temperature, inoculum level and race of *Fusarium oxysporum f. sp. cubense* on the disease reaction of banana cv. Cavendish. Crop Pasture Sci. 46: 673–685.

Chaves, N. 2019. Epidemiología y manejo del mal de Panamá (*Fusarium* oxysporum F.SP. Cubense) y *Radopholus similis* en banano "Gros Michel" (AAA) en cafetales bajo sombra en Turrialba, Costa Rica. Tesis de Doctorado. Universidad de Costa Rica. 158 p.

Chavez, N; Schilly, A; Salcedo, AI; Staver, C. 2015. Marchitez por Fusarium en Costa Rica, resultados de investigación. *In* Memorias III Congreso Latinoamericano y del Caribe de Plátanos y Bananos. St. Catarina, Brazil.

Díaz-Romeu, R; Hunter, A. 1978. Metodología de muestreo de suelos, análisis químico de suelos y tejido vegetal y de investigación en invernadero. CATIE, Turrialba, CR. 62 pp.

Dijkshoorn, K; Huting, J; Temple, P. 2005. Update of the 1: 5 million soil and terrain database for Latin America and the Caribbean (SOTERLAC; version 2.0). Report 2005/01, ISRIC – World Soil Information, Wageningen, The Netherlands

Dita, MA; Waalwijk, C; Buddenhagen IW; Souza Jr, MT; Kema GHJ. 2010. A molecular diagnostic for tropical race 4 of the banana Fusarium wilt pathogen. Plant Pathol. 59: 348–357.

Domínguez, J; Negrín, M; Rodríguez, C., 2001. Aggregate water-stability, particle-size and soil solution properties in conducive and suppressive soils to Fusarium wilt of banana from Canary Islands Spain. Soil Biol. Biochem. 33: 449–455.

Elementar Analysensysteme GmbH., 2011. Vario macro cube elemental analyzer condensed manual. Elementar Analysensysteme GmbH, Germany. 4 p.

Escobedo, A. 2010. Cadena productiva de banano criollo ("Gros Michel") de Costa Rica. CATIE. Turrialba, CR. 28 p.

Fick, SE; Hijmans, RJ. 2017. Worldclim 2: New 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology, 37: 4302-4315.

García-Bastidas, F; Ordóñez, N; Konkol, J; Al-Qasim, M; Naser, Z; Abdelwali, M; Salem, N; Waalwijk, C; Ploetz, RC; Kema, GHJ. 2014. First report of *Fusarium oxysporum f. sp. cubense* tropical race 4 associated with Panama disease of banana outside Southeast Asia. Plant Dis. 98: 694.

García-Bastidas, FA; Quintero-Vargas JC; Ayala-Velasquez, M; Schemer, T; Seidl, MF; Santos-Paiva, M; Noguera, AM; Aguilera-Galvez, C; Wittenberg, A; Hofstede, R., Sørensen, A; Kema GHJ. 2014. First report of Fusarium wilt Tropical Race 4 in Cavendish bananas caused by*Fusarium odoratissimum* in Colombia. APS publications. Available in https://doi.org/10.1094/PDIS-09-19-1922-PDN

Geense, P; Pattison, AB; Kukulies, TL; Scholberg, JMS; Molina, AB. 2015. Can changes in soil properties in organic banana production suppress Fusarium wilt? Nat. Resour. 6: 181–195.

Ghorbani, R; Wilcockson, S; Koocheki, A; Leifert, C. 2008. Soil management for sustainable crop disease control: a review. Environ. Chem. Lett. 6: 149–162.

Gibson, D; Connnolly, J; Hartnett, D; Weidenhamer, J. 1999. Designs for greenhouse studies of interactions between plants. Journal of Ecology. 87: 1–16.

Heyman, F; Lindahl, B; Persson, L; Wikström, M; Stenlid, J. 2007. Calcium concentrations of soil affect suppressiveness against Aphanomyces root rot of pea. Soil Biol. Biochem. 39: 2222–2229.

Holdridge, RL. 1987. Ecología basada en zonas de vida. Instituto Interamericano de Cooperación para la Agricultura. San José, CR, 216 p.

Janvier, C; Villeneuve, F; Alabouvette, C; Edel-Hermann, V; Mateille, T; Steinberg, C. 2007. Soil health through soil disease suppression: Which strategy from descriptors to indicators? Soil Biol. Biochem. 39, 1–23.

Kass, DHL; Jimenez, M; Kauffman, JH; Herrera-Reyes, C. 1995. Costa Rica: Reference soils of the Turrialba valley and slopes of the Irazú volcano. Soil Brief Costa Rica 2, CATIE-ISRIC. Wageningen, The Netherlands.

Klinkert, S. 2014. A Mechanistic approach to soil variability at different scale levels: A case study for the Atlantic Zone of Costa Rica. MSc. Thesis, Wageningen University, Wageningen. The Netherlands. http://edepot.wur.nl/310077

Mazzola, M. 2002. Mechanisms of natural soil suppressiveness to soil borne diseases. Antonie van Leeuwenhoek 81: 557–564.

Marschner, P. 2012. Marschner's Mineral nutrition of higher plants. Elsevier. 672 p. Available in https://doi.org/10.1016/C2009-0-63043-9

Mehlich, A. 1984. Mehlich 3 soil test extractant: a modification of Mehlich 2 extractant. Soil Plant Anal. 15, 1409–1416.

NASA JPL. 2009. ASTER global digital elevation model. NASA JPL. Available in https://doi.org/10.5067/ASTER/ASTGTM.002

Ordoñez, N; Garcia, FA; Laghari, H; Akkary, M; Harfouche, EN; Awar, al Awar, BN; Kema, GHJ. 2015. First report of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 causing Panama disease in Cavendish bananas in Pakistan and Lebanon. Plant Dis. 100: 209.

