Relationship between the nutritional status of banana plants and black sigatoka severity in the Magdalena region of Colombia

Relación entre estado nutricional de plantas de banano y la severidad de sigatoka negra en el Magdalena - Colombia

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ABSTRACT

The association between the severity (average percentage of infection-API) by *Mycosphaerella fijiensis* Morelet and the plant nutrient content in the banana growing zone of the department of Magdalena (Colombia) was established. Between 2011 and 2012, the foliar contents of N, P, K, Ca, Mg, Na, S, Cu, Fe, B, Zn, and Mn were determined in sectors with high, medium, and low incidences in order to establish their relationships with the API. Severity was determined with the Stover and Dickson methodology, modified by Gauhl for bananas, in order to obtain sanitary information for the zone. With the obtained data, a correlation analysis was completed and the ordination technique was utilized to establish the relationships between farms and variables using an Euclidean distance. The differences between the farms and years were estimated with a two way analysis of variance with permutations and a canonical discrimination analysis in order to differentiate the farms using the measured foliar variables. The results highlighted the importance of the appropriate and balanced management of site-specific nutritional plans for the management of black sigatoka.

Key words: *Mycosphaerella fijiensis* Morelet, *Musa* sp., integrated disease management, leaf tissue analysis.

RESUMEN

En la zona bananera del departamento del Magdalena (Colombia), se determinó la asociación entre parámetros nutricionales y la severidad (porcentaje promedio de infección-PPI) producido por *Mycosphaerella fijiensis* Morelet. Entre 2011 y 2012 en sectores de alta, media y baja incidencia del patógeno, se determinó contenidos foliares de N, P, K, Ca, Mg, Na, S, Cu, Fe, B, Zn y Mn para establecer su relación con el PPI. La severidad se determinó con la metodología de Stover y Dickson, modificado por Gauhl para banano, obteniéndose información sanitaria de la zona. Con los datos se realizó análisis de correlación, se empleó la técnica de ordenación para establecer las relaciones entre fincas y variables mediante la distancia euclidiana. Las diferencias entre fincas y años se estimaron mediante análisis de varianza a dos vías con permutaciones y análisis discriminante canónico para determinar las diferencias entre fincas a partir de las variables foliares medidas. Los resultados obtenidos ponen de manifiesto la importancia del manejo sitio-específico de planes nutricionales adecuados y balanceados para reducir la severidad de la sigatoka negra.

Palabras clave: *Mycosphaerella fijiensis* Morelet, *Musa* sp., manejo integrado de enfermedades, análisis de tejido foliar.

Introduction

The banana (Musa AAA Simmonds) is cultivated in tropical and subtropical regions (Marín *et al*., 2003; Blomme *et al*., 2011; Churchill, 2011). It is a staple food in many countries, a source of income for farmers, and an important export product that generates foreign exchange income in developing countries (Cordoba and Jansen, 2014). In Colombia, 48,325 ha of banana are cultivated (36,325 ha in the Uraba zone and 12,000 ha in the Magdalena zone), with an average production of 95 million exported boxes per year, with a value of US$736 million per year, making it the third-largest exporter in the world (DANE, 2013).

Mycosphaerella fijiensis Morelet is the causal agent of black sigatoka (black leaf streak) in the majority of the cultivars of edible banana (Blomme *et al*., 2011; Churchill, 2011; Niño *et al*., 2011; Cordoba and Jansen, 2014); in favorable conditions, it is destructive and reduces yield by between 35-50% in susceptible cultivars. Annually, a typical plantation is submitted to 24-50 sprayings of fungicides, which is equivalent to approximately 30% of the production costs (Hall *et al*., 2006; Churchill, 2011).

Over time, this pathogen has increased its resistance to chemicals and its distribution in productive areas (Ganry *et al*., 2012; Guzmán, 2012). Management strategies that

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allow for the aggressiveness of the pathogen to be confronted and for the chemical load to be reduced are necessary (Orozco-Santos *et al*., 2013). *Pseudomonas*, *Bacillus*, and *Serratia* sp. have been used as biological alternatives for this purpose, but investigations on their effectiveness are in the preliminary phases and are not yet viable for commercial use (Hall *et al*., 2007; Blomme *et al*., 2011; Gutiérrez-Román *et al*., 2015).

