The cause and potential solution to the Fusarium wilt disease in banana plants
La causa y potencial solución a la marchitez de plantas de plátano

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SUMMARY

The Fusarium wilt disease of banana caused by the soil-borne fungal pathogen Fusarium oxysporum f. sp. cubense is currently considered the most prominent disease that threatens the global fruit production. Control of this pathogen is essential to guarantee the supply of banana fruits. The use of chemical pesticides is a common practice for its control; however, this causes contamination of soils, water and atmosphere, and also affects the human health. The knowledge of natural antagonists of Fusarium oxysporum f. sp. cubense is a reliable alternative to develop crop protection strategies. Concerning this, the genus Trichoderma comprises mycoparasitic species adapted to distinct environmental and soil conditions. The features of Trichoderma have enabled its use as an effective biological control agent, but this potential must be extended in order to prevent the economic losses caused by the Fusarium wilt. In this review we present essential information about two contrasting fungal inhabitants of the soil: a group of phytopathogens that produces deleterious consequences in banana plants and its potential antagonists that will provide novel methods of biological control.

Index words: biological control, Panama disease, Trichoderma.

RESUMEN

La fusariosis en plátano causada por el hongo patógeno Fusarium oxysporum f. sp. cubense es considerada actualmente la enfermedad más prominente que amenaza la producción mundial del cultivo del plátano por lo que el control de este patógeno es esencial para garantizar el suministro de un fruto de calidad al mercado. Actualmente para su control se emplean pesticidas químicos los cuales causan contaminación de los suelos, del agua y la atmósfera, además de afectar la salud humana. El conocimiento de los antagonistas naturales de Fusarium oxysporum f. sp. cubense es una alternativa confiable para desarrollar estrategias de protección de los cultivos. Entre los antagonistas, el género Trichoderma comprende especies micoparasitarias adaptadas a distintas condiciones ambientales y del suelo. Las características de Trichoderma han permitido su uso como un agente de control biológico efectivo y con un uso potencial para prevenir las pérdidas económicas causadas por Fusarium. En esta revisión presentamos información esencial sobre dos habitantes fúngicos del suelo que presentan contrastantes: por una parte, un grupo de fitopatógenos que producen consecuencias perjudiciales en las plantas de plátano y sus antagonistas que proporcionarán nuevos métodos potenciales de control biológico.

Palabras clave: control biológico, enfermedad de Panamá, Trichoderma.
INTRODUCTION

Bananas (*Musa* spp.) are one of the most common staple foods, their importance as crop ranks next to rice, wheat and maize (Perrier *et al*., 2011). Southeastern Asia is considered as the center of its origin and domestication, but the climate conditions in Latin America, including Mexico, facilitate the plant development and fruit production (FAO, 2017). Banana production represents an important income to farmers and all the industry associated to the its food chain. Food safety is a factor that provides a value added because guarantees the consumption of healthy products; it has to be considered as part of the supply chain of fruit production (Carvalho, 2006). Implementation of strategies for crop protection is always required to prevent diseases, however, the production of healthy and innocuous fruits must be assured by using environmentally friendly alternatives. Use of chemical pesticides is a common practice in the fields. Unfortunately, contamination the soil, groundwater and the atmosphere have been a consequence of the recurrent use of such chemicals (Gavrilescu, 2005). Pesticides that act in unspecific manner can also affect the natural diversity of beneficial microbes and even they can affect human health (Singh *et al*., 2016).

In the case of banana plants, the protection is indispensable as diseases are the most prominent factors that harms their global production. Panama disease or Fusarium wilt is currently devastating the production of bananas and plantains, affecting the incomes of thousands of farmers all over the world (Ordonez *et al*., 2015). Due to the risks associated to the use of pesticides, and the difficulty to generate and to select resistant cultivars, screening of native antagonist of phytopathogens will be helpful to design novel methods based on biological control agents in order to avoid the use of toxic compounds.

It is widely documented that soil-borne *Trichoderma* fungus species are mycoparasites and natural antagonists of fungal phytopathogens (Harman *et al*., 2004). The adaptation of these fungi to a myriad of ecosystems allows the selection of strains that can potentially protect crops growing under different soil and environmental conditions. Furthermore, their intrinsic antagonism towards phytopathogenic fungi prevents the selective resistance pressure. These features open the possibility to implement *Trichoderma* genus as a biocontrol agent against the Fusarium wilt disease of banana. Given its importance, in this contribution we present essential considerations about the Fusarium wilt, the mode of action of *Trichoderma* and the status of its use as biocontrol agent in banana plants.

A Recalcitrant Soil Inhabitant is the Major Threat for Banana Production

*Fusarium oxysporum* f. sp. *cubense* (*Foc*) is the causal agent of the Fusarium wilt, also known as Panama disease (Figure 1). The hyphae of *Foc* accedes to the plant by the roots, once inside the plant, the fungus is incorporated into the xylem vessels and disseminates to the aerial tissue (Ploetz, 2015). Yellowing of the leaves and bending of their petioles are typical symptoms of Panama disease (Pérez-Vicente *et al*., 2014) (Figure 1). Browning of the pseudostem and necrosis of whole leaves are late disease symptoms. At this stage, *Foc* invades both xylem vessels and photosynthetic tissue. Three types of asexual spores are produced by *Foc*: macroconidia, microconidia and chlamydospores. Chlamydospores are spores produced when the plant decomposes, they are highly resistant to hostile conditions such as high radiation and temperature. Once chlamydospores are produced, they can persist in the soil for years in absence of the banana host, but they germinate and start its infectious cycle when banana plants are again placed in the fields (Ploetz, 2015).

*Foc* is a specialist pathogen of *Musa* plants, it is further classified into three races according to the host cultivar. *Foc* race 1 (FocR1) is pathogen of plants from the Gros Michel subgroup, which dominated the global market during XIX and early XX Centuries. Epidemic caused by *Foc1* eliminated the production of Gros Michel. Recovery of banana production was achieved by the introduction cultivars from the FocR1-resistance Cavendish subgroup. *Foc* race 2 (FocR2) is pathogen of Bluggoe bananas. *Foc* race 4 in divided into tropical race 4 (FocTR4) and subtropical race 4 (FocSTR4), depending on the climate conditions where pathogen cause disease. They are pathogens of the bananas from the FocR1-resistant Cavendish subgroup, and shows increased virulence towards host of FocR1. *Foc* race 3 (FocR3) is rather pathogen of plants the genus *Heliconia* and it does not affect *Musa* plants (Ploetz, 2015). *3Foc* can invade weeds or species that coexist in the banana plantations (Pérez-Vicente *et al*., 2014), which contributes to the persistency of this pathogen.
The outbreak of *FocTR4* started in 1986 when *FocR1*-resistant Cavendish plants grown in Southeast Asia displayed Fusarium wilt, since then, *FocTR4* has caused dramatic losses in producers in Southeast Asia, Australia, Africa (Ordonez *et al.*, 2015). *FocTR4* has been recently reported in Israel (EPPO, 2018), which entailed total destruction of the plants, however, due to the durability of the chlamydospores in the soil, the destruction of the plants does not eradicate the problem. The high similarity between the isolate from Israel and an isolate form the neighbor country Jordan is an evidence of the pathogen dispersion. The dispersion of *FocTR4* has entailed a decrease in the global production and import restrictions in producers such as Philippines and Mozambique (FAO, 2017). There are no reports of the presence in Latin America and the Caribbean, but the expansion of the pathogen by climatic events or infected rhizomes are factors that alert the danger of a future establishment of *FocTR4* in Latin America. Furthermore, cultivars susceptible to *FocR1* and *FocR2* are still produced in Mexico (Nadal-Medina *et al.*, 2009). This scenario forces us to seek methods of management in the field, and thus ensure the supply of bananas and the welfare of the people involved in this production chain. The physical resistance of chlamydospores together with the ability to produce other two types of spores that contribute to preserve the fungus under unfavorable conditions, suggest that the methods of disease control must attack vegetative mycelium before the accession into the root. Therefore, native microbes already adapted to the soil that compete with this type of pathogens are reliable candidates of biological control agents.

### The Soil and Rhizosphere are Source of Sustainable Agents for Protection of Banana Plants

The total amount of carbon in the soils is higher than the carbon pools in vegetation and the atmosphere (Tarnocai *et al.*, 2009). Atmospheric CO$_2$ is fixed in plants as unique source of carbon, and distinct organic compounds are produced by the plant’s metabolism, which together with the availability of organic nitrogen serves as nutrient sources that boosts the generation and selection of the microbial biodiversity in the soils. Fungal diversity in soil play important roles in modulating the soil carbon cycling, plant nutrition and pathology. Due to the eco-physiological relationship between plants and fungi, the fungal diversity positively correlates to the plant diversity (Tedersoo
The fraction of the soil influenced by the roots is known as rhizosphere (Toal et al., 2000). At the rhizosphere, microbial population is even higher than in plant-free portions of soil. The high microbial diversity implies that such microbes play distinct roles, either providing benefits to plants such as sources of organic nitrogen and phosphorus or causing detrimental effects as in the case of Foc and other pathogens. In both cases, the response of the fungus is induced after the sensing of roots exudates produced by the root metabolic activity (Bais et al., 2006). Accumulation of root exudates in soil is a consequence of the metabolic flux of carbon. Organic compounds produced from the fixation of atmospheric CO$_2$ by the plants are incorporated in primary and secondary metabolism pathways that generates a combination of compounds secreted by the roots. Composition of the exudates is specific for every plant species, and they directly promotes the selection of specific phylotypes of the fungal community (Broeckling et al., 2008). Hence, the biodiversity of the rhizosphere is plant-specific, including saprophytes, beneficial and pathogenic fungi. Foc is adapted to interact with diverse microbes at the rhizosphere of banana plants, and it is also able to sense the metabolism of the living roots necessary to start with its infective process. Survival of beneficial species such as endophytes depends on healthy banana roots; they might compete against Foc to directly colonize root tissue, and then, decrease the root surface prone to get infected. Competition for carbon or nitrogen sources between saprophytes and Foc at the rhizosphere can also affect their respective populations. Foc has to be quite adapted to the micro-environment of the rhizosphere however, introduction of adapted saprophytes in plantations of banana might be helpful to restrain the abundance of this pathogen.

Fungal inhabitants of the soil known as mycoparasites can take nutrients by the hyphae of another fungi, have the ability to destroy hyphae by mechanical force and production of cell wall degrading enzymes, produce antibiotics that inhibit growth of the host, furthermore, they can also protect plants by stimulation of the plant immune system (Brimmer and Boland, 2003). All this features have to be considered to select novel biological control agents in the soil of banana plants. In particular, several species of the genus Trichoderma –a ubiquitous soil fungus- fulfills the features that allow their implementation to protect plants against Fusarium wilt.

**Soil Inhabitants from the Genus Trichoderma as Biological Control Agents**

Species in the genus Trichoderma are mycoparasite of many fungal phytopathogens, some are endophytes and others also plays beneficial roles to the plants providing soil nutrients, plant growth promotion and induction of resistance to biotic and abiotic factors (Contreras-Cornejo et al., 2016) (Figure 1). The first evidence of Trichoderma as mycoparasite was reported in 1932, and the first commercial formulation was registered in 1989, since then, over the 60% of the biopesticides are based on conidia or mycelia of Trichoderma spp. (Mukherjee et al., 2013). Trichoderma harzianum has been used mainly to control leaf fungal pathogens (Samuelian, 2016), however, its characteristics as a fungus adapted to the soil and rhizosphere, suggest that it could be a reliable alternative to prevent the Fusarium wilt of banana. Here, we describe and discuss the features of Trichoderma as a microorganism that offers multiple beneficial effects to plants.

**Antifungal activity.** Secondary metabolites are extensively produced by Trichoderma spp. (Reino et al., 2008). Those compounds confer adaptive advantages, and contribute to the colonization of Trichoderma into very different ecological niches. It is well documented that diverse biosynthetic pathways in Trichoderma spp. produce metabolites such as peptabiols, polyketides and terpenes with antifungal activity (Keswani et al., 2014). Production of antifungal metabolites by Trichoderma might restrict the growth of surrounding fungi, which facilitates the consumption of free nutrients and dispersion in the rhizosphere. Furthermore, direct antifungal activity might be required as part of the process of mycoparasitism. Likewise, Trichoderma can uptake and utilize nutrients directly from other fungi by mycoparasitism. Fungal recognition by Trichoderma leads the attachment to the hyphae of the host and formation of appressoria-like structures that exert a physical force over the host (Harman et al., 2004). During mycoparasitism, the production of cell wall degrading enzymes such as chitinases, glucanases and proteases is necessary to utilize host macromolecules as carbon and nitrogen source (Gruber and Seidl-Seiboth, 2012). The ability to sequester fungi by appressoria-like structures, production of antifungal compounds and activity of cell wall degrading enzymes are adaptations acquired by Trichoderma that place it as
a potent fungal antagonist. Also its persistency in the soil could be potentiated by roots exudates produced by plants that took advantage of its antifungal character.

**Iron uptake.** Iron is an essential micronutrient, nevertheless, most of the iron present in the soil is insoluble. The absorption of available iron is mediated by the secretion of siderophores that chelate and carry Fe$^{3+}$ into the cell, which is later reduced to Fe$^{2+}$. Harzianic acid is a Fe$^{3+}$ siderophore produced by *Trichoderma harzianum* that promotes the growth of canola seedlings, possibly by facilitating the Fe$^{3+}$ uptake to the roots, and also showing *in vitro* antifungal activity against phytopathogens such as *Rhizoctonia solani* (Vinale *et al*., 2009). Over 10 different siderophores are produced by the *Trichoderma* spp. (Lehner *et al*., 2013); which suggest the prominent role of *Trichoderma* in the mineral relocation between organism of the rhizosphere. During saprophytic growth, *Foc* and other phytopathogens might not have efficient systems of iron uptake, hence, colonization of plant could facilitate the acquisition of this micronutrient. Fe$^{3+}$ chelation in the rhizosphere might have a simultaneous effect as provider of Fe$^{3+}$ to the plants and as inhibitor of Fe$^{3+}$ uptake by the phytopathogens.

**Promotion of plant growth.** Mutual benefit between *Trichoderma* and plant species is favored by the physical contact between roots and this fungus, thus, the exchange of nutrients and signal molecules is facilitated. Moreover, the presence of *Trichoderma* at the root surface or inside the roots functions as a barrier that impedes the access of pathogens such as *Foc* (Contreras-Conejo *et al*., 2016). In addition to colonizing the epidermis of cucumber roots, *Trichoderma harzianum* can penetrate and grow in intracellular spaces of outer cortical cells (Yedidia *et al*., 1999). Roots colonized by *Trichoderma* adopt a barrier that protect them against subsequent pathogens invasion. Under hydroponic conditions, *Trichoderma harzianum* promoted an increased biomass production of cucumber plants as well as the germination rate of the seeds (Yedidia *et al*., 2001). Several reports reviewed by López-Bucio *et al*. (2015) illustrate the positive effect of *Trichoderma* spp. as stimulator of growth of plant aerial tissue or roots. This stimulation of plant growth is consequence of changes in the hormonal balance (Guzmán-Guzmán *et al*., 2018). Hormones produced by *Trichoderma* at the contact interface of the root can be efficiently recognized by the root cells, and consequently modulate cell proliferation and differentiation in the roots or get transported to induce a response in distant parts of the plant. Auxins, well-known phytohormones, are produced by *Trichoderma virens* (Contreras-Cornejo *et al*., 2009). Auxins produced by *Trichoderma virens* are biologically active since auxin-dependent responses in the roots were also reported in the same research (Contreras-Cornejo *et al*., 2009). Root branching induced by *Trichoderma virens* broaden the volume of soil explored, thus, increases the uptake of nutrients and water required to growth. Analysis of the *Trichoderma atroviride* genome identifies genes encoding biosynthetic enzymes of cytokinins, gibberellins, ethylene and abscisic acid, whose crosstalk mediates plant development (Guzmán-Guzmán *et al*., 2018). The production of other fungal hormones has been demonstrated, but this finding suggests that the concentration of phytohormones in the root is directly altered by *Trichoderma* as a mechanism to potentiate endogenous signals of development. Besides the typical phytohormones, volatile compounds of low molecular weight are produced by *Trichoderma atroviride*, they enhanced the fresh weight and the production of secondary roots in the model plant *Arabidopsis thaliana* (Nieto-Jacobo *et al*., 2017). All these results show that *Trichoderma* is endowed with biostimulant activity that complements its use as a biocontrol agent.

**Induction of plant defense response.** Conserved molecules among microbes, either phytopathogens or saprophytes can be recognized by the plant to induce defense responses (Boller and Felix, 2009). Likewise, the effect produced by phytohormones, the attachment and penetration of *Trichoderma* spp. inside the roots facilitates identification of the chemical composition of the hyphae (for example, chitin oligomers and secreted proteins), that triggers a local defense response that might not be strong enough to kill the microbiota of the rhizosphere and *Trichoderma* itself, but can limit invasion of root phytopathogens such as *Fusarium* (Jogaiah *et al*., 2018). The activity of cell wall degrading enzymes releases small fragments of cell wall constituents that can also be perceived by the plant and boost a defense response. The presence of *Trichoderma* in the root induces signaling mediated by of stress-related phytohormones such as salicylic acid and jasmonic acid (Salas-Marina *et al*., 2011), and promotes establishment of preventive defense responses in distal parts of the plant, known as induced systemic resistant (ISR), that protect the plant against
subsequent pathogen attacks, and it is also triggered by beneficial rhizobacteria (Mathys et al., 2012; Pieterse et al., 2014). Enzymes related to biosynthesis of salicylic acid have been identified in Trichoderma atroviride (Guzmán-Guzmán et al., 2018), which might be required to increase the salicylic acid pools during specific stage of the interaction. Promotion of the ISR by inoculation of Trichoderma hamatum in the roots of Arabidopsis thaliana suppressed necrotic lesions in leaves inoculated with the pathogen Botrytis cinerea (Mathys et al., 2012). Same protective effect mediated by different Trichoderma spp. has been reported in soybean (Zhang et al., 2016), tomato (Martínez-Medina et al., 2013), and pearl millet (Siddaiah et al., 2017). These evidences suggest that ISR has a general effect produced by Trichoderma spp. that enabled its selection as inhabitant of the rhizosphere. Beneficial microorganisms such as the species of Trichoderma also induce a priming effect. Priming consists on the induction of a physiological state of enhanced sensitivity to the presence of pathogens, and hyperactivation of induced defense responses when pathogens invade the plant (Martínez-Medina et al., 2016). This effect is also promoted by Trichoderma spp. in different plants, it is effective against the fungus Botrytis cinerea (Martínez-Medina et al., 2013), the oomycete Plasmopara viticola (Perazzolli et al., 2011) and the nematode Meloidogyne incognita (Martínez-Medina et al., 2017). The profitable ability of Trichoderma to induce general preventive defense responses should be explored in Trichoderma-banana interactions to develop methods to control the Fusarium wilt and other diseases.

CONCLUSIONS

- Bananas are important staple foods whose global production is threatened by the Fusarium wilt, the emergence of highly virulent FocTR4 aggravates the current problem. Foc produces chlamydospores that persist in soil for years, which makes the crop rotation and the use of chemical fungicides ineffective alternatives of control. Chemical treatments for fungal diseases contaminate soil, water and represent a risk for human health. The soil-borne species of Trichoderma directly inhibit phytopathogens and induce general defense responses that protect plants against pathogens in local and systemic tissues. The growth promotion of Trichoderma which is mediated by production of phytohormones and increased iron supply is also beneficial to the plant.
- Bioprospection and characterization of native strains of Trichoderma spp. isolated from rhizosphere of banana plants which are adapted to interact with plants and microbiota, might be the basis for the implementation of novel and sustainable biological control formulations to cope with the Fusarium wilt.

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