Orr, R; Nelson, P; 2018. Impacts of soil abiotic attributes on Fusarium wilt, focusing on bananas. Applied Soil Ecology. 132: 20-33.

Pattison, AB; Moody, PW; Badcock, KA; Smith, LJ; Armour, JA; Rasiah, V; Cobon, JA; Gulino, L.-M; Mayer, R. 2008. Development of key soil health indicators for the Australian banana industry. Appl. Soil Ecol. 40: 155–164.

Peng, H.X; Sivasithamparam, K;Turner, DW. 1999. Chlamydospore germination and Fusarium wilt of banana plantlets in suppressive and conducive soils are affected by physical and chemical factors. Soil Biol. Biochem. 31: 1363–1374.

Ploetz, RC; Churchill, C. 2011. Fusarium wilt: the banana disease that refuses to go away. Acta Hortic. 897: 519–526.

Ploetz, R; Freeman, S; Konkol, J; Al-Abed, A; Naser, Z; Shalan, K; Barakat, R; Israeli, Y. 2015. Tropical race 4 of Panama disease in the Middle East. Phytoparasitica, 43: 283-293.

Poveda, L. 2013. Evaluación de tratamientos biológicos y químicos para el combate del mal de Panamá en banano Gros Michel (*Musa* AAA, cv. Cocos). Tesis de Licenciatura. Universidad de Costa Rica. Turrialba, CR. 66 p.

Ramírez, C; Tapia, A; Calvo P. 2010. Quality evaluation of the banana of height produced in the area of Turrialba, Costa Rica. InterSedes. XI (20): 114-136

Rishbeth, J. 1955. Fusarium wilt of bananas in Jamaica. II. Some aspects of host-parasite relationships. Ann Bot NS XXI: 215–245.

Rivero, C; Chirenje, T; Ma, L; Martínez, G. 2004. Influence of compost on soil organic matter quality under tropical conditions. Geoderma 123: 355-361.

Riveros, A; Rosales, F; Romero, J; Romero, C; Jiménez, M; Jiménez, R; Acuña, O; Tabora, P; Segura, R; Pocasangre, L; Villalobos, M. 2006. Estandarización de enmiendas orgánicas para banano en América Latina y el Caribe. Págs. 234-240. *In* Memorias de la XVII Reunión Internacional ACORBAT. Joinville, Brasil.

Scher, FM. 1980. Mechanism of biological control in a Fusarium-suppressive soil. Phytopathology 70: 412.

Segura, RA; Serrano, E; Pocasangre, L; Acuña, O; Bertsch, F; Stoorvogel, JJ; Sandoval, JA. 2015. Chemical and microbiological interactions between soils and roots in commercial banana plantations *Musa* AAA, cv. Cavendish. Sci. Hortic. 197: 66–71.

Segura, RA; Stoorvogel, JJ; Samuels, JZ; Sandoval. JA. 2018. Managing the interactions between soil abiotic factors to alleviate the effect of Fusarium wilt in bananas. Acta Hortic. 1196: 163–168.

Shen, Z; Ruan, Y; Wang, B; Zhong, S; Su, L.; Li, R; Shen, Q. 2015. Effect of biofertilizer for suppressing Fusarium wilt disease of banana as well as enhancing microbial and chemical properties of soil under greenhouse trial. Appl. Soil Ecol. 93: 111–119.

Smith, LD; Smith, KM; Tree, D; O'Keefe, D; Galea,VJ. 2008. Development of a small-plant bioassay to assess banana grown from tissue culture for consistent infection by *Fusarium oxysporum* f. sp. cubense. Australian Plant Pathology 37: 171–179.

Stoorvogel, JJ; Segura, RA. 2018. Nutrition and soil management in banana cultivation. Pags. 223-234. *In* Achieving sustainable cultivation of bananas. Vol. 1. Burleigh Dodds Science Publishing. Cambridge, UK.

Stover, RH. 1962. Fusarial wilt panama disease of bananas and other Musa species. The Commonwealth Mycological Institute, Kew Surrey, UK. 117 p.

Stover, R.H., Simmonds, NW. 1987. Bananas. Longman Scientific & Technical, Harlow, UK. 468 p.

Sugimoto, T; Watanabe, K; Yoshida, S; Aino, M; Matsuyama, M; Maekawa, K Irie, K. 2007. The effects of inorganic elements on the reduction of Phytophthora stem rot disease of soybean, the growth rate and zoospore release of Phytophthora sojae. J. Phytopathol. 155, 97–107.

Sun, J; Liangping, Z; Wenbin L; Jinghao Y; Yuguang W; Qiyu X; Ming P. 2018. Rhizosphere soil properties and banana Fusarium wilt suppression influenced by combined chemical and organic fertilizations. Agric. Ecosys. Environ. 254: 60–68.

Tapia, AC; Vargas, M; Brenes, S. 2009. Evaluación de sistemas orgánicos y tradicionales de banano de altura en Turrialba, Costa Rica. Rev. Bras. De Agroecología 4: 464-468.

Tripp, R; Woolley, J. 1989. The Planning of on-farm research: Identifying for experimentation. CIMMYT, Mexico. 85 p.

Weller, DM; Raaijmakers, JM; Gardener, BBM; Thomashow, LS. 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu. Rev. Phytopathol. 40: 309–348.

Zhang, M.-X; Zhang, M.-C; Chen, P; Ruan, Y; Zhu, Y.-Y; Shen, Q.-R. 2013. Influence of nitrate/ammonium ratio on the plant growth of banana and related wilt disease development. J. Plant Nutr. Fert. 19: 1241–1247.