Another strategy is to increase the resistance of the host, but high sterility levels and large generation times (from seed to seed) are obstacles for breeding programs (Irish *et al*., 2013).

Until recently, plant nutrition was considered to be an exogenous factor, with production as the main objective; but, today, it is considered to be a complementary strategy in disease management used to obtain products that are beneficial, since nutriments participate in the growth and survival of pests, predispose or increase plant resistance to pathogens, alter plant systems of defense, and increase the formation of mechanical barriers (lignification) and the synthesis of phytoalexins (Moreira *et al*., 2008). As such, the effect a nutrient element can have on diseased plants depends on the species, phenological stage, pathogen, environment, agricultural management, and nutriment availability (Jones and Huber, 2007; Marschner, 2012; Aguirre *et al*., 2012).

Although resistance is genetically controlled, it is mediated by complexes and physiological processes that are affected by the nutritional state of the plant (Datnoff *et al*., 2007). Although biotic diseases cannot be completely eliminated due to the presence of a specific nutrient, their severity or incidence may be reduced.

In this study, the relationship between the nutrient content in banana plants and the percentage of infection (API) produced by black sigatoka (*M. fijiensis*) was evaluated, as part of a macroproject that determined the associations between climate, soil properties and foliar content with black sigatoka (Aguirre, 2014).

Materials and methods

Study area. This study took place in the Magdalena Department, geographical coordinates: 10°46'00" N and 74°8'00" W, in an area composed of 11,000 ha situated at 20 m a.s.l., with an annual mean temperature of 27°C; relative humidity of 82%, evaporation of 1,500 mm year^{-1}, and precipitation of 1,371.7 mm year^{-1} (IGAC, 2009). The study area is classified as Tropical Dry Forest and Tropical Very Dry Forest in accordance with the Holdridge life zones (Chica *et al*., 2004). The region is recognized for its export banana production: 60% of the territory of the Banana Growing Zone has optimal soils for agriculture, with the influence of the Tucurinca, Sevilla, and Frio rivers providing water for most of the year. In the low zone, the water table is near the surface and close to the Cienaga Grande (large swamp) and, in the rainy season, there are floods caused by the overflow of water ways (IGAC, 2009). The areas in which the study was conducted belong to eight farms: La Vega, Eva and Manuel in the low incidence zone (Aguja), Olga and Eufemia in the middle incidence zone (Orihueca); and Don Faud, Fortuna, and Ovidio in the high incidence zone (Sevilla). The Spatial location of transects and the Edaphic and climatic characteristics of the region were described by Aguirre *et al*. (2012).

Soils in the zone correspond to Dystric Haplustepts, Typic Dystrustepts, and Typic Endoaquepts and are mineralogically dominated by kaolinite (50%), quartz (5-15%), feldspar (5-15%), interbedded (trace), micas (5-30%), montmorillonite (30-50%) and vermiculite (5-30%) (IGAC 2009; Aguirre *et al*., 2012).

Measurement of average percent of infection (API). The cultivars most representative of the Cavendish (Large, dwarf and Valery) banana were used in the study due to their production and adaptability. Ten plants per farm were used to determine the API weekly during 2011 and 2012, with the Stover methodology as modified by Gauhl (1989) (Fouré, 1985). The sanitary information for the youngest leaves (1, 2 and 3), growing normally, as measured by visual estimation as a function of six degrees of disease development in plants close to flowering, was registered using the Biological Prewarning System (Marín *et al*., 2003). The foliar emission (number of leaves) of the plants; state of the leaf primordium; level of leaf infection (II, III, and IV); and state of development of black sigatoka were evaluated, with the objective of the estimating the weighted percentage of infection (API).

Measurement of foliar variables. For the foliar analysis, samples of healthy fresh leaves and leaves in different states of disease were collected. These were processed and analyzed in the CIAT (Valle del Cauca) and Western Hemisphere Analytical Laboratory - Honduras laboratories, where the foliar element content was determined (Tab. 1).

Information analysis. With the records from the production units, a database matrix of the health and foliar

TABLE 1. Determined element contents and methods used in the foliar samples of banana plats in the Magdalena region of Colombia.

Element	Symbol	Unit	Method
Nitrogen	N	mg kg^{-1}	Acid digestion, automated spectrophotometry
Phosphorous	P	mg kg^{-1}	
Potassium	K	mg kg^{-1}	Atomic absorption spectrophotometry
Calcium	Ca	mg kg^{-1}	
Magnesium	Mg	mg kg^{-1}	
Sodium	Na	mg kg^{-1}	
Sulfur	S	mg kg^{-1}	Manual spectrophotometry
Iron	Fe	mg kg^{-1}	Atomic absorption spectrophotometry
Manganese	Mn	mg kg^{-1}	
Zinc	Zn	mg kg^{-1}	
Copper	Cu	mg kg^{-1}	
Boron	B	mg kg^{-1}	Manual spectrophotometry

analysis was constructed. With the objective of determining the existing relationships between the foliar variables and API, a graphic and numerical interpretation of the API was made for the different variables tested on the eight farms during the periods 2011 and 2012. A multivariate analysis was used, as well as its spatial-temporal variation with a factorial design in which the factors were constituted of the eight farms and the years 2011 and 2012. The API was considered to be the biotic variable and values for the twelve foliar variables were established (Tab. 1). As the assumptions of normality and homogeneity were not met, non-parametric tests were run. For the characterization of the farms and sample periods, exploratory multivariate techniques were utilized, followed by multivariate analysis of variance PERMANOVA (McCune *et al.*, 2002).

-**Spearman's Correlation Analysis:** statistical procedure applied to determine the level of association between the API and foliar variables.

-**Ordination of farms and variables:** the multivariate ordination technique was utilized, applying Euclidian distances and the R function scale in order to standardize the magnitudes and units of measure of the evaluated variables.

-**Analysis of hypothesis checking (PERMANOVA):** due to a lack of compliance with the supposed parameters of Multivariate Normality and Homogeneity of Covariance required for a multivariate variance analysis (MANOVA), a two way analysis of multivariate variance with permutations (PERMANOVA) was utilized to evaluate the differences between farms and years (factors).

-**Canonical Discriminant Analysis (CDA):** done with data obtained from a lineal multivariate model. Transformation of continuous variables was done in a canonical space controlling the terms of the model. The R statistical program, version 3.02, was utilized for the analyses (R Development Core Team *2015*).

Results

Relationships between the foliar nutrient content and API were established. The data showed (Tab. 2) that the zone with the lowest API showed the lower values of foliar levels of Mg (2.56-2.98 mg kg^{-1}) in comparison with 3.046 mg kg^{-1}, the average shown by the zone with the highest API. On the other hand, the analyses showed that the zone with the lowest API showed higher foliar levels of N (27.97 mg kg^{-1}), P (1.99 mg kg^{-1}), K (39.44 mg kg^{-1}), Ca (9.004 mg kg^{-1}), Fe (583.67 mg kg^{-1}), B (22.71 mg kg^{-1}), and Zn (30.72 mg kg^{-1}), values with direct relationships between these nutrients and the API (Tab. 3), suggesting that these nutrients play a fundamental part in the resistance and tolerance of the banana to black sigatoka.

Ordination of farms and variables. The ordination done permitted the farms to be associated with variables that characterized them. The variance explained by the

TABLE 2. Levels of the nutrients (mg kg^{-1}) and average percent of infection (API) in the banana leaves with presence of black sigatoka in farms the Magdalena region of Colombia.

	Farms							
	Donfuad	Fortuna	Ovidio	Olga	Eufemia	Eva	Manuel	La Vega
API	3.22±0.53	3.68±0.67	3.04±0.54	3.89±0.94	3.49±0.93	1.99±0.43	1.97±0.4	2.35±0.35
K	36.2±4.4	29.7±4.4	32.87±4.16	32.7±1.42	38.49±2.04	39.44±3.88	40.5±2.3	44.62±5.59
N	29.2±2.2	24.57±4.54	23.94±4.23	27.17±1.35	26.15±1.08	27.85±2.44	27.65±2.96	28.43±2.61
P	1.79±0.16	1.56±0.37	1.51±0.29	1.92±0.13	1.74±0.12	1.82±0.17	1.74±0.27	1.99±0.15
Ca	8.26±1.3	8.17±0.76	6.8±1.03	7.97±1.0	6.56±0.55	8.69±1.78	9.06±1.27	9.26±1.01
Mg	3.03±0.59	3.13±0.34	3.13±0.49	3.2±0.18	2.74±0.17	2.56±0.34	2.98±0.45	2.62±0.28
Fe	108.0±43.21	164.9±94	166.2±15.7	88.79±26.4	82.87±29.05	166.6±62.03	1364.2±136.5	220.2±23.7
Zn	24.53±7.17	34.5±15.5	28.15±10.16	16.06±1.1	16.65±2.8	32.91±14.54	31.36±11.54	27.9±10.33
B	18.83±6.9	18.9±5.46	18.49±5.64	12.47±2.12	14.02±2.06	17.5±1.96	29.95±13.38	20.69±3.96

Means±SD.

two first principal components (inertia) was 52.3%, and showed significant patterns of ordination (Fig. 1) in which the smallest API was established, which was found on the Eva, Manuel, and Vega farms whit 1.99, 1.97 and 2.35%, respectively, and was determined by greater foliar contents of K, Ca, Mg, and B.

TABLE 3. Correlational analysis between the average percent of infection (API) and foliar nutrient contents in the banana leaves with presence of black sigatoka in farms the Magdalena region of Colombia.

Var 1	Var 2	Spearman	*P*-value
API	K	-0.47	<0.0001
	N	-0.15	<0.0001
	P	-0.08	0.0402
	Ca	-0.28	<0.0001
	Mg	0.26	<0.0001
	Fe	-0.41	<0.0001
	Zn	-0.16	<0.0001
	B	-0.38	<0.0001

Correlation is significant at the 0.01 level

Analysis of hypothesis testing (PERMANOVA). Significant differences were found in the studied foliar variables between the farms and years, as in the interactions of the following factors (farm:year). In summary, it was shown that there are nutritional variables that influence the API, but they must be identified, which is why the canonical discriminant analysis (CDA) was run.

Canonical discriminant analysis (CDA). In the first discriminant function (Can1), or canonical axis, 73.9% of the variation was captured (Fig. 2). The Manuel, Eva, and Vega farms, which had the lowest API, were completely differentiated from the rest. The variables that most clearly differentiated these farms were the foliar contents of K, Fe, B, and Ca. The Don Fuad, Fortuna, Ovidio, Olga and Eufemia farms, with the higher APIs (3.22, 3.68, 3.04, 3.89 and 3.49%, respectively), were differentiated from the rest of the farms and were directly associated with the Mg content.

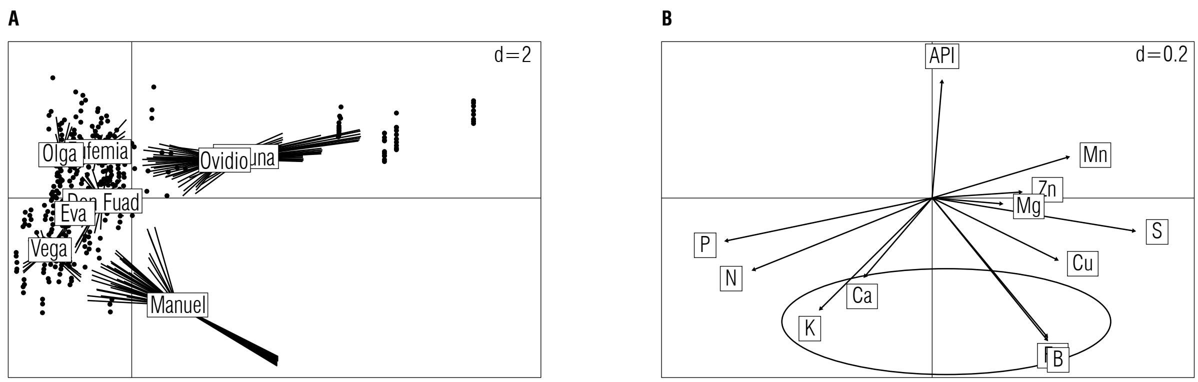

FIGURE 1. Ordination of the farms and variables for the years 2011-2012 with presence of black sigatoka in banana plantation the Magdalena region of Colombia. The average percent of infection (API) was inversely correlated to the foliar contents of K, Ca, B, and Fe. The left side shows the level of relationship of the farms based on different samples in linear relations, while the right shows the vectorial projection of the measured variables and their relationships with the API.

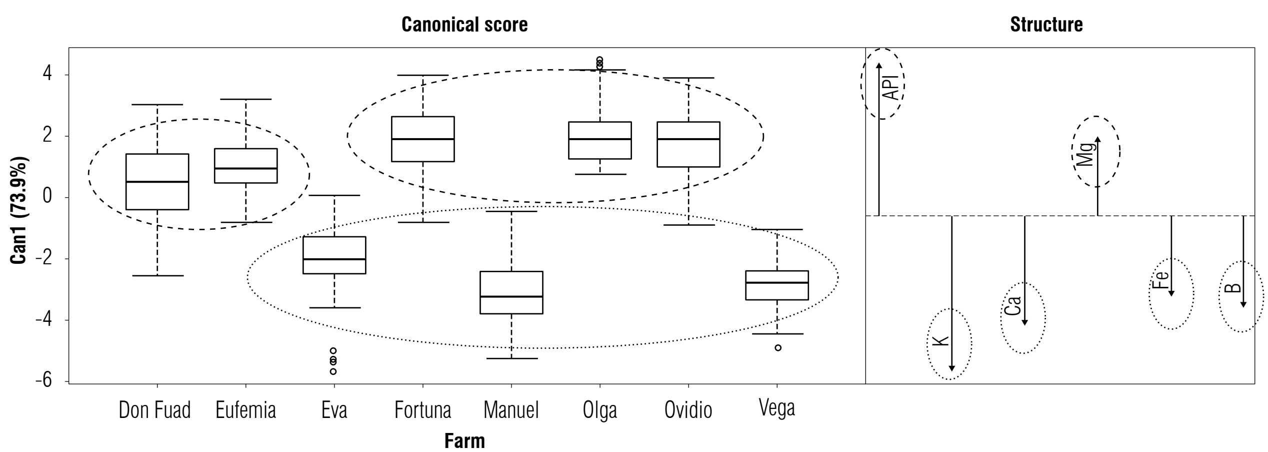

FIGURE 2. Contribution of the variables to the discriminant capacity of the analysis between the presence of black sigatoka and the foliar nutrient content in banana plantations of the Department of Magdalena. Canonical punctuation (left) represents their differences. Structure (right) corresponds with vectorial projections of foliar variables to identify their relationships with the farms.

Discussion

The dynamics of growth and development of the banana are a product of the relationships that occur during its cycle with different biotic and abiotic conditions; thus, the yield is determined by interactions between the genotype and the environment (Hay and Porter, 2006; Turner *et al*., 2007) and it is precisely in this last aspect where nutrition is found, immersed as a productive factor (Marschner, 2012).

As a result of the pathogenic infection, physiological damage that affects the absorption, assimilation, transport, and redistribution of nutrients may happen (Marschner, 2012, Wang *et al*., 2013). Adequate nutrition diminishes the development of diseases through changes in plant physiology or direct effects on the pathogen (Dordas, 2008; Spann and Schumann, 2013).

In this study, it was shown that a high foliar content of K is associated with a low API in the field. Many crops, among them banana, are strong nutrient extractors, requiring the application of K in every fertilization program (Bernstein *et al*., 2011). It is the most abundant cation in higher plants and plays an important role in essential processes, such as enzymatic activation (Cakmak, 2005; Zafar and, 2013), protein synthesis, photosynthesis, transport of solutes by the phloem, osmoregulation (Moreira *et al*., 2008), and stomatic regulation (Blatt, 2000; Cochrane and Cochrane, 2009). Because the pathogen enters the plant through the stomata (Manzo *et al*., 2012; Li *et al*., 2010; Manzo *et al*., 2005), proper attention must be given to potassium fertilization.

The authors found that the incidence of black sigatoka was low when the K: Mg ratio was high in the soil, whereas a low K: Mg ratio increased the disease (Aguirre, 2014). Furthermore, vermiculite clays, as reported by Aguirre *et al*. (2012), may be fix potassium and a high Mg content could be generating a K induced deficiency, a phenomenon explained by Marschner (2012) and Sharma *et al*. (2005) and expanded in the study area.

Other authors have reported that this element helps to reduce the incidence of pests and diseases (Cakmak, 2005; Datnoff *et al*., 2007; Dordas, 2008; Sharma *et al*., 2006; Sharma and Duveiller, 2004; Sharma *et al*., 2005). Reports have been made on the existence of a complex relationship between potassic nutrition and specific metabolic functions that alter the compatibility of the host-parasite-environment relationship, which thus reduces the susceptibility of at least 140 diseases caused by fungi, bacteria, viruses, and nematodes as long as K remains in the tissues and helps thicken cellular walls (Wang *et al*., 2013; Römheld and Kirkby, 2010). Furthermore, Perrenoud (1977) revised 2,450 references and concluded that the use of K decreases the incidence of fungal diseases in more than 70% of the cases.

The low API value obtained in the Aguja zone (including: Manuel, Eva and Vega) and that which showed the highest content leaf K, may be associated to better control of the permeability of the cytoplasmic membrane, thus avoiding the output of sugars and amino acids (pathogens feeds) to the apoplast or intercellular space and formation of phenolic compounds with different fungistatic properties. Furthermore, it may be highlighted that, in photosynthesis, K regulates the opening and closing of stomata and thus the absorption of CO_2, triggering the activation of enzymes and is essential for the production of adenosine triphosphate (ATP), a source of energy for chemical processes in plant cells. It also plays an important role in water regulation in terms of absorption through the roots and loss through stomata, and may be measured by the K content in the plant (Aguilar *et al*., 2003). In this sense, and using as a premise that the pathogen enters the plant through the stomata (Manzo *et al*., 2012; Manzo *et al*., 2005), it is necessary to emphasize care in potassium fertilization.

Likewise, for the synthesis of proteins and starches, plants require K. Deficiencies in this element bring about the accumulation of amino acids (contributing to the degradation of phenols) and soluble sugars (nutrients for pathogens), and its deficiency slows down the scarring of wounds, favoring the entrance of pathogens (Römheld and Kirkby, 2010; Wang *et al*., 2013). Therefore, K addition is only effective in disease control if it alleviates K deficiency (Huber and Jones, 2013).

The results of this study showed that an increase in Mg at the foliar level increases the API. Little information exists about the role of Mg in plant diseases. However, its role is attributed to indirect effects (ex. the absorption of other nutrients such as K) and direct effects on physiological functions in which the element acts (Huber and Jones, 2013; Jones and Huber, 2007). Dordas (2008) explained its link to the metabolism of other nutrients, in that it diminishes the content of calcium in peanuts and thus predisposes the plant to *Rhizoctonia* and *Pythium*. In the same sense, Persson and Olsson (2000) affirmed that increases in Mg may inhibit the absorption of K, Ca, and Mn. In this respect, Piraneque *et al*. (2007) and Aguirre *et al*. (2012), reported that an increased Mg content in onion bulbs reduced K uptake and increased the damage caused by *Sclerotium* *cepivorum*. Thus, it is generally accepted that the optimal nutrient supply improves disease resistance in plants (Huber and Jones, 2013, Marschner, 2012; Katan, 2009), a situation that could also be reflected in the case of *M. fijiensis* in the high API zone, where there are possible K induced deficiencies that should be investigated.

Higher levels of foliar Ca were correlated with a reduction in the API for black sigatoka, which was shown in the ordination of the farms and in the canonical discriminant analysis (Figures 1 and 2). This is a vital element with many functions and essential in the development of a firm structure due to its role in the construction of the cellular membrane (Marschner, 2012; Merhaut, 2006; Sugimoto *et al*., 2010; Sugimoto *et al*., 2008; Springer, 2009; Azofeifa, 2007). If there is not a sufficient concentration of the element in the plant tissues, it causes losses of cellular compounds and consequently the death of the plant. Calcium functions as a secondary messenger for the absorption of other nutrients and is linked with processes that allow the plant to escape environmental stress (Marschner, 2012), an example is the heat stress that leads to long stems and small leaves, a circumstances present in the study area where temperatures exceeded 28°C.

Boron is the least understood essential micronutrient in the development and growth of plants, but at the same time, among the micronutrients, its deficiency is the most extensive throughout the world (Blevins and Lukaszewski, 1998; Brown *et al*., 2002). It has a direct function in the structure and stability of cell walls and cellular membranes, in the metabolism of phenols and lignin, and in diminishing the severity of diseases (Brown *et al*., 2002). Also, this element is essential for the optimal functioning of ATPase and the redox systems of the plasmatic membrane, in which the activity of polyphenol oxidase and peroxidase is increased. Rajaratnam and Lowry (1974), working with seedlings of oil palm, demonstrated that an increase in the content of foliar B reduced the infestation of red mites (*Tetranichus-pioroei*) and reported that there is a correlation between B and the production of cyanide, a toxic polyphenol that forms complexes with nitrogenated compounds that are indigestible for mites.

These results correspond with Marschner (2012), who affirmed that this micronutrient diminishes the severity of diseases caused by *Plasmodiophora brassicae* in crucifers, *Fusarium solani* in beans, *Verticillium albo-atrum* in tomato and cotton, mosaic virus in tobacco and beans, yellow stripe virus in tomato, and *Blumeria graminis* in wheat; and by Rolshausen and Gubler (2005), who affirmed that applications of B are an alternative to the use of Benlate.

Little is known about the role of Fe in resistance to plant pathogens. Certain pathogens, such as *Fusarium,* have high requirements for this element, but there is still no certainty on the extent of iron contribution to the resistance (Dordas, 2008). However, Expert *et al*. (2012), and Kieu *et al*. (2012) made clear that new regulation cascades and virulence mechanisms influenced by iron levels have been discovered and pointed out the importance of the strict control of iron levels in the plant-pathogen subsystem, as this element is related to metabolic activities during infection.

Graham (1983) and Graham and Webb (1991) reported a diminished severity of diseases such as rust, wheat rot, *Sphaeropsis malarum* in apples and pears, *Olpium brassicae* in cabbage, and *Colletotrichum musae* in banana after an increased absorption of Fe. Kieu *et al*. (2012) and Expert *et al*. (2012) posited that Fe promotes antimycosis without interfering in the synthesis of lignin although it is a fundamental part of peroxidase and stimulates other enzymes involved in the biosynthetic pathway that is responsible for generating compounds charged with the defense of the plant, such as polyphenols.

Conclusion

High levels of foliar potassium, calcium, boron, and iron are associated with a low disease severity, whereas high levels of magnesium correlate with a high disease severity. Also, the evaluated farms were differentiated, where the higher APIs and higher Mg contents characterized the Don Fuad, Fortuna, Eufemia, Olga and Ovidio farms, while the lower APIs and higher contents of K, Ca, Fe and B characterized the Manuel, Vega and Eva farms. These results highlight the importance of an appropriate and balanced management and site-specific nutritional plans to manage black sigatoka.

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