Fusarium wilt of banana in Cuba:

Pathogen diversity and implications of a wider host range

Einar Martínez de la Parte

Propositions

1. Without a fully resistant cultivar, exclusion is the only feasible management practice for TR4.
   (this thesis)

2. Redefining the *Fusarium* race nomenclature requires a molecular and genetic analysis of the plant-pathogen- interaction.
   (this thesis)

3. Microbial diversity in soil and its potential applications in agriculture is underestimated due to a lack of data.

4. The negative bias against research from least-developed regions is a hurdle for scientists from these regions to publish in high-impact journals.

5. Globalization perpetuates environmental degradation in vulnerable regions of the global south.

6. Inclusive leadership styles are crucial in managing the cultural diversity of multinational working groups.

Propositions belonging to the thesis, entitled:
"Fusarium wilt of banana in Cuba: pathogen diversity and implications of a wider host range"

Einar Martinez de la Parte
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Thesis

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CHAPTER 1

General Introduction

INTRODUCTION

Bananas originated in Southeast Asia where their domestication began about 7,000 years ago (D'hont *et al.*, 2012). Since then, they have been used for food, and many other purposes such as medicine, shelter and fiber (Kennedy, 2009). From the center of origin, bananas were disseminated to other regions. They were brought to East Africa, where they rapidly became an important food crop (Perrier *et al.*, 2011). In the Americas, bananas and plantains were introduced in the 16th century after the arrival of the Spanish and Portuguese. The arrival occurred in 1516, when friar Tomás de Berlanga, of the Order of Preachers, brought a banana plant from the Canary Islands to the Hispaniola Island, which is now the Dominican Republic, and then they were taken to Cuba in 1529, and later to other countries of the region (Marin *et al.*, 1998). However, it has also been suggested that bananas were introduced to the Americas by the Portuguese via the Cape Verde Islands (Ashe *et al.*, 1971; Bradley, 1992). In any case, the fruit became an important part of the local diet soon after its arrival. Furthermore, bananas have been commercially grown and eventually exported in Central and South America, since the 1870´s, gaining significant attention in the 20th century when they became one of the most traded fruit crops (Koeppel, 2008). Today, bananas (including dessert banana, plantain and cooking banana) are among the world's leading food crops, with an annual total production of more than 170 million tons of which only less than 12% (more than 20 million tonnes) is exported (FAO, 2022 a,b) which indicates the high importance of these crops for local diets and food security in all producing countries.

Bananas are perennial monocotyledonous herbs of the order Zingiberales, family Musaceae and genus *Musa*, which is divided into two sections, namely *Callimusa* and *Musa* (Häkkinen, 2013). Edible bananas are predominantly related to the *Musa* section and include a number of diploid and triploid hybrids derived from two wild and seeded species, *M. acuminata* and *M. balbisiana*, with the former contributing to the so-called "A" genome and the latter contributing the "B" genome (Perrier *et al.*, 2011). Cultivated hybrids also include tetraploid bananas, which have essentially been developed through breeding programs in different parts of the world (Amorim *et al.*, 2020). However, despite of the existence of about 300 distinct cultivars of edible bananas, only a few have characteristics suitable for commercial exploitation, such as the sweet cultivars of the Gros Michel (AAA) and Cavendish (AAA) subgroups. As a result, these bananas have been traditionally propagated vegetatively or as tissue-cultured plantlets, and distributed throughout the different producing regions of the world. This has led to a massive genetic uniformity in the crop, making bananas more prone to rapidly succumb to pathogen and pest epidemics (Drenth & Kema, 2021).

Banana production is affected by numerous diseases and pests around the world (Jones, 2019). Among them, Fusarium wilt of banana (FWB), is considered to be one of the most destructive diseases affecting the crop (Stover, 1962; Ploetz & Pegg, 1999; Ploetz *et al.*, 2015). The disease, was first reported in Australia but gained notoriety from the epidemic of race 1 (R1) of the FWB pathogen which severely impacted Gros Michel export plantations in Latin American during the first half of the 20th century (Wardlaw, 1961; Stover, 1962; Ploetz, 2005). At present,

the tropical race 4 (TR4) currently known as *Fusarium odoratissimum* is the biggest threat to the sustainability of the global banana industry, which is based on R1 resistant Cavendish cultivars. TR4 also affects a wide range of bananas (Zuo *et al.*, 2018; Chen *et al.*, 2019; García-Bastidas, 2019), including regionally important cultivars, threatening food security in regions that rely on bananas as one of their main staple foods (Drenth & Kema, 2021; van Westerhoven *et al.*, 2022b).

Bananas: cultivar diversity and production

Despite the great genetic diversity of the genus *Musa* in its center of origin, the history of the global distribution of bananas illustrates that only a tiny fraction of this genus is used in modern commercial banana production (Drenth & Kema, 2021). Overall, banana cultivars include both dessert types and cooking types, but some cultivars can have dual uses. Cavendish dessert cultivars (genome group AAA), are currently the most widely grown banana (Lescot, 2020) and the preferred cultivars of major export companies. However, Cavendish bananas are also increasingly grown by smallholders in all regions, thereby contributing to the extinction of traditional cultivars and local landraces (Chase *et al.*, 2023).

Globally, the most popular bananas found in local markets belong to the Cavendish, Plantain and Mutika Lujugira, subgroups, of which the top five cultivars are Grande Naine (AAA), Gros Michel (AAA), Williams (AAA), Manzano (AAB) and Poyo (AAA). However, the proportion of the most popular subgroups varies by region. In Latin America and the Caribbean (LAC), Cavendish and Plantain are the most popular, but in Africa, Cavendish, Plantain, and East African Highland bananas (EAHB, Mutika Lujugira subgroup), whereas in Asia and the Pacific region Cavendish and Pisang Awak dominate local markets (Chase *et al.*, 2023). Furthermore, the range of banana cultivars used in production is specific to each country. For example, in Ecuador 89% of bananas produced are Cavendish, whereas in Uganda, which is the leading banana producer in Africa, Cavendish accounted for only 4% of total national banana production in 2018 (Lescot, 2020).

In Cuba, the clonal composition of banana fields has historically been shaped by international market demands, local preferences and the impact of diseases (Table 1). Nowadays, the clonal composition of Cuban fields is Cavendish and other AAA cultivars-11.3%, Plantains and Pisang Ceylan-34.4% and Bluggoes and Pisang Awak-46%, with Plantain (AAB) and Bluggoe (ABB) accessions dominating (79%) the country´s banana acreage, and Cavendish representing only 11 % of national production (unpublished data; DSV, 2014). In this scenario, another ABB banana, Pisang Awak is one of the most popular cultivars in Cuba due to its semi-acid taste and drought tolerance. In addition, Cuba is one of the countries with the largest area planted with FHIA (Fundación Hondureña de Investigación Agrícola) hybrids (Frison & Sharrock, 2000).

Bananas are predominantly produced in Asia, Africa and Latin America (Figure 1). The largest banana producers for domestic consumption are India and China with more than 10 million of tonnes produced annually (FAO, 2022a,b), while only 1% of this production is exported (Lescot, 2020). In Africa, banana is a very important cash crop and important staple food for millions of people, particularly in East Africa, the region with the highest per capita consumption of bananas in the world (Petsakos *et al.*, 2019; Matthews *et al.*, 2020). The LAC

region grows around 23% of the global banana production, exports 78% of the global banana trade (FAO, 2022a,b), and includes seven of the top 10 banana exporting countries, with Ecuador being the global prime exporter with almost 7 million tonnes exported in 2021 (FAO, 2022b). Nevertheless, 62% of banana and plantain production in LAC is consumed locally, which indicates the high importance of these crops in local diets and food security throughout the region (Dita *et al.*, 2013). Particularly in Cuba, bananas have for long been one of the country´s most important crops, as a major export commodity between 1857 and 1950 (Pérez-Ponce & Orellana, 1995; García, 2000, 2001), and nowadays an important cash crop for smallholders and indispensable staple food representing approximately 28% of all starchy fruit and roots staple foods produced in the country (ONEI, 2022). The fruit is produced in all provinces of the island, including the special municipality "Isla de la Juventud" (Figure 1). The provinces with higher areas planted with bananas are Artemisa and Matanzas in the Western region, Las Tunas, Granma and Guantánamo in the Eastern region of the country, while the leading producing provinces are Artemisa in the Western, Cienfuegos in the Center and Santiago de Cuba in the Eastern part of the island (ONEI, 2022).

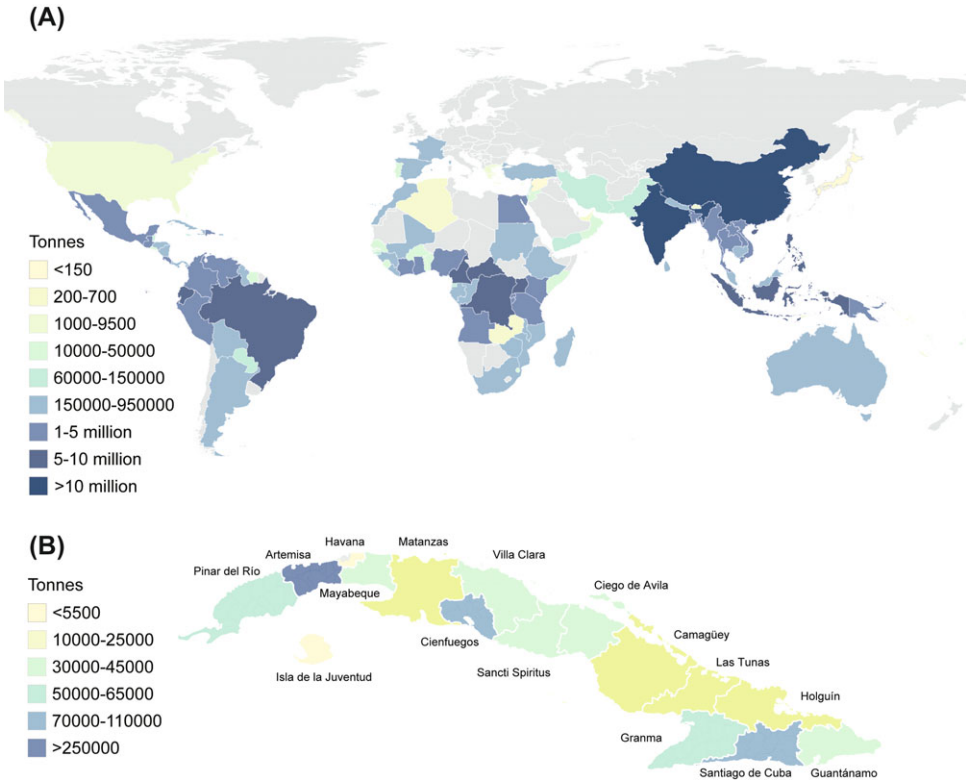

FIGURE 1. Bananas are grown in over 150 countries of tropical and subtropical regions. (A) World banana production in 2021 according to FAO (2022b). Major producers are India, China, Indonesia, Brazil, Ecuador, and the Philippines, while Ecuador and the Philippines are the world's leading exporters. (B) Bananas are produced in Cuba throughout the country. The background shading of the provinces represents the quantities (in metric tons) produced in 2021, according to ONEi (2022). Maps created with QGIS (Version 3.30.3, https://qgis.org/en/site/).

TABLE 1. Clonal composition of Cuban banana and plantain fields over time (Minneman, 1943a; Acuña, 1966; Rodriguez, 1980; Rodríguez & Rodríguez, 1983; González *et al.*, 1997; Alvarez, 2011).

Type	Genome	Period					
		Until 1945	**1946-1969**	**1970-1982**	**1983-1989**	**1990-1999**	**2000-2015**
Banana	AAA	Gros Michel Dwarf Cavendish Robusta Lacatán Red bananas	Gros Michel Dwarf Cavendish Robusta Lacatán Others	Giant Cavendish Robusta Parecido al Rey Dwarf Cavendish Valery Others	Giant Cavendish Dwarf Cavendish Parecido al Rey Grand Naine Others	Grand Naine Giant Cavendish Parecido al Rey Williams Others	Grand Naine Giant Cavendish Dwarf Cavendish Williams Robusta Others
	AAB	Silk	Silk	Silk	Silk	Silk	Pisang ceylan Manzano INIVIT
	ABB					Pisang Awak	Pisang Awak
	AAAA					SH-3436 FHIA 23	FHIA 23
	AAAB					FHIA 01 FHIA 18	FHIA 01 FHIA 18
Plantain	AAB	Macho Criollo Macho 3/4 Hembra Others	Macho 3/4 Macho enano M. de Baracoa[1] Hembra Navolean Zancudo Others	Macho 3/4 CEMSA 3/4 Macho Criollo Navolean Others	CEMSA 3/4 Macho 3/4 E. Guantanamero[2] Cueto 250 Zanzibar M. de Santa Lucia[3] Others	CEMSA 3/4 Macho 3/4 E. Guantanamero[2] Macho 7/8 Hembra Zanzivar Others	CEMSA 3/4 E. Guantanamero[2] INIVIT PV-0630 INIVIT PV-2011 Macho ¾ M. de Santa Lucia[3] Others
	AAAB						FHIA 04 FHIA 21
Cooking banana	ABB	Burro Criollo Burro Enano	Burro Criollo Burro Enano	Burro Criollo Burro Enano Burro Cenizo	Burro CEMSA Burro Criollo Burro Enano Burro Cenizo Pelipita	Burro CEMSA Burro Criollo Saba Pelipita	Burro CEMSA Burro Enano Pelipita Saba FHIA 25 INIVIT PB-2009 INIVIT PB-2012
	AABB					FHIA 03	FHIA 03

[1]Montaña de Baracoa, [2]Enano Guantanamero, [3]Macho de Santa Lucía or Vianda de Santa Lucía.

In Cuba, as in other producing countries, FWB is a major concern for banana cultivation (Ploetz *et al.*, 2015; Pérez-Vicente *et al.*, 2020; Staver *et al.*, 2020; Drenth & Kema, 2021). This devastating vascular disease withers banana plants, causing a reduction of yields and considerable economic losses (Ploetz, 2005; Drenth & Kema, 2021).

The FWB causal agents are pathogenically and genetically diverse

The soilborne fungus causal agent of FWB belongs to the *Fusarium oxysporum* species complex (FOSC), which comprises anamorphic, filamentous, and morphologically undifferentiated fungal species (O'Donnell *et al.*, 1998). Members of FOSC have the ability to cause disease in certain plant genera and families, and hence are classified in *formae speciales* that specialize on a particular host. However, once the FWB pathogen was recognized as a variant of *F. oxysporum* by Wollenweber and Reinking in 1935, the species was named as *F. oxysporum* f. sp. *cubense* (Ploetz, 2005). The addition of *cubense* is unusual because the name is not taken from the host but from the geographic origin of the first characterized strains (Cuba).

The FWB pathogen produces macroconidia, microconidia and chlamydospores for asexual reproduction and dispersal (Figure 2). The sexual stage (teleomorph) has not been found, although the mating type idiomorphs *MAT-1* and *MAT-2* have been detected in isolates from different populations of the pathogen (Fourie *et al.*, 2009; Ordóñez, 2018; Magdama *et al.*, 2020). Traditionally, isolates of the pathogen have been classified in races and vegetative compatible groups (VCG). To date, three different races of the pathogen have been described based on the pathogenicity to reference host cultivars (Pérez-Vicente, 2004; Ploetz, 2015a; Dita *et al.*, 2018). Race 1 (R1) affects Gros Michel (AAA), Manzano/Apple/Latundan (Silk subgroup, AAB), Pisang Awak (Pisang Awak subgroup, ABB), and Maqueño (Mai'a Maoli–Popoulu subgroup, AAB); while Race 2 (R2) affects cooking bananas of the Bluggoe (ABB) subgroup. Race 4 (R4) affects all cultivars in the Cavendish (AAA) subgroup in addition to those susceptible to R1 and R2, and has been split into two sub-groups (Ploetz, 2015a): Subtropical race 4 (STR4), which causes FWB on Cavendish in the subtropics under abiotic stress conditions and TR4, which affects many of the same cultivars as STR4, independent of abiotic conditions in the subtropics as well as in the tropics.

Due to the lack of a genetic basis for the discrimination of the causal agent into races, heterocompatibility or heterokaryon development between isolates has been used to characterize populations (Pérez-Vicente *et al.*, 2014). At present, 24 VCGs or VCG complexes have been identified (Ordóñez *et al.*, 2015; Mostert *et al.*, 2017; Dita *et al.*, 2018). The distribution and relationships among the various VCGs have been used as phenotypic markers to provide insight into the dissemination and evolution of populations of the FWB pathogen.

Strains belonging to diverse VCGs can belong to the same race, suggesting that pathogenicity towards a specific cultivar evolved convergently (O'Donnell *et al.*, 1998; Ploetz, 2006; Fourie *et al.*, 2009), is the product of past sexual recombination (Drenth *et al.*, 2019), or results from horizontal transfer of mini chromosomes among members of the *F. oxysporum* complex (Ma *et al.*, 2010). However, from a genetic point of view, race designations in Foc are

influenced by environmental conditions and their use as phenotypic markers is inappropriate to understand the spread and the evolution of this pathogen (Dita *et al.*, 2020).

While vegetative compatibility gives a measure of phenotypic diversity, molecular genetic markers and specific DNA sequences provide more detailed information into the genetic relationships among isolates within each VCG and among different VCGs. Different genetic markers have been used, such as translation elongation factor-1α (*tef1*), the mitochondrial small subunit (mtSSU), the Intergenic Spacer (IGS), and Internal Transcribed Spacer (ITS) of the ribosomal DNA, the RNA polymerase II largest subunit (*rpb1*), the RNA polymerase II second largest subunit (*rpb2*), and a repeat region encoded in the mitochondrial genome (MtR). These have shown that the fungus is polyphyletic, consisting of several clonal lineages (Bentley *et al.*, 1998; Fourie *et al.*, 2009, 2011; Mostert *et al.*, 2017, 2022; Maryani *et al.*, 2019). Moreover, a recent study based in multigene phylogeny confirmed these genetically distinct lineages that were consequently recognized as individual *Fusarium* species (Maryani *et al.*, 2019). Among these species, *F. odoratissimum* corresponds with TR4, while R1, R2 and STR4 isolates belong to the other species. However, the species composition of the FWB-causing pathogen population in Cuba and most Latin American countries is unknown.

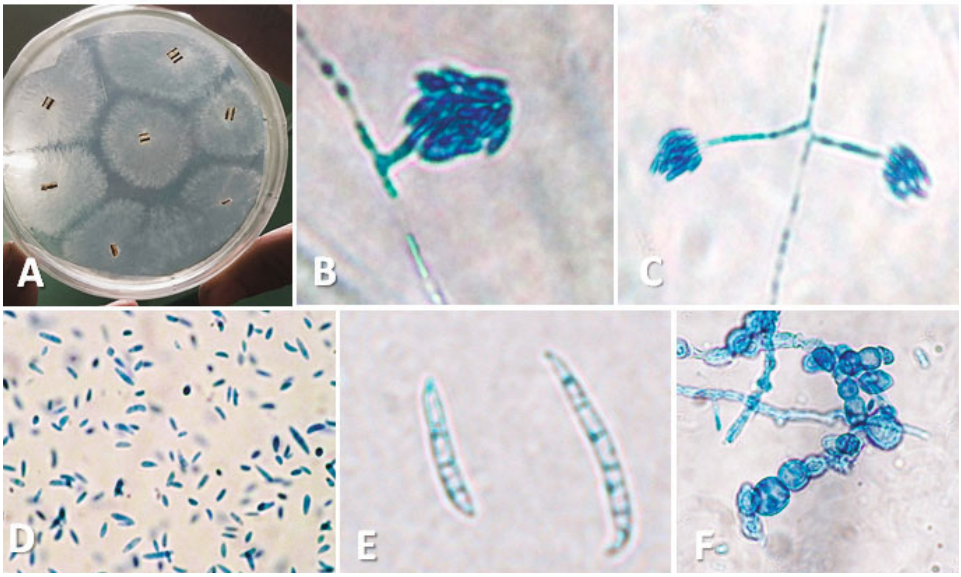

FIGURE 2. Characteristics of the causal agents of FWB: A) colony growths from pieces of infected vascular tissues; B) and C) false heads of microconidia developed in monophialides; D) microconidia; E) macroconidia; F) chlamydospores in chains.

Host–Pathogen Interactions

Spores of the FWB pathogen remain dormant until stimulated to germinate by host or non-host root exudates or by direct contact with the root tissue of susceptible plants (Stover, 1962;

Ploetz & Pegg, 1999; Guo *et al.*, 2015). Spores germinate, and produce germ tubes that attach to the roots, hyphae grow along the grooves at junctions of epidermal cells until they reach the xylem vessels of banana roots (Guo *et al.*, 2015).

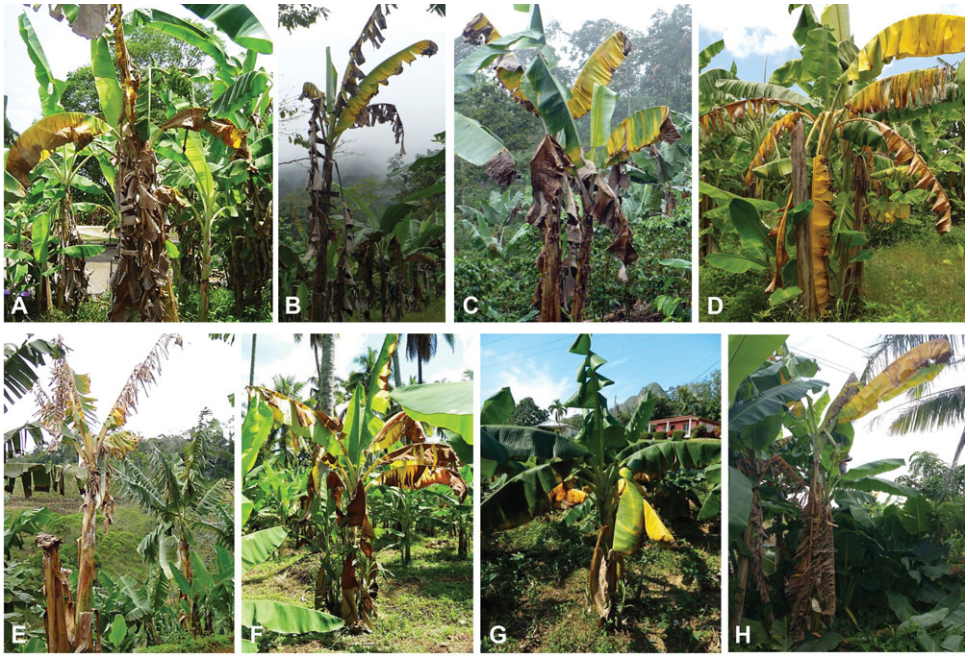

FIGURE 3. Symptoms of Fusarium wilt of banana in different cultivars. There are no differences among symptoms caused by different races of the pathogen. Symptoms caused by R1 on (A) Apple, (B) Gros Michel, (C) Cocos, (D) Pisang Awak and (E) Prata Ana. Symptoms caused by TR4 on (F) Lakatan, and symptoms caused by R2 on (G) Dwarf Bluggoe, and (H) Burro Cemsa.

Once the fungus reaches the xylem vessels inside the lateral roots, it is well-established and will eventually colonize the rhizome of susceptible banana varieties (Rishbeth, 1955). Colonization of the vascular bundles in the rhizome is a crucial step of disease development as it blocks the vessels and thus interferes with nutrient uptake and upward water transport to the pseudostem and leaves (Li *et al.*, 2017). However, xylem vessels are also blocked by host defense responses which involve the production of gels, tyloses, and lignification, leading to vascular occlusion aimed to stop the pathogen's advance (Pegg *et al.*, 2019a). After the pathogen has systemically invaded the xylem vessels with appreciable colonization of the rhizome, a severe water shortage develops in susceptible banana plants due to vascular plugging. This limitation in water movement leads to a reduction in transpiration and the expression of external symptoms (Figure 3). In resistant cultivars, infection is restricted by the rapid deployment of the host defenses in the rootlets, roots, or at the root base (MacHardy & Beckman, 1981).

During colonization and to overcome plant defenses, *Fusarium* pathogens produce a diverse array of effector proteins that determine the infection capacity in bananas (Meldrum *et al.*, 2012; Guo *et al.*, 2014; Czislowski *et al.*, 2017; Chang *et al.*, 2020; Wang *et al.*, 2020a).

Among these effectors, those secreted in host xylem sap, termed *SIX* (Secreted in Xylem) effectors have received great attention. *SIX* genes were originally described in *F. oxysporum* f. sp. *lycopersici*, and have been particularly useful for distinguishing different races and clonal lineages that exist in a *formae speciales* (Lievens *et al.*, 2009b; Taylor *et al.*, 2016; van Dam *et al.*, 2016; Czislowski *et al.*, 2018). Homologues of *SIX1* and *SIX9* have been identified in all studied *Fusarium* isolates pathogenic on banana, whereas other identified *SIX* genes include *SIX2, SIX6, SIX7, SIX10, SIX13,* and a pseudogeneised homolog of *SIX4*, while *SIX8* was only identified in race 4 isolates (Fraser-Smith *et al.*, 2014; Guo *et al.*, 2014; Czislowski *et al.*, 2018; Mostert *et al.*, 2022). Differences between TR4 and R1 isolates regarding the composition and copy numbers of *SIX* genes seem to justify their differential virulence pattern (Guo *et al.*, 2014; Czislowski *et al.*, 2018). However, their role in the virulence to banana is not clear, as only the involvement of *SIX1* and *SIX8* homologs has so far been experimentally demonstrated (Widinugraheni *et al.*, 2018; An *et al.*, 2019).

During the infection process, the causal agents of FWB also produce other effectors like binding proteins, hydrolases, oxidoreductases, proteases, transferases, and rapid alkalinization factors (Chang *et al.*, 2020; Wang *et al.*, 2020b). Interestingly, a differential expression pattern of the encoding genes for these effectors has also been found among different races of the FWB pathogen, suggesting that their expression is race-specific and plays different roles in each race (Chang *et al.*, 2020; Wang *et al.*, 2020). However, these studies only compare the level of expression of effector genes in a limited number of R1 or TR4 isolates and do not include R2 isolates, and hence do not take into account the diversity found in the pathosystem. Furthermore, comparison of R1 isolates regarding effector genes expression need to consider that they can belong to different lineages or species.

Disease occurrence and impact

Virtually all banana-producing areas for export in the Americas and Africa were affected by FWB during the past century (Ploetz, 2019). Currently, R1 and R2 are present in most banana-producing regions of the world (Figure 4), while TR4 continues to spread (van Westerhoven *et al.*, 2022).

In LAC, R1 and R2 still cause serious losses in subsistence agriculture, mainly in Gros Michel, which is still grown in agroforestry systems or intercropped with coffee (Dita *et al.*, 2013b; Magdama *et al.*, 2020). Other affected varieties are the preferred and highly susceptible Prata-type (AAB) cultivars and Apple (Silk, AAB), which account for more than 70% of the total acreage (~500,000 ha) in Brazil. In Northern Paraná (Santa Mariana, Urai, Assaí, Andirá), losses of up to 100% are recorded each year in Silk plantations, and in Corupá and the Ribeira Valley, losses in Prata can exceed 30% (Dita *et al.*, 2020). Pisang Awak and Bluggoe (ABB) types are grown due to their abiotic stress tolerance factors and adaptability to subsistence farming but are heavily affected by R1 and R2, respectively. The cultivars Maqueño and Mai'a Maoli (Mai'a Maoli' subgroup, AAB), Mysore (Mysore subgroup, AAB), Yangambi (Ibota subgroup, AAA), Isla (Iholena subgroup, AAB, in Peru), and FHIA 03 (AABB hybrid in Cuba) are sometimes affected (Stover & Waite, 1960; Battle & Pérez-Vicente, 2009; Pocasangre *et al.*, 2011). With the recent

incursions of TR4 in South America, Cavendish plantations in Colombia, Perú and Venezuela have been affected by the disease (Garcia-Bastidas *et al.*, 2020; Acuña *et al.*, 2021; Herrera *et al.*, 2023).

Similarly to LAC, Bluggoe, Gros Michel, Silk and, Pisang Awak are the banana subgroups more affected by R1 and R2 in Africa, together with Pome (AAB) and Ney Poovan (AB) (Blomme *et al.*, 2013; Karangwa *et al.*, 2016). In Kenya, losses of up to 100% of 'Wang'ae' (Ney Poovan) plantations have been reported and it is estimated that FWB directly affects the livelihood of more than 9.5 million people (Kung'u *et al.*, 2001). In Tanzania, R1 caused the destruction of 75% of the Ney Poovan plantations in an area of the Arusha/Kilimanjaro region, and gradually destroyed the widely planted Pisang Awak and Gros Michel cultivars in the Kagera district (Blomme *et al.*, 2013). In Uganda, FWB caused an estimated yield loss of more than 60% in dessert bananas (Tushemereirwe *et al.*, 2000). Cavendish bananas are also affected in Africa, under the subtropical conditions of Canary Island and South Africa, while TR4 affects Cavendish plantation in Mozambique and Mayotte (Viljoen, 2002; Blomme *et al.*, 2013; Aguayo *et al.*, 2020; van Westerhoven *et al.*, 2022a).

In India, R1 has been commonly observed to affect bananas of the Pisang Awak, Silk, Pome and Ney Poovan subgroups, causing yield reductions up to 50%-70% (Ghag, 2019) and under subtropical conditions, Cavendish bananas are affected in Gujarat and Madhya Pradesh (Thangavelu *et al.*, 2022). More recently, TR4 was introduced and affects Cavendish cultivars in the states of Bihar, Uttar Pradesh and Madhya Pradesh (Damodaran *et al.*, 2019; Thangavelu *et al.*, 2019).

More to the South, in Australia, R1 is widespread throughout the entire east coast banana production area, affecting mainly Lady Finger (Pome, AAB) but also Ducasse (Pisang Awak, ABB), Gros Michel, and Mysore (AAB) cultivars. Among these, Lady Finger is the only non-Cavendish cultivar of commercial importance (Pegg *et al.*, 1996; Bentley *et al.*, 1998). Cavendish plantations in subtropical Eastern Australia currently suffer significant damage from FWB (Pegg *et al.*, 2019). In addition, TR4 occurred in 1997 in the Northern Territory, where it destroyed the small local industry, and 18 years later it was identified in Tully, in the heart of the main production area of Northern Queensland (Drenth & Kema, 2021).

The relevance of FWB was first demonstrated by its impact on the intensive export production of Gros Michel in Central American plantations between the 1920s and 1950s. According to Stover (1962), the losses caused by R1 were calculated at US$2 billion (equal to about US$2.74 billion in 2021) (Ploetz, 2005; Drenth & Kema, 2021). However, these figures only include data registered by exporting companies and not the losses to existing smallholder production systems and cultivars. A more recent FWB damage projection, including losses caused by TR4 in Cavendish plantations, estimates a global loss of 17% of the contemporary banana acreage, which equals an annual production of 36 million metric tons worth approximately US$10 billion over the next 20 years (Scheerer *et al.*, 2018b). These numbers underscore the threat that TR4 represents for food security and sustainability of the economy of banana-producing countries.

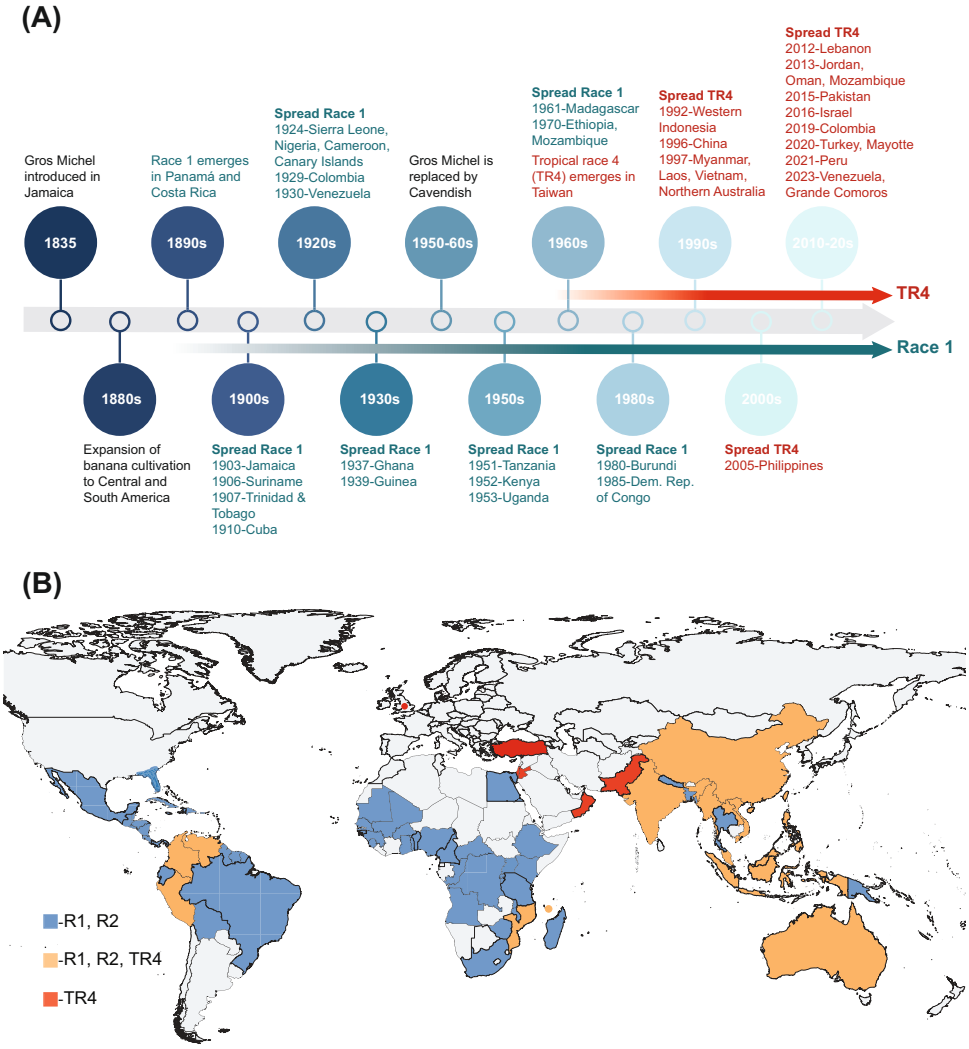

FIGURE 4. Fusarium wilt of banana is present in all producing regions of the world. (A). Timeline of the first FWB epidemic caused by Race 1 strains which decimated the Gros Michel-based banana production and the contemporary pandemic caused by the spread of TR4 that threatens global banana production and the food security of millions of people living in tropical areas (Stover, 1990; Blomme *et al.*, 2013; Drenth & Kema, 2021; Herrera *et al.*, 2023). Adapted from Dirkmaat *et al.* (2023). (B) Geographic distribution of races of the FWB pathogen.

Is the host range of the FWB pathogen limited to edible bananas?

As mentioned before *Fusarium* spp. cause FWB in different banana cultivars. Overall, FWB occurs across *Musa acuminata*, *M. balbisiana*, *M. schizocarpa*, *M. textilis* and their hybrids (Ploetz and Pegg, 1999). However, little is known about the susceptibility of ornamental *Musa* spp. (e.g. *M. velutina*, *M. coccinea*). Furthermore, *Heliconia* spp., another member of the order Zingiberales, has also been reported as a host of the fungus, with *H. caribaea, H. crassa, H. collinsiana, H.*

latispatha, H. mariae, H. rostrata, and *H. vellerigera* (Stover, 1962). Initially, these *Fusarium* strains were considered to belong to Race 3, which is currently no longer considered a causal agent of FWB, as they were either weakly pathogenic or avirulent on Gros Michel and Bluggoe (Ploetz, 2019). Additionally, it is not clear whether FWB-causing strains can infect *Heliconia* spp.

Finally, some weed and grass species have been reported as secondary hosts of *Fusarium* isolates that cause FWB. R1 isolates have been found in the roots of weeds such as *Paspalum fasciculatum, Panicum purpurescens, Ixophorus unisetus* (Poaceae), and *Commelina diffusa* (Commelinaceae) in Central America (Waite and Dunlap, 1953). Similarly, TR4 has been detected under Australian field conditions, in *Chloris barbata* (Poaceae), *Euphorbia heterophylla* (Euphorbiaceae), and *Cyanthillium cinereum* and *Tridax procumbens* L. (Asteraceae) (Hennessy *et al.*, 2005). In all cases, these weeds showed no symptoms of infection. These findings suggest that weed hosts can be reservoirs of inoculum of FWB pathogens in the absence of susceptible bananas, contributing to the longterm survival of the fungus in the soil. However, it is unknown whether the pathogenicity of recovered *Fusarium* strains from weeds are still pathogenic to banana.

THESIS OUTLINE

The first chapter provides a general introduction and addresses the history, production, cultivar diversity, and importance of bananas as an export commodity and staple food. Additionally, the chapter provides an overview of the fungal causal agents of FWB revolving around two major epidemics. The first was during the 1920s - 1950s in the Gros Michel-based industry and the second currently developing pandemic of TR4. Furthermore, it includes details of their life cycle, typical symptoms of the disease, host range, impact, classification, and the genetic diversity of pathogen populations.

The second chapter describes the exploration of FWB across all production zones of Cuba, and provides an overview of which banana cultivars are affected by the disease. Samples from these plants resulted in a collection of 170 *Fusarium* isolates. Genotyping-by-sequencing and whole-genome comparisons, show the genetic diversity across this collection in comparison with a global *Fusarium* panel, as well as isolates from LAC.

The third chapter is about the phenotyping of 18 important Cuban banana cultivars with two *Fusarium* strains, representing TR4 and Race 1, under greenhouse conditions. These varieties constitute 72,8% of the national banana acreage in Cuba and are also widely distributed in LAC. Our results underscore that TR4 potentially threatens nearly 56% of the contemporary Cuban banana production area, and call for a preemptive evaluation of new varieties obtained in the national breeding program and the strengthening of quarantine measures to prevent the introduction of TR4 into the country.

The fourth chapter takes us to The Philippines where a survey of three banana farms showed that *F. odoratissimum* can survive between crop cycles as an endophyte in weed species from seven botanical families. Subsequent greenhouse experiments showed that the pathogen is able to progress into different plant tissues besides the roots system, and the pathogenicity

of the retrieved isolates toward Cavendish banana plants varied depending on these hosts. This information broadens the perspective of effective disease management of FWB.

The fifth chapter compiles the results of greenhouse experiments to investigate whether *F. phialophorum* (Race 1) and *F. odoratissimum* (TR4) can infect *Heliconia* species, ornamental bananas or *Musa textilis* (abacá). *Heliconia* spp. and *M. velutina* were more susceptible to Race 1 than to TR4 and both strains can colonize the above-ground tissues of these plants, but the pathogenicity to banana was not compromised after reisolation from these alternative hosts. The data question the current race nomenclature and containment protocols for FWB pathogens.

The sixth chapter focuses on the genome structure, effector repertoire, and pathogenic phenotypes of *Fusarium* spp. causing FWB in Cuba. For this purpose, three new (near) chromosome-level genome assemblies of Race 1, and two Race 2 strains were compared with the existing genome assemblies of TR4 and Race 1 reference strains. Race 2 strains share accessory regions that are absent in other races. In addition, Race 1 and Race 2 strains have diverse candidate effector profiles which does not correlate with their phenotypes in pathogenicity tests that involved nine banana cultivars. The data underscore the relevance of functional analyses to elucidate the molecular mechanisms of *Fusarium* spp. to infect bananas.

The seventh chapter is the general discussion of the entire thesis, which puts the collected data in the broader context of sustainable banana production and the required research to achieve that goal.

CHAPTER 2

Dissecting the genetic diversity of the Fusarium wilt pathogens of bananas in Cuba and Latin American countries

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ABSTRACT

Fusarium wilt of banana (FWB) is a devastating plant disease that causes significant economic losses to banana production worldwide and is one of the major concerns for Cuban banana cultivation. The disease is caused by soil-borne *Fusarium* species. However, the genetic diversity among *Fusarium* species infecting bananas in Cuba is unknown. We conducted a comprehensive survey of symptomatic banana plants across all production zones of the island and built a collection of 170 *Fusarium* isolates. Using genotyping-by-sequencing and whole-genome comparisons, we investigated the genetic diversity across this suite of isolates and compared it with the genetic diversity in a global *Fusarium* panel. Typical FWB symptoms were observed in varieties of the Bluggoe cooking banana and Pisang Awak subgroups in 14 provinces. Phylogenetic analysis revealed that *F. purpurascens, F. phialophorum,* and *F. tardichlamydosporum* cause FWB in Cuba, with the latter dominating the population. Furthermore, we identified between five and seven genetic clusters, with *F. tardichlamydosporum* isolates divided into at least two distinct subgroups, indicating a high genetic diversity of *Fusarium* spp. causing FWB in the Americas. Our study provides unprecedented insights into the population genetic structure and diversity of the FWB pathogen in Cuba and the Latin American and Caribbean regions.

INTRODUCTION

Bananas are among the most produced, traded, and consumed fruits globally (FAO, 2022a). In terms of global production, bananas directly follow behind major crops such as wheat, rice and maize (Perrier *et al.*, 2011). With more than 1,000 cultivars, bananas are a staple food for more than 400 million people who rely on the commodity for food security and income. Approximately 84% of the crop is grown by smallholders and delivered to domestic markets, while the remaining 16% goes to international markets with an estimated annual export volume of approximately 20 million tones (FAO, 2022a). Bananas arrived in Cuba in the 16th century and are among the most produced fruits in the country, required for food security and providing an important source of income for small growers (ONEI, 2022; Martínez-de la Parte *et al.*, 2023).

The global production of bananas, including plantains, is threatened by a range of different globally spreading plant diseases (Drenth & Kema, 2021). Among these, Fusarium wilt of banana (FWB) is one of the most destructive and widespread diseases of the crop (Ploetz, 2015b). It wiped out the famed Gros Michel variety in the previous century and currently devastates Cavendish plantations, and many other banana cultivars consumed and traded locally around the world (Ploetz, 2019; Staver *et al.*, 2020; van Westerhoven *et al.*, 2022b). The disease is caused by diverse, soil-borne anamorphic fungi belonging to the *Fusarium oxysporum* species complex (FOSC), which have been traditionally classified as *Fusarium oxysporum* f. sp. *cubense* (*Foc*). Genetic diversity was previously described by using vegetative compatibility groups (VCGs; (Ploetz & Correll, 1988; Brake *et al.*, 1990; Ploetz, 1990), a system where genetically related individuals exchange genetic material through heterokaryosis (Pérez-Vicente *et al.*, 2014). Strains with identical alleles at vegetative or heterokaryon incompatibility (*het*/*vic*) loci belong to the same VCG (Correll, 1991). Physiological specialization towards particular banana varieties resulted in the definition of a core set of races; Race 1, Race 2 and Race 4, the latter being divided into Subtropical Race 4 (STR4) and Tropical Race 4 (TR4). Race 1 strains have been identified in no less than nine VCGs, are avirulent to Cavendish under regular conditions, but can cause FWB under abiotic stress and are then categorized as STR4 (Buddenhagen, 2009; Ploetz, 2015b; Mostert *et al.*, 2017). This illustrates that there is no strict correlation between VCGs and races in the FWB-banana pathosystem (Ordóñez *et al.*, 2015). Nevertheless, VCGs (up to 24) have been used to gain insight into the dissemination and evolution of the causal agents of FWB (Fourie *et al.*, 2009; Ordóñez *et al.*, 2015; Mostert *et al.*, 2022), although they do not provide a measure of genetic distance between phenotypes (Bentley *et al.*, 1998; Fourie *et al.*, 2009). Thus, neither VCGs nor physiological races have contributed to a thorough understanding of the phylogeny and dissemination of the causal fungi of FWB (Dita *et al.*, 2020).

DNA-based techniques provide detailed information about the genetic distance of different pathogen populations (Freese & Beyhan, 2023; Feurtey *et al.*, 2023). Previously, phylogenetic analyses revealed that *Foc* is polyphyletic and divided into three FOSC clades, which can be further divided into eight to ten individual lineages (Bentley *et al.*, 1998; O'Donnell *et al.*, 1998; Groenewald *et al.*, 2006; Fourie *et al.*, 2009; Mostert *et al.*, 2017; Ordóñez, 2018). High-resolution

genotyping-by-sequencing analyses using Diversity Array Technology (DArTseq; Cruz *et al.*, 2013, Kilian *et al.*, 2003) is a genome-wide method that validated and extended these findings (Ordóñez *et al.*, 2015; Mostert *et al.*, 2022) It has also been used to study genetic diversity and resistance mapping in many crop species, such as cassava, chickpea, pea, tea, and wheat (Sohail *et al.*, 2015; Malebe *et al.*, 2019; Adu *et al.*, 2021; Sampaio *et al.*, 2021; Ahmed *et al.*, 2021) as well as in banana (Amorim *et al.*, 2009; Risterucci *et al.*, 2009; Sardos *et al.*, 2016; Ahmad *et al.*, 2020) and their pathogens (Sharma *et al.*, 2014; Ordóñez *et al.*, 2015; Mostert *et al.*, 2022).

Fusarium wilt of banana has been present in Cuba since the end of 19th century (Battle & Pérez-Vicente, 2009) and contributed significantly to the collapse of the exporting activities during the first-mid of the last century. After the substitution of the susceptible 'Gros Michel' (AAA) by resistant Cavendish (AAA) varieties during the 1950s and the large-scale cultivation of plantain (AAB) varieties, FWB lost its economical relevance (Battle & Pérez-Vicente, 2009). However, in smallholder farms and backyard gardens with conducible soils, planted with susceptible cultivars, the disease is still present. In a previous survey, FWB was observed in eight provinces across a suite of important banana cultivars in Cuba, which resulted in a small collection of 52 isolates that were characterized and divided into four VCGs and two races (Battle & Pérez-Vicente, 2009). Here, we extend this collection by conducting a nationwide survey of many geographically and environmentally different locations, resulting in a collection of 170 isolates that were subsequently characterized by DArTseq and whole-genome sequencing and compared with a global panel of isolates to investigate the diversity of the FWB causing *Fusarium* complex in Cuba.

MATERIALS AND METHODS

Survey and sampling

A comprehensive survey of Fusarium wilt of banana was undertaken in Cuba between 2016-2018. In total, locations in 58 municipalities of 15 provinces were visited, representing the main banana-producing regions in Cuba. Plants with FWB symptoms - including chlorotic and wilted leaves, collapsed leaves at the petioles, pseudostem discoloration and splitting - were sampled from large banana plantations, smallholder farms, and backyard gardens (Figure 1). At each site, global positioning coordinates and altitude data were collected, and the varieties were identified. The pseudostems of the diseased plants were cut and discolored vascular strands were collected, placed on sterile filter paper to dry, and packed in a paper envelope until further analyses.

Strain isolation, purification and maintenance

Dried pseudostem samples were cut into pieces of ~0.8 cm^2, surface sterilized with ethanol (70%) and commercial bleach (1%) for 1 min each, rinsed with sterile distilled water, plated on 2% water agar (WA) supplemented with streptomycin sulphate (100 µg/mL) in 9 cm-diameter

Petri dishes, and incubated at 25°C in complete darkness. After approximately two days, fungal colonies resembling *Fusarium* were transferred to potato dextrose agar plates (PDA, 24g/L; Thermo Scientific™ Oxoid™, Landsmeer, the Netherlands). A total of 40 isolates from a previous survey in 1993-1994 (Battle-Viera and Pérez-Vicente, 2009) were also included in the collection, resulting in a Cuban panel of in total 170 isolates (Supplementary Table S1).

Single-spore colonies were obtained for each isolate and these were maintained in PDA for short-term use and as seven-day-old spore suspensions in glycerol (2mL, 15%) stocks for long-term preservation at -80°C in Nunc® CryoTubes® (Sigma-Aldrich, Roskilde, Denmark). The entire collection was deposited in the microbial culture collection of the Instituto de Investigaciones de Sanidad Vegetal (INISAV, Cuba) with a copy at the Laboratory of Phytopathology of Wageningen University and Research (Supplementary Table S1). The total data set that was analyzed in this study comprised the Cuban panel of 170 isolates, complemented by a collection of 210 isolates from the Americas and a set of 33 isolates from a global panel that was analyzed earlier (Ordóñez *et al.*, 2023).

DNA isolation and PCR amplifications

Fungal isolates were grown on PDA plates for seven days at 28°C. Then, conidiospores were collected and transferred to a flask with 100 mL potato dextrose broth (PDB, 34g/L) (Thermo Scientific™ Oxoid™, Landsmeer, the Netherlands, Landsmeer, the Netherlands) and incubated by shaking at 140 rpm and 25°C for 5 days. The mycelium was obtained by filtering the inoculum through two layers of sterile Miracloth and washed at least twice with sterile MQ water. Subsequently, the mycelium was freeze-dried overnight in a 2 mL Eppendorf tube and ground in a mortar with a pestle using liquid nitrogen. Genomic DNA of each isolate was extracted using the MasterPure™ Yeast DNA Purification Kit (LGC Biosearch Technologies, Halle-Zoersei, Belgium), according to manufacturer´s protocol. Genomic DNA size, concentration and integrity were assessed by gel electrophoresis, NanoDrop™ One/OneC Microvolume UV-Vis Spectrophotometer (Thermo Scientific™, Landsmeer, the Netherlands) and Qubit analysis (Qubit™ Flex Fluorometer, Thermo Fisher Scientific, Landsmeer, the Netherlands).

Primer pairs PFO2 (5'-CCCAGGGTATTACACGGT-3') and PFO3 (5'-CGGGGGATAAAGGCGG-3') (Edel *et al.*, 2000), FocTR4-F (CACGTTTAAGGTGCCATGAGAG) and Foc TR4-R (5'-GCACGCCAG-GACTGCCTCGTGA-3') (Dita *et al.*, 2010), Six1a_266-F (5'-GTGACCAGAACTTGCCCACA-3') and Six1a_266-2R (5'-CTTTGATAAGCACCATCAA-3') and Six6b_210-F (5'-ACGCTTCCCAATACCGTCT-GT-3') and Six6b_210-R (5'-AAGTTGGTGAGTATCAATGC-3') (Carvalhais *et al.*, 2019) were used to identify the isolates, using the described conditions for PCR in 25 µL using GoTaq® G2 DNA Polymerase and Master Mix (Promega Benelux BV, Leiden, the Netherlands), 10 µM of each primer, 10–20 ng of DNA, and sterile deionized water. The amplicons were resolved by electrophoresis using 1.5% agarose gel, stained with ethidium bromide, visualized and photographed using the Chemidoc™ MP image system (Bio-Rad Laboratories, Veenendaal, the Netherlands). Amplicon sizes were estimated using a 100-bp ladder as reference.

Mating type idiomorphs were determined by PCR using the primer set Falpha 1 (5´-CGGTCAYGAGTATCTTCCTG-3´) and Falpha 2 (5´-GATGTAGATGGAGGGTTCAA-3´) for *MAT1-1* (Arie *et al.*, 2000), and FF1(5'-GTATCTTCTGTCCACCACAG-3´) and Gfmat2c (5´-AGCGTCATTATTCGATCAAG-3´) for *MAT1-2* (Fourie *et al.*, 2009). The cycling conditions for *MAT1-1* amplification were: initial denaturation at 95°C for 15 min; 35 cycles at 94°C for 1 min., 55°C for 30 s, 72°C for 1 min. and a final extension at 72°C for 10 min. The cycling conditions for *MAT1-2* were an initial denaturation at 95°C for 2 min; 35 cycles at 94°C for 1 min., 54°C for 40 s, 72°C for 2 min. and a final extension at 72°C for 7 min.

Pathogenicity tests

Eleven strains representative of different provinces and banana cultivars were tested for their pathogenicity towards Grand Naine (Cavendish-AAA, ITC0180), Gros Michel (Gros Michel- AAA, ITC1122) and Burro Cemsa (Bluggoe-ABB, ITC1259). Traditionally, race designations are based on the pathogenicity towards the aforementioned banana cultivars, where isolates causing FWB on 'Gros Michel' are considered as Race 1, on 'Burro CEMSA' as Race 2, and on 'Grand Naine' as TR4. Tissue culture plants, approximately 2.5-month-old, were inoculated with the selected strains following a previously published protocol (García-Bastidas *et al.*, 2019). The *F. odoratissimum* reference strain II-5 (NRRL 54006, VCG01213) was included as a positive control, and plants treated with water were used as negative controls. All the assays were conducted in an environmentally controlled greenhouse compartment (28±2°C, 16h light, and ~85% relative humidity) at the Unifarm greenhouse facility of Wageningen University & Research (WUR, The Netherlands). Twelve weeks after inoculation, the corms of the plants were cut transversally, photographed and the Rhizome Discolored Area (RDA) was calculated using ImageJ 1.52r software (National Institutes of Health, Bethesda, MD, USA). At the end of the phenotyping experiments, a random banana plant per treatment was selected for re-isolation and (molecular) diagnosis of the FWB causal agent to complete Koch's postulates.

Diversity Array Technology by sequencing (DArTseq) analyses

DArTseq is a genome complexity reduction method by establishing genomic markers, which are scored as binary data to represent either the presence or absence of the marker sequence in each isolate (Jaccoud, 2001). Based on the DArTseq markers, single nucleotide polymorphisms (SNP) can be called and similarly encoded as binary data in a matrix. Isolated DNAs from monosporic cultures of each isolate of the Cuban panel were sent to Diversity Arrays Technology Pty. Ltd. (http://www.diversityarrays.com), Canberra, Australia, for genome complexity reduction and Illumina sequencing (Kilian *et al.*, 2003), which was previously adapted for FOSC (Ordóñez *et al.*, 2015). Additionally, we included markers that were generated in a collection of 243 strains from a global *Fusarium* diversity panel (Table S3), which represents the species identified by Maryani *et al.* (2019).

The DArTseq marker and SNPs were analyzed using the R package dartR v.1.8.3 (Gruber *et al.*, 2018; R Core Team, 2021), where ploidy was set as haploid, monomorphic loci were removed from both data sets, and only isolates with a repeatability index above 1.0 were maintained. Both the remaining loci and isolates were kept for downstream analyses when their call rates were higher than 0.95 (0.98 for SNPs) and >=10 positive markers or a MAF>=0.05 in the case of SNPs were present. Based on the filtered binary presence/absence matrix we constructed a distance matrix using the Dice similarity coefficient and inferred the phylogenetic relationships using the Neighbor Joining algorithm (bootstrap=1,000) and the previously generated distance matrix in R (R Core Team, 2021). Additionally, we performed a principal component analysis (PCA) with the filtered matrix to verify our results using factoextra and FactoMineR (Lê *et al.*, 2008). To calculate the genetic diversity within each species in our dataset, we calculated the Bray-Curtis and Jaccard distances in R (R Core Team, 2021) to provide insight into diverse genotypes within some species. Thus, based on Jaccard and Bray-Curtis distances, we determined the optimal number of clusters based on the GAP statistic. Furthermore, we disentangled the number of genetic clusters in each species by Discriminant Analysis of Principal Components analysis (DAPC (Pritchard *et al.*, 2000; Grünwald & Goss, 2011). We set the maximum number of possible cluster combinations to ten and subsequently determined the optimal number based on the BIC statistic and the posterior membership probability (Grünwald & Goss, 2011).

Illumina sequencing and whole-genome comparisons

The genomes of a subset of 22 Cuban strains that capture the diversity uncovered by the DArTseq analysis were sequenced using Illumina HiSeq PE150 (BGI, Hong Kong) and *de novo* assembled with Spades (Bankevich *et al.*, 2012; version 3.13.0, with default settings). Contigs shorter than 500 bp were subsequently removed. To assess the assembly quality, the genome assemblies were analyzed with QUAST (Gurevich *et al.*, 2013; version 5.0.2), and genome completeness was estimated based on the presence of conserved single-copy genes using BUSCO (Simão *et al.*, 2015; version 5.1.2) with the sequencing odb10 database. To determine the relationship of the sequenced Cuban isolates with other banana-infecting *Fusarium* spp., we included whole-genome sequencing data of 58 previously sampled FWB pathogen isolates from different species and geographical locations (Table S4). To identify sequence variation, the sequencing reads were aligned against the *F. odoratissimum* II5 (NRRL 54006) reference strain (van Westerhoven *et al.*, 2023) using BWA-mem (v. 0.7.17) (Li, 2013). Single nucleotide polymorphisms (SNPs) of the 80 isolates compared with the reference genome assembly were called based on the GATK best practices (Auwera *et al.*, 2013), using GATK4 (v. 4.2.0.0). To account for the presence/absence variation between the genomes, we excluded SNPs located on the variable genomic regions (chromosome 1, pos. 0-1.2 Mb, and chromosome 12) (van Westerhoven *et al.*, 2023) as well as SNPs with any missing values. The maximum likelihood phylogenetics tree of the concatenated SNPs was inferred using RAxML (v. 8.2.12, -m GTRCAT).

RESULTS

Fusarium wilt of banana is widespread across Cuba

To obtain a nationwide overview of the incidence of FWB in Cuba, we completed a comprehensive survey from 2016 to 2018 that included all the provinces of the country and sampled bananas in large commercial fields, small farms, backyards, and roadsides. During the survey, plants with FWB symptoms were observed in 14 of the 15 provinces in Cuba, and in 43 of the 66 surveyed municipalities (Figure 1). In the province Las Tunas, we did not observe affected plants since most of the visited and investigated plantations were relatively young due to rejuvenation after the devastating hurricane 'Irma'. In total, 130 isolates were obtained from discolored vascular pseudostem strands sampled from symptomatic banana plants (Supplementary Table S1). The FWB-affected banana varieties comprised 'Manzano vietnamita' from the Pisang Awak subgroup (ABB), and the cooking banana varieties 'Burro Criollo', 'Burro Enano' (Dwarf), and 'Burro CEMSA' (Figure 1B) from the Bluggoe subgroup. Diseased plants showed the typical FWB symptoms but in 'Burro CEMSA' symptoms were occasionally limited to marginal leaf chlorosis and splitting of the pseudostem. In total, 37% and 43 % of the observed FWB cases were in fields of 'Manzano vietnamita' and 'Burro CEMSA', respectively, while the remaining 20% of FWB cases were in the other Bluggoe varieties (ABB). We did not observe any FWB in 'Pisang Ceylan' (AAB), 'FHIA-18' (AAAB), 'FHIA-21' (AAAB), and those of the Cavendish (AAA) and Plantain (AAB) subgroups, which are also widely cultivated in Cuba (Supplementary Table S2), suggesting that these banana varieties may be resistant to the population of the pathogen present in the island.

All isolates were initially identified as members of the FOSC based on morpho-cultural characteristics (Leslie & Summerell, 2006), like the production of oval to kidney-shaped microconidia in false heads on short monophialides, which were typically single-celled. Moreover, the different isolates produced chlamydospores which were single or in pairs and produced at hyphal ends or within the hyphae. Colonies on PDA plates varied in color, from white or pinkish to dark purple (Supplementary Figure S1). To further substantiate that the isolates belonged to the FOSC, all isolates were characterized by PCR following the protocols described by Edel *et al.* (2000), Dita *et al.* (2010), and Carvalhais *et al.* (2019), which confirmed that they belonged to FOSC and that they were either Race 1 or Race 2 strains. Importantly, all isolates tested negative with diagnostic TR4 primers (Supplementary Table S3), which explains why FWB was not detected during the survey on bananas of the Cavendish and Plantain subgroups, which are resistant to Race 1 and Race 2.

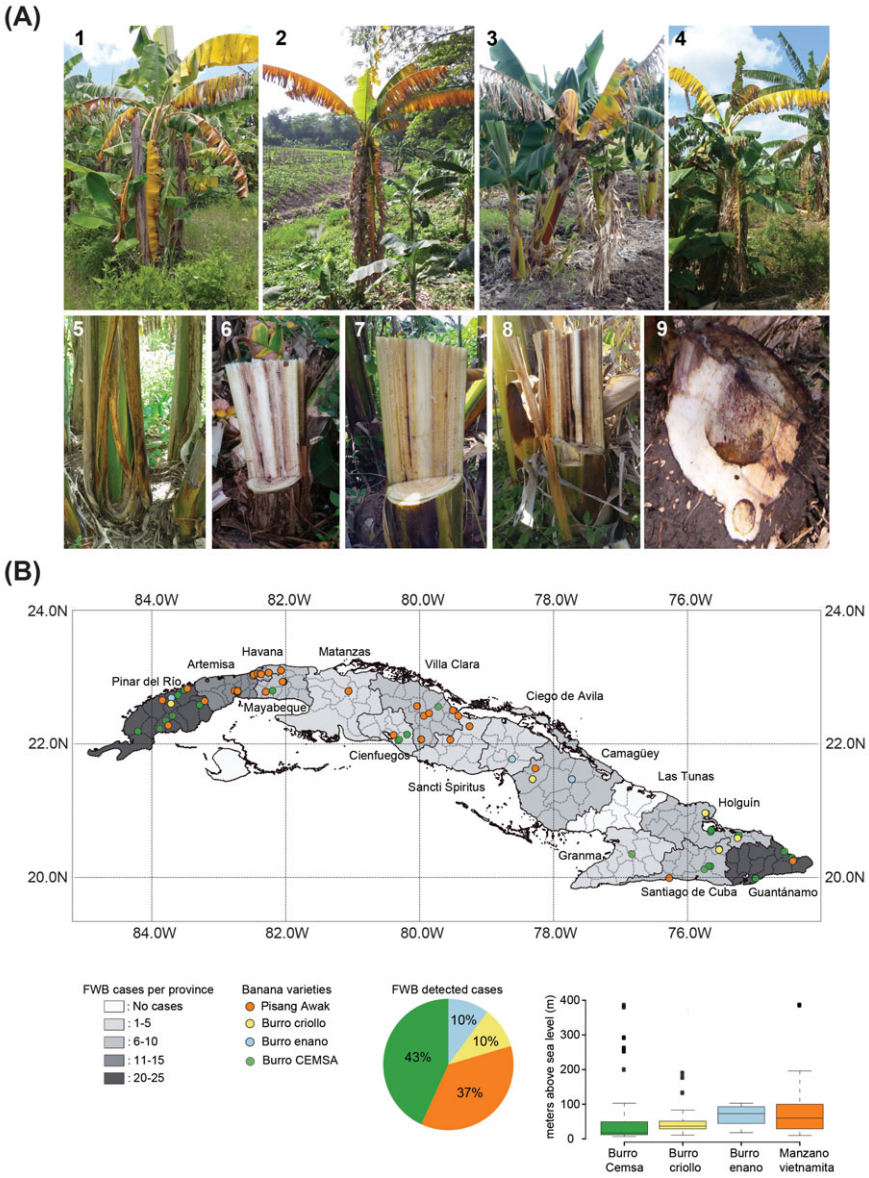

FIGURE 1. Fusarium wilt of banana (FWB) is widely distributed across Cuba. (A) Typical symptoms of FWB detected during the survey; leaf chlorosis or collapse in banana cultivars 'Pisang Awak' [1], 'Burro Criollo' [2], 'Burro Enano' [3], and in 'Burro CEMSA' [4]. Pseudostem splitting in Burro CEMSA [5], internal pseudostem symptoms (discolored vascular strands) in 'Pisang Awak' [6] and in 'Burro CEMSA' [7, 8]. Corm necrosis in 'Burro Criollo' [9]. (B) Overview of the 2016-2018 survey. The FWB incidence is indicated by the shading of the provinces and colored dots represent different banana varieties, orange dots are 'Pisang Awak' fields with FWB symptoms, yellow dots are fields with FWB affected 'Burro Criollo', blue dots are locations with FWB-affected 'Burro Enano' plants, and green dots represent FWB cases in 'Burro CEMSA'. The pie chart indicates the proportion of FWB observed in four banana varieties that are cultivated in Cuba, which collectively represent 44.4% on the national banana acreage. The boxplot shows the altitude of the sampling locations where diseased plants were detected.

Fusarium isolates causing FWB in Cuba are exclusively belonging to race 1 and race 2

To corroborate the pathogenicity of the Cuban isolates to a subset of banana cultivars under standardized greenhouse conditions, we selected 11 isolates representative of various provinces and surveyed hosts, and tested them with differential cultivars for Race 1 ('Gros Michel'), Race 2 ('Burro CEMSA'), and Race 4 ('Grand Naine'). All isolates tested caused characteristic FWB symptoms at 12 weeks after inoculation, on either Race 1 or Race 2 susceptible cultivars (Figure 2). The rhizome discolored area (RDA) scores in each banana cultivar varied according to the inoculated isolate. Race 1 isolates (cub0012, cub0035, cub0129, cub0130, cub0140) caused RDA scores ranging from 27.9 to 61.3% in 'Gros Michel' but in 'Burro CEMSA' their RDA scores were lower than 16.1%. In contrast, 'Gros Michel' plants inoculated with Race 2 isolates (cub0022, cub0034, cub0088) scored RDA values from 0 to 19.3%. However, these isolates caused RDA scores ranging from 25.2 to 64.4 % in 'Burro CEMSA' plants. Interestingly, three isolates (cub0043, cub0145, and cub0150) caused symptoms in both 'Gros Michel' and 'Burro CEMSA' plants, and can therefore be classified as either Race 1 or Race 2 isolates. Importantly, all inoculated 'Grand Naine' plants and the water controls remained free of FWB symptoms, which corroborated the PCR results, and confirms that Race 4 is likely not present in Cuba at the time of the survey.

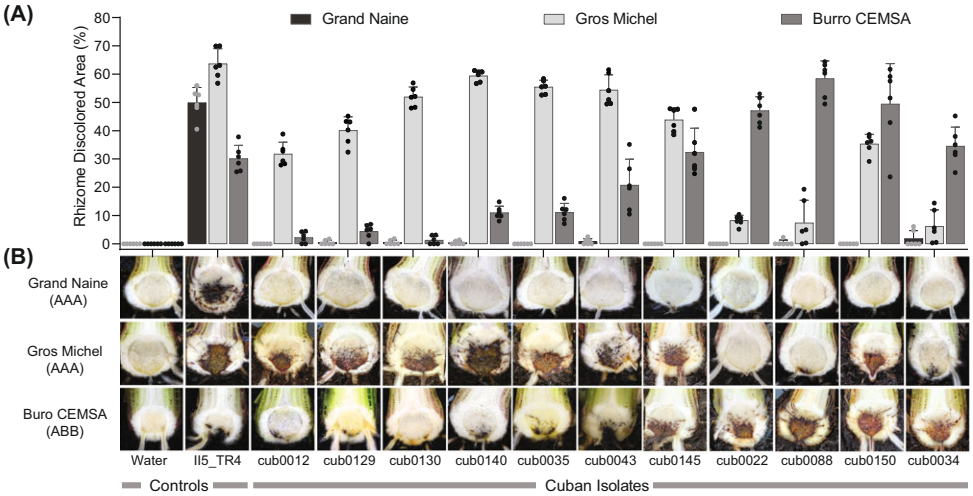

FIGURE 2. Cuban *Fusarium* strains are pathogenic in greenhouse infections assays, causing FWB on banana varieties that are susceptible to the so-called Race 1 ('Gros Michel') and Race 2 ('Burro CEMSA') strains. (A) Rhizome discolored area scores, calculated with ImageJ, were isolate-dependent and ranged from 0.2% to 61.3% in 'Gros Michel' and from 4.9% to 69.4% in 'Burro CEMSA'. (B) Representative overview of FWB severity in the corms of banana cultivar 'Grand Naine', 'Gros Michel', and 'Burro CEMSA' 12 weeks after inoculation. Note that none of the Cuban strains caused FWB symptoms in the Cavendish cultivar 'Grand Naine'.

Both mating types occurred in Cuban populations of *Fusarium* spp. causal agent of FWB

The mating type frequency in fungal populations provides an indication of possible sexual recombination. Random mating usually results in a 1:1 ratio between the two mating type alleles in fungi with a bipolar, heterothallic mating system, and deviation from this ratio either indicates mating irregularities or even the absence of mating (Heitman *et al.*, 2017). Although *Fusarium* spp. that cause FWB are thought to be asexual pathogens, they do carry the classical mating type idiomorphs *mat1-1* and *mat1-2* (Fourie *et al.*, 2009). Furthermore, mating types have indirectly been associated with genetic diversity as a determinant of a possible recombination frequency in a population of asexually reproducing *Fusarium* (Kerényi *et al.*, 2004; Kashyap *et al.*, 2016) and has been used to study the evolution of different *formae speciales* of *F. oxysporum* (Fourie *et al.*, 2009; Lievens *et al.*, 2009a). Thus, we sought to determine the *mat1-1* : *mat 1-2* ratio among the Cuban isolates. Similar to earlier findings in *Fusarium* spp. (Fourie *et al.*, 2009; Visser *et al.*, 2010; Ordóñez, 2018; Magdama *et al.*, 2020), we observed that all isolates either contained the *mat1-1* or the *mat1-2* idiomorph. However, as expected from isolates that do not undergo regular sexual recombination, the ratio of *mat1-1* : *mat1-2* was 15:155 (Table S3). Thus, we conclude that even though both mating types occurred across Cuba, and no strains with both mating type alleles were sampled, the skewed mating type frequency suggests the absence of a sexual cycle during the evolution of Cuban populations of the pathogen.

Fusarium spp. causing FWB in Cuba are genetically diverse

Recently, the causal causal agent of FWB has been reclassified and divided into 11 distinct species, with TR4 described as a new species *F. odoratissimum,* while Race 1, Race 2 and STR4 strains comprise a suite of different *Fusarium* species (Maryani *et al.*, 2019). To determine which of the recently described *Fusarium* species causing FWB are present in Cuba and to uncover the genetic diversity of Cuban isolates, we generated genotyping-by-sequencing DArT markers across the 170 Cuban isolates, and added these to DArT markers that have been previously obtained from additional 243 isolates (Table S4). In total, we obtained 33,298 high-quality DArT markers (presence/absence) with a mean call rate of 99.3% and 6,600 single-nucleotide polymoprhisms (SNPs) across the 413 FOSC isolates, with *Fusarium fujikuroi* (CBS 221.76) as an outgroup.

We performed all analyses using either the DArT presence/absence markers or the SNPs dataset, and in both cases, the results were consistent (Figure S2). Hierarchical clustering based on DArT presence/absence markers showed that the *Fusarium* isolates could be divided into FOSC clades 1, 2, and 3, and then into ten independent lineages, which overall correspond with the phylogenetic species described by Maryani *et al.* (2019) (Figure 3). In clade 1, isolates of *F. odoratissimum* were grouped with those of *F. purpurascens* and *F. phialophorum*. Isolates of *F. duoseptatum, F. grosmichelii, F. tardicrescens* and *F. tardichlamydosporum* were grouped

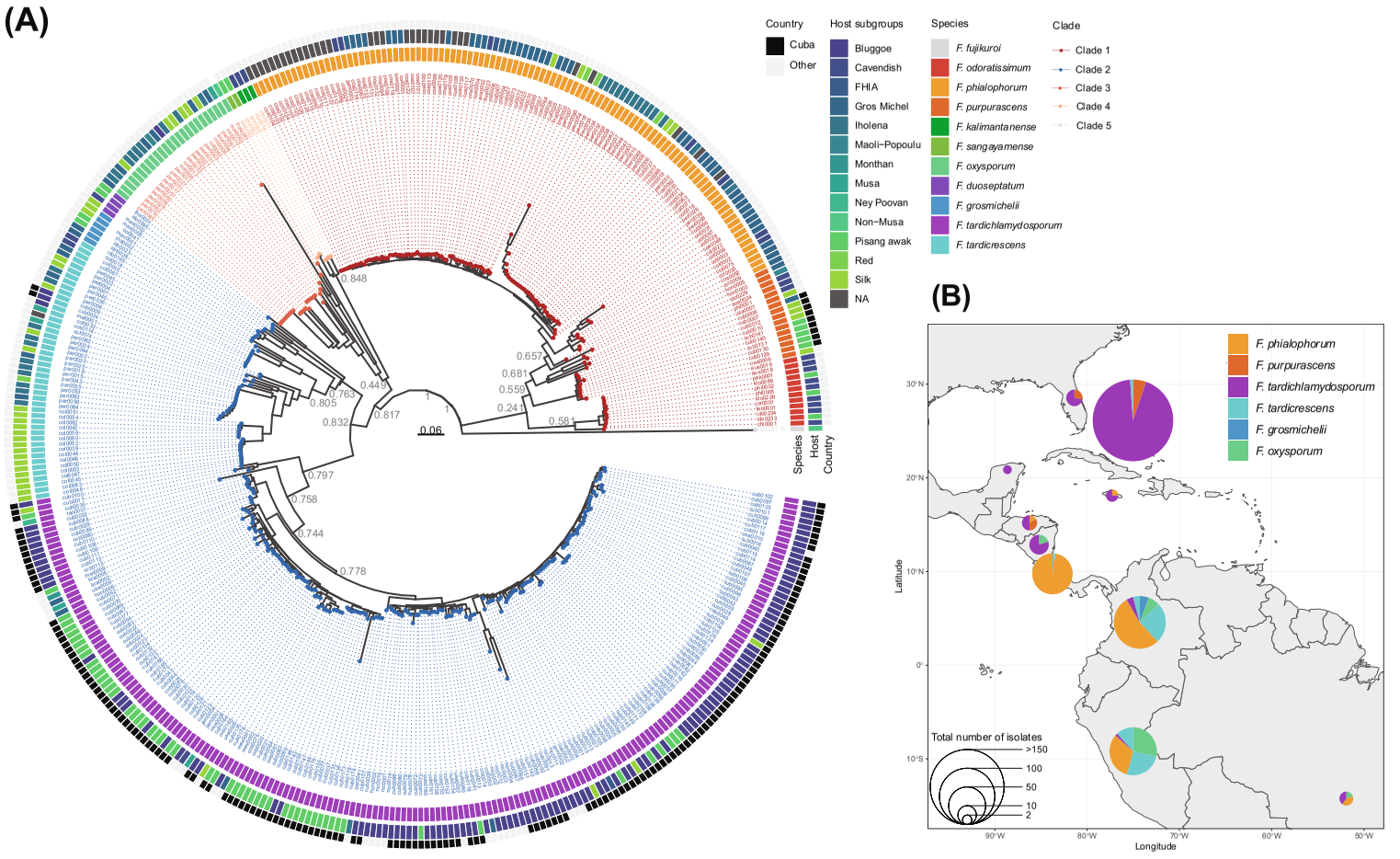
(A)
Country
Cuba
Other
Host subgroups
Bluggoe
Cavendish
FHIA
Gros Michel
Iholena
Maoli−Popoulu
Monthan
Musa
Ney Poovan
Non−Musa
Pisang awak
Red
Silk
NA
Species
F. fujikuroi
F. odoratissimum
F. phialophorum
F. purpurascens
F. kalimantanense
F. sangayamense
F. oxysporum
F. duoseptatum
F. grosmichelii
F. tardichlamydosporum
F. tardicrescens
Clade
Clade 1
Clade 2
Clade 3
Clade 4
Clade 5
0.848
0.657
0.681
0.559
0.241
0.581
0.449
0.762
0.805
0.832
0.817
1
1
0.06
0.797
0.758
0.744
0.778
Species
Host
Country
(B)
F. phialophorum
F. purpurascens
F. tardichlamydosporum
F. tardicrescens
F. grosmichelii
F. oxysporum
30°N
20°N
10°N
0°
10°S
Latitude
Total number of isolates
>150
100
50
10
2
90°W
80°W
70°W
60°W
50°W
Longitude

FIGURE 3. *Fusarium* isolates causing Fusarium wilt of banana (FWB) in the Americas are genetically diverse. A) Cuban isolates clustered in the lineages of *F. purpurascens*, *F. tardicrescens,* and *F. tardichlamydosporum* based on genotyping-by-sequencing (DArT) markers from a total of 414 isolates from 22 countries that were analyzed by the Neighbor-Joining method. The association of isolates to clades are shown as colored dots, red corresponds with clade 1, blue with clade 2, orange with clade 3, and yellow with clade 4. Species are represented as colored blocks in the inner circle, the middle circle corresponds with host and black or grey blocks in the outer circle indicate Cuban vs. non-Cuban isolates, respectively. The entire tree is rooted by *Fusarium fujikuroi* (CBS 221.76). B) Variable compositions of FWB-causing *Fusarium* spp. per country according to (DArTseq) data. The species *F. tardichlamydosporum* and *F. purpurascens* are primarily present in the North Caribbean, whereas *F. phialophorum* and *F. tardicrescens* occur predominantly in Costa Rica and South American countries.

in clade 2. Clade 3 included a group of 29 *F. oxysporum* isolates from Brazil, Colombia, Nicaragua, and Perú (Table S3), which did not cluster with any of the representatives of the previously defined lineages. Finally, an small group of isolates grouped in clade 4 which encompasses *F. kalimantanense* and *F. sangayamense* lineages, these isolates are endemic to Indonesia, and non-pathogenic on 'Gros Michel' and 'Grand Naine' (Maryani *et al.*, 2019).

The great majority (95%) of the Cuban isolates grouped in the lineages corresponding with the species *F. tardichlamydosporum* and *F. tardicrescens* in clade 2, whereas only 5% grouped in the *F. purpurascens* lineage of clade 1. The *F. tardichlamydosporum* isolates include both Race 1 and Race 2 strains (Figure 2, Table S3) and dominate (94%) the Cuban isolates, which affect 'Burro Criollo', 'Burro CEMSA', 'Burro Enano', 'FHIA-03', 'Silk' and 'Pisang Awak' (Table S2). Only two isolates belong to *F. tardicrescens* and were obtained from wilted 'Burro Criollo' plants in the Villa Clara province. The *F. purpurascens* isolates were phenotyped as Race 1 and affected 'Gros Michel', 'Silk' and 'Pisang Awak' bananas in the Central provinces Camagüey, Sancti Spiritus and Villa Clara, as well as in the Western Mayabeque province.

Having determined the species composition of the Cuban FWB-causing *Fusarium* population, we were interested to compare it with the diversity of the FWB pathogen populations in the Americas. We noticed that *F. tardichlamydosporum* and *F. purpurascens* are mostly present in the North Caribbean (from Nicaragua to Florida), whereas *F. phialophorum* and *F. tardicrescens* are primarily distributed across South America, but also in Costa Rica (Figure 3). Furthermore, we calculated the Jaccard and Bray-Curtis indices based on the DArT markers matrix and identified at least two subgroups within all the *F. tardichlamydosporum* isolates (Figure S3). It is worth noticing that these clusters are present in Cuba, one mostly in the Northwest of the island and primarily associated with 'Pisang Awak', whereas the other is present in the Southeast and predominantly associated with Bluggoe cultivars (Figure 3, 4, Table S2), which suggests the presence of distinct genetic sub-clusters within the FWB causing species. Therefore, to investigate the putative number of *Fusarium* genotypes present in the Americas, we performed a discriminant analysis of principal components (DAPC; Grünwald & Goss, 2011), which revealed an optimum of six and a maximum of seven genetically distinct clusters (Figure S3). Similar as the Jaccard and Bray-Curtis indices, the number of genetic clusters exceeds the six species that we described for the American continent. As expected, based on the asexual nature of the FOSC, we did not observe any signs of admixture (Figure 4), hence each isolate is grouped into a discrete genetic cluster. The species *F. grosmichelli, F.*

phialophorum, F. purpurascens, and *F. oxysporum* form discrete clusters, but it was also noted that Honduran isolates of *F. purpurascens* cluster closely to *F. phialophorum* isolates (Figure 4) which indicate a complex relationship between both species.

To corroborate the DArT-based taxonomic classification, we compared whole-genome short-read sequences of 22 Cuban isolates with 58 *Fusarium* isolates from a global panel of fully sequenced isolates (van Westerhoven *et al.*, 2023; Table S5). The 22 Cuban isolates represent the range of diversity in terms of race, lineage, host, and geographic origin across the country.

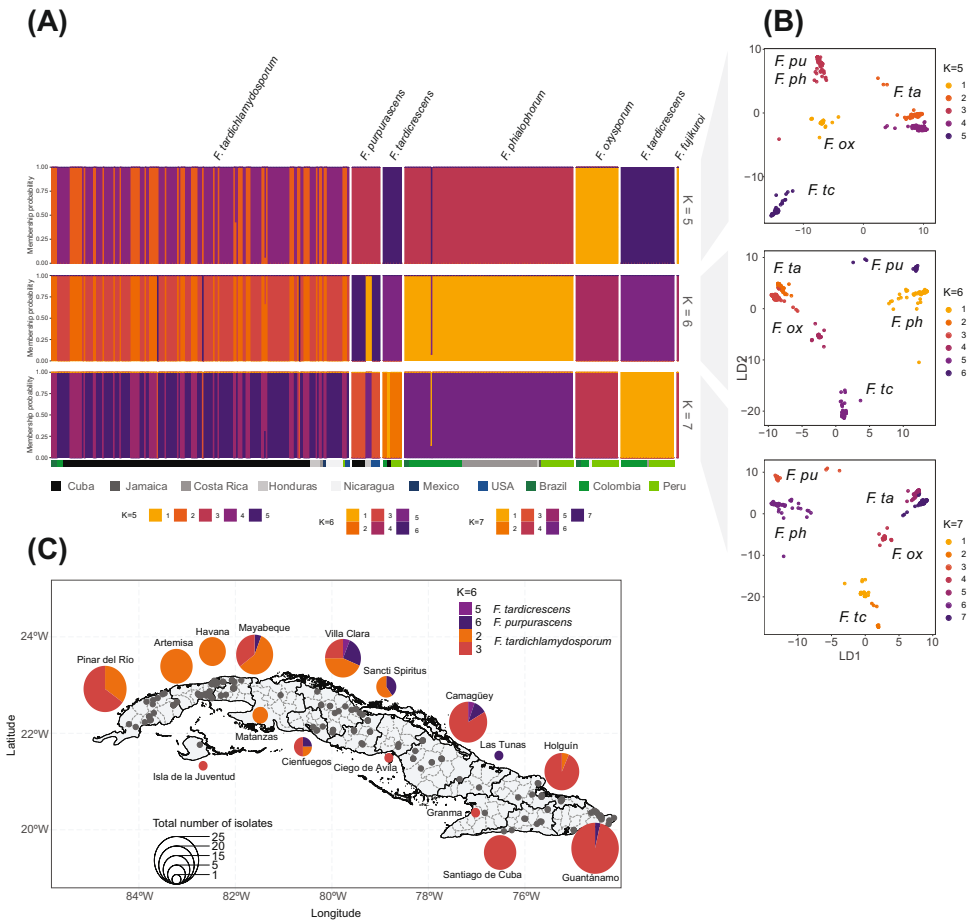

FIGURE 4. Discriminant analysis of principal components (DAPC) revealed the presence of an optimum of six genetically distinct clusters within *Fusarium* spp. that cause Fusarium wilt of banana (FWB) in the Americas. A) Structure of FWB-causing *Fusarium* isolates according to DAPC for *K*=5-7, the bottom colors depict the country of origin. B) Spatial clustering of the isolates based on *K*=5-7 highlights at least two genetic sub-clusters in *F. tardichlamydosporum.* Abbreviations: *F.ox = F. oxysporum, F.pu = F. purpurascens, F.ph = F. phialophorum, F.ta = F. tardichlamydosporum, and F.tc = F. tardicrescens*. C) Two genetically diverse sub-groups of *F. tardichlamydosporum* are present in Cuba. One group (light orange) is more abundant in the Center to the Northwest of the country, whereas the second group (maroon) is mostly present in the Southeast.

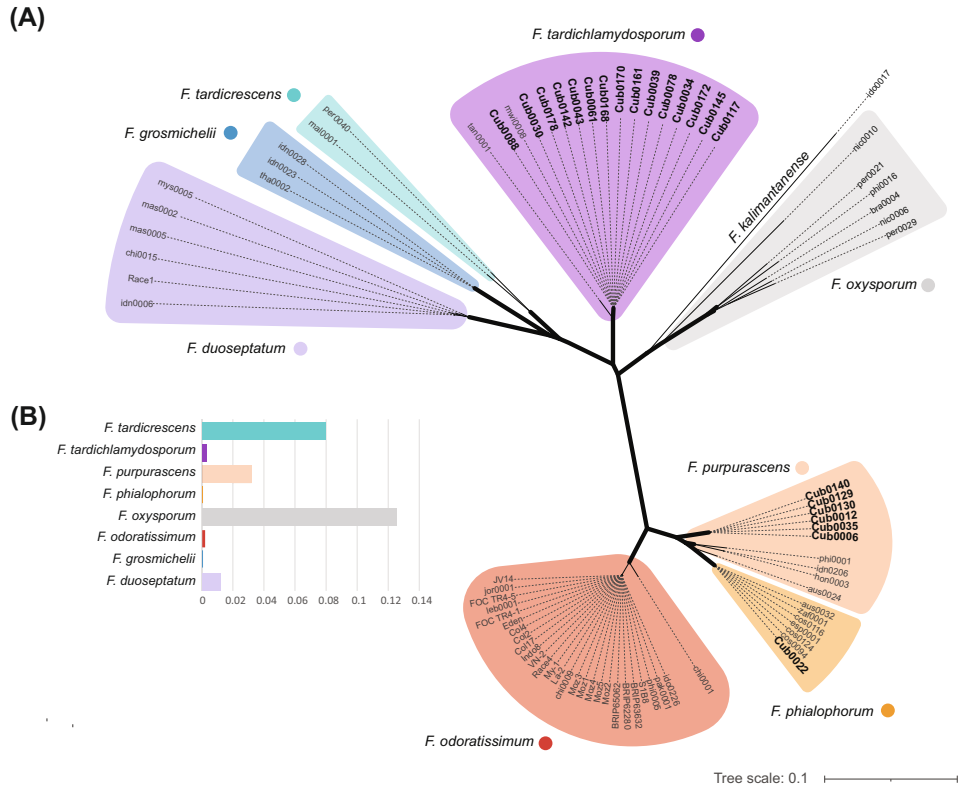

FIGURE 5. *Fusarium* isolates that cause Fusarium wilt of banana grouped in distinct lineages irrespective of their geographical origin. (A) The unrooted Maximum-Likelihood phylogenetic tree based on 1,391,348 high-quality SNPs, inferred from mapping sequence data of a subset of 80 isolates onto the reference genome of *F. odoratissimum* strain II5, shows that the identified groups are consistent with species naming mostly consistent with species naming. Thick branches indicate a bootstrap support of 100%. Cuban isolates clustered in the lineages of *F. purpurascens*, *F. phialophorum*, and *F. tardichlamydosporum*. Background shading delineates different *Fusarium* lineages. The isolate codes of those from Cuba are in bold. (B) Bar plot with the nucleotide diversity values (π) for each species determined between isolates for each *Fusarium* lineages.

We analyzed nearly 1,4 high-quality SNPs using a maximum-likelihood based phylogenetic analyses and confirmed that FWB-causing isolates are grouped in ten independent lineages. Overall, the DArTseq and the whole-genome analyses were in accord with two exceptions; isolate cub0034 clustered with isolates of *F. tardichlamydosporum* according to the SNP data but was considered as *F. tardicrescens* by DArTseq. Similarly, isolate cub0022 is the only Cuban *F. phialophorum* isolate according to the SNP data, but DArTseq identified it as *F. tardichlamydosporum*. However, the lineages determined by SNPs whole-genome analysis were supported by high bootstrap values (Figure 5) and correspond with the phylogenetic species described by Maryani *et al.* (2019). Thus, we conclude that the distinct *Fusarium* lineages, only *F. purpurascens*, *F. phialophorum* and *F. tardichlamydosporum* are present in Cuba. Notably, within these lineages, Cuban isolates clustered with isolates from other countries indicating that they did not undergo independent speciation and showing that these species can be found globally.

DISCUSSION

Roots, tubers, and bananas (including dessert bananas, plantains, and cooking bananas) are important food crops and valuable traded commodities in numerous developing countries where they play a critical role in ensuring food security and income (Scott, 2021). The importance of these crops for food security is due to their high yields and carbohydrate content which translates into a higher daily energy supply per cultivated hectare than cereals (Petsakos *et al.*, 2019). Particularly, bananas are the most popular fruit worldwide and a primary staple food in tropical and subtropical regions where most bananas are produced (FAO, 2022a). The importance of banana for food security is markedly relevant for East Africa, a region with the world's highest per capita banana consumption (Akankwasa *et al.*, 2021). Meanwhile for Latin America and the Caribbean (LAC), bananas are a very important cash crop with five countries of the region are among the top 10 banana exporting nations (FAO, 2022a). Furthermore, as in East Africa, bananas are also important in LAC as a staple food in local diets, with approximately 62% of the regional production consumed locally (Dita *et al.*, 2013b). In Cuba, bananas are produced in all provinces including the special municipality 'Isla de la Juventud' and are the most produced fruits nationally along with mango and guava, representing nearly 30% of all fruit and starchy root staples produced in the country (ONEI, 2022).

In the past, Cuban banana production was threatened by yellow Sigatoka and FWB, which destroyed the national export activities during the first half of the last century. However, when Cavendish bananas and plantains were adopted, FWB lost importance (Martínez-de la Parte *et al.*, 2023), but when Black Leaf Streak Disease entered the country in the 1990s, the overall assembly of the national banana acreage changed. Since then, areas grown with susceptible Cavendish and plantain cultivars were significantly reduced and replaced by more resistant FHIA hybrids and Bluggoe cultivars (Pérez-Vicente *et al.*, 2002), and 'Pisang Awak' became popular due to its higher rusticity and semi-acid taste. These changes caused a reemergence of FWB in Cuba, which is corroborated by our analyses that demonstrated that this disease is widespread across the entire country.

We built a nationwide collection of 170 isolates from symptomatic bananas primarily (80%) from 'Burro CEMSA' and 'Pisang Awak', which are extensively grown in Cuba (Martínez-de la Parte *et al.*, 2023). Although diagnostic PCRs and phenotyping assays showed that Race 1 and Race 2 prevailed in the collection, some isolates (isolates cub0043, cub0145, and cub0150) caused symptoms in both 'Gros Michel' and 'Burro CEMSA' which is in accord with previous field experiments in Cuba and greenhouse results with isolates from Brazil, East Africa, Florida, Nepal and Puerto Rico (Ploetz & Churchill, 2011; Garcia *et al.*, 2018; García-Bastidas, 2019; Martínez-de la Parte *et al.*, 2023; Pant *et al.*, 2023). Hence, this indicates that the current race concept in FWB is inadequate because these isolates do not fit in the classical race nomenclature (i.e., race 1, race 2, and race 4). Thus, we anticipate that more FWB races will be recognized with expanded phenotyping, including more banana accessions, similar to observations in *Fusarium* spp. causing Fusarium wilt in lettuce and watermelon (Zhou *et al.*, 2009; Gilardi *et al.*, 2017).

Phylogenetic analyses, based on several DNA-based techniques, have consistently shown that *Foc* isolates are divided in three major clades, which are further subdivided into eight to ten lineages (Bentley *et al.*, 1998; O'Donnell *et al.*, 1998; Groenewald *et al.*, 2006; Fourie *et al.*, 2009; Mostert *et al.*, 2017; Maryani *et al.*, 2019). Furthermore, multi-locus genotyping and genome-by-sequencing using DArTseq showed that *Foc* is genetically complex, and it was previously proposed to rename the recognized and new *Foc* lineages into new *Fusarium* species (Maryani *et al.*, 2019). Thus, the hitherto determined genetic lineages that comprise the various physiological races are now considered to be different species. However, these analyses focused on Southeast Asia, where *Musa* has its center of origin, and thus far there is no information about these species in the Latin American context. Previous studies used DNA fingerprinting (Bentley *et al.*, 1998), multigene phylogeny (Fourie *et al.*, 2009; Maryani *et al.*, 2019; Magdama *et al.*, 2020), DArTseq (Mostert *et al.*, 2022), or whole-genome sequence comparisons (Garcia-Bastidas *et al.*, 2020; Acuña *et al.*, 2021; Leiva *et al.*, 2022; Reyes-Herrera *et al.*, 2022), but included only a limited number of isolates from the Americas or focused on an individual countries (Magdama *et al.*, 2020; Batista *et al.*, 2022). Hence, no conclusions could yet be drawn on the phylo-geography of FWB-causing fungi in the Americas. Our current study provides an unparalleled insight into the genetic diversity across a large set of 380 isolates obtained from ten American countries, including 170 isolates from Cuba. Therefore, contrary to the aforementioned studies, our data is able to resolve the structure of the FWB pathogens in the Americas. We conclude that FWB-causing fungi comprise five to seven genetic clusters, as isolates of *F. tardichlamydosporum* can be split into two distinct genetic clusters suggesting a population differentiation according to the type of banana cultivar from which they were sampled. Furthermore, some *F. purpurascens* isolates cluster closely with *F. phialophorum*, despite their position in the DArT/SNPs-based phylogenetic tree and the PCA support. Hence, the phylogenetic relationship between both species is intricate and requires further analysis. Our data on the ratio between the *mat 1-1* or *mat 1-2* ideomorphs are also in accordance with previous reports (Fourie *et al.*, 2009; Visser *et al.*, 2010; Ordóñez, 2018; Magdama *et al.*, 2020). Taken together, our analyses provide a high-resolution and genome-wide dataset to describe genetic diversity in FWB-causing *Fusarium* spp. in an unparalleled manner that matches recent genome-wide analyses (van Westerhoven *et al.*, 2023: Ordóñez *et al.*, 2023).

The genotyping data showed that the Cuban isolates are grouped in Clades 1 and 2 and in three lineages of which none clustered with the *F. odoratissimum* lineage, which is in line with the pathogenicity tests – no strain caused disease in Cavendish - and PCR data that were all negative for TR4 (Dita *et al.*, 2010). Nevertheless, Cuban banana production is threatened by the recent expansion of TR4 in Latin America (Garcia-Bastidas *et al.*, 2020; Acuña *et al.*, 2021; Herrera *et al.*, 2023). The discrepancy between the phylogenies based on DArT and whole-genome SNPs for two Cuban isolates is likely a technical issue due to different genome representations. Therefore, we conclude that FWB pathogens in Cuba belong to the species *F. purpurascens, F. tardichlamydosporum,* and *F. phialophorum*. We also showed through Discriminant Analysis of Principal Components that isolates of a single species are closely related, across different

geographic locations, compared to their relationship with isolates of other species present in Cuba. This suggests several independent introductions of *Fusarium* since the inception of banana cultivation in Cuba. Bananas arrived in Cuba in 1529 from Hispaniola Island, now Dominican Republic, and soon became one of the most important staple foods for the population (García, 2001). Over time, different banana cultivars from different origins were introduced to Cuba at different times, such as Gros Michel from Martinique, Grand Naine from Panama, and plantains from the Dominican Republic and Saint Lucia (Marin *et al.*, 1998; García, 2008; Alvarez, 2011). Additionally, movement of banana planting material in LAC occurred (Marin *et al.*, 1998), which may explain the observed close relationship and similar species composition of *Fusarium* populations in Central America and Caribbean compared to populations from South America (Brazil, Colombia, Perú). It is also possible that the introduction of planting material from different producing areas facilitated the introduction of genetically diverse pathogen strains. This is in line with the currently observed diversity in Cuba, which is also illustrated by the similarity between isolates from Cuba and Florida, USA. Both countries harbour a unique *Fusarium* population comprising isolates of VCG01210 that is only found here and in the Cayman Islands (Ploetz, 1990; Pérez-Vicente, 2004a; Mostert *et al.*, 2017), which could be explained by the historical ties between Cuba and the USA through trade and migration. FWB was first described in Cuba in 1910 and occurred in Florida decades later (1986), suggesting that VCG01210 isolates may have been introduced from Cuba via infected Silk banana plants.

Collectively, our results represent the hitherto broadest analyses of genetic diversity of FWB-causing *Fusarium* spp. in the Americas, which is primarily associated with the presence of two genotypes of *F. tardichlamydosporum* in Cuba. However, our data did not include strains of *F. odoratissimum* from the recent outbreaks in the American continent (Garcia-Bastidas *et al.*, 2020; Acuña *et al.*, 2021; Herrera *et al.*, 2023). Despite the strategies implemented by regional and national plant protection organization, *F. odoratissimum* (TR4) arrived to the Americas and continue spreading (Reyes-Herrera *et al.*, 2022), underscoring the failure of current management strategies for this pathogen, which rely in the detection of wilting symptoms in Cavendish. In the absence of a fully resistant cultivar, strict quarantine measures that take into account the host range of TR4, as well as a continuous surveillance with protocols that allow rapid and accurate identification of the pathogen, are the best options to limit the spread of TR4 in the region.

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SUPPLEMENTARY MATERIAL

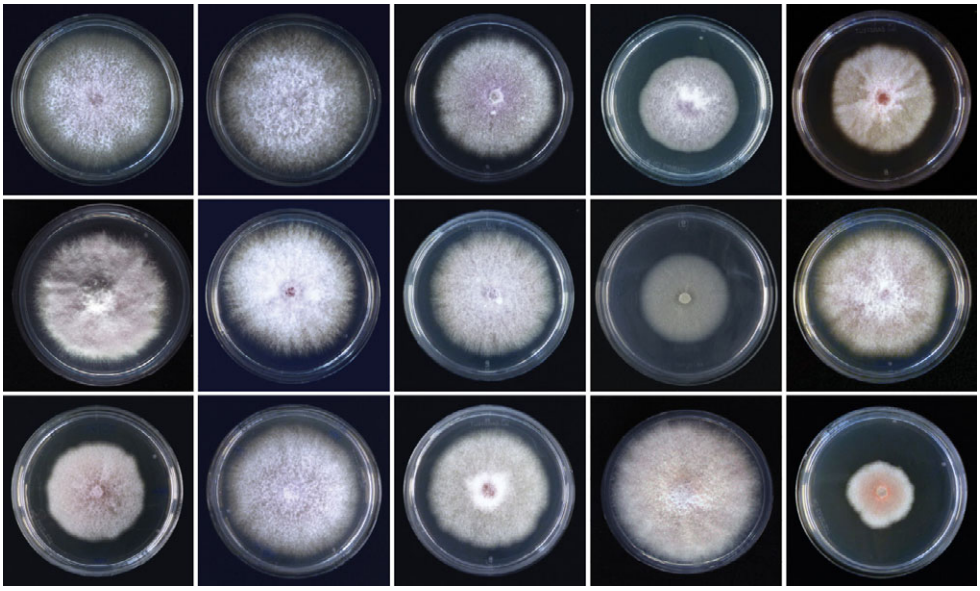

FIGURE S1. Cultural characteristics of Cuban isolates of *Fusarium* spp. causal agent of FWB. Colonies from different isolates differ in color, texture and colony diameter after one week incubation in PDA plates at 25°C.

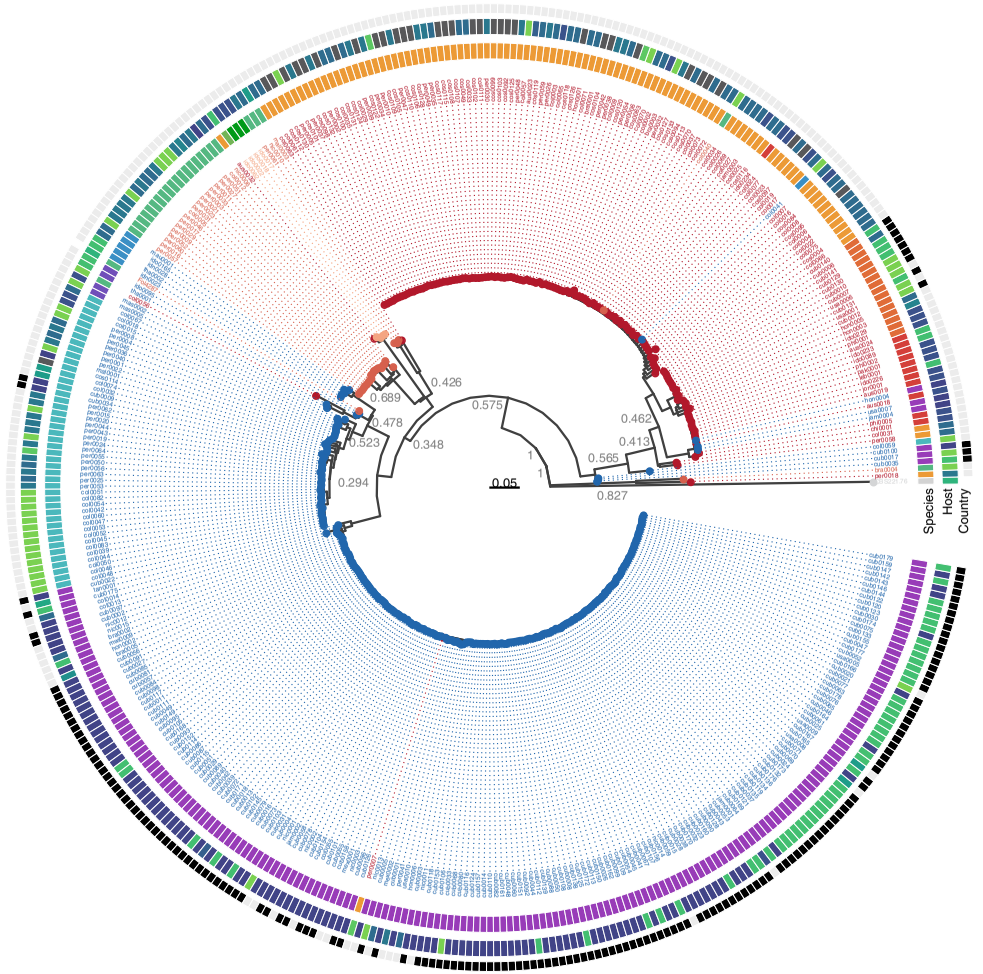

FIGURE S2. *Fusarium* isolates causing Fusarium wilt of banana (FWB) in the Americas are genetically diverse as shown by a Neighbor-Joining tree inferred from single-nucleotide polymorphisms derived from the DArT markers. The association of isolates to clades are shown as colored dots, red corresponds with clade 1, blue with clade 2, orange with clade 3, and yellow with clade 4. Species as in Maryani *et al.* (2019) are shown as colored blocks in the inner circle, the middle circle corresponds with host and black or grey blocks in the outer circle indicate Cuban vs. non-Cuban isolates, respectively. The entire tree is rooted by *Fusarium fujikuroi* (CBS 221.76).

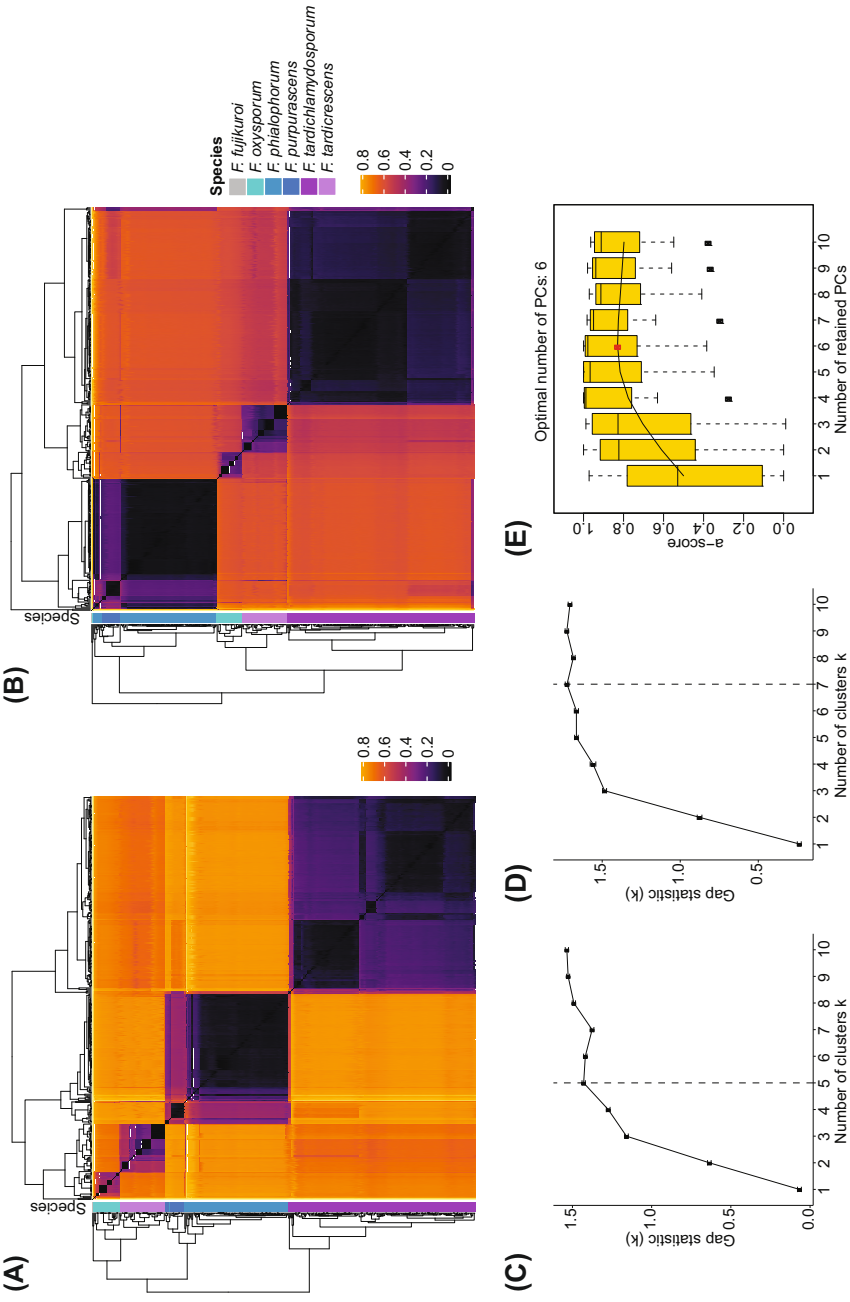

FIGURE S3. American strains of the causal agent of FWB are grouped between five to seven genetically distinct clusters. A-B) Heatmap of the Jaccard distance (A) and Bray-Curtis dissimilarity (B) between 380 *Fusarium* strains. C-E) The optimal number of clusters was determined according to Jaccard distance (C), Bray-Curtis distance (D), or a Discriminant Analysis of Principal Components (E) with the aim to reduce variation within groups and to maximize variation between groups.

TABLE S1. Origen of Cuban *Fusarium* isolates that cause Fusarium wilt of banana and that were characterized in this study.

DArT id	Isolate Code[1]	Geographical origin			
		Province	Municipality	coordinates	MASL[2]
cub0001	IJ-1	Isla de la Juventud	Isla de la Juventud	21.761454, -82.736489	39
cub0002	SC-3	Santiago de Cuba	Palma Soriano	20.226192, -75.989683	133
cub0003	C.Esm1.3	Camaguey	Esmeralda	n.d.	n.d.
cub0004	Cca1.1	Camaguey	Camaguey	n.d.	n.d.
cub0005	R.Go	Havana	Playa	n.d.	n.d.
cub0006	Cub9	Villa Clara	n.d.	n.d.	n.d.
cub0007	M3	Villa Clara	n.d.	n.d.	n.d.
cub0008	C.Esm1.1	Camaguey	Esmeralda	21.851445, -78.116708	37
cub0009	CGua 1.1	Camaguey	Guaimaro	21.049077, -77.353706	83
cub0010	C.V 2.1	Camaguey	Vertientes	21.270623, -78.155012	58
cub0011	ESB-1	Camaguey	Esmeralda	21.843757, -78.123587	38
cub0012	Cam3	Camaguey	Camaguey	21.397476, -77.929907	95
cub0013	SC-2	Santiago de Cuba	Palma Soriano	20.230811, -76.011663	136
cub0014	GuCUM	Guantanamo	Maisi	20.191915, -74.251045	380
cub0015	GuBaCu	Guantanamo	Maisi	20.214089, -74.410481	72
cub0016	GuMa	Guantanamo	Maisi	20.244781, -74.153092	17
cub0017	TUPP-1	Las Tunas	Puerto Padre	21.183148, -76.621987	18
cub0018	GuBaPo	Guantanamo	Baracoa	20.291000, -74.464333	29
cub0020	MoGu 1	Artemisa	Guira de Melena	n.d.	n.d.
cub0021	MoGu 3	Artemisa	Guira de Melena	n.d.	n.d.
cub0022	WaHa	La Habana	Boyeros	23.008254, -82.419289	71
cub0023	PaGu	Artemisa	Alquízar	22.816530, -82.622965	23
cub0024	AlqHa 1	Artemisa	Alquízar	n.d.	NA
cub0025	GuHa	Artemisa	Guira de Melena	22.780703, -82.529753	12
cub0026	PeBa	Guantanamo	Baracoa	20.352072, -74.630209	46
cub0027	ToaGu	Guantanamo	Baracoa	n.d.	NA
cub0028	Bar 1	Guantanamo	Baracoa	20.3606683, -74.5144867	29
cub0029	MaHo 1	Holguin	Mayarí	n.d.	n.d.
cub0030	BaHo 1	Holguin	Banes	20.958913, -75.720306	36
cub0031	BaHo 2	Holguin	Banes	20.964810, -75.734556	41
cub0032	SaHo	Holguin	Sagua de Tánamo	20.625285, -75.230387	7
cub0033	Bar 3	Guantanamo	Baracoa	20.356750, -74.516493	19
cub0034	R-2	Villa Clara	Santo Domingo	22.586179, -80.219636	48
cub0035	JCien	Cienfuegos	Cienfuegos	22.1332, -80.3882	33
cub0037	CaVC	Villa Clara	Caibarién	22.4192, -79.4228	18
cub0038	ToBaGu	Guantanamo	Baracoa	20.386083, -74.569061	22
cub0039	NarBaGu	Guantanamo	Baracoa	20.3906, -74.5489	10
cub0040	PGGua1	Santiago de Cuba	Guamá	19.991499, -76.272205	11
cub0041	PGGua2	Santiago de Cuba	Guamá	19.991499, -76.272205	11
cub0042	BaGra	Granma	Bayamo	20.3491, -76.8307	40
cub0043	PunBra1	La Habana	La Lisa	23.02041, -82.489242	60
cub0044	PunBra2	La Habana	La Lisa	23.019867, -82.487798	61
cub0045	BatMay1	Mayabeque	Batabanó	22.7801, -82.2996	33
cub0046	BatMay2	Mayabeque	Batabanó	22.7806, -82.2984	33
cub0047	Min1Art	Artemisa	Artemisa	22.77132, -82.723665	14
cub0048	MaySC1	Santiago de Cuba	Segundo Frente	20.424554, -75.525176	190

Host			Survey
Cultivar	Subgroup	genotype	
Burro CEMSA	Bluggoe	ABB	1996-2000
Burro criollo	Bluggoe	ABB	1996-2000
Burro criollo	Bluggoe	ABB	1996-2000
Manzano	Silk	AAB	1996-2000
Manzano	Silk	AAB	1996-2000
Gros Michel	Gros Michel	AAA	-
Manzano	Silk	AAB	-
Burro criollo	Bluggoe	ABB	1996-2000
Burro criollo	Bluggoe	ABB	1996-2000
Manzano	Silk	AAB	1996-2000
Burro criollo	Bluggoe	ABB	1996-2000
Manzano	Silk	AAB	1996-2000
FHIA-03	FHIA hybrid	AABB	1996-2000
Burro CEMSA	Bluggoe	ABB	1996-2000
Burro Criollo	Bluggoe	ABB	1996-2000
Burro Criollo	Bluggoe	ABB	1996-2000
Manzano	Silk	AAB	1996-2000
Burro Criollo	Bluggoe	ABB	1996-2000
Manzano vietnamita	Pisang Awak	ABB	1996-2000
Manzano vietnamita	Pisang Awak	ABB	1996-2000
Burro CEMSA	Bluggoe	ABB	1996-2000
Manzano vietnamita	Pisang Awak	ABB	1996-2000
Manzano vietnamita	Pisang Awak	ABB	1996-2000
Manzano vietnamita	Pisang Awak	ABB	1996-2000
Burro CEMSA	Bluggoe	ABB	1996-2000
Burro criollo	Bluggoe	ABB	1996-2000
Burro criollo	Bluggoe	ABB	1996-2000
Burro CEMSA	Bluggoe	ABB	1996-2000
Burro criollo	Bluggoe	ABB	1996-2000
Burro criollo	Bluggoe	ABB	1996-2000
Burro CEMSA	Bluggoe	ABB	1996-2000
Manzano	Silk	AAB	1996-2000
Burro criollo	Bluggoe	ABB	1996-2000
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Burro Criollo	Bluggoe	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Burro enano	Bluggoe	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Burro Criollo	Bluggoe	ABB	2016-2018

TABLE S1. (*Continued*).

DArT id	Isolate Code[1]	Geographical origin			
		Province	Municipality	coordinates	MASL[2]
cub0049	MaySC2	Santiago de Cuba	Segundo Frente	20.414686, -75.527427	177
cub0050	CumCF1	Cienfuegos	Cumanayagua	22.054055, -80.320488	11
cub0051	CumCF2	Cienfuegos	Cumanayagua	22.064173, -80.303424	27
cub0052	BoyHa	La Habana	Boyeros	23.043357, -82.366863	82
cub0053	CotHa	La Habana	Cotorro	23.065313, -82.255912	86
cub0054	GuaHa1	La Habana	Guanabacoa	23.066299, -82.253049	100
cub0055	C.Esm1.2	Camaguey	Esmeralda	21.851445, -78.116708	37
cub0056	C.Esm2.1	Camaguey	Esmeralda	21.851445, -78.116708	37
cub0057	ESB-5	Camaguey	Esmeralda	21.843757, -78.123587	38
cub0058	SCGu	Santiago de Cuba	Guamá	19.968256, -76.433774	11
cub0059	Bar 2	Guantanamo	Baracoa	20.3606683, -74.5144867	29
cub0060	ColCA	Ciego de Avila	Baragua	21.77283, -78.61835	18
cub0061	Min2Art	Artemisa	Artemisa	22.772401, -82.723595	14
cub0063	Art1Art-1	Artemisa	Artemisa	22.790584, -82.720201	29
cub0064	Art1Art-2	Artemisa	Artemisa	22.790584, -82.720201	29
cub0065	Art2Art-1	Artemisa	Artemisa	22.790572, -82.719580	29
cub0067	MelArt1.1	Mayabeque	Melena del Sur	22.798744, -82.196981	43
cub0068	MelArt1.2	Mayabeque	Melena del Sur	22.798744, -82.196981	43
cub0069	Front1Art1	Artemisa	Artemisa	22.778835, -82.721711	33
cub0070	Front1Art2	Artemisa	Artemisa	22.778835, -82.721711	33
cub0071	Front2Art	Artemisa	Artemisa	22.779190, -82.721217	33
cub0072	FloCam1	Camaguey	Florida	21.472639, -78.315359	44
cub0073	CesCam1	Camaguey	Cespedes	21.633698, -78.272799	42
cub0075	LisHa2	La Habana	La Lisa	23.039357, -82.475418	43
cub0076	TolHa	La Habana	Marianao	23.054845, -82.420970	52
cub0077	AldHa	La Habana	Boyeros	23.053978, -82.38377	68
cub0078	FloCam2	Camaguey	Florida	21.47199, -78.313663	51
cub0079	FloCam3	Camaguey	Florida	21.47199, -78.313363	51
cub0080	FloCam4	Camaguey	Florida	21.472675, -78.313992	51
cub0081	CCam1	Camaguey	Camaguey	21.471365, -77.726914	83
cub0082	CCam2	Camaguey	Camaguey	21.471778, -77.726673	83
cub0084	CCam3.2	Camaguey	Camaguey	21.471907, -77.726643	83
cub0085	MaHo2	Holguin	Mayarí	20.683855, -75.654668	12
cub0086	MaHo3	Holguin	Mayarí	20.683829, -75.654668	12
cub0087	MaHo4	Holguin	Mayarí	20.683874, -75.654712	12
cub0088	MaHo5	Holguin	Mayarí	20.709818, -75.647436	9
cub0089	MaHo6	Holguin	Mayarí	20.710014, -75.647722	9
cub0090	MaHo7	Holguin	Mayarí	20.709832, -75.6478	9
cub0091	PaHo1	Holguin	Sagua de Tánamo	20.615969, -75.248517	12
cub0092	PaHo2	Holguin	Sagua de Tánamo	20.615971, -75.248511	12
cub0093	SaHo2	Holguin	Sagua de Tánamo	20.589202, -75.252072	16
cub0095	SaHo3.2	Holguin	Frank Pais	20.62353, -75.232295	10
cub0096	SaHo4	Holguin	Frank Pais	20.633719, -75.233427	10
cub0097	MosGu1	Guantanamo	Baracoa	20.261361, -74.422292	13
cub0098	MosGu2	Guantanamo	Baracoa	20.261302, -74.422075	13
cub0099	MosGu3.1	Guantanamo	Baracoa	20.261177, -74.421611	14

Host			Survey
Cultivar	Subgroup	genotype	
Burro Criollo	Bluggoe	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	1996-2000
Burro criollo	Bluggoe	ABB	1996-2000
Burro criollo	Bluggoe	ABB	1996-2000
Burro criollo	Bluggoe	ABB	1996-2000
Burro criollo	Bluggoe	ABB	1996-2000
Burro criollo	Bluggoe	ABB	1996-2000
Burro enano	Bluggoe	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Burro enano	Bluggoe	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Burro criollo	Bluggoe	ABB	2016-2018
Burro criollo	Bluggoe	ABB	2016-2018
Burro criollo	Bluggoe	ABB	2016-2018
Burro enano	Bluggoe	ABB	2016-2018
Burro enano	Bluggoe	ABB	2016-2018
Burro enano	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro Criollo	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018

TABLE S1. (*Continued*).

DArT id	Isolate Code[1]	Geographical origin			
		Province	Municipality	coordinates	MASL[2]
cub0100	MosGu3.2	Guantanamo	Baracoa	20.261177, -74.421611	14
cub0101	MosGu8	Guantanamo	Baracoa	20.261212, -74.421655	14
cub0102	MosGu4	Guantanamo	Baracoa	20.257265, -74.414429	12
cub0103	MosGu5	Guantanamo	Baracoa	20.257234, -74.414065	12
cub0104	MosGu6	Guantanamo	Baracoa	20.246432, -74.423753	12
cub0105	MosGu7	Guantanamo	Baracoa	20.245011, -74.426939	12
cub0106	SaLGu1	Guantanamo	Baracoa	20.294545, -74.436374	14
cub0107	SaLGu2	Guantanamo	Baracoa	20.294402, -74.436478	14
cub0108	SaSGu1.1	Guantanamo	San Antonio del Sur	19.994528, -74.98861	17
cub0109	SaSGu1.2	Guantanamo	San Antonio del Sur	19.994528, -74.98861	17
cub0110	SaSGu2	Guantanamo	San Antonio del Sur	19.994029, -74.987754	17
cub0111	SaSGu3.1	Guantanamo	San Antonio del Sur	19.987371, -74.982173	14
cub0112	SaSGu3.2	Guantanamo	San Antonio del Sur	19.987371, -74.982173	14
cub0113	SaSGu4	Guantanamo	San Antonio del Sur	19.986947, -74.983302	14
cub0114	LaMSC1.1	Santiago de Cuba	Songo La Maya	20.167602, -75.656472	253
cub0115	LaMSC1.2	Santiago de Cuba	Songo La Maya	20.167602, -75.656472	253
cub0116	LaMSC2.1	Santiago de Cuba	Songo La Maya	20.16981, -75.684817	262
cub0117	LaMSC2.2	Santiago de Cuba	Songo La Maya	20.16981, -75.684817	262
cub0118	CriSC	Santiago de Cuba	Santiago de Cuba	20.12074, -75.749968	200
cub0119	JaMay1.1	Mayabeque	Jaruco	23.10003, -82.07	68
cub0120	JaMay1.2	Mayabeque	Jaruco	23.10003, -82.07	68
cub0121	JaMay2	Mayabeque	Jaruco	23.100081, -82.069980	68
cub0122	JaMay3.1	Mayabeque	Jaruco	23.09985, -82.0701	68
cub0123	JaMay3.2	Mayabeque	Jaruco	23.09985, -82.0701	68
cub0124	SJMay1	Mayabeque	San José	22.931547, -82.027809	103
cub0125	SJMay2.1	Mayabeque	San José	22.932981, -82.039293	103
cub0126	SJMay2.2	Mayabeque	San José	22.932981, -82.039293	103
cub0128	SJMay3.2	Mayabeque	San José	22.932295, -82.045842	103
cub0129	SJMay4	Mayabeque	San José	22.933316, -82.046872	103
cub0130	CabSS1	Sancti Spiritus	Cabaiguan	22.062181, -79.542205	152
cub0131	CabSS2	Sancti Spiritus	Cabaiguan	22.062141, -79.542425	152
cub0132	YagSS1	Sancti Spiritus	Yaguajay	22.263664, -79.255669	196
cub0133	YagSS2	Sancti Spiritus	Yaguajay	22.263654, -79.255779	196
cub0134	YagSS3	Sancti Spiritus	Yaguajay	22.263668, -79.255651	196
cub0135	StCVC1	Villa Clara	Santa Clara	22.419466, -79.938231	114
cub0136	StCVC2	Villa Clara	Santa Clara	22.457706, -79.859251	99
cub0137	CaVC2	Villa Clara	Caibarién	22.502725, -79.502266	10
cub0138	CaVC3	Villa Clara	Caibarién	22.502241, -79.503412	10
cub0139	CamVC1	Villa Clara	Camajuani	22.556938, -79.722614	11
cub0140	CiVC1	Villa Clara	Cifuentes	22.565426, -80.039095	119
cub0141	CiVC2	Villa Clara	Cifuentes	22.565523, -80.038918	119
cub0142	CumCF3.1	Cienfuegos	Cumanayagua	22.139874, -80.189647	292
cub0143	CumCF3.2	Cienfuegos	Cumanayagua	22.139874, -80.189647	292
cub0144	ManVC1	Villa Clara	Manicaragua	22.068554, -79.976483	386
cub0145	MAnVC1-1	Villa Clara	Manicaragua	22.068554, -79.976483	386
cub0146	ManVC2	Villa Clara	Manicaragua	22.068520, -79.976453	386

Host			Survey
Cultivar	Subgroup	genotype	
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro Criollo	Bluggoe	ABB	2016-2018
Burro Criollo	Bluggoe	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Burro enano	Bluggoe	ABB	2016-2018
Burro enano	Bluggoe	ABB	2016-2018
Burro enano	Bluggoe	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018

TABLE S1. (*Continued*).

DArT id	Isolate Code[1]	Geographical origin			
		Province	Municipality	coordinates	MASL[2]
cub0147	ManVC2-1	Villa Clara	Manicaragua	22.068520, -79.976453	386
cub0149	PalPR1	Pinar del Rio	Los Palacios	22.583139, -83.281703	49
cub0150	PalPR2	Pinar del Rio	Los Palacios	22.583167, -83.281708	49
cub0151	PalPR3	Pinar del Rio	Los Palacios	22.582946, -83.282194	46
cub0152	PalPR4	Pinar del Rio	Los Palacios	22.583670, -83.283490	47
cub0153	PalPR5	Pinar del Rio	Los Palacios	22.583811, -83.283559	50
cub0154	PalPR6	Pinar del Rio	Los Palacios	22.583817, -83.283649	50
cub0155	PalPR7	Pinar del Rio	Los Palacios	22.641865, -83.21056	71
cub0156	PalPR8	Pinar del Rio	Los Palacios	22.64187, -83.210544	71
cub0157	ViPR2	Pinar del Rio	Viñales	22.689105, -83.70567	63
cub0158	ViPR3	Pinar del Rio	Viñales	22.689105, -83.70567	63
cub0159	PalmPR1	Pinar del Rio	La Palma	22.730729, -83.610914	67
cub0160	PalmPR2	Pinar del Rio	La Palma	22.730729, -83.610914	67
cub0161	PalmPR3.1	Pinar del Rio	La Palma	22.808993, -83.511016	16
cub0162	PalmPR3.2	Pinar del Rio	La Palma	22.808993, -83.511016	16
cub0164	GuaPR1	Pinar del Rio	Guane	22.188265, -84.205815	12
cub0165	GuaPR2	Pinar del Rio	Guane	22.188265, -84.205815	30
cub0166	SanJPR1	Pinar del Rio	San Juan y Martínez	22.228536, -83.877718	30
cub0167	SanJPR2	Pinar del Rio	San Juan y Martínez	22.65457, -83.842405	15
cub0168	SanJPR3	Pinar del Rio	San Juan y Martínez	22.65457, -83.842405	26
cub0169	PinPR	Pinar del Rio	Pinar de Río	22.420475, -83.689995	26
cub0170	SanLPR1	Pinar del Rio	San Luis	22.267129, -83.769663	45
cub0171	SanLPR2	Pinar del Rio	San Luis	22.276589, -83.75275	18
cub0172	SanLPR3	Pinar del Rio	San Luis	22.347291, -83.789534	21
cub0173	PerMat1	Matanzas	Perico	22.790785, -81.063242	30
cub0174	PerMat2.1	Matanzas	Perico	22.790563, -81.062872	31
cub0175	PerMat2.2	Matanzas	Perico	22.790563, -81.062872	31
cub0176	Art3Art1	Artemisa	Artemisa	22.795743, -82.763285	33
cub0177	Art3Art2	Artemisa	Artemisa	22.795743, -82.763285	33
cub0178	Art3Art3	Artemisa	Artemisa	22.795743, -82.763285	33
cub0179	ManVC3	Villa Clara	Manicaragua	22.068519, -79.976387	386
cub0180	R-1	Villa Clara	Santo Domingo	22.586431, -80.224707	48

[1]-Isolate code in the microbial culture collection of Instituto de Investigaciones de Sanidad Vegetal (Cuba). [2]- MASL: Meters above sea level.

Host			Survey
Cultivar	Subgroup	genotype	
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Burro enano	Bluggoe	ABB	2016-2018
Burro enano	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro Cemsa	Bluggoe	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Burro enano	Bluggoe	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Burro CEMSA	Bluggoe	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano vietnamita	Pisang Awak	ABB	2016-2018
Manzano	Silk	AAB	1996-2000

TABLE S2. Composition of the total banana acreage (180596 ha.) of Cuba according to the last national survey (Plant Health Directorate of the Cuban Ministry of Agriculture, DSV 2014, not published).

TYPE	Subgroup	Variety	Genome composition	Planted Area (ha)
Dessert	Cavendish	Gran Naine	AAA	6847
Dessert	Cavendish	Robusta (Valery)	AAA	12223
Dessert	Gros Michel	Gros Michel	AAA	513
Dessert	Cavendish	Bungulan	AAA	85
Dessert	Cavendish	Giant Cavendish	AAA	65
Dessert	Cavendish	Dwarf Cavendish	AAA	42
Dessert	Ibota	Yangambi Km5	AAA	3
Dessert	FHIA hybrids	FHIA-02	AAAA	3
Dessert	FHIA hybrids	SH-3436-9	AAAA	1
Dessert	FHIA hybrids	FHIA-23	AAAA	48
Dessert	FHIA hybrids	FHIA-01	AAAB	274
Dessert	FHIA hybrids	FHIA-18	AAAB	6811
Dessert	Mysore	Pisang ceylan	AAB	409
Dessert	Silk	Manzano INIVIT	AAB	327
Dessert	Silk	Manzano	AAB	6
Dessert	Pisang Awak	Manzano vietnamita	ABB	1043
Cooking	Bluggoe	Burro Cemsa	ABB	77801
Cooking	Bluggoe	Burro Criollo	ABB	858
Cooking	Bluggoe	Burro Enano	ABB	415
Cooking	FHIA hybrids	FHIA-25	AAB	371
Cooking	Bluggoe	Other Bluggoe	ABB	39
Cooking	FHIA hybrids	FHIA-03	AABB	2
Plantain	Plantain	INIVIT PV-0630	AAB	1428
Plantain	Plantain	CEMSA3/4	AAB	37087
Plantain	Plantain	Macho3/4	AAB	19054
Plantain	Plantain	Enano Guantanamero	AAB	6236
Plantain	FHIA hybrids	FHIA-21	AAAB	8549
Plantain	FHIA hybrids	FHIA-20	AAAB	2
Plantain	Plantain	Other Plantain	AAB	50
Other	Others	Others	-	5

TABLE S3. Geographical and sampling details of 170 Cuban *Fusarium* isolates that cause Fusarium wilt of banana, their proposed *Fusarium* species name derived from genotyping-by-sequencing (DArTseq) data and attributed clade, vegetative compatibility group, and race identification, either by PCR or phenotyping.

DArT id	Isolate Code[1]	Origin			Identification							Mating type
		Province	Cultivar	Subgroup	Species[2]	Clade[3]	VCG[4]	Race[5]	Fox[a]	TR4[b,c]	R1/R2[d]	
cub0001	IJ-1	Isla de la Juventud	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	0124	R2$^+$	+	-	+	*MAT1-2*
cub0002	SC-3	Santiago de Cuba	Burro criollo	Bluggoe	*F. tardichlamydosporum*	c2	0128	R2$^+$	+	-	+	*MAT1-2*
cub0003	C.Esm1.3	Camaguey	Burro criollo	Bluggoe	*F. tardichlamydosporum*	c2	0124/5	R2$^+$	+	-	+	*MAT1-2*
cub0004	Cca1.1	Camaguey	Manzano	Silk	*F. tardichlamydosporum*	c2	01210	R1$^+$	+	-	+	*MAT1-2*
cub0005	R.Go	Havana	Manzano	Silk	*F. tardichlamydosporum*	c2	01210	R1$^+$	+	-	+	*MAT1-2*
cub0006	Cub9	Villa Clara	Gros Michel	Gros Michel	*F. purpurascens*	c1	01210	R1	+	-	+	*MAT1-1*
cub0007	M3	Villa Clara	Manzano	Silk	*F. purpurascens*	c1	01210	R1	+	-	+	*MAT1-2*
cub0008	C.Esm1.1	Camaguey	Burro criollo	Bluggoe	*F. tardichlamydosporum*	c2	0124/5	R2$^+$	+	-	+	*MAT1-2*
cub0009	CGua 1.1	Camaguey	Burro criollo	Bluggoe	*F. tardicrescens*	c2	01210	R2$^+$	+	-	+	*MAT1-1*
cub0010	C.V 2.1	Camaguey	Manzano	Silk	*F. purpurascens*	c1	01210	R1$^+$	+	-	+	*MAT1-1*
cub0011	ESB-1	Camaguey	Burro criollo	Bluggoe	*F. tardichlamydosporum*	c2	01210	R2$^+$	+	-	+	*MAT1-2*
cub0012	Cam3	Camaguey	Manzano	Silk	*F. purpurascens*	c1	01210	R1*	+	-	+	*MAT1-1*
cub0013	SC-2	Santiago de Cuba	FHIA-03	FHIA hybrid	*F. tardichlamydosporum*	c2	01210	n.d.	+	-	+	*MAT1-2*
cub0014	GuCUM	Guantanamo	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	0124	R2$^+$	+	-	+	*MAT1-2*
cub0015	GuBaCu	Guantanamo	Burro Criollo	Bluggoe	*F. tardichlamydosporum*	c2	0124	R2$^+$	+	-	+	*MAT1-2*
cub0016	GuMa	Guantanamo	Burro Criollo	Bluggoe	*F. tardichlamydosporum*	c2	0124	R2$^+$	+	-	+	*MAT1-2*
cub0017	TUPP-1	Las Tunas	Manzano	Silk	*F. tardichlamydosporum*	c2	01210	R1$^+$	+	-	+	*MAT1-1*
cub0018	GuBaPo	Guantanamo	Burro Criollo	Bluggoe	*F. tardichlamydosporum*	c2	0124	R2$^+$	+	-	+	*MAT1-2*
cub0020	MoGu 1	Artemisa	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	0124	R2$^+$	+	-	+	*MAT1-2*
cub0021	MoGu 3	Artemisa	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	0124	R2$^+$	+	-	+	*MAT1-2*
cub0022	WaHa	La Habana	Burro CEMSA	Bluggoe	*F. phialophorum*	c2	n.d.	R2*	+	-	+	*MAT1-2*
cub0023	PaGu	Artemisa	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	0124	R2$^+$	+	-	+	*MAT1-2*
cub0024	AlqHa 1	Artemisa	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0025	GuHa	Artemisa	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0026	PeBa	Guantanamo	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	0124	R2	+	-	+	*MAT1-2*
cub0027	ToaGu	Guantanamo	Burro criollo	Bluggoe	*F. tardichlamydosporum*	c2	0124	R2$^+$	+	-	+	*MAT1-2*
cub0028	Bar 1	Guantanamo	Burro criollo	Bluggoe	*F. tardichlamydosporum*	c2	0124	R2$^+$	+	-	+	*MAT1-2*
cub0029	MaHo 1	Holguin	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	0124	R2$^+$	+	-	+	*MAT1-2*

TABLE S3. (*Continued*).

DArT id	Isolate Code[1]	Origin			Identification							Mating type
		Province	Cultivar	Subgroup	Species[2]	Clade[3]	VCG[4]	Race[5]	Fox[a]	TR4[b,c]	R1/R2[d]	
cub0030	BaHo 1	Holguin	Burro criollo	Bluggoe	*F. tardichlamydosporum*	c2	0124	R2$^+$	+	-	+	*MAT1-2*
cub0031	BaHo 2	Holguin	Burro criollo	Bluggoe	*F. tardichlamydosporum*	c2	0124	R2$^+$	+	-	+	*MAT1-2*
cub0032	SaHo	Holguin	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	0124	R2$^+$	+	-	+	*MAT1-2*
cub0033	Bar 3	Guantanamo	Manzano	Silk	*F. tardichlamydosporum*	c2	01210	R1$^+$	+	-	+	*MAT1-2*
cub0034	R-2	Villa Clara	Burro criollo	Bluggoe	*F. tardicrescens*	c2	01210	R2*	+	-	+	*MAT1-2*
cub0035	JCien	Cienfuegos	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1*	+	-	+	*MAT1-1*
cub0037	CaVC	Villa Clara	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0038	ToBaGu	Guantanamo	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0039	NarBaGu	Guantanamo	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0040	PGGua1	Santiago de Cuba	Burro Criollo	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0041	PGGua2	Santiago de Cuba	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0042	BaGra	Granma	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0043	PunBra1	La Habana	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1*	+	-	+	*MAT1-2*
cub0044	PunBra2	La Habana	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0045	BatMay1	Mayabeque	Burro enano	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0046	BatMay2	Mayabeque	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0047	Min1Art	Artemisa	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0048	MaySC1	Santiago de Cuba	Burro Criollo	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0049	MaySC2	Santiago de Cuba	Burro Criollo	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0050	CumCF1	Cienfuegos	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0051	CumCF2	Cienfuegos	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0052	BoyHa	La Habana	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0053	CotHa	La Habana	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0054	GuaHa1	La Habana	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0055	C.Esm1.2	Camaguey	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	0124/5	R2$^+$	+	-	+	*MAT1-2*
cub0056	C.Esm2.1	Camaguey	Burro criollo	Bluggoe	*F. tardichlamydosporum*	c2	0124/5	R2$^+$	+	-	+	*MAT1-2*
cub0057	ESB-5	Camaguey	Burro criollo	Bluggoe	*F. tardichlamydosporum*	c2	0124	R2$^+$	+	-	+	*MAT1-2*
cub0058	SCGu	Santiago de Cuba	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	0124	R2$^+$	+	-	+	*MAT1-2*
cub0059	Bar 2	Guantanamo	Manzano	Silk	*F. tardichlamydosporum*	c2	0124	R2$^+$	+	-	+	*MAT1-2*

TABLE S3. (*Continued*).

DArT id	Isolate Code[1]	Origin			Identification							Mating type
		Province	Cultivar	Subgroup	Species[2]	Clade[3]	VCG[4]	Race[5]	Fox[a]	TR4[b,c]	R1/R2[d]	
cub0060	ColCA	Ciego de Avila	Burro criollo	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0061	Min2Art	Artemisa	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0063	Art1Art-1	Artemisa	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0064	Art1Art-2	Artemisa	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0065	Art2Art-1	Artemisa	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0067	MelArt1.1	Mayabeque	Burro Criollo	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0068	MelArt1.2	Mayabeque	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0069	Front1Art1	Artemisa	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0070	Front1Art2	Artemisa	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0071	Front2Art	Artemisa	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-1*
cub0072	FloCam1	Camaguey	Burro enano	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0073	CesCam1	Camaguey	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0075	LisHa2	La Habana	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0076	TolHa	La Habana	Burro Criollo	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0077	AldHa	La Habana	Burro Criollo	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0078	FloCam2	Camaguey	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0079	FloCam3	Camaguey	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0080	FloCam4	Camaguey	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-1*
cub0081	CCam1	Camaguey	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0082	CCam2	Camaguey	Burro enano	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0084	CCam3.2	Camaguey	Burro enano	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0085	MaHo2	Holguin	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0086	MaHo3	Holguin	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0087	MaHo4	Holguin	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0088	MaHo5	Holguin	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2*	+	-	+	*MAT1-2*
cub0089	MaHo6	Holguin	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0090	MaHo7	Holguin	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0091	PaHo1	Holguin	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0092	PaHo2	Holguin	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*

TABLE S3. (*Continued*).

DArT id	Isolate Code[1]	Origin			Identification							Mating type
		Province	Cultivar	Subgroup	Species[2]	Clade[3]	VCG[4]	Race[5]	Fox[a]	TR4[b,c]	R1/R2[d]	
cub0093	SaHo2	Holguin	Burro Criollo	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0095	SaHo3.2	Holguin	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0096	SaHo4	Holguin	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-1*
cub0097	MosGu1	Guantanamo	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0098	MosGu2	Guantanamo	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0099	MosGu3.1	Guantanamo	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0100	MosGu3.2	Guantanamo	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0101	MosGu8	Guantanamo	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0102	MosGu4	Guantanamo	Burro Criollo	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0103	MosGu5	Guantanamo	Burro Criollo	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0104	MosGu6	Guantanamo	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0105	MosGu7	Guantanamo	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0106	SaLGu1	Guantanamo	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0107	SaLGu2	Guantanamo	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0108	SaSGu1.1	Guantanamo	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0109	SaSGu1.2	Guantanamo	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0110	SaSGu2	Guantanamo	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0111	SaSGu3.1	Guantanamo	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0112	SaSGu3.2	Guantanamo	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0113	SaSGu4	Guantanamo	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0114	LaMSC1.1	Santiago de Cuba	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0115	LaMSC1.2	Santiago de Cuba	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0116	LaMSC2.1	Santiago de Cuba	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0117	LaMSC2.2	Santiago de Cuba	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0118	CriSC	Santiago de Cuba	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0119	JaMay1.1	Mayabeque	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0120	JaMay1.2	Mayabeque	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0121	JaMay2	Mayabeque	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0122	JaMay3.1	Mayabeque	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0123	JaMay3.2	Mayabeque	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*

TABLE S3. (*Continued*).

DArT id	Isolate Code[1]	Origin			Identification							Mating type
		Province	Cultivar	Subgroup	Species[2]	Clade[3]	VCG[4]	Race[5]	Fox[a]	TR4[b,c]	R1/R2[d]	
cub0124	SJMay1	Mayabeque	Burro enano	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0125	SJMay2.1	Mayabeque	Burro enano	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0126	SJMay2.2	Mayabeque	Burro enano	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0128	SJMay3.2	Mayabeque	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0129	SJMay4	Mayabeque	Manzano vietnamita	Pisang Awak	*F. purpurascens*	c1	n.d.	R1*	+	-	+	*MAT1-1*
cub0130	CabSS1	Sancti Spiritus	Manzano vietnamita	Pisang Awak	*F. purpurascens*	c1	n.d.	R1*	+	-	+	*MAT1-1*
cub0131	CabSS2	Sancti Spiritus	Manzano vietnamita	Pisang Awak	*F. purpurascens*	c1	n.d.	R1	+	-	+	*MAT1-1*
cub0132	YagSS1	Sancti Spiritus	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-1*
cub0133	YagSS2	Sancti Spiritus	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0134	YagSS3	Sancti Spiritus	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0135	StCVC1	Villa Clara	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0136	StCVC2	Villa Clara	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0137	CaVC2	Villa Clara	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0138	CaVC3	Villa Clara	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0139	CamVC1	Villa Clara	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0140	CiVC1	Villa Clara	Manzano vietnamita	Pisang Awak	*F. purpurascens*	c1	n.d.	R1*	+	-	+	*MAT1-1*
cub0141	CiVC2	Villa Clara	Manzano vietnamita	Pisang Awak	*F. purpurascens*	c1	n.d.	R1	+	-	+	*MAT1-1*
cub0142	CumCF3.1	Cienfuegos	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0143	CumCF3.2	Cienfuegos	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0144	ManVC1	Villa Clara	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0145	MAnVC1-1	Villa Clara	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1*	+	-	+	*MAT1-2*
cub0146	ManVC2	Villa Clara	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0147	ManVC2-1	Villa Clara	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0149	PalPR1	Pinar del Rio	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0150	PalPR2	Pinar del Rio	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2*	+	-	+	*MAT1-2*
cub0151	PalPR3	Pinar del Rio	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0152	PalPR4	Pinar del Rio	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0153	PalPR5	Pinar del Rio	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0154	PalPR6	Pinar del Rio	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*

TABLE S3. (*Continued*).

DArT id	Isolate Code[1]	Origin			Identification							Mating type
		Province	Cultivar	Subgroup	Species[2]	Clade[3]	VCG[4]	Race[5]	Fox[a]	TR4[b,c]	R1/R2[d]	
cub0155	PalPR7	Pinar del Rio	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0156	PalPR8	Pinar del Rio	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0157	ViPR2	Pinar del Rio	Burro enano	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0158	ViPR3	Pinar del Rio	Burro enano	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0159	PalmPR1	Pinar del Rio	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0160	PalmPR2	Pinar del Rio	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0162	PalmPR3.2	Pinar del Rio	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0164	GuaPR1	Pinar del Rio	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0165	GuaPR2	Pinar del Rio	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0166	SanJPR1	Pinar del Rio	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0167	SanJPR2	Pinar del Rio	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0168	SanJPR3	Pinar del Rio	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0169	PinPR	Pinar del Rio	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0170	SanLPR1	Pinar del Rio	Burro enano	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0171	SanLPR2	Pinar del Rio	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0172	SanLPR3	Pinar del Rio	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	c2	n.d.	R2	+	-	+	*MAT1-2*
cub0173	PerMat1	Matanzas	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0174	PerMat2.1	Matanzas	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0175	PerMat2.2	Matanzas	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0176	Art3Art1	Artemisa	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0177	Art3Art2	Artemisa	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0178	Art3Art3	Artemisa	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0179	ManVC3	Villa Clara	Manzano vietnamita	Pisang Awak	*F. tardichlamydosporum*	c2	n.d.	R1	+	-	+	*MAT1-2*
cub0180	R-1	Villa Clara	Manzano	Silk	*F. tardichlamydosporum*	c2	0124	R1[+]	+	-	+	*MAT1-2*

[1]- Code in the microbial culture collection of the Instituto de Investigaciones de Sanidad Vegetal (INISAV, Cuba); [2,3]- According to DArT analysis; [4]- According to Battle and Pérez-Vicente (2009); [5]-Race determined based on the host of isolation or according to Battle and Pérez-Vicente (2009)(+) or determined in the present study by pathogenicity test (*), Race 1 (R1), Race 2 (R2), Tropical Race 4 (TR4). Fox[a]-results according to the protocol described by Edel *et al.* (2000) with primers PFO2/PFO3; TR4[b]- results of the PCR reaction with primers FocTR4-F/FocTR4-R and TR4[c]- with primers Six1a_266-F/ Six1a_266-R (Dita *et al.*, 2010; Carvalhais *et al.*, 2019); R1/R2[d]-identified with the protocol described by (Carvalhais *et al.*, 2019) and with primers Six6b_210-F/Six6b_210-R.

TABLE S4. *Fusarium* isolates whose genome sequences were analyzed in this study.

Isolate Code	Alternative code	Country	Host	*Musa* subgroup	Species	VCG	Race	Sequencing t echnology	Database IDs	Reference
aus0024	NRRL36110	Australia	Mons	Cavendish	*F. purpurascens*	0129	STR4	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
aus0032	NRRL36101	Australia	Mons Mari	Cavendish	*F. phialophorum*	0120	R1	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
BRIP62280	-	Australia	Cavendish	Cavendish	*F. odoratissimum*	01213	TR4	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
BRIP63632	-	Australia	Williams	Cavendish	*F. odoratissimum*	01213	TR4	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
BRIP65062	-	Australia	Cavendish	Cavendish	*F. odoratissimum*	01213	TR4	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
bra0004	R1	Brazil	Silk	Silk	*F. oxysporum*	n.d.	n.d.	Short-read (Illumina/BGISeq)	PRJNA1005897	This study
chi0001	NRRL36102	China	Cavendish	Cavendish	*F. odoratissimum*	0121	n.d.	Long-read(Nanopore)	PRJNA979975	van Westerhoven, 2023
chi0009	Chi1.1A	China	n.d.	n.d.	*F. odoratissimum*	01213	TR4	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
chi0015	F9129	China	Latundan	Silk	*F. duoseptatum*	0123	R1	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
Race1	N2	China	Brazil	-	*F. duoseptatum*	n.d.	R1	Short-read (Illumina/BGISeq)	PRJNA174275	Guo *et al.*, 2014
Race4	B2	China	Pisang Awak	Pisang Awak	*F. odoratissimum*	n.d.	R4	Short-read (Illumina/BGISeq)	PRJNA174275	Guo *et al.*, 2014
Col17	-	Colombia	Williams	Cavendish	*F. odoratissimum*	01213	TR4	Short-read (Illumina/BGISeq)	PRJNA565135	García-Bastidas *et al.*(2019)
Col2	-	Colombia	Williams	Cavendish	*F. odoratissimum*	01213	TR4	Short-read (Illumina/BGISeq)	PRJNA565135	García-Bastidas *et al.*(2019)
Col4	-	Colombia	Williams	Cavendish	*F. odoratissimum*	01213	TR4	Short-read (Illumina/BGISeq)	PRJNA565135	García-Bastidas *et al.*(2019)
cos0094	BIF-04	Costa Rica	n.d.	n.d.	*F. phialophorum*	0120/15	n.d.	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
cos0116	CR1.1A	Costa Rica	Gros Michel	Gros Michel	*F. phialophorum*	0120	R1	Long-read(Nanopore)	PRJNA979975	van Westerhoven, 2023
cos0124	FocP1	Costa Rica	n.d.	n.d.	*F. phialophorum*	0120/15	n.d.	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
cub0006	Cub9	Cuba	Gros Michel	Gros Michel	*F. purpurascens*	01210	n.d.	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
cub0012	Cam3	Cuba	Silk	Silk	*F. purpurascens*	01210	R1	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
cub0022	WaHa	Cuba	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	n.d.	R2	Short-read (Illumina/BGISeq)	PRJNA1005897	This study
cub0030	BaHo 1	Cuba	Burro criollo	Bluggoe	*F. tardichlamydosporum*	0124	R2	Short-read (Illumina/BGISeq)	PRJNA1005897	This study
cub0034	R-2	Cuba	Burro criollo	Bluggoe	*F. tardichlamydosporum*	01210	R2	Long-read(Nanopore)	PRJNA979975	van Westerhoven, 2023
cub0035	JCien	Cuba	Manzano vietnamita	Pisang awak	*F. purpurascens*	n.d.	n.d.	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
cub0039	NarBaGu	Cuba	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	n.d.	n.d.	Short-read (Illumina/BGISeq)	PRJNA1005897	This study
cub0043	PunBra1	Cuba	Manzano vietnamita	Pisang awak	*F. tardichlamydosporum*	n.d.	n.d.	Long-read(Nanopore)	PRJNA979975	van Westerhoven, 2023
cub0061	Min2Art	Cuba	Manzano vietnamita	Pisang awak	*F. tardichlamydosporum*	n.d.	n.d.	Short-read (Illumina/BGISeq)	PRJNA1005897	This study
cub0078	FloCam2	Cuba	Burro criollo	Bluggoe	*F. tardichlamydosporum*	n.d.	n.d.	Short-read (Illumina/BGISeq)	PRJNA1005897	This study
cub0088	MaHo5	Cuba	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	n.d.	n.d.	Long-read(Nanopore)	PRJNA979975	van Westerhoven, 2023

TABLE S4. (*Continued*).

Isolate Code	Alternative code	Country	Host	*Musa* subgroup	Species	VCG	Race	Sequencing t echnology	Database IDs	Reference
cub0117	LaMSC2.2	Cuba	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	n.d.	n.d.	Short-read (Illumina/BGISeq)	PRJNA1005897	This study
cub0129	SJMay4	Cuba	Manzano vietnamita	Pisang awak	*F. purpurascens*	n.d.	n.d.	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
cub0130	CabSS1	Cuba	Manzano vietnamita	Pisang awak	*F. purpurascens*	n.d.	n.d.	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
cub0140	CiVC1	Cuba	Manzano vietnamita	Pisang awak	*F. purpurascens*	n.d.	n.d.	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
cub0142	CumCF3.1	Cuba	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	n.d.	n.d.	Short-read (Illumina/BGISeq)	PRJNA1005897	This study
cub0145	MAnVC1-1	Cuba	Manzano vietnamita	Pisang awak	*F. tardichlamydosporum*	n.d.	n.d.	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
cub0161	PalmPR3.1	Cuba	Burro Cemsa	Bluggoe	*F. tardichlamydosporum*	n.d.	n.d.	Short-read (Illumina/BGISeq)	PRJNA1005897	This study
cub0168	SanJPR3	Cuba	Manzano vietnamita	Pisang awak	*F. tardichlamydosporum*	n.d.	n.d.	Short-read (Illumina/BGISeq)	PRJNA1005897	This study
cub0170	SanLPR1	Cuba	Burro enano	Bluggoe	*F. tardichlamydosporum*	n.d.	n.d.	Short-read (Illumina/BGISeq)	PRJNA1005897	This study
cub0172	SanLPR3	Cuba	Burro CEMSA	Bluggoe	*F. tardichlamydosporum*	n.d.	n.d.	Short-read (Illumina/BGISeq)	PRJNA1005897	This study
cub0178	Art3Art3	Cuba	Manzano vietnamita	Pisang awak	*F. tardichlamydosporum*	n.d.	n.d.	Short-read (Illumina/BGISeq)	PRJNA1005897	This study
hon0003	NRRL36107	Honduras	Maqueno	Plantain	*F. purpurascens*	0126	STR4	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
idn0006	InaCC F829	Indonesia	Pisang Awak	Pisang Awak	*F. duoseptatum*	n.d.	n.d.	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
idn0023	InaCC F833	Indonesia	Pisang Awak	Pisang awak	*F. grosmichelii*	n.d.	R1	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
idn0028	InaCC F867	Indonesia	P. Ambon Kuning	Gros Michel	*F. grosmichelii*	n.d.	n.d.	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
idn0206	InaCC F823	Indonesia	Pisang Kepok	Pisang Awak	*F. purpurascens*	n.d.	n.d.	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
ido0017	InaCC F917	Indonesia	Pisang Ambon	Cavendish	*F. kalimantanense*	n.d.	n.d.	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
ido0226	II5	Indonesia	Pisang Manurung	Pisang awak	*F. odoratissimum*	01213	TR4	Long-read(Nanopore)	PRJNA979975	van Westerhoven, 2023
FOC TR4-1	-	Israel	n.d.	Cavendish	*F. odoratissimum*	01213	TR4	Short-read (Illumina/BGISeq)	PRJNA563197	Maymon *et al.* (2020)
FOC TR4-5	-	Israel	n.d.	Cavendish	*F. odoratissimum*	01213	TR4	Short-read (Illumina/BGISeq)	PRJNA563197	Maymon *et al.* (2020)
jor0001	JV11	Jordan	Cavendish	Cavendish	*F. odoratissimum*	01213	TR4	Short-read (Illumina/BGISeq)	PRJNA472880	Zheng *et al.*(2018)
JV14	-	Jordan	n.d.	Cavendish	*F. odoratissimum*	01213	TR4	Short-read (Illumina/BGISeq)	PRJNA563197	Maymon *et al.* (2020)
La-2	-	Laos	Brazilian	Cavendish	*F. odoratissimum*	01213	TR4	Short-read (Illumina/BGISeq)	PRJNA472880	Zheng *et al.*(2018)
leb0001	Leb1.2C	Lebanon	Cavendish	Cavendish	*F. odoratissimum*	01213	TR4	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
mal0001	NRRL36113	Malawi	Harare	Bluggoe	*F. tardicrescens*	01214	R2	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
mwi0008	NRRL36117	Malaysia	P. Awak Legor	Pisang awak	*F. tardichlamydosporum*	01222	R1	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
mas0002	NRRL36115	Malaysia	Pisang Ambon	Cavendish	*F. duoseptatum*	01224	n.d.	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
mas0005	Mal43	Malaysia	Pisang Rastali	Silk	*F. duoseptatum*	01217	n.d.	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023

TABLE S4. (*Continued*).

Isolate Code	Alternative code	Country	Host	*Musa* subgroup	Species	VCG	Race	Sequencing t echnology	Database IDs	Reference
mys0005	NRRL36116	Malaysia	Pisang Keling	n.d.	*F. duoseptatum*	01223	n.d.	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
Moz1A	-	Mozambique	n.d.	Cavendish	*F. odoratissimum*	n.d.	TR4	Short-read (Illumina/BGISeq)	PRJNA805401	van Westerhoven, 2023
Moz2A	-	Mozambique	n.d.	Cavendish	*F. odoratissimum*	n.d.	TR4	Short-read (Illumina/BGISeq)	PRJNA805401	van Westerhoven, 2023
Moz3A	-	Mozambique	n.d.	Cavendish	*F. odoratissimum*	n.d.	TR4	Short-read (Illumina/BGISeq)	PRJNA805401	van Westerhoven, 2023
Moz4A	-	Mozambique	n.d.	Cavendish	*F. odoratissimum*	n.d.	TR4	Short-read (Illumina/BGISeq)	PRJNA805401	van Westerhoven, 2023
Moz5A	-	Mozambique	n.d.	Cavendish	*F. odoratissimum*	n.d.	TR4	Short-read (Illumina/BGISeq)	PRJNA805401	van Westerhoven, 2023
My-1	-	Myanmar	Brazilian	Cavendish	*F. odoratissimum*	01213	TR4	Short-read (Illumina/BGISeq)	PRJNA472880	Zheng *et al.*(2018)
nic0006	Foc16	Nicaragua	Gros Michel	Gros Michel	*F. oxysporum*	newVCG13	n.d.	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
nic0010	Foc8	Nicaragua	Gros Michel	Gros Michel	*F. oxysporum*	newVCG12	n.d.	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
pak0001	Pak1.1A	Pakistan	Cavendish	Cavendish	*F. odoratissimum*	01213	TR4	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
per0021	P20a	Peru	Manzano	Silk	*F. oxysporum*	newVCG16	n.d.	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
per0029	P26c	Peru	Seda	Gros Michel	*F. oxysporum*	newVCG17	n.d.	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
per0040	P41	Peru	Seda	Gros Michel	*F. tardicrescens*	newVCG8	n.d.	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
phi0001	NRRL36103	Philippines	Cavendish	Cavendish	*F. purpurascens*	0122	STR4	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
phi0005	Phi2.6C	Philippines	GCTCV-218	Cavendish	*F. odoratissimum*	01213	TR4	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
phi0016	Phi6.6a	Philippines	UNIC29	n.d.	*F. oxysporum*	newVCG20	n.d.	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
S1B8	-	Philippines	n.d.	STR4	*F. odoratissimum*	01213	TR4	Short-read (Illumina/BGISeq)	PRJNA563197	Maymon *et al.* 2020
zaf0001	NRRL36112	South Africa	n.d.	Cavendish	*F. phialophorum*	01215	STR4	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
esp0001	FocST4-98	Spain	Dwarf Cavendish	Cavendish	*F. phialophorum*	0120	STR4	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
tan0001	NRRL36108	Tanzania	Ney Poovan	Ney Poovan	*F. tardichlamydosporum*	01212	n.d.	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
tha0001	NRRL36118	Thailand	Kluai nam wa	Pisang awak	*F. cugenangense*	01221	n.d.	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
tha0002	NRRL36120	Thailand	Kluai nam wa	Pisang awak	*F. grosmichelii*	01218	R1	Short-read (Illumina/BGISeq)	PRJNA979975	van Westerhoven, 2023
Eden	-	UK	n.d.	n.d.	*F. odoratissimum*	01213	TR4	Short-read (Illumina/BGISeq)	PRJNA556111	Warmington *et al.* (2019)
VN-2	-	Vietnam	Giujiao No 6	Cavendish	*F. odoratissimum*	01213	TR4	Short-read (Illumina/BGISeq)	PRJNA472880	Zheng *et al.*(2018)

CHAPTER 3

The vulnerability of Cuban banana production to Fusarium wilt caused by Tropical Race 4

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ABSTRACT

Bananas are major agricultural commodities in Cuba. One of the main constraints of banana production worldwide is Fusarium wilt of banana (FWB). Recent outbreaks in Colombia, Perú and Venezuela raise widespread concern in Latin America due to the potential devastating impact on the sustainability of banana production, food security, and livelihoods of millions of people in the region. Here, we phenotyped 18 important Cuban banana and plantain varieties with two *Fusarium* strains; Tropical Race 4 (TR4) and Race 1 under greenhouse conditions. These varieties represent 72,8% of the national banana acreage in Cuba and are also widely distributed in Latin America and the Caribbean region. A broad range of disease responses from resistant to very susceptible was observed against Race 1. On the contrary, not a single banana variety was resistant to TR4. These results underscore that TR4 potentially threatens nearly 56% of the contemporary Cuban banana production area, which is planted with susceptible and very susceptible varieties, and call for a preemptive evaluation of new varieties obtained in the national breeding program and the strengthening of quarantine measures to prevent the introduction of TR4 into the country.

INTRODUCTION

Recently, the United Nations declared 2021 the International Year of Fruit and Vegetables to highlight the critical role of plants for human nutrients, food security, and health (FAO, 2020). Bananas (*Musa* spp.) are among the most produced, globally traded and consumed fruits. Since their arrival in Cuba in 1529 (Marin *et al.*, 1998), bananas became an important staple food and developed into a profitable export commodity between 1857 and 1950 (García 2000, 2001; Pérez-Ponce and Orellana, 1995). Fusarium wilt of banana (FWB) impacted the production in the previous century and together with yellow Sigatoka caused by *Pseudocercospora musae* (Zimm.) Deighton (Jones, 2003) provoked the collapse of national export activities. Since then, Cuban banana production is entirely destined for the domestic market. Bananas are an important cash crop for small growers and are indispensable as staple food for the Cuban population. According to the National Office for Statistics and Information (ONEI) they represent nearly 30% of all fruit and starchy root staples produced in the country (ONEI, 2022).

Initially, two major groups of edible bananas were cultivated in Cuba. The first group comprised popular dessert-type bananas including the main varieties Gros Michel (3x = AAA) and its dwarf variants, together with Manzano (Silk, 3x = AAB), Indio, Morados (red banana, 3x = AAA) and Datil (2x = AA). The second group included plantains (subgroup Plantain, 3x = AAB) represented by the main varieties Macho and Hembra (Horn and French types, respectively) and several cooking banana varieties known as Burro (subgroup Bluggoe, 3x = ABB). The dessert bananas were also divided into two groups: those cultivated for domestic consumption, mainly Gros Michel and Manzano, and those cultivated for export, dominated by Gros Michel (Minneman, 1943; García, 2001, 2008).

However, fungal pathogens altered the diversity of the national production. The FWB epidemic in Latin America in the previous century was caused by genetically diverse lineages of the fungus that was hitherto known as *Fusarium oxysporum* f. sp. *cubense* that were collectively named Race 1 (Ordóñez *et al.*, 2015; Maryani, 2019) and destroyed plantations of Gros Michel and Manzano. These were therefore gradually replanted since the 1950s by the resistant Cavendish varieties (3x = AAA). Initially by Robusta (Valery or Poyo), but later other Cavendish varieties such as "Parecido al rey" and Grand Naine were introduced from Vietnam in the 1970s and Panamá in 1981, respectively (Alvarez, 2011). In addition, during the 1990s, black leaf streak disease (BLSD) or black Sigatoka, caused by *Pseudocercospora fijiensis* (M. Morelet) Deighton (Crous *et al.*, 2021), greatly affected the production of the susceptible Cavendish varieties and plantains. This disease demands higher disease management costs, which along with the declining socialist partner markets, again altered the diversity of the national banana production in Cuba. Therefore, the contemporary Cavendish production is mostly directed to the tourism sector but the national production gradually turned towards BLSD-resistant banana hybrids developed by the Fundación Hondureña de Investigación Agrícola (FHIA), particularly the dessert types FHIA-23 (4x = AAAA) and FHIA-18 (4x = AAAB), the plantains FHIA-20 and 21 (4x = AAAB) and the cooking bananas FHIA-03 (AABB) and Burro CEMSA (ABB, Bluggoe) (Pérez-Vicente *et al.*, 2002). At the same time and despite its susceptibility to Race 1, Pisang Awak (3x

= ABB) became popular due to its higher rusticity and semi-acid taste, reminiscent of the once favored Manzano (Battle and Pérez-Vicente, 2009). Nowadays, Pisang Awak and other cultivars of the Bluggoe subgroups (3x = ABB) are commonly grown in backyards and small plots across the country. Consequently, FWB surfaced again in locations where Pisang Awak or Bluggoe cultivars were cultivated on soils infested with Race 1 and the so-called Race 2, respectively.

Fusarium wilt of banana is a typical vascular wilt disease. The pathogen is considered to be a hemibiotroph, which initially establishes a biotrophic interaction with the plant, by colonizing the root surface and growing between epidermal root cells (Guo *et al.*, 2015), but eventually turns into necrotrophy that kills host tissue (Dita *et al.*, 2018). Initial infection occurs at the tips of secondary and tertiary roots and penetration can be either direct or through wounds. Proliferating mycelium reaches the vascular system of the rhizome and later colonizes the pseudostem, which eventually kills the plant. In resistant cultivars, the host defenses arrest pathogen colonization in the root system (Pegg *et al.*, 2019)Pome subgroup.

For this project, we adopted recent taxonomical analyses, which showed that FWB is caused by a genetically diverse *Fusarium* species complex (Maryani *et al.*, 2019). Hence, the hitherto determined genetic lineages that comprise the various pathogenic races, including Tropical Race 4 (TR4), are considered to be different species. Though this conclusion is being disputed (Torres-Bedoya *et al.*, 2021), it is based on solid genomic analyses (Maryani, 2019). The international dissemination of TR4 is a serious threat to the global banana industry (Pérez-Vicente, 2004; Dita *et al.*, 2013; Maryani *et al.*, 2019; van Westerhoven *et al.*, 2022a, 2022b), and particularly for Latin America and the Caribbean region after its emergence in Colombia (García-Bastidas *et al.*, 2020), Perú (Acuña *et al.*, 2021) and Venezuela (Herrera *et al.*, 2023). Therefore, we evaluated the resistance to FWB caused by TR4 and Race 1 of 18 banana varieties that are crucial for Cuba, and many other countries in Latin America and the Caribbean.

MATERIALS AND METHODS

Plant Material

All banana varieties (Table 1) were multiplied at the Instituto de Biotecnología de las Plantas (IBP, Villa Clara) in Cuba. Upon transport and arrival at Wageningen University and Research (WUR, The Netherlands), plants were retrieved from the plastic containers and transferred to individual 1L pots containing a standard soil (Swedish sphagnum peat 20%, Baltic peat 30%, garden peat 30%, beam structure 20%, grinding clay granules 40.6 Kg/m^3, Lime + MgO 2.5 Kg/m3, PG-Mix-15-10-20 0.8 Kg/m^3) from the WUR-Unifarm greenhouse facility.

The potted plants were acclimatized under plastic for two weeks to maintain high humidity in an environmentally controlled greenhouse compartment (28±2°C, 16h light, and ~85% relativity humidity) and were thereafter grown for ~ 2.5 months prior to inoculation and evaluation. Plants were watered daily and fertilized (NH_4^+-1.2 mM/L, K^+-7.2 mM/L, Ca^{2+}-4 mM/L, Mg^{2+}-1.82 mM/L, NO_3^--12.4 mM/L, SO_4^{2-}-3.32 mM/L, $H_2PO_4^-$-1.1 mM/L, Mn^{2+}-10 µMol/L, Zn^{2+}-5

µMol/L, B-30 µMol/L, Cu^{2+}-0.75 µMol/L, Mo-0.5 µMol/L, Fe/DTPA-50/3%, Fe-EDDHSA-50/3%, pH=5.8) three times per week.

TABLE 1. Banana germplasm phenotyped for resistance to Fusarium wilt.

Name	Accession code[1]	Subgroup	Ploidy	Type
FHIA-01	MUSA200	FHIA hybrid	AAAB	Dessert
FHIA-17	MUSA352	FHIA hybrid	AAAA	Dessert
FHIA-18	MUSA206	FHIA hybrid	AAAB	Dessert
FHIA-23	MUSA211	FHIA hybrid	AAAA	Dessert
Gran Enano[2]	ITC0180	Cavendish	AAA	Dessert
Grand Naine[3]	-	Cavendish	AAA	Dessert
Gros Michel	ITC0484	Gros Michel	AAA	Dessert
Pisang Awak	ITC0213	Pisang Awak	ABB	Dessert
Pisang Ceylan	MUSA269	Mysore	AAB	Dessert
Manzano	MUSA30	Silk	AAB	Dessert
SH-3640	ITC1307	FHIA hybrid	AAAB	Dessert
Yangambi Km5	MUSA317	Ibota	AAA	Dessert
Burro CEMSA	MUSA198	Bluggoe	ABB	Cooking banana
FHIA-25	MUSA212	FHIA hybrid	AAB	Cooking banana
CEMSA3/4	MUSA31	Plantain	AAB	French Horn
Curare	ITC1165	Plantain	AAB	False Horn
FHIA-04	MUSA204	FHIA hybrid	AAAB	Plantain
FHIA-20	MUSA208	FHIA hybrid	AAAB	Plantain
FHIA-21	MUSA209	FHIA hybrid	AAAB	Plantain

[1]ITC = International *Musa* germplasm Transit Center, MUSA = Cuban banana germplasm collection. [2]Grand Naine from the Cuban germplasm collection. [3]Provided by Rahan Meristem (http://www.rahan.co.il).

Fungal isolates and inoculations

We used two strains for phenotyping: a Brazilian *Fusarium oxysporum* isolate originating from Cruz das Almas in Brazil, strain CNPMF 000-8-01-R1 (unknown and hence new vegetative compatibility group (VCG) according to Ordóñez, 2018), which represents Race 1 and the *F. odoratissimum* reference strain II-5 (also known as NRRL54006, VCG 01213), originating from Indonesia (Maryani *et al.*, 2019), which represent TR4. These strains have also been used in previous FWB phenotyping studies under greenhouse conditions (Dita *et al.*, 2011; Ribeiro *et al.*, 2011; Rebouças *et al.*, 2018; García-Bastidas, 2019). Sporulation media were prepared by autoclaving 500 ml water in 1 L Erlenmeyer flasks supplemented with 2 gr Mung beans. The flasks were closed with cotton plugs and sterilized at 120°C for 20 min and after cooling inoculated with mycelium plugs from a freshly grown potato dextrose agar Petri dish (incubated at 28°C for five days) and subsequently incubated in a rotary shaker at 140 rpm and 28°C for five days. Inoculum was produced by passing the obtained spore suspension through two layers of sterile cheesecloth to remove hyphal fragments and the final concentration was adjusted to 10^6 conidia/mL. Inoculations were conducted by pouring 200 mL of inoculum directly on the soil of root-wounded potted banana plants (García-Bastidas *et al.*, 2019).

Disease assessment and experimental design

For disease assessment, we followed the protocol of García-Bastidas *et al.* (2019) and determined the Rhizome Discolored Area (RDA) as a percentage of the total rhizome area by ImageJ 1.52r software (National Institutes of Health, Bethesda, MD, USA) and plotted individual RDA-values by using the web-tool BoxPlotR (Spitzer *et al.*, 2014).

Alternatively, and for comparative reasons, we also used the RDA values to categorize the symptoms into the commonly used 1–6 scale of the Rhizome Discoloration Index (RDI). On this scale 1 = No discoloration in the corm (RDA=0), 2 = isolated points (RDA<5%), 3 = 5%<RDA<30%, 4 = 30%<RDA<50%, 5 = 50%<RDA<90%, and 6 = plant totally decayed (RDA>90%) (García-Bastidas *et al.*, 2019). Disease Indexes (DIs) were calculated following McKinney (1923) where DI= [Σ(score in the scale x frequency)/(total number of plants x maximum class in the scale)] x 100%, which were then used to classify the germplasm as resistant (R= 0 < DI ≤ 24±1 %); moderately susceptible (MS=25 < DI ≤ 44±1 %); susceptible (S=45 < DI ≤ 64±1%); very susceptible (VS=65 < DI ≤ 84±1 %) or extremely susceptible (XS= DI ≤ 85%).

The phenotyping assays were carried out following a partially balanced incomplete block design and comprised four separate sub-trials. This facilitates variable numbers of varieties and replicates per trial to cope with the varying success of tissue culture. Data analysis was conducted using R 4.0.3 (R Core Team, 2020) and functions from the package lmerTest (Kuznetsova *et al.*, 2017; version 3.1-3) in a linear mixed model with the square root transformed RDA percentage as a function of the accession and as fixed factor, and the experiment and the accession within the experiment as random effects. Grand Naine plants were used as controls for all comparative analyses and water-treated (200 mL) plants of the 18 varieties were used as mock.

RESULTS

Here we report a comprehensive greenhouse evaluation of the response to FWB across banana germplasm that is crucial for Cuba and many other countries and compare our data with various other reports (see also Supplementary Table S1). We used tissue culture plants across all experiments. Multiplying Cavendish plants through tissue culture is easy but for many other varieties, it is complicated and prone to failure, which is a common setback in banana research that evaluates wider germplasm collections or segregating populations (Ahmad *et al.*, 2020). Eventually, we were able to include between 2-12 replicates per variety in each of the four sub-trials. During the entire experiment, control plants never showed any external or internal FWB symptoms and the reference interactions (Gros Michel vs. Race 1 and Cavendish vs. Race 1 or TR4) showed the typical differential response (Figure 1). Not all the tested susceptible plants showed noticeable external symptoms but internal discoloration of their rhizomes was consistent (Figures 1 and 2).

The response to Race 1 was diverse, median RDA values were 0 for resistant varieties CEMSA ¾, Curare, FHIA-20, FHIA-21, FHIA-25, Grand Naine, Gran Enano (Grand Naine from the Cuban

germplasm collection) and SH-3640, whereas those of susceptible germplasm ranged from 1.3 to 62.6% (Figure 2). Burro CEMSA is known for its susceptibility to Race 2 but also showed RDA values up to 37.6 % (Figure 2) for Race 1.

Phenotyping with TR4 showed that none of the tested varieties was resistant since RDA median values ranged from 1.2 to 71.4%, except for FHIA-18. The RDA median value of this hybrid was 0 but 13 of the 29 tested plants had RDA values between 1.5-22.6%, hence its DI was 25.9% which classifies it as moderately susceptible (Supplementary Figure S1, Supplementary Table S2). The reference variety Grand Naine (as well as Gran Enano) was very susceptible to TR4, with RDA scores up to 67.6% and 88.8%, respectively (Supplementary Table S2).

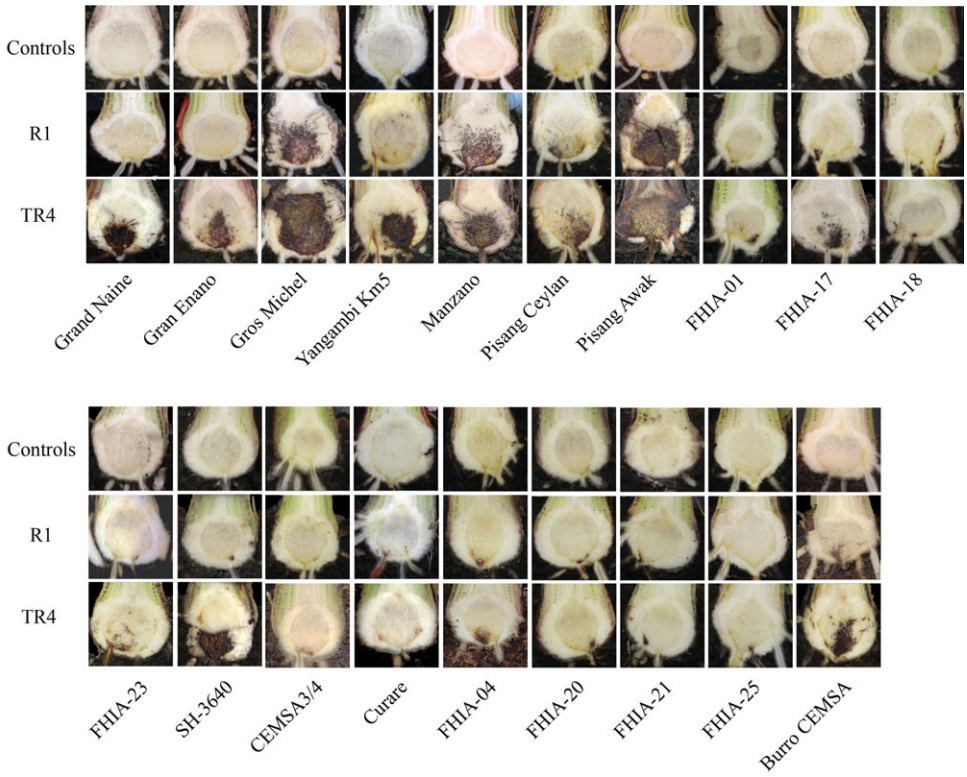

FIGURE 1. Cross sections of corms of banana accessions showing a differential response after inoculations with Race 1 (strain CNPMF 000-8-01-R1, *Fusarium oxysporum*) and TR4 (strain II-5, *F. odoratissimum*).

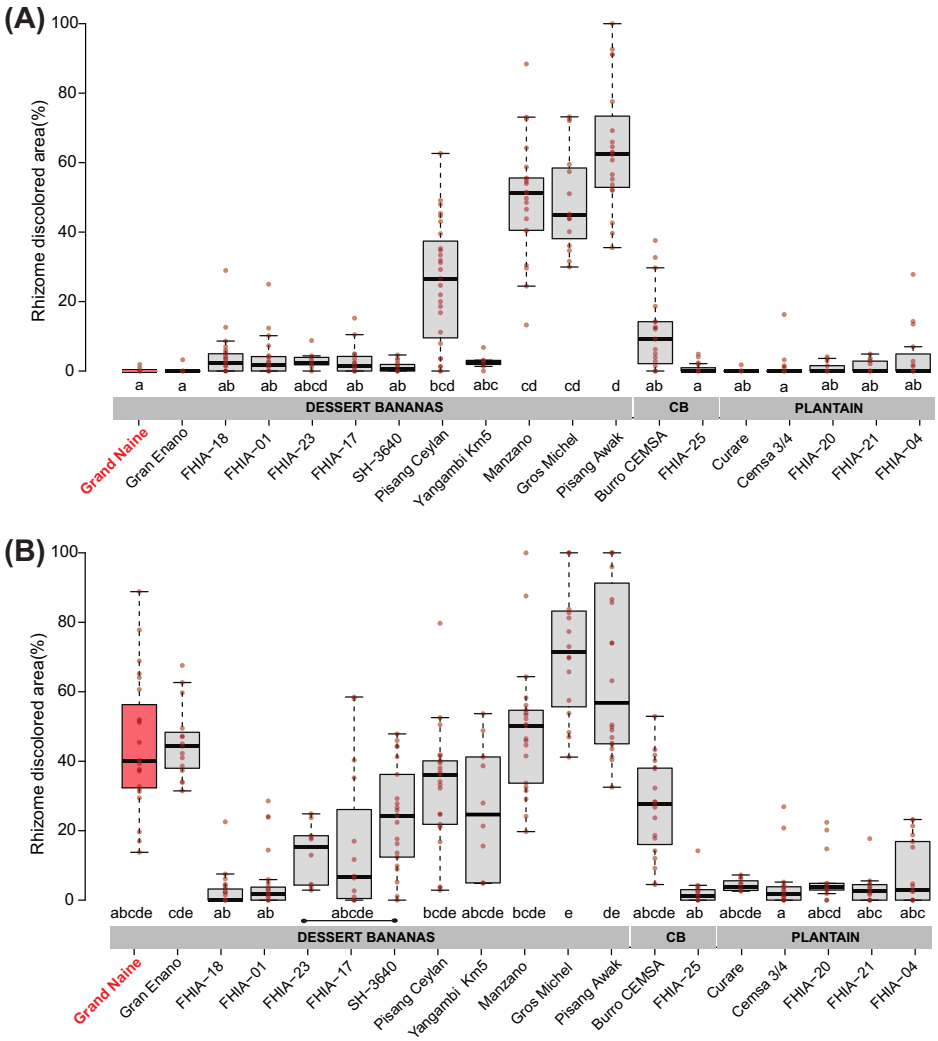

FIGURE 2. Response of banana and plantain cultivars to (A) Race 1 (strain CNPMF 000-8-01-R1, *Fusarium oxysporum*) and (B) TR4 (strain II-5, *F. odoratissimum*) expressed as percentage of Rhizome Discolored Area (RDA). The reference Grand Naine is indicated in red. CB = Cooking Banana. Different letters indicate estimated marginal means of the square root transformed RDA values that are significantly different ($p < 0.05$) according to Tukey's multiple comparisons test.

The non-Cavendish triploid AAA varieties such as Yangambi Km5 and Gros Michel were also susceptible to TR4 (Figure 2) with RDA-values ranging from 5-54% to 41.2-100%, respectively. The AAB banana varieties Manzano, Pisang Ceylan and Pisang Awak were susceptible to both Race 1 and TR4. In particular, Pisang Awak had very high RDA-values ranging between 35.5% and 100% for Race 1 and up to 100% for TR4 (Figure 2).

The eight FHIA hybrids differed in their levels of resistance. FHIA-20, FHIA-21 (plantain types) and FHIA-25 (cooking banana) were resistant to Race 1 (median RDA 0%) and moderately susceptible to TR4 (median RDA between 1.2% to 3.8%). However, FHIA-04 was moderately

susceptible to both Race 1 and TR4 (Supplementary Table S2). The dessert type hybrids FHIA-01, FHIA-18 and FHIA-23 were moderately susceptible to both races (median RDA between 1.8% to 15.3%), while FHIA-17 was also moderately susceptible to Race 1 (median RDI 1.3%) but susceptible to TR4 (median RDI 6.7%). Across all FHIA hybrids, SH-3640 was the most susceptible to TR4 (median RDA 24.4%) and resistant to Race 1 (RDA 0%).

The cooking banana, Burro CEMSA (ABB, Bluggoe subgroup) is susceptible to TR4 (median RDA 27.6%).

Finally, the plantain subgroup (AAB) was significantly less affected by FWB compared to all other varieties. CEMSA$^{3}/_{4}$ and Curare were resistant to Race 1 (median RDA 0%) but moderately susceptible to TR4 (median RDA 25.2% and 3.8%, respectively) (Figure 2, Supplementary Table S2).

The more conventional Rhizome Discoloration Index (RDI) showed that on the 1-6 scale, resistant varieties ranged between 1-2, while all others had various levels of susceptibility ranging from 2 to 5 (Supplementary Figure S1). However, there are a few exceptions; CEMSA$^{3}/_{4}$ which was classified as resistant to Race 1 (DI 21.4%) showed an RDI of 3 in some plants (Supplementary Figure S1). Similarly, FHIA-18 and FHIA-25 despite their RDI median values of 1, had DIs >25%, hence were classified as moderately susceptible to TR4 (Supplementary Figure S1, Supplementary Table S2).

In conclusion, Figure 3 shows the varietal composition of the total area planted to banana in Cuba and the overall vulnerability to FWB based on the results of the present study and the results of Zuo *et al.* (2018) and García (2019) for the cultivars Dwarf Cavendish, Giant Cavendish, Valery (AAA, Cavendish subgroup), Pelipita (ABB, Bluggoe subgroup), FHIA-02 (AAAA) and FHIA-03 (AABB), which represent nearly 85% of the national banana acreage of Cuba.

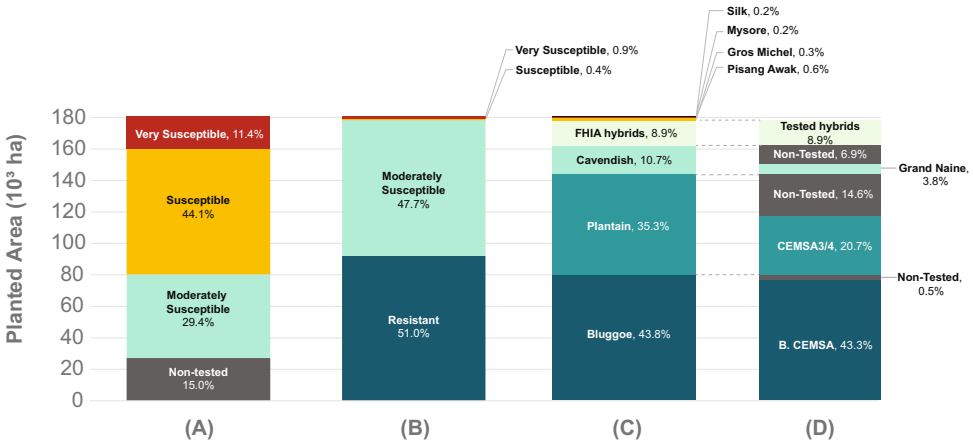

FIGURE 3. The overall vulnerability of cultivated bananas in Cuba to Fusarium wilt caused by TR4 (A) or Race 1 (B) based on the current study together with the results from Zuo *et al.* (2018) and García (2019), Subgroups composition (C) in relation with the total national banana acreage of Cuba, according to the last national survey (Plant Health Directorate of the Cuban Ministry of Agriculture, DSV 2014, not published) and proportion within subgroups of the tested and non-tested cultivars of the present study (D).

DISCUSSION

The global spread of pests and diseases is a great concern for food security (Bebber *et al.*, 2014). The developing pandemic of TR4 is no exception, particularly for countries where banana is a staple food (van Westerhoven, 2022b). In this study, we evaluated the resistance of all major Cuban banana varieties that cover 72.8% of the national banana acreage (DSV 2014; unpublished) to FWB caused by Race 1 and TR4. It is a great concern that all varieties have various degrees of susceptibility to TR4. The recent, likely independent, incursions in Latin America (Garcia-Bastidas *et al.*, 2020; Acuña *et al.*, 2021; IPPC, 2023) and local expansions (Fruitrop, 2022; Nakasato-Tagami *et al.*, unpublished) are therefore worrisome for Cuban food security because they underscore the risk for uncontained spread across the entire region.

Our data corroborate the susceptibility of different varieties of the Gros Michel and Silk subgroups to TR4 and Race 1 under greenhouse conditions (García-Bastidas, 2019; Rebouças *et al.*, 2018; Chen *et al.*, 2019; Zhan *et al.*, 2022) and in the field (Orjeda, 2000; Pérez-Vicente *et al.*, 2009; Smith *et al.*, 2018; Zuo *et al.*, 2018; Zhan *et al.*, 2022). Like Gros Michel and Silk, the high level of susceptibility of Pisang Awak to Race 1 is well known (Stover, 1962; Ploetz, 2006) but only a few studies showed that it is also very susceptible to TR4 (García-Bastidas, 2019; Mintoff *et al.*, 2021). This variety - locally known as "Manzano vietnamita" or "Burro manzano" or "Ducasse" - is very popular in Cuba and commonly found in backyards and small plots for local markets and family consumption. In a recent national survey, FWB-affected Pisang Awak plants represented 42% of all FWB cases across the country (13 of the 16 provinces) (Martínez de la Parte *et al.*, unpublished). The susceptibility of Pisang Awak to Race 1 and TR4 complicates preemptive surveillance and early detection of a possible TR4 incursion, not only in Cuba but also in Indonesia, Laos, Thailand, Vietnam, and in East African countries where it is widely grown and appreciated (Ploetz and Churchill, 2011; Maryani, 2019; Chittarath *et al.*, 2022).

Similarly, Bluggoes are very popular as staple food and consequently provide a source of income for many farmers across Latin America and Africa (Dita *et al.*, 2013; Blomme *et al.*, 2013). In Cuba, the Bluggoe acreage expanded after the year 2000 at the expense of plantains, which are very susceptible to BLSD (Pérez-Vicente *et al.*, 2002). Currently, Burro CEMSA nearly represents the entire Bluggoe area (96.6%) and covers 43.8% of the national banana acreage. This dominance and the susceptibility to TR4 – showed for the first time in this study - are a serious risk to food security upon a possible TR4 incursion in Cuba.

The plantain varieties which cover 35.5% of the national banana acreage, were more resistant to Race 1 and TR4 than all other germplasm. However, the collective data show they should not be considered as one coherent and unanimous group (García-Bastidas, 2019; Zuo *et al.*, 2018; Molina *et al.*, 2016b; Li *et al.*, 2020; Zhan *et al.*, 2022), despite that only a limited number of plantain varieties has been evaluated with TR4. In our study, CEMSA $^{3}/_{4}$ - which dominates the national plantain acreage (58.5%) - is moderately susceptible to TR4, whereas Zhan *et al.* (2022) reported it as resistant. Unfortunately, the response of other important plantains varieties such as Enano guantanamero and Macho ¾ is still unknown. Hence, due to their importance for

the entire Caribbean and Latin American region, it is strongly recommended to proactively evaluate all plantain varieties for their resistance to TR4.

Finally, the FHIA hybrids cover nearly 9% of the commercial national banana acreage, with FHIA-01, FHIA-04, FHIA-18 and FHIA-21 as the most important representatives. They were introduced to Cuba in 1994 (Alvarez, 1997) because of their resistance to BLSD and to the burrowing nematode *Radopholus similis*. We tested eight FHIA hybrids and their level of resistance to TR4 accords with the results of Zuo *et al.* (2018), except for FHIA-25 which has been reported as resistant to TR4 (Walduck and Daly, 2007; Zuo *et al.*, 2018; Chen *et al.*, 2019; Mintoff *et al.*, 2021;Zhan *et al.*, 2022). Our data, however, show that it is moderately susceptible to TR4 which accords with Garcia-Bastidas (2019).

The above treatise on a range of FWB evaluations points to a more general aspect of banana disease trials. Apart from a comprehensive analysis of the vulnerability of Cuban banana germplasm to FWB, our data also underscore an inconsistency of the scores for various specific cultivars, not only compared with our data, but also across literature reports. For instance, and in addition to the abovementioned data, FHIA-18 is moderately susceptible in our experiments as well as under field conditions in Cuba (Pérez-Vicente *et al.*, 2009) but Smith *et al.* (2014), report resistance to Race 1 in Australia. Similarly, Yangambi Km5 and Pisang Ceylan were at least moderately susceptible to Race 1 in our trials, but Orjeda (2000) reported that Yangambi Km5 is resistant in the field in various countries. This contrasts again with Cuban field data where Yangambi Km5 was susceptible to Race 1 and Race 2 (Pérez-Vicente *et al.*, 2009). Finally, Burro CEMSA is moderately susceptible to Race 1 in our experiments, but Bluggoe is according to the current FWB race nomenclature only susceptible to Race 2 strains (Ploetz, 2006; Pérez-Vicente *et al.*, 2014; Stover, 1962). Recently, Maryani *et al.* (2019) reported that Race 1 strains are phylogenetically diverse, which might explain these contrasting observations. Conflicting data might also result from different experimental conditions and protocols, as well as comparisons between field trials and greenhouse evaluations. For instance, field trials showed that Curare enano, French Sombre, Intokatoke, Ihitisim, Kazirakwe, Kofi, Orishele, Pisang Ceylan, Pisang Rajah, Obino l'Ewai, and Rukumamb were resistant to FWB, but they showed internal symptoms in the greenhouse (Zuo *et al.*, 2018; Li *et al.*, 2020; Zhan *et al.*, 2022). Taken together, such inconsistencies hamper reliable information about the vulnerability of tested varieties toward FWB, particularly for those with an intermediate response (Li *et al.*, 2015; Zuo *et al.*, 2018; Mintoff *et al.*, 2021), which in turn affect overall strategies for disease management, particularly after a TR4 outbreak. Therefore, we advocate the adoption of greenhouse trials in addition to field trials that frequently result in conflicting data as outlined above. Field trials take longer, have limited throughput, and are prone to varying environmental conditions, contrary to greenhouse assays that are shorter, environmentally stable and hence reproducible (Zuo *et al.*, 2018; Smith *et al.*, 2008; Ribeiro *et al.*, 2018; García-Bastidas *et al.*, 2019). Moreover, hidden experimental factors, such as irregular inoculum distribution and identity (Ribeiro *et al.*, 2011; Zuo *et al.*, 2018; Chen *et al.*, 2019; Mintoff *et al.*, 2021), or possible compound effects from

interactions with pests such as nematodes and banana weevils (Meldrum *et al.*, 2013; Dita *et al.*, 2018) are a source of variation that is usually not considered.

Our experiments comprised 18 banana cultivars that cover 72.8% of the national banana acreage (~ 105.100 ha., FAO 2022b) and show that nearly 56% of the acreage is planted to varieties that are susceptible or very susceptible to TR4. Indeed, many popular varieties are also susceptible to Race 1 and Race 2 but have not succumbed to FWB. This is likely due to the overall smallholder mixed cropping settings of these livelihoods as is also seen for Gros Michel. Despite its susceptibility to Race 1 and the demise in the previous century (Marquardt, 2001), Gros Michel is still cultivated on small patches across Latin America and Africa (Blomme *et al.*, 2013; Magdama *et al.*, 2020). The susceptibility of many popular banana varieties to Race 1 and Race 2 also poses a risk for a stealthy introduction of TR4 in Cuba, as FWB symptoms caused by TR4, Race 1 or Race 2 are indistinguishable.

Therefore, a Fusarium wilt national task force has been established to provide guidance to the Cuban National Plant Protection Organization in case of an outbreak of TR4. Since 2000, awareness campaigns started for growers and biosecurity officers along with training on disease recognition, diagnostics, and on-farm biosecurity measures. However, similar strategies in various other countries have not stopped the dissemination of TR4 (van Westerhoven *et al.*, 2022b). Hence, careful surveillance and rapid diagnosis are required to reduce the risk of a TR4 incursion in Cuba, which would strongly impact national banana production, food security and farmers' livelihoods.

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SUPPLEMENTARY MATERIAL

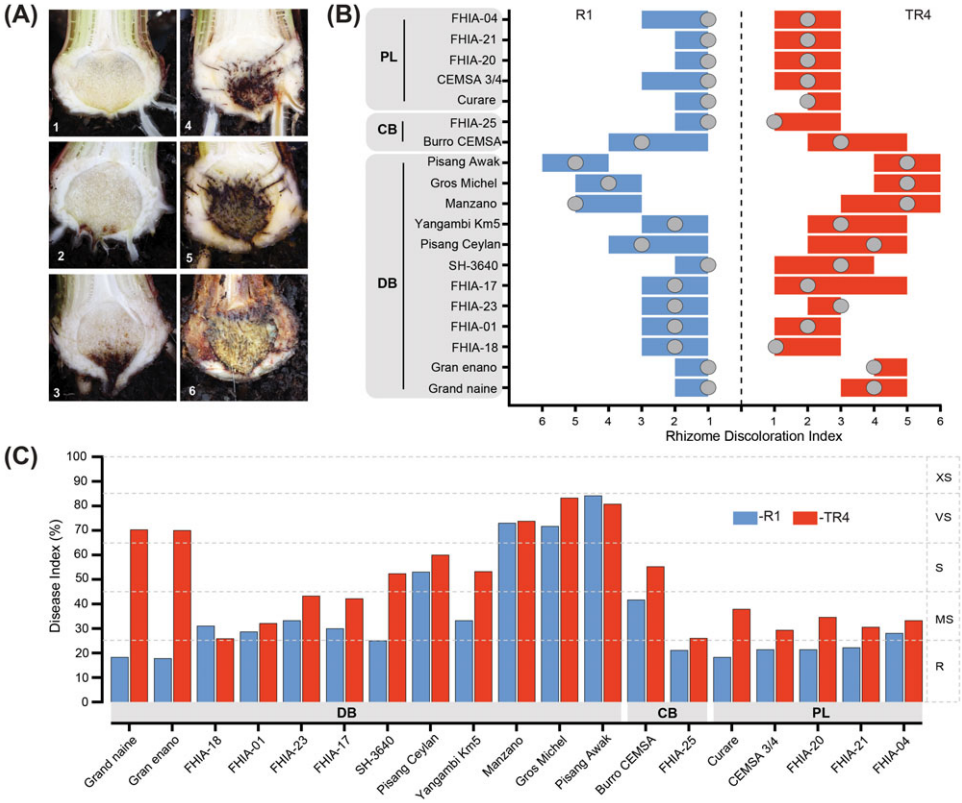

FIGURE S1. Evaluation of FWB of analyzed Cuban banana germplasm. A. Representation of the grades of the scale used to assess disease severity; B. Response of tested accessions against Race 1 and TR4, gray dots represent median values; C. Disease Index of tested banana accessions. DB: Dessert type, CB: Cooking Banana, and PL: Plantain. The level of resistance is expressed as R: Resistant, MS: Moderately Susceptible, S: Susceptible, and VS: Very Susceptible.

TABLE S1. Comparison of the response[1] of banana cultivars to TR4 strains under greenhouse conditions in five studies.

Accession name	Subgroup / Type	Zuo *et al.*, 2018	Chen *et al.*, 2019	Garcia-Bastidas, 2019	Zhan *et al.*, 2022	Present study
Grande Naine	Cavendish	S	-	VS-XS	-	VS
FHIA-01	FHIA hybrid	-	R	VS	-	MS
FHIA-17	FHIA hybrid	S	-	MS	-	S
FHIA-18	FHIA hybrid	-	R	S	-	MS
FHIA-23	FHIA hybrid	MR	SS	MS	-	MS
SH-3640	FHIA hybrid	-	-	VS	-	S
Gros Michel	Gros Michel	HS	-	VS	-	VS
Yangambi Km5	Ibota	-	-	-	-	S
Pisang Ceylan	Mysore	R	-	S	I	S
Manzano vietnamita	Pisang Awak	-	-	-	-	VS
Manzano	Silk	-	-	VS	-	VS
Burro CEMSA	Bluggoe	-	-	-	-	S
FHIA-25	FHIA hybrid	R	R	MS	-	MS
CEMSA ¾	Plantain, False Horn	-	-	-	R	MS
Curare	Plantain, False Horn	-	-	-	R	MS
FHIA-04	FHIA hybrid	-	-	R	-	MS
FHIA-20	FHIA hybrid	-	-	MS	-	MS
FHIA-21	FHIA hybrid	R	-	-	R	MS
EXPERIMENTAL CONDITIONS						
Type of plants		tissue culture	tissue culture	tissue culture	tissue culture	tissue culture
Age of plants		Height 30-50 cm with 6-7 true leaves	Height 30 cm with 5-6 true leaves	2.5 months	4 months	2.5 months
Strain tested		ACCC 37997	NTPDc 35673	NRRL5006 (II-5)	ACCC 37997	NRRL5006 (II-5)
Inoculation method		Pouring	Pouring	Pouring and wounding	Root dipping	Pouring and wounding
Inoculum		10^3 conidia/g soil	40 g of infested millet grains	200 mL of 10^6 conidia/mL	10^6 conidia/mL	200 mL of 10^6 conidia/mL
Incubation[2]		45 dpi	10-12 wpi	10-12 wpi	35 dpi	10-12 wpi
Disease severity determination		Rhizome Discolored Area (rating scale)	Rhizome Discolored Area (rating scale)	Rhizome Discolored Area (rating scale)	Rhizome Discoloration Index (rating scale)	Rhizome Discolored Area (quantified with ImageJ)

[1]R = resistant, MR= moderately resistant, I = intermediate, SS = slightly susceptible, S = susceptible, MS = moderately susceptible, VS = very susceptible, HS = highly susceptible. [2]dpi = days post inoculation, wpi = weeks post inoculation

TABLE S2. Response of banana germplasms to Race 1 and TR4.

Accession name	E[1]	Race 1										TR4								
		n[2]	RDA[3] (%)				RDI[4]			DI (%)	LR	n	RDA[3] (%)				RDI[5]			DI[6] (%)	LR[7]
			Min	Max	Med	S[4]	Min	Max	Med				Min	Max	Med	S[4]	Min	Max	Med		
FHIA-01	3	25	0	12.4	1.8	ab	1	3	2	28.7	MS	28	0	28.5	1.8	ab	1	3	2	32.1	MS
FHIA-17	2	15	0	15.2	1.3	ab	1	3	2	30.0	MS	15	0	58.5	6.7	abcde	1	5	2	42.2	S
FHIA-18	2	30	0	29	2.5	ab	1	3	2	31.1	MS	29	0	22.6	0	ab	1	3	1	25.9	MS
FHIA-23	1	10	0	8.8	2.2	abcd	1	3	2	33.3	MS	10	2.9	24.9	15.3	abcde	2	3	3	43.3	MS
Gran Enano	2	15	0	3.3	0	a	1	2	1	17.8	R	15	31.5	67.6	44.3	cde	4	5	4	70.0	VS
Grand Naine	2	21	0	1.9	0	a	1	2	1	18.3	R	23	13.8	88.8	40.1	abcde	3	5	4	70.3	VS
Gros Michel	2	15	30	73.2	44.9	cd	3	5	4	71.7	VS	16	41.2	100	71.4	e	4	6	5	83.3	VS
Pisang Awak	2	20	35.5	100	62.6	d	4	6	5	84.2	VS	20	32.5	100	56.8	de	4	6	6	80.8	VS
Pisang Ceylan	3	27	0	62.6	26.6	bcd	1	4	3	53.1	S	25	2.9	79.7	36.1	bcde	2	5	4	60.0	S
Manzano	2	21	13.3	88.4	51.4	cd	3	5	5	73.0	VS	21	19.7	100	50.3	bcde	3	5	6	73.8	VS
SH-3640	2	20	0	2.4	0	ab	1	2	2	25.0	R	21	0	47.9	24.4	abcde	1	4	3	52.4	S
Yangambi Km5	1	10	0	6.8	2.7	ab	1	3	2	33.3	MS	10	4.9	53.7	24.7	abcde	2	5	3	53.3	S
Burro CEMSA	3	20	0	37.6	9.2	ab	1	4	3	41.7	MS	19	4.5	52.9	27.6	abcde	2	5	3	55.3	S
FHIA-25	2	19	0	4.9	0	a	1	2	1	21.1	R	23	0	14.2	1.2	ab	1	3	1	26.1	MS
CEMSA3/4	3	21	0	16.3	0	a	1	3	1	21.4	R	21	1.5	52.9	25.2	a	1	3	2	29.4	MS
Curare	1	10	0	1.79	0	ab	1	2	1	18.3	R	10	2.6	7.3	3.8	abcde	2	3	2	37.9	MS
FHIA-04	2	16	0	27.8	0	ab	1	3	1	28.1	MS	17	0	23.2	2.81	abc	1	3	2	33.3	MS
FHIA-20	2	14	0	4.1	0	ab	1	2	1	21.4	R	13	0	22.4	3.8	abcd	1	3	2	34.6	MS
FHIA-21	3	18	0	4.9	0	ab	1	2	1	22.2	R	18	0	17.7	2.5	abc	1	3	2	30.6	MS

[1] E = number of experiments each in which an accession was tested. [2] n = total number of plants tested. [3] Rhizome Discolored Area (RDA) calculated with ImageJ software. [4] S = Different letters indicate significantly different estimated marginal means of the square root transformed RDA values ($p < 0.05$) according to Tukey's multiple comparisons test. [5] Rhizome Discoloration Index (RDI) according to scale proposed by Garcia-Bastidas *et al.* (2019); Min = Minimum, Max = Maximum, Med = Median. [6] DI = Disease Index. [7] LR = Level of resistance expressed as R = Resistant, MS = Moderately Susceptible, S = Susceptible and VS =Very Susceptible

CHAPTER 4

Endophytic colonization of non-host plants by the banana Fusarium wilt pathogen *Fusarium odoratissimum* contributes to inoculum persistence and modulates pathogenic fitness on banana

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ABSTRACT

Fusarium wilt of banana (FWB) is caused by several *Fusarium* spp. formerly known as *F. oxysporum* f. sp. *cubense* (Foc) and poses a significant threat to the global cultivation of bananas. The prevailing dogma is that these fungi persist in the soil via chlamydospores. However, some reports describe the presence of soilborne *Fusarium* spp. in weeds. Here we extend these studies and describe a survey of three banana farms in The Philippines and show that *F. odoratissimum,* commonly known as Tropical Race 4 (TR4), and one of the most notorious causal agents of FWB, also can survive between crop cycles as an endophyte in eight weed species from seven botanical families and that the pathogenicity of the retrieved isolates toward Cavendish banana plants varied depending on these hosts. In subsequent greenhouse trials *Ageratum houstonianum*, *Amaranthus spinosus, Chamaesyce hirta, Cyperus involucratus, Digitaria, Echinochloa colona,* and *Momordica charantia* were also infected with TR4 which could be reisolated from various parts of these plants, showing that infection is not restricted to the root system. These findings suggest an endophytic behaviour of TR4 in weeds that may play a significant role in its long-term survival, next to the survival of chlamydospores in soil. Subsequent pathogenicity tests showed that passing TR4 through *Portulaca oleracea* and *Arachis pintoi* significantly reduced its pathogenicity on Grand Naine plants. Identifying the host range of *F. odoratissimum* is crucial for effective disease management of FWB, also when banana cropping is terminated due to severe infestations.

INTRODUCTION

Fusarium wilt of banana (FWB) is considered one of the most destructive diseases in history (Stover & Simmonds 1987). In the past, FWB destroyed the Gros Michel-based banana cultures in Central America (Ploetz, 2005). Currently with its expansion in Africa (Viljoen *et al.* 2020; van Westerhoven *et al.* 2022) and Latin America (Garcia-Bastidas *et al.*, 2020; Acuña *et al.*, 2021; Herrera *et al.*, 2023), FWB threatens all major banana-producing regions of the world. In the last twenty years, the costs of biological invasions, including those caused by FWB, have increased significantly compared to the previous century and are similar in magnitude to those caused by natural disasters (Turbelin *et al.*, 2023). Thus, FWB constitutes a historical example of the danger of emerging diseases to devastate crops and a highly topical threat to a modern, globally traded commodity (Fones *et al.*, 2020).

Fusarium wilt of banana is caused by soil-borne fungi traditionally identified as *F. oxysporum* f. sp. *cubense*, despite its well-known diversity (Ploetz, 2005; Fourie *et al.*, 2009; Ordóñez *et al.*, 2015; Mostert *et al.*, 2022). However, recent studies based on genotyping analyses confirmed several genetically distinct lineages that were consequently recognized as individual *Fusarium* species (Maryani *et al.*, 2019). For instance, the Race 1 strains, which affect dessert types of the subgroups Gros Michel, Silk, Pome and Pisang Awak (Ploetz, 2015b) but not Cavendish, actually comprise a suite of different *Fusarium* species (Maryani *et al.*, 2019; van Westerhoven *et al.*, 2023). Meanwhile, the strain known as Tropical Race 4 (TR4), which affects Cavendish and a wide range of other banana cultivars, was described as the new species *Fusarium odoratissimum* (Maryani *et al.*, 2019). Though this nomenclature is being disputed (Torres-Bedoya *et al.*, 2021), recent genomic analyses of more than 555 strains from 32 countries support the proposed taxonomic revision (Ordóñez *et al.*, unpublished data; van Westerhoven *et al.*., 2023). Henceforward, we use the proposed new nomenclature.

The causal agents of FWB are soil-borne *Fusarium* spp., well adapted to long-term survival in the soil by the formation of persistent propagules called chlamydospores, which remain dormant in the remnants of decayed host tissue until stimulated to germinate by root exudates from banana (Stover, 1962). Although chlamydospores are generally accepted as the primary means of the survival of *Fusarium*, the persistence may also be due to the capacity of the fungus to colonize the outer root cells of the cortex or root epidermis of grasses and weeds as asymptomatic endophytes (Ploetz, 2015b). Hence, other plants may act as reservoirs for *Fusarium* spp. to persist in the absence of banana plants (Hennessy *et al.*, 2005). This potentially contributes to the endured contamination of soils with the pathogen, and - after run-off - irrigation sources and rivers (Pegg *et al.*, 2019b), which can pose a significant challenge for disease management and pathogen containment strategies (Nakasato Tagami *et al.*, 2023).

Understanding the host range of *F. odoratissimum* is crucial for developing effective FWB management strategies such as the prevention of incursions into new areas and secondary expansion once it is endemic, or re-emergence once banana cropping is continued after abandoning infested plantations. Here, we identified 12 non-host weed species of *F. odoratissimum* and evaluated whether passing TR4 through these plants affects its pathogenicity on banana plants.

MATERIALS AND METHODS

Surveying weeds in banana plantations in The Philippines

Weed plant species were surveyed in three banana farms in The Philippines, one with no FWB incidence (7° 28'18" N 125° 35'55" E), a second farm with FWB incidence (7° 29'45" N 125° 37'28" E) and an abandoned banana farm (7° 33'16" N 125° 42'49" E) due to severe FWB infestations. The collected weed species were taxonomically identified following Moody *et al.* (2014).

Healthy and symptomless weed plants were uprooted, loose soil was removed and, in the laboratory, each plant was washed with distilled water and the aboveground parts were cut or segmented and surface sterilized with 70% ethanol for one minute, rinsed three times with distilled water, then placed on Komada medium in 9 cm Petri dishes and incubated for five days (±27°C). Mycelial colonies similar to *Fusarium* spp. were transferred to fresh Komada plates and single spore cultures of each isolate were generated. DNA extraction and diagnostic TR4 LAMP-PCR identification were performed as described by Ordóñez *et al.* (2019).

Tested plant material under greenhouse conditions

Plant materials used for the greenhouse experiments were obtained from different sources. Seeds of *Amarantus spinosus* L., *Chamaesyce hirta* (L.) Millsp., *Digitaria setigera* Roth, *Echinocloa colona* (L.) Link, *Momordica charanthia* L. and *Portulaca oleracea* L. were collected from banana farms in the Philippines. Seeds from *Agerantum houstonianum* Mill., as well as three to four weeks old cuttings from *Arachis pintoi* Krapov. & W.C. Greg. and *Cyperus involucratus* Rottb., were in the collection of the Unifarm greenhouse facility of Wageningen University & Research (Supplementary Table S1). Tissue culture, *cv*. Grand Naine (Cavendish subgroup, AAA) plants were obtained from Rahan Meristem (http://www.rahan.co.il).

Seeds of the selected weeds were surface sterilized (70% ethanol solution for five minutes) and sowed in 1L pots containing a standard soil used at Unifarm (Swedish sphagnum peat 20%, Baltic peat 30%, garden peat 30%, beam structure 20%, grinding clay granules 40.6 Kg/m3, Lime + MgO 2.5 Kg/m3, PG-Mix-15-10-20 0.8 Kg/m3). Plants were maintained, in an environmentally controlled greenhouse compartment (28±2°C, 16h light, and ~85% relativity humidity), for three to four weeks before inoculation.

Tissue culture banana plants were grown individually in 1L pots containing the standard soil. Potted plants were first acclimatized under plastic for two weeks at 28±2°C to maintain high humidity conditions in the aforementioned greenhouse compartment and were thereafter grown for ~2.5 months until inoculation.

Fungal inoculum production and inoculation methods

Inoculum of the *F. odoratissimum* reference strain II5 (NRRL 54006) was produced according to the procedure described by García-Bastidas *et al.* (2019) using mung beans broth (2 g mung beans per 500 ml water) and the conidial concentration was adjusted to 1.10^6 conidia/mL. Two inoculation methods were tested: i) wounding of the roots in the soil and subsequent drenching with inoculum and ii) seed immersion. For the first method, we used a soil scoop at two opposite sides of a plant within the pot and then drenched the soil with 200 ml of inoculum per litre of soil. For negative controls, we drenched the soil with 200 ml water per litre of soil. Positive controls comprised Grand Naine plants inoculated with *F. odoratissimum*. Seed immersion was performed by placing the seeds in 50 ml tubes with inoculum supplemented with Tween 80 at 0,1% (v/v). For controls, the inoculum was replaced by water. The tubes were incubated at 25 °C for 12-16 hours and then the seeds were planted. All species were inoculated by both methods except *A. pintoi* and *C. involucratus* which were inoculated only by wounding and drenching because we could not obtain seeds of these species (Supplementary Table S1). The experiments were designed following a randomized complete block design with two independent experiments comprising four plants of each species per treatment and per inoculation method as experimental units.

Detection of *Fusarium odoratissimum* in inoculated plants

Plants were inspected on a weekly basis for any noticeable disease symptoms, such as wilting, chlorosis or necrosis, compared to the controls. At 12 weeks post-inoculation, three pieces (~1 cm^2) of roots, stems, leaves and flowers were collected of each plant, subsequently extensively washed with demineralized water, and surface sterilized with a 70% ethanol solution for five minutes in a laminar flow cabinet. The pieces were then rinsed three times with sterile demineralised water and placed on Komada medium (Komada, 1975). After six days, mycelia that resembled *F. odoratissimum* morphology were collected and transferred individually to new plates with potato dextrose agar (PDA). Mycelium grown on the PDA plates was collected, immediately frozen with liquid nitrogen, and ground in a mortar with a pestle. Genomic DNA of each isolate was extracted using the MasterPure™ Yeast DNA Purification Kit, according to the manufacturer´s protocol. The size, concentration, and integrity of extracted DNAs were assessed by gel electrophoresis, NanoDrop™ One/OneC Microvolume UV-Vis Spectrophotometer (Thermo Scientific™), and Qubit analysis (Qubit™ Flex Fluorometer, Thermo Fisher Scientific, Switzerland).

Duplex PCRs on the extracted DNAs were performed using the procedure developed by Dita *et al.* (2010), with primers FocTR4-F / TR4-R for the diagnosis of TR4, while the primers EF-1 / EF-2 were used as an internal control to detect the TEF-1α gene (Table 1). The total reaction volume was 25 *µl*, and the thermal cycling program was used as described by Dita *et al.* (2010).

Pathogenicity of recovered endophytic *F. odoratissimum* isolates

Fusarium odoratissimum isolates, re-isolated from the uppermost organ of the inoculated weeds, were confirmed by the aforementioned diagnostic PCRs, and tested for pathogenicity starting from a monosporic isolate. We used the first procedure – wounding and drenching – and evaluated disease severity at 11 weeks after inoculation, by quantifying the Rhizome Discoloured Area (RDA) with ImageJ 1.52r software (National Institutes of Health, Bethesda, MD, USA). Individual RDA-values for different isolates were plotted using the web-tool BoxPlotR (Spitzer *et al.*, 2014) and compared with the Kruskal-Wallis nonparametric analysis followed by Dunn's multiple comparisons test, at 0.05 ($p < 0.05$).

TABLE 1. Primers used in this study.

Target	Primer name	Sequence (5´-3´)	Reference
F. odoratissimum	SeqA_F3	AATAGTAAAGATGCTGAACTTCT	Ordóñez *et al.* (2019)
	SeqA_B3	ACTCTTGTGAGAGGTCGA	
	SeqA_FIP	TGGGAGGAAGAACTTTCTAGTATGAGAAAGGATAAGGGATGTAATGTTG	
	SeqA_BIP	TTGCTCAATTTCCTTGTGTTTCGCAGGATTCACGATAGTAGAGTT	
	SeqA_Loop F	ACCAAAAGCCTAGGAGAGGATT	
	SeqA_Loop B	TCTTCTTCTTCGCCGTACCTCATCA	
F. odoratissimum	FocTR4-F	CACGTTTAAGGTGCCATGAGAG	Dita *et al.* (2010)
	FocTR4-R	GCCAGGACTGCCTCGTGA	
Elongation Factor-1α	EF-1	ATGGGTAAGGAGGACAAGAC	O'Donnell *et al.* (1998)
	EF-2	GGAGGTACCAGTGATCATGTT	

Two isolates, retrieved from the corm (AP2.41) and the roots (AP2.12) of an inoculated *A. pintoi* plant were also tested for pathogenicity, using the above-mentioned inoculation procedure, in two independent experiments. Individual RDA-values of these isolates were compared with those of the *F. odoratissimum* TR4 reference strain II5, with two-tailed unpaired t-test with Welch´s correction ($p<0.0001$), and plotted using the web-tool BoxPlotR.

RESULTS

Fusarium odoratissimum (TR4) is an endophyte of weeds in banana plantations in The Philippines

In the present study, weeds in three banana farms in North Davao, Mindanao, the Philippines were surveyed for the presence of TR4 (Table 2). One farm was abandoned due to TR4, one farm was affected by TR4 and the third farm had no previous TR4 infestations. None of the collected weeds showed any external symptoms of disease or wilting. In the latter farm, no *F. odoratissimum* was recovered from any plant. The survey of the FWB-affected farm resulted in only one positive TR4 diagnosis from *Echinochloa colona* (L.) Link, but all weeds from the abandoned farm tested positive for TR4.

Colonization of weeds and cover crops by *Fusarium odoratissimum*

To further investigate the potential role of *F. odoratissimum* persistence in other weed species and cover crops, phenotyping experiments were conducted in the greenhouse. The species that were challenged with the pathogen were *Ageratum houstonianum*, *Cyperus involucratus, Digitaria setigera, Echinochloa colona,* and *Portulaca oleracea* and the species *A. spinosus, C. hirta* and *M. charantia*- that were previously found to be infected by *F. odoratissimum* under field conditions-, as well as *Arachis pintoi* (Table 3) a common cover crop in various banana production systems (Jhons, 1994; Espindola *et al.*, 2006; Ramos-Hernández *et al.*, 2011).

TABLE 2. Incidence of *F. odoratissimum* in eight weeds species, commonly growing in infested banana plantations in Mindanao, Philippines.

Family	Botanical name[a]	Common name[a]	I		II		III	
			N	P	N	P	N	P
Acanthaceae	*Asystasia gangetica* (L.) T. Anderson	Chinese violet	9	0	0	0	2	2
Amaranthaceae	*Amaranthus spinosus* L.	spiny amaranth	9	0	4	0	2	2
Asteraceae	*Ageratum conyzoides* L.	tropical whiteweed	8	0	0	0	2	1
Convolvulaceae	*Ipomoea triloba* L.	littlebell	10	0	0	0	2	1
Cucurbitaceae	*Momordicha charanthia* L.	balsampear	10	0	5	0	2	1
Euphorbiaceae	*Chamaesyce hirta* (L.) Millsp.	pillpod sandmat	0	0	7	0	2	2
Poaceae	*Echinochloa colona (*L.) Link	jungle rice	9	0	4	1	0	0
Poaceae	*Paspalum conjugatum* P.J. Bergius	hilograss	10	0	0	0	2	1

[a]- Botanical and common name according to The PLANTS database (https://plants.usda.gov/home). I-banana farm with no wilt incidence, II-banana farm with disease incidence and III-abandoned banana farm and currently cropped to maize. N-total number of sampled plants, P-Plants on which the presence of *F. odoratissimum* was confirmed by LAMP-PCR (Ordóñez *et al.*, 2019).

TABLE 3. Differential recovery of *F. odoratissimum* from inoculated plants. Total number of positive TR4 isolates, per inoculation method and plant organ, from each weed species.

Family	Plant species	Seed inoculation / Wounding-drenching			
		Roots	Stem	Leaf	Flower
Amaranthaceae	*Amaranthus spinosus* (L.) T. Anderson	2 / 0	2 / 0	1 / 0	0 - 0
Asteraceae	*Ageratum houstonianum* Mill.	0 / 2	0 / 1	0 / 0	0 / 0
Cucurbitaceae	*Momordica charantia* L.	0 / 2	0 / 2	0 / 1	0 / 0
Cyperaceae	*Cyperus involucratus* Rottb.	NT / 3	NT / 3	NT / 0	NT - 0
Euphorbiaceae	*Chamaesyce hirta* (L.) Millsp.	3 / 1	1 / 0	0 / 0	1 / 0
Leguminosae	*Arachis pintoi* Krapov. & W.C. Greg.	NT / 7	NT / 2	NT / 1	NT / 1
Poaceae	*Digitaria setigera* Roth	5 / 0	3 / 0	0 / 0	2 / 0
Poaceae	*Echinochloa colona* (L.) Link	7 / 0	3 / 0	0 / 0	0 / 0
Portulacaceae	*Portulaca oleracea* L.	2 / 0	2 / 0	0 / 0	0 / 0

NT-non tested.

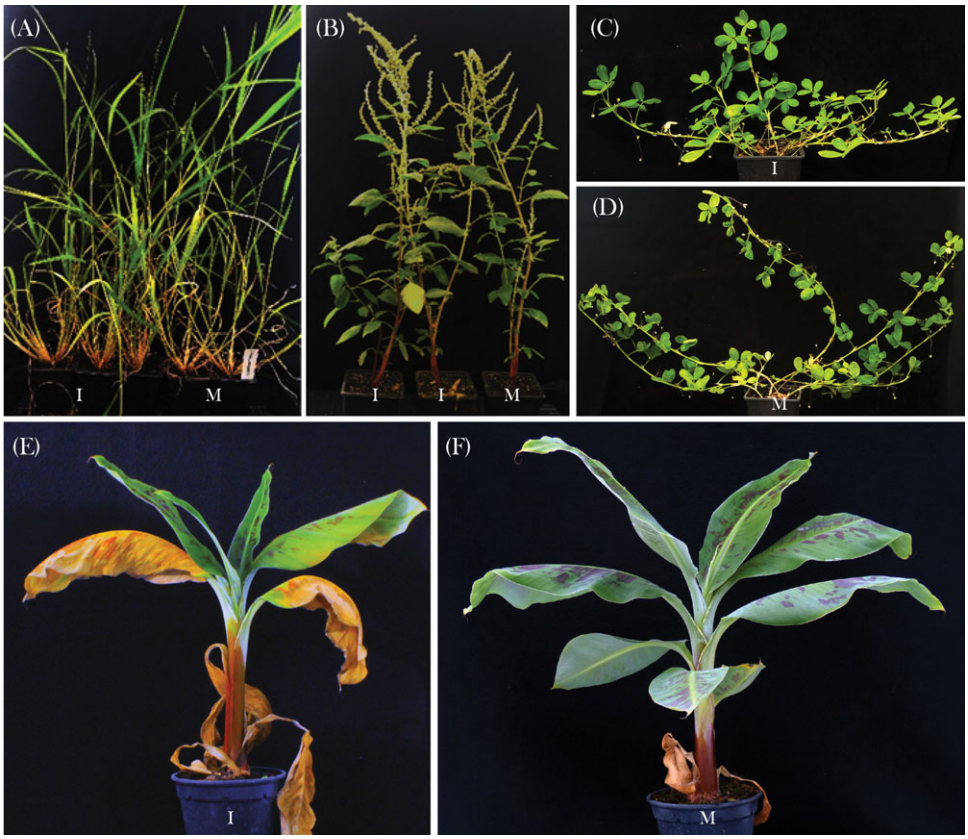

FIGURE 1. Phenotypes of three weed species inoculated with *Fusarium odoratissimum* isolate II5 (TR4) and various controls. (A) *Echinocloa colona,* (B) *Amaranthus spinosus,* (C) and (D) *Arachis pintoi,* (E) positive control Grand Naine inoculated with TR4, (F) Grand Naine negative water control. All panels: I-Inoculated plants, M-Mock, non-inoculated plants.

In total, 60 isolates were recovered from the inoculated weeds and cover crops and confirmed as TR4 by diagnostic PCR (Supplementary Figures S1 and S2). Overall, this confirmed the presence of the pathogen in roots and stems of all tested plant species, which is consistent with previous studies reporting that several weed species were infected by *F. odoratissimum* (Supplementary Table S2). None of the inoculated plants showed any symptoms or growth delay at 12 weeks after inoculation compared to the controls, but the positive control Grand Naine plants showed the typical FWB symptoms (Figure 1). However, the two used inoculation methods – wounding and drenching vs. seed immersion – returned dissimilar results. No *F. odoratissimum* isolate was obtained from any plant parts of *A. houstonianum* and *M. charantia* after seed immersion or from *A. spinosus, D. setigera, E. colona* and *P. oleracea* after wounding and drenching (Table 3). Seed immersions mostly resulted in the recovery of TR4 from the roots and the stems, while leaves only tested positive for *A. spinosus* and flowers for *E. hirta* and *D. setigera*. Wounding and drenching resulted in TR4 recovery from the leaves of *M. charantia* and *A. pintoi*, as well as from the flowers of the latter. In contrast, samples from *E. hirta* always tested positive, irrespective of the used inoculation method.

Virulence differences of endophytic *F. odoratissimum* retrieved from non-hosts

To understand whether the colonization of non-hosts affected the pathogenicity on banana we selected seven recovered and confirmed TR4 isolates and examined them in phenotyping assays on Grand Naine banana plants. Each isolate originated from a different weed species and from the uppermost organs that tested positive for TR4. Contrary to the isolates obtained from *A. spinosus, D. setigera, E. colona, E. hirta* and *M. charantia,* isolates from *A. pintoi* and *P. oleracea* caused a significantly different disease severity compared with the positive control (Figure 2). Retesting with additional monosporic isolates from other or lower plant organs of *A. pintoi* confirmed these results (Figure 3). None of the *A. pintoi* re-isolates tested showed a different growth rate or conidia production level compared to the *F. odoratissimum* (TR4) reference strain II5 (data not shown).

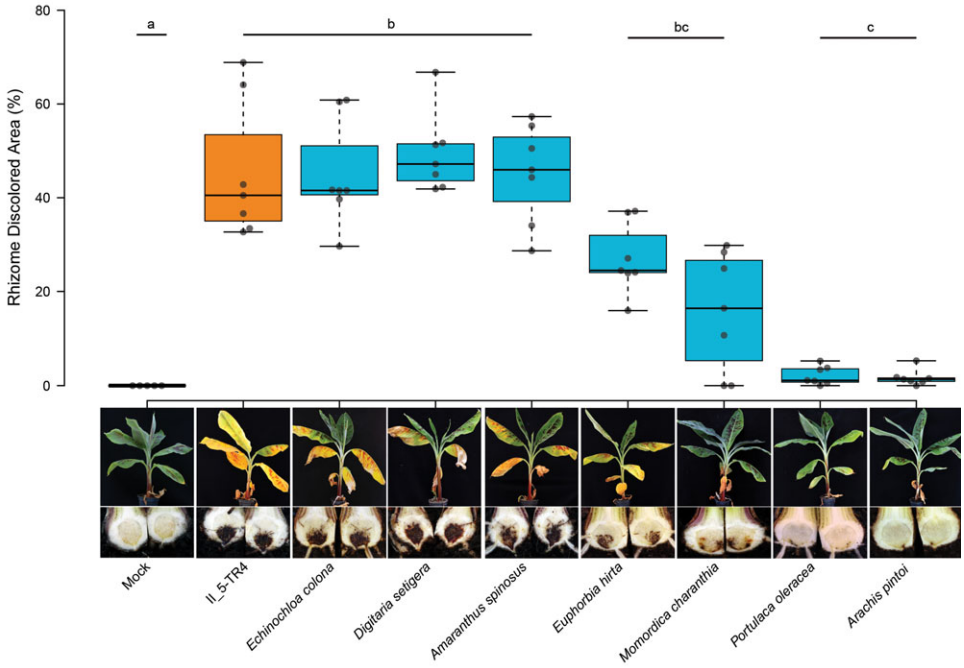

FIGURE 2. Recovered TR4 isolates from weeds show variable pathogenicity on banana. The Rhizome Discolored Area (RDA) of Cavendish Grand Naine plants, quantified with ImageJ at 11 weeks after inoculation, with recovered TR4 isolates from seven weed species compared to the negative water control and the *F. odoratissimum* (TR4) reference strain II5. Different letters indicate significantly different ($p < 0.05$) median RDA values according to Kruskal-Wallis and Dunn's multiple comparison tests.

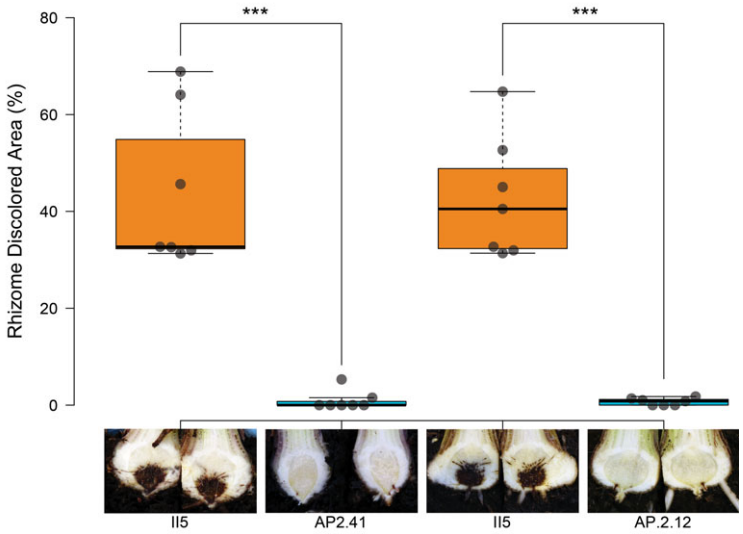

FIGURE 3. Two independent experiments confirmed that re-isolates from *A. pintoi* shows a diminished pathogenicity to Grand Naine compared with the *F. odoratissimum* (TR4) reference strain II5. AP2.41-re-isolate from the corm and AP2.12-re-isolate from the roots. Asterix on top of the box plots indicate statistical differences (two-tailed unpaired t-test with Welch´s correction; N=7, ***=p<0.0001).

DISCUSSION

The Philippines is globally the second largest banana exporter with a volume of 2,383,000 metric tonnes during 2021 (FAO, 2022a), and the North Davao Region accounts for 35.5% of the total national production (Philippine Statistics Authority, 2023). Since the discovery of TR4 in 2005 (Molina *et al.*, 2008), FWB has become increasingly important in the Philippines. The number of cases of TR4 per year increased dramatically from 700 cases in 2005 to 15,000 cases in 2007 (Molina *et al.*, 2008), and it is estimated that 34% of the banana production area in the Philippines, could be lost to the disease by 2027(Scheerer *et al.*, 2018a). Until now, chlamydospores have been considered to be the major persistent *Fusarium* propagules in infested soils, making them unfit for prolonged banana cultivation (Pegg *et al.*, 2019b), despite several reports of infected weeds as potential reservoirs of inoculum (Hennessy *et al.* 2005; Su *et al.* 1986; Waite & Dunlap 1953). In this study, we report the extended presence of TR4 in eight weed species, representing seven botanical families under field conditions in the Davao region, which is characterized by intensive banana production and high TR4 incidence (Molina *et al.*, 2016a). The reported species are among the most common weeds in banana fields in the Philippines, as well as in Latin America and the Caribbean (Quénéhervé *et al.*, 2006; Moody *et al.*, 2014; Lanza *et al.*, 2017; Oliveira *et al.*, 2019; Quintero-Pertúz *et al.*, 2020). Furthermore, we identified four other species of the Asteraceae, Cyperaceae and Poaceae families that can be colonized by *F. odoratissimum* in greenhouse experiments. Taken together, *A. houstonianum*,

A. spinosus, C. hirta, C. involucratus, D. setigera, E. colona, and *M. charantia*, were shown to be infected by *F. odoratissimum* by using two different inoculation methods.

In the past, TR4 was found in eight weed species of the Asteraceae, Cyperaceae, Euphorbiaceae and Poaceae families in Australia and Taiwan (Su *et al.*, 1986; Hennessy *et al.*, 2005), and other causal agents of FWB were also isolated from grasses or herbs in Honduras (Waite & Dunlap 1953). However, in all these studies the pathogen was only isolated from the root systems. Here, we show that *F. odoratissimum* can also be isolated from above-ground plant organs, although the incidence of *F. odoratissimum* is lower in the leaves and flowers. We did not find *Eleusine indica* (L.) Gaertn. (Poaceae) in the surveyed farms, and therefore could not confirm the result of Catambacan & Cumagun (2022) who tested this species under greenhouse conditions. Hennessy *et al.* (2005) surveyed a limited number of plants and, therefore, may have underestimated the presence of TR4 in other plant species. In addition, our phenotyping procedures, such as the timing of the inoculation and sampling under greenhouse conditions, may have inadvertently influenced the presence of TR4 in some weed organs, particularly in the aboveground parts. Therefore, we also examined eight weed species in the field for three months and diagnosed TR4 from aboveground parts of 99 plants with LAMP (Ordóñez *et al.*, 2019) and examined another four weed species and *A. pintoi* under greenhouse conditions, using validated protocols (García-Bastidas *et al.*, 2019; Martínez-de la Parte *et al.*, 2023). Nevertheless, we acknowledge that our data may not fully capture the complexities of field conditions. For instance, we did not test seed transmission of TR4 in the field and extended surveillance may have compensated the manifold environmental variables and natural interactions (Corredor-Moreno & Saunders, 2020), which can significantly impact the behaviour and prevalence of *F. odoratissimum* in weed populations.

Arachis pintoi is a leguminous plant, commonly known as pinto peanut, and is widely used as a cover crop in banana plantations because it fixes nitrogen from the air and competes with harmful weeds (Dita *et al.* 2018). Our work shows *F. odoratissimum* can colonize the root system, stems, leaves and flowers. Similar to recovered isolates from *P. oleracea* the pathogenicity of the recovered *F. odoratissimum* isolates from *A. pintoi* were significantly reduced. We cannot explain this result, but it may have contributed to the 20% reduction of FWB in Ducasse bananas (Pisang Awak subgroup, ABB) grown with pinto peanut as a cover crop (Pattison *et al.*, 2014). Indeed, the observed differences in pathogenicity of some recovered *F. odoratissimum* isolates are analogous to the altered pathogenic fitness of *F. graminearum* after passing through different alternative hosts (Akinsanmi *et al.*, 2007). These observations require further studies to explain alleviated pathogenicity after non-host passaging particularly since strains may regain their pathogenicity after passaging through bananas, as demonstrated in *in vitro* assays (Wu *et al.*, 2021).

Taken together, our data show and confirm the various strategies of *F. odoratissimum* for long-term survival which increase its persistence in the absence of banana plants (Pittaway *et al.*, 1999; Hennessy *et al.*, 2005). Weeds can act as reservoirs for lateral transmission of the pathogen to uninfected areas, even across continents (Jones & Diekmann 2000). This should be

considered in disease and weed management strategies, particularly after abandoning banana plantations, which is a common practice once the incidence of TR4 has passed the critical level of economic feasibility.

ACKNOWLEDGEMENTS

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SUPPLEMENTARY MATERIAL

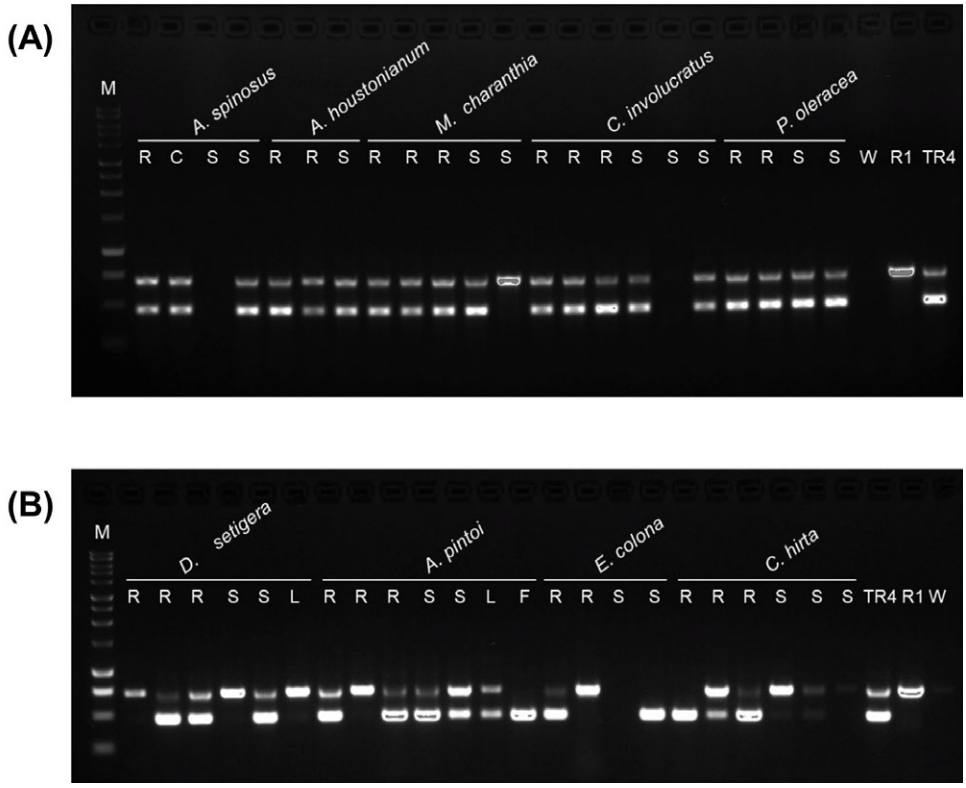
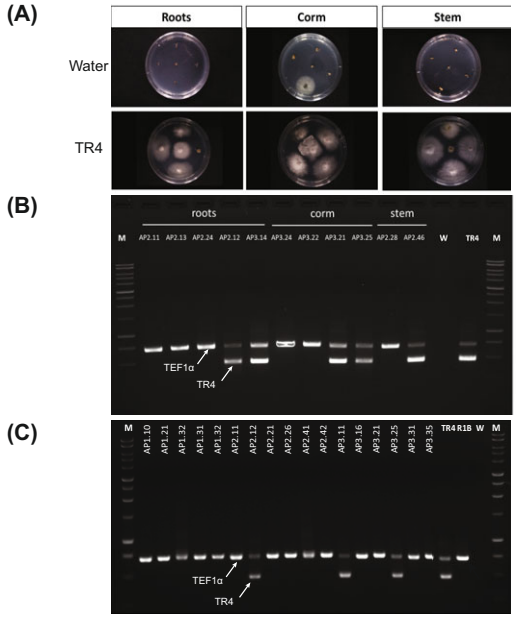


FIGURE S1. Identification of recovered TR4 isolates from different weed species. (A) and (B) Agarose gel (1.5%) showing PCR amplicons following the molecular diagnosis of Dita *et al.* (2010), with the internal TEF1α amplicon and the TR4 diagnostic amplicon of 462 bp, M-1Kb ladder. Samples obtained from roots (R), stem (S), leaf (L) or flower (F). Controls comprise *Fusarium odoratissimum* strain II5 (TR4), *F. oxysporum* strain CNPMF 000-8-01-R1 (R1) and water (W).



(D)

Treatment	Localization	Isolates
Water	Root	-
	Corm	AP1.10
	Stem	-
R1B	Root	AP1.11, AP1.12, AP1.13, AP1.14
	Corm	AP1.21, AP1.22, AP1.23, AP1.24
	Stem	AP1.31, AP1.32, AP1.33,
TR4-Plant 1	Root	AP2.11, **AP2.12**, AP2.13, AP2.14
	Corm	AP2.21, AP2.22, AP2.23, AP2.24, AP2.25, AP2.26, AP2.27, AP2.28, AP2.29, AP2.30
	Stem	**AP2.41**, AP2.42, AP2.43, AP2.44, AP2.45 **AP2.46**, AP2.47, AP2.48, AP2.49, AP2.50
TR4-Plant 2	Root	**AP3.11**, **AP3.12**, AP3.13, **AP3.14**, AP3.15 AP3.16, AP3.17, AP3.18, AP3.19
	Corm	**AP3.21**, AP3.22, AP3.23, AP3.24, **AP3.25** AP3.26, AP3.27, AP3.28
	Stem	AP3.31, AP3.32, AP3.33, AP3.34 AP3.35, AP3.36, AP3.37, AP3.38, AP3.39

FIGURE S2. Colonization of *Arachis pintoi* by *Fusarium odoratissimum* isolate II5 (TR4). A. Komada plates with fungal colonies isolated from roots, corms and stems. (B) and (C) Agarose gel (1.5%) showing PCR amplicons following the molecular diagnosis of Dita *et al.* (2010), with the internal TEF1α amplicon (650bp) and the TR4 diagnostic amplicon (462 bp). Isolates (AP1.11-AP3.35) retrieved from different plant parts of *A. pintoi* plants inoculated with the TR4 strain. AP1.10 isolate from water-treated plant did not amplify with TR4 specific primers (C).

TABLE S1. Inoculation method tested for each plant species. This depended mainly on the original plant material obtained for the experiment.

Family	Botanical name	Common name	Origin	Inoculation method	
				Seed inoculation	Wounding & drenching
Amaranthaceae	*Amaranthus spinosus* L.	spiny amaranth	Philippines	X	X
Asteraceae	*Ageratum houstonianum* Mill.	bluemink	Unifarm, WUR	X	X
Cucurbitaceae	*Momordica charantia* L.	balsam pear	Philippines	X	X
Cyperaceae	*Cyperus involucratus* Rottb.	umbrella palm	Unifarm, WUR	NT	X
Euphorbiaceae	*Chamaesyce hirta* (L.) Millsp.	asthma plant	Philippines	X	X
Fabaceae	*Arachis pintoi* Krapov. & W.C.Greg.	pinto peanut	Unifarm, WUR	NT	X
Poaceae	*Digitaria setigera* Roth ex Roem. & Schult.	East Indian crabgrass	Philippines	X	X
Poaceae	*Echinochloa colona (*L.) Link	jungle rice	Philippines	X	X
Portulacaceae	*Portulaca oleracea* L.	little hogweed	Philippines	X	X

NT-non tested.

TABLE S2. Weed species reported as secondary host of the Fusarium wilt pathogen.

Family	Botanical name	Common name [a]	Secondary host of:		Reference
			R1 [b]	TR4 [c]	
Amaranthaceae	*Amaranthus spinosus* L.	spiny amaranth		X	Current study
Asteraceae	*Ageratum houstonianum* Mill.	bluemink		X	Current study
Asteraceae	*Cyanthillium cinereum* (L.) H. Rob.	little ironweed		X	Hennessy *et al.* (2005)
Asteraceae	*Gamochaeta pensylvanica* (Willd.) Cabrera	Pennsylvania everlasting		X	Su *et al.* (1986)
Asteraceae	*Tridax procumbens* L.	coatbuttons		X	Hennessy *et al.* (2005)
Commelinaceae	*Commelina diffusa* Brum.	climbing dayflower	X		Waite & Dunlap (1953)
Cucurbitaceae	*Momordica charantia* L.	balsam pear		X	Current study
Cyperaceae	*Cyperus involucratus* Rottb.	umbrella palm		X	Current study
Cyperaceae	*Cyperus iria* L.	ricefield flatsedge		X	Su *et al.* (1986)
Cyperaceae	*Cyperus rotundus* L.	nutgrass		X	Su *et al.* (1986)
Cyperaceae	*Fimbristylis littoralis* Gaudich.	fimbry		X	Su *et al.* (1986)
Euphorbiaceae	*Chamaesyce hirta* (L.) Millsp.	asthma plant		X	Current study
Euphorbiaceae	*Euphorbia heterophylla* L.	Mexican fireplant		X	Hennessy *et al.* (2005)
Poaceae	*Chloris barbata* Sw.	swollen fingergrass		X	Hennessy *et al.* (2005)
Poaceae	*Digitaria setigera* Roth ex Roem. & Schult.	East Indian crabgrass		X	Current study
Poaceae	*Echinochloa colona* (L.) Link	jungle rice		X	Current study
Poaceae	*Eleusine indica* (L.) Gaertn.	Indian goosegrass		X	Catambacan & Cumagun (2022)
Poaceae	*Ixophorus unisetus* (Presl.) Schlecht.,	Mexican grass	X		Waite & Dunlap (1953)
Poaceae	*Paspalum fasciculatum* Willd. ex Flueggé	Mexican crowngrass	X		Waite & Dunlap (1953)
Poaceae	*Urochloa mutica* (Forssk.) T.Q. Nguyen	para grass	X		Waite & Dunlap (1953)
Portulacaceae	*Portulaca oleracea* L.	little hogweed		X	Current study

[a]- Botanical and common name according to The PLANTS database (https://plants.usda.gov/home). [b]- R1- *Fusarium* spp. that are causal agents of Fusarium wilt of banana (previously identified as Race 1).[c]-TR4- *Fusarium odoratissimum.*

CHAPTER 5

Species causing Fusarium wilt of banana infect and survive in *Heliconia* species and ornamental bananas

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ABSTRACT

Fusarium wilt of banana (FWB), caused by soilborne *Fusarium* spp., is a major global threat to the cultivation of bananas. In addition to persistent chlamydospores, weeds are a reservoir of the causal agents. However, it remains unclear whether other Zingiberales species, which are grown in the same geographic regions, also can serve as hosts for *Fusarium* spp. that cause FWB. Greenhouse assays were conducted to investigate whether *Fusarium phialophorum* (Race 1; pathogenic to Gros Michel banana) and *Fusarium odoratissimum* (TR4; pathogenic to Cavendish banana) can infect three *Heliconia* species, two ornamental banana species or *Musa textilis* (abacá). *Heliconia latispatha, Musa balbisiana,* and *Musa coccinea* displayed external symptoms after inoculation with TR4, while inoculation with Race 1 caused symptoms in *H. latispatha*, *H. psittacorum, M. coccinea,* and *M. velutina*. Isolates recovered from distinct organs of all studied plant species were characterized and re-isolated strains caused FWB symptoms in Gros Michel and Cavendish banana plants, and their scores for rhizome discolored areas were similar to the reference strains. The susceptibility of some ornamental species and the presence of *Fusarium* strains as asymptomatic endophytes in others, with remaining pathogenicity, call for a revision of the race nomenclature and the current containment protocols for FWB.

INTRODUCTION

The genus *Musa* belongs to the Musaceae family of the order Zingiberales and comprises many wild and seeded banana species as well as all seedless and therefore edible varieties. Cultivated bananas are a major staple food for millions of people in many countries and dominate the global fruit commerce (FAO 2022a; Ploetz 2015). The genus *Musa* is divided into the sections *Callimusa* and *Musa*, with the latter including most edible banana cultivars (Häkkinen, 2013), which are diploid or triploid hybrids of *Musa acuminata* (AA, 2n=22) or from hybridization with *Musa balbisiana* (BB, 2n=22) (Ploetz 2006). Only a minor group of cultivars, which includes Fe'i bananas, is derived from species of the *Callimusa* section (Häkkinen 2013). This section also includes *M. textilis* (also known as abacá) which is appreciated as source of cordage fiber. Ornamental banana species can be found in both of the aforementioned sections and typically have relatively few fruits and are best known for their brightly coloured bracts (Häkkinen 2013; Häkkinen and Väre 2008). *Heliconia* spp. are ornamental plants that also belong to the Zingiberales, but to the family of the Heliconeaceae. They are very popular neotropical ornamentals characterized by colorful inverted flowers (Gómez-Merino *et al*., 2018).

Fusarium wilt of banana (FWB) is a devastating vascular disease that severely impacts international banana production (Staver *et al.* 2020) and jeopardizes food security and livelihoods in regions that rely on banana cultivation (Fones *et al.* 2020; Steinberg and Gurr 2020). The disease is caused by a diverse array of *Fusarium* species (Maryani *et al.* 2019; Mostert *et al.* 2022; Ordóñez *et al.* 2015). They invade the vascular system of banana plants after root infection, obstructing water and nutrient transport and ultimately leading to wilting, chlorosis and plant death (Pegg *et al.* 2019). To date, three different races of the FWB pathogen have been described based on the pathogenicity to reference banana cultivars: Race 1, Race 2 and Race 4, with the latter subdivided into Tropical Race 4 (TR4) and Subtropical Race 4 (Pérez-Vicente 2004; Ploetz 2015). A pathogen population causing wilt in *Heliconia* spp., was originally described as Race 3 (Waite 1963), but it is no longer considered part of *Fusarium* complex infecting banana (Ploetz 2015). The FWB pathogens also reside and survive in non-banana plant species (Chapter 4; Hennessy *et al.* 2005; Pittaway *et al.* 1999; Waite and Dunlap 1953) which contributes to their long-term survival in the field. Some *M. velutina* and *M. textilis* cultivars have been included in germplasm screenings for resistance to TR4 (García-Bastidas 2019; Li *et al.* 2015; Zuo *et al.* 2018), but others, such as *M. coccinea*, have never been tested. Additionally, *Fusarium* isolates infecting *M. textilis* have not been used in disease evaluations of bananas (Borines *et al.* 2007; Dyah Purwati and Hidayah 2008). Thus, the understanding of the potential role of *Heliconia* spp. and the aforementioned non-edible *Musa* spp., in addition to nonrelated weed species (Chapter 4), in the epidemiology and survival of FWB pathogens remains limited. Since these Zingiberales species are grown in the same geographical areas as bananas it is crucial to understand their role in the FWB epidemiology to support improved disease management strategies.

MATERIALS AND METHODS

Plant material

Tissue culture plants of *Heliconia latispatha* Benth., *Heliconia psittacorum* L., *Heliconia rostrata* Ruiz & Pav., *Musa coccinea* Andrews, *M. velutina* H. Wendl. & Drude, *M. textilis* Nee, and *cv*. Gros Michel (AAA) were propagated at the tissue culture laboratory of the Corporación Bananera Nacional (CORBANA S.A.), Costa Rica. Tissue culture banana plants *cv*. Grand Naine (AAA) were obtained from Vitropic (Saint Mathieu-de-Tréviers, France). Upon arrival at Wageningen University & Research (WUR, The Netherlands), the plants were transferred from transport plastic boxes to 1L pots containing a standard soil (Swedish sphagnum peat 20%, Baltic peat 30%, garden peat 30%, beam structure 20%, grinding clay granules 40.6 Kg/m^3, Lime + MgO 2.5 Kg/m^3, PG-Mix-15-10-20 0.8 Kg/m^3) from the WUR-Unifarm greenhouse facility. The potted plants were then acclimatized under plastic to maintain high humidity conditions for two weeks in an environmentally controlled greenhouse compartment and thereafter grown for ~2.5 months prior to inoculation (28±2°C, 16h light, and ~85% relativity humidity). During the experiment, plants were watered daily and fertilized three times per week (NH_4^+-1.2 mM/L, K^+-7.2 mM/L, Ca^{2+}-4 mM/L, Mg^{2+}-1.82 mM/L, NO_3^--12.4 mM/L, SO_4^{2-}-3.32 mM/L, $H_2PO_4^-$-1.1 mM/L, Mn^{2+}-10 µMol/L, Zn^{2+}-5 µMol/L, B-30 µMol/L, Cu^{2+}-0.75 µMol/L, Mo-0.5 µMol/L, Fe/DTPA-50/3%, Fe-EDDHSA-50/3%, pH=5.8).

Inoculum production and inoculation methods

To produce inoculum conidial suspensions of the reference strains for TR4 and Race 1, *Fusarium odoratissimum* strain II5 and *F. phialophorum* strain CR1.1A (van Westerhoven *et al.* 2023), respectively, were produced in flasks containing 100 mL of mung beans broth (2 g mung beans per 500 ml water) according to García-Bastidas *et al.* (2019). The flasks were incubated at 25°C, 150 rpm for five days, after which the concentration was adjusted to 1×10^6 conidia·ml^{-1} prior to inoculation.

Inoculations were performed by wounding the roots in the soil using a soil scoop at two opposite sides of the plant and subsequent drenching with a 200 ml suspension of 10^6 conidia/L per pot (Supplementary Figure 1). For negative controls (Mock), we drenched each pot with 200 ml of water after damaging the roots, and inoculated Grand Naine and Gros Michel plants were used as positive controls for TR4 and Race 1, respectively. During the experiment, six plants of each species were inoculated with Race 1 and nine with TR4, while three to five plants were used as negative control (Supplementary Table S1). The experiment was designed according to a Randomized Complete Block Design with two repetitions.

Disease progress and diagnosis of reisolated strains

Plants were weekly inspected for any noticeable FWB symptoms compared to the non-inoculated and the positive controls. Five plants per species were randomly selected 12 weeks post-inoculation, and three pieces (~1 cm^2) of plant material were collected from the roots, rhizome, corm or pseudostem of each plant. Sampled plant parts were extensively washed with demineralized water and then surface sterilized with 70% ethanol for five minutes in a laminar flow cabinet, then three times rinsed with sterile demineralized water and placed on Komada´s semi-specific media for *Fusarium* spp. (Komada 1975). After six days, small fragments of the edges of fungal colonies with *Fusarium* morphology were collected and transferred to fresh potato dextrose agar (PDA) plates.

For diagnosis, fragments of the mycelium on PDA plates (five to seven days old) were collected with a sterile toothpick and transferred into a tube containing 20 µL of dilution buffer included in the direct Thermo Scientific Phire Plant Direct PCR Master Mix PCR master mix kit, which does not require separate DNA extraction (Thermo Scientific, Landsmeer, the Netherlands). The tube was vortexed for 10 s and incubated at 95°C for 5 min. One µL of the solution without mycelia was used as template in 20-µL PCR, which contains 10 µL of the Master Mix (2X) and 0.5 µL of each primer (10mM). The PCR program comprised an initial denaturation at 98°C for 5 min, followed by 35 or 40 cycles of denaturation at 98°C for 5 s, annealing at 62°C or 55°C (Table1) for 5 s and extension at 72°C for 20 s, and a final extension at 72°C for 1 min. We used the primers of Dita *et al.* (2010), and Carvalhais *et al.* (2019) (Table 1) for final molecular diagnosis. The resulting amplicons were visualized after electrophoresis with a 100-bp ladder as a reference on 1.5% agarose gel by staining with ethidium bromide, and gels were photographed using the ChemidocTM MP image system (Bio-Rad Laboratories, Veenendaal, The Netherlands).

TABLE 1. Primers for molecular diagnosis of re-isolated *Fusarium* strains.

Target	Primer name	Sequence (5´-3´)	Annealing Temperature (°C)	Reference
TR4	FocTR4-F	CACGTTTAAGGTGCCATGAGAG	60	Dita *et al.*, 2010
	FocTR4-R	GCCAGGACTGCCTCGTGA		
Elongation Factor-1α	EF-1	ATGGGTAAGGAGGACAAGAC	60	O´Donnell *et al.*, 1998
	EF-2	GGAGGTACCAGTGATCATGTT		
Race 1	Six6b_210-F	ACGCTTCCCAATACCGTCTGT	55	Carvalhais *et al.*, 2019
	Six6b_210-R	AAGTTGGTGAGTATCAATGC		

Pathogenicity of reisolated *Fusarium* isolates

From each plant species, we selected one re-isolated strain of Race 1 and TR4 that tested positive with the abovementioned molecular diagnostics and was reisolated from the uppermost colonized part of the plant for inoculations following the aforementioned protocols. Twelve-week-old plants of Gros Michel and Grand Naine were inoculated with these re-isolated strains,

using the reference strains CR1.1A (Race 1) or II5 (TR4) as positive controls, and water-treated plants as negative controls (mock). For each isolate, we inoculated in total seven Gros Michel plants and seven Grand Naine, in two separate experiments following a Randomized Complete Block Design. The rhizomes of the inoculated plants were cut transversally 11 weeks after inoculation, photographed and the Rhizome Discoloured Area (RDA) was determined using ImageJ 1.52r software (National Institutes of Health, Bethesda, MD, USA). Individual RDA values were plotted using the web-tool BoxPlotR (Spitzer *et al.*, 2014). The Kruskal-Wallis test was used to compare RDA values on Grand Naine and Gros Michel plants caused by the various isolates and Dunn's multiple comparisons test was applied for multiple comparisons of variables at a P value of <0.05.

RESULTS

To investigate the host range of the FWB pathogen among *Heliconia* spp. and the level of susceptibility of non-edible bananas used as ornamental or fiber crop, we conducted greenhouse tests where *H. latispatha*, *H. psittacorum*, *H. rostrata*, *M. coccinea*, *M. velutina* and *M. textilis* were challenged with inoculum of two reference isolates for Race 1 and TR4. Throughout all the individual phenotyping experiments conducted in this study, negative water controls never showed any external or internal FWB symptoms. The compatible TR4/Grand Naine plants and Race 1/Gros Michel interactions used as positive controls, displayed the typical FWB symptoms, whereas the incompatible Race 1/Grand Naine interaction did not result in any external or internal FWB symptoms (Figure 1).

All non-edible *Musa* spp. showed FWB symptoms typical for susceptible responses at 12 weeks after inoculation with Race 1 (Figure 1) with an average RDA value higher than 38% with *M. coccinea* being the most susceptible (RDA =62%). Both *M. coccinea* and *M. textilis* were also susceptible to TR4 with average RDA values of 77.8% and 53.2%, respectively. However, 35% of the *M. velutina* plants did not show any external or internal symptoms while the remainder of the plants showed the lowest scores among the tested *Musa* spp. (Figure 1) with an average RDA value of 3.8% at 12 weeks after inoculation.

Inoculation of the three *Heliconia* spp. with Race 1 caused wilting symptoms in *H. latispatha* and *H. psittacorum* but not in *H. rostrata*. The symptoms included leaf chlorosis, drying of leaves, weakened pseudostems that easily dislodged from the base upon pulling. However, all Race 1 inoculated *Heliconia* plants, including those of *H. rostrata*, showed necrosis in the roots and rhizomes (Figure 1). TR4 caused rhizome discoloration in all *Heliconia* species but never necrosis and only caused external symptoms in *H. latispatha,* similar to the symptoms caused by Race 1. Taken together, these results show that the *Heliconia* spp. and *M. velutina* plants are more susceptible to Race 1 than to TR4.

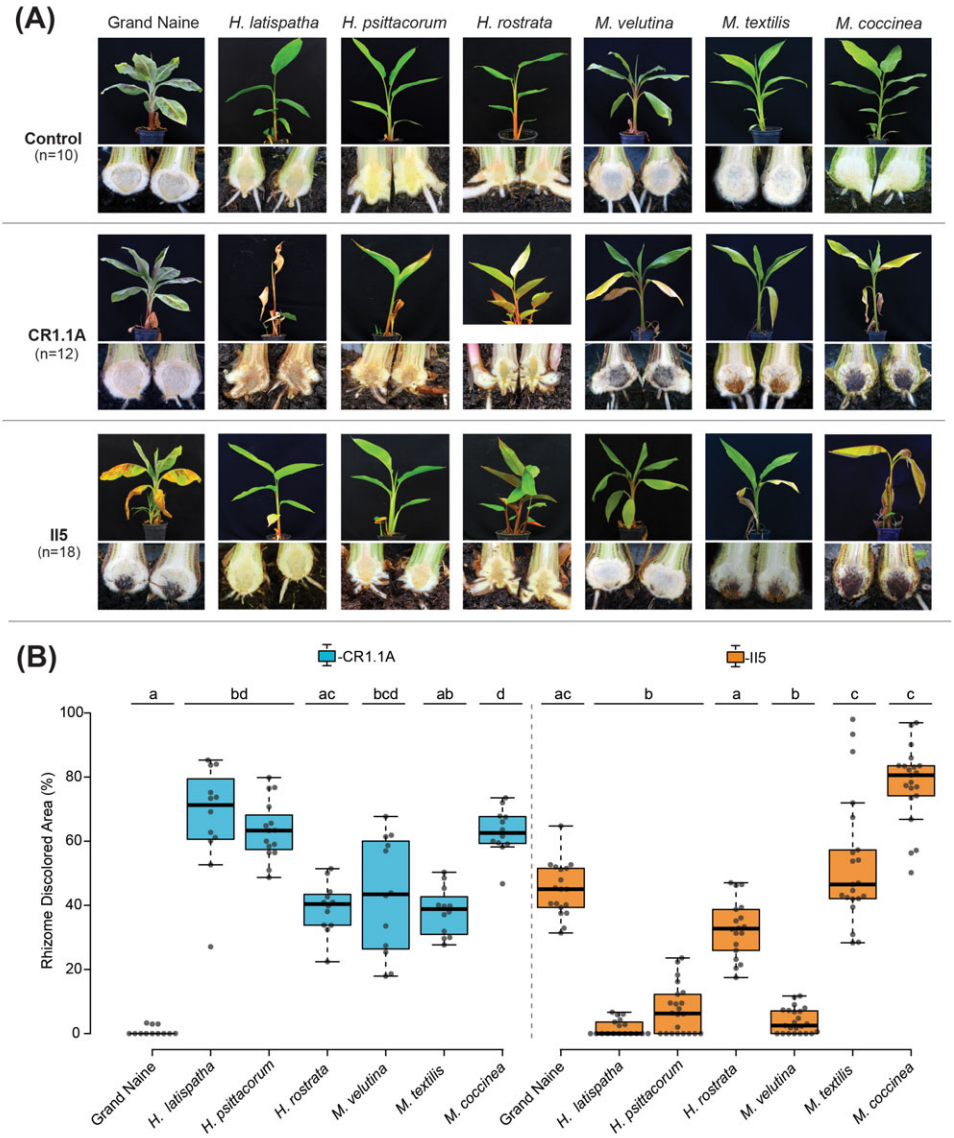

FIGURE 1. Differential responses of three *Heliconia* spp. and three non-edible *Musa* spp. after inoculations with *F. phialophorum* isolate CR1.1A representing Race 1 or *F. odoratissimum* isolate II5 representing TR4. A. External symptoms (upper rows) and cross sections of rhizomes (bottom rows) from representative plants 12 weeks after inoculation with mock (upper panel), Race 1 (middle panel) or TR4 (lower panel). B. RDA scores of inoculated plants. Statistically significant differences ($p < 0.05$) between median RDA values are indicated with different letters, according to Dunn's multiple comparison test.

TABLE 2. The number of recovered isolates from different plant parts of three *Heliconia* and *Musa* species after inoculation with Race 1 and TR4.

Plant species	Race 1			TR4		
	Roots	Rhizome	Pseudostem	Roots	Rhizome	Pseudostem
Heliconia latispatha	25	24	15	24	8	5
Heliconia psittacorum	20	20	5	21	9	-
Heliconia rostrata	18	6	-	14	7	-
Musa coccinea	31	31	22	32	30	29
M. velutina	35	35	30	31	21	-
Musa textilis	31	32	17	34	28	10
Gros Michel	23	20	19	24	25	21
Grand Naine	-	-	-	34	32	30

To further investigate the colonization of the tested *Heliconia* spp and *Musa* spp. by Race 1 or TR4, various parts of inoculated plants were sampled to re-isolate the *Fusarium* strains. A total of 928 isolates was obtained (Table 2), re-isolates representative of the different plant organs per species were confirmed as Race 1 or TR4 by diagnostic PCRs (Figure S1). The presence of Race 1 or TR4 strains was confirmed in the roots, rhizomes and pseudostems of *H. latispatha, M. coccinea* and *M. textilis.* We could not recover TR4 from the pseudostems of *H. psittacorum, H. rostrata* or *M. velutina.* Similarly, no Race 1 isolates could be recovered from the pseudostem of inoculated *H. rostrata* plants or from any tissue obtained from Grande Naine plants (Supplementary Figure S2). Thus, we have demonstrated that causal agents of FWB are able to colonize the aboveground tissues of *H. latispatha, H. psittacorum, M. coccinea* and *M. textilis* plants.

To determine whether the pathogenicity of the recovered isolates was altered by passage through these hosts, we used one re-isolated and confirmed Race 1 and TR4 per plant species to inoculate Gros Michel and Grand Naine banana plants. Isolates were selected from the uppermost colonized part and their pathogenicity did not significantly differ from the reference strains CR1.1A and II5, except the TR4 isolate recovered from *H. rostrata* which scored significantly lower than the reference strain *F. odoratissimum* II5 strain with an average RDA of 14.9%, (Figure 2).

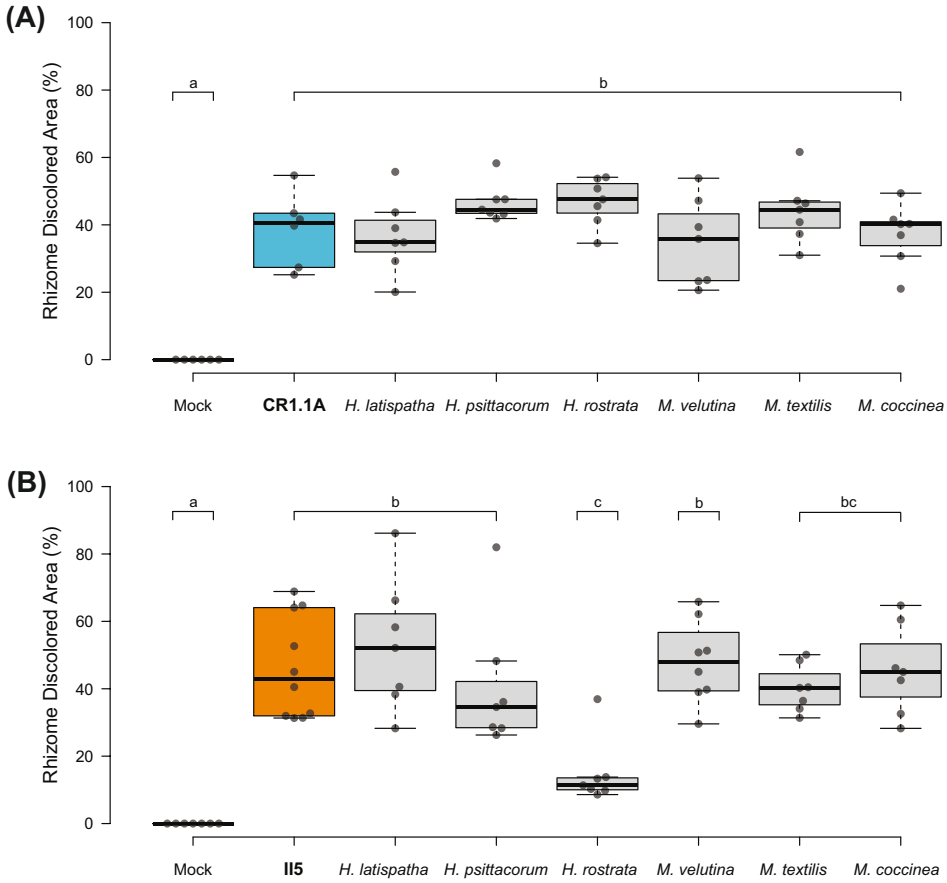

FIGURE 2. Phenotyping results of six re-isolated *Fusarium* strains from three *Heliconia* and three *Musa* spp. at 11 weeks post-inoculation. (A) Race 1 isolates tested on Gros Michel and compared with the *F. phialophorum* Race 1 reference strain CR1.1A. (B) TR4 isolates tested on Grand Naine and compared with the *F. odoratissimum* TR4 reference strain II5. Different letters indicate statistically significant differences ($p < 0.05$) between median RDA values, according to Dunn's multiple comparison test.

DISCUSSION

Host plants and plant products are traded around the globe, often facilitating the unintended transport of associated pathogens or endophytes (Fones *et al.* 2020). The intensified movement and volume of traded plants and plant products over the last decades has resulted in an unprecedented spread of pathogens at a global scale, with the current dissemination of TR4 as one of the clearest examples (Drenth & Kema 2021). This worrisome global spread, particularly the recent cases in Latin America and Mozambique (Acuña *et al.* 2021; Garcia-Bastidas *et al.* 2020; Herrera *et al.* 2023; van Westerhoven *et al.* 2022), shows that FWB successfully disseminates despite implemented quarantine and prevention strategies (van Van Westerhoven *et al.* 2022). The recommended eradication and containment strategies, (Dita *et al.* 2018; Viljoen *et al.* 2020),

largely ignore the presence of secondary hosts of the pathogen (Pegg *et al.* 2019). Thus, the identification of such alternative hosts could be useful in understanding pathogen persistence, inoculum accumulation, and subsequent spread to other areas (Hennessy *et al.* 2005).

Heliconia spp. are native to the tropical regions of the Americas, primarily Central and South America, with some species also found in the Caribbean and the Pacific islands (Gómez-Merino *et al.* 2018). In those regions, they are often present in rainforests, along riverbanks, and naturally growing alongside or near banana plantations. In addition, they are cultivated to produce cut flowers for the export in Latin-American and in some African countries (Linares-Gabriel *et al.* 2020), and are also used in landscaping parks and gardens (Gómez-Merino *et al.* 2018). Similar to bananas, Fusarium wilt is one of the most common diseases in *Heliconia* spp. (Castro *et al.* 2010). The causal agent of wilt in *Heliconia* spp. was initially described as Race 3 of the FWB pathogen (Waite 1963) but is no longer considered as part of the causative *Fusarium* complex infecting banana (Ploetz 2015)Recent studies evaluating the pathogenicity of *Fusarium* strains from *Heliconia* genotypes did not include testing on bananas (Castro *et al.* 2008, 2010). Nevertheless, only limited information is available on the potential infection of *Heliconia* by FWB-causing *Fusarium* spp. This is a significant knowledge gap when considering the potential impact of global dissemination of these species, particularly since it has been shown that weeds can contribute to FWB spread which requires altered disease management strategies (Chapter 4; Hennessy *et al.* 2005; Pittaway *et al.* 1999; Su *et al.* 1986; Waite and Dunlap 1953).

Our results are mostly consistent with, but significantly extend, early studies which reported that Race 1 strains caused symptoms on *H. psittacorum* (Rishbeth 1957), *H. caribea* and *H. latispatha* (Waite 1961) and that re-isolated strains were pathogenic on Gros Michel. Then, the re-isolated strains were obtained exclusively from the belowground plant organs (Rishbeth, 1957; Waite 1961), and information on the genotypes of the studied isolates is lacking. Moreover, recent phylogenetic studies confirmed that Race 1 is polyphyletic and comprises a suite of *Fusarium* spp. (Maryani *et al.* 2019; van Westerhoven *et al.* 2023). Here, we demonstrated that passing Race 1 through *H. lathispatha* and *H. psittacorum* did not change the phenotype on Gros Michel. However, under our experimental conditions, *Heliconia* spp. exhibited higher susceptibility to Race 1 than to TR4. This could be attributed to the prolonged co-evolution of Race 1 with a plethora of *Heliconia* species in the Latin American and Caribbean region, which is considered the center of origin for these plants (Gómez-Merino *et al.* 2018; Malakar *et al.* 2022). We also showed for the first time that TR4 can colonize three *Heliconia* species, causing a range of symptoms. It is important to underscore that, in contrast to the other *Heliconia* spp., TR4 did not cause any external symptoms in *H. psittacorum* and *H. rostrata,* similar to the non-symptomatic colonization of various weeds and cover crops (Chapter 4; Hennessy *et al.* 2005; Pittaway *et al.* 1999; Su *et al.* 1986; Waite and Dunlap 1953), which urges adaptation of contingency and containment strategies upon the detection of TR4.

The *Musa* spp. we tested showed a varied reaction to the inoculation with the Race 1 and TR4 strains. The susceptibility to FWB of *M. textilis* was consistent with previous reports (García-Bastidas 2019; Zuo *et al.* 2018), but we also observed that *M. coccinea* was equally susceptible to

TR4 as to Race 1, and that *M. velutina* plants showed no wilting symptoms after inoculation with TR4, which confirms a previous report (Li *et al.* 2015). However, these authors neither examined the colonization of the different plant organs, nor phenotyped recovered strains from this species. We show that passage of FWB-causing *Fusarium* spp. through these hosts generally does not affect pathogenicity to banana, except for strains recovered from *M. velutina,* similar to the recently reported reduction of pathogenicity after passing TR4 through *Arachis pintoi* and *Portulaca oleracea* (Chapter 4). The attenuated pathogenicity of TR4 after colonization of non-host plants and *M. velutina* requires further studies, but these data nevertheless underscore the need to consider the wider host range of TR4 in disease management strategies.

In conclusion, our results evidenced that the tested non-edible *Musa* spp. and *Heliconia* spp. commonly used as fiber and ornamental crops, are vulnerable to FWB-causing *Fusarium* spp. Until more data will be available, the cultivation, propagation, and trade of these species should be avoided in FWB-infested areas, as they may serve as unrecognized *Fusarium* spp. reservoirs that inadvertently contribute to FWB dissemination into new areas. Moreover, *Heliconia* spp. have also been reported as hosts of the important banana bacterial pathogen *Ralstonia solanacearum* and banana bunchy top virus, which cause Moko and bunchy top disease, respectively (Blomme *et al.* 2017; Hamim *et al.* 2017) improved quarantine regulations.

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SUPPLEMENTARY MATERIAL

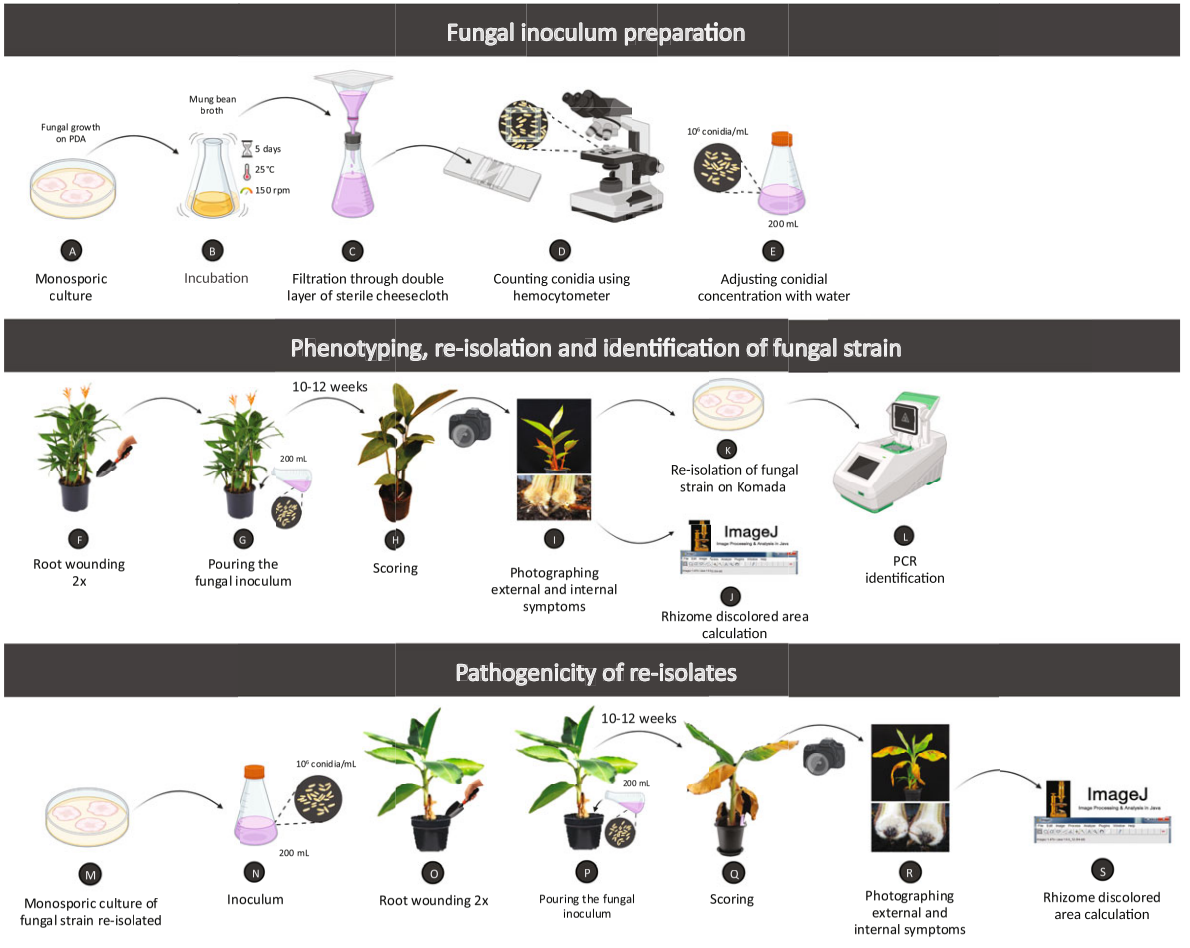

FIGURE S1. The general workflow of the experiment. Inoculum production and inoculation method according to García-Bastidas *et al.* (2019).

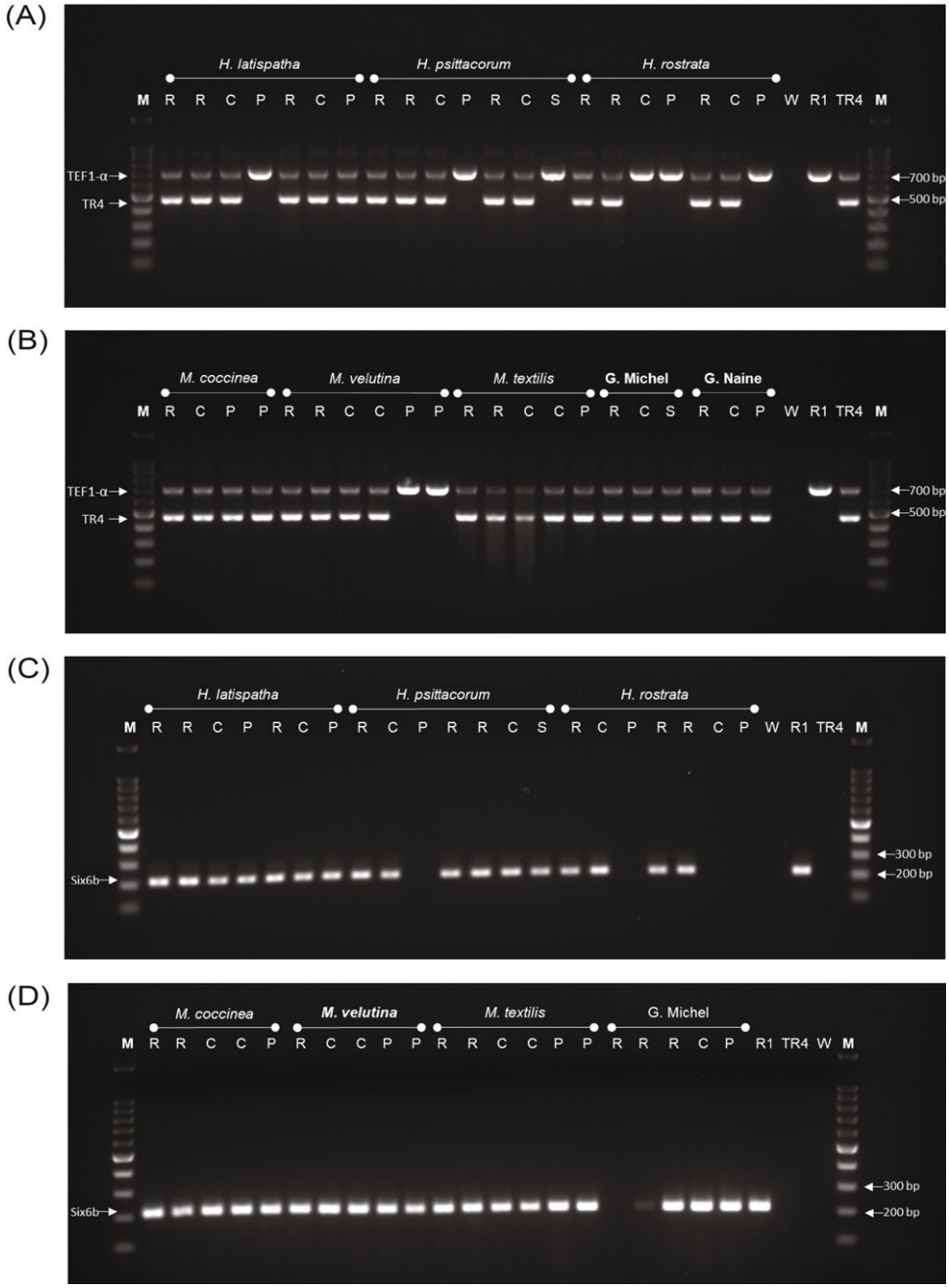

FIGURE S2. PCR identification of re-isolates obtained from different *Heliconia* and *Musa* species. (A) and (B) Agarose gel (1.5%) showing PCR amplicons following the molecular diagnosis of Dita *et al.* (2010), with the internal TEF1α amplicon and the TR4 diagnostic amplicon, corresponding with re-isolates from plants inoculated with TR4. (C) and (D) Agarose gel (1.5%) showing the results of PCR identification, according to the molecular diagnosis of Carvalhais *et al.* (2019), of the re-isolates obtained from plants inoculated with Race 1. Samples obtained from roots (R), corm (C), pseudostem (P). Controls comprise *Fusarium odoratissimum* strain II5 (TR4), *F. phialophorum* strain CR1.1A (R1) and water (W).

TABLE S1. Total number of plants used in the experiments. Mock-plants treated with water, Race 1-plants inoculated with *F. phialophorum* strain CR1.1A from Costa Rica, TR4-plants inoculated with the *F. odoratissimum* strain II5 from Indonesia.

Plant	Treatment		
	Mock	Race 1	TR4
Grand naine	10	12	18
Gros Michel	6	10	10
H. latispatha	10	12	18
H. psittacorum	10	12	18
H. rostrata	6	12	18
M. velutina	10	12	18
M. textilis	10	12	18
M. coccinea	10	12	18

CHAPTER 6

Comparing genome structures, effector repertoires, and pathogenicity profiles of the races that cause Fusarium wilt of banana

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ABSTRACT

Fusarium wilt of banana (FWB) is a destructive plant disease that has resulted in devastating economic losses to banana production worldwide. The disease is caused by a suite of genetically distinct *Fusarium* species, which strains are classified into pathogenic races based on their capacity to infect a subset of banana cultivars. Effector gene repertoires present in accessory regions of the *Fusarium* genome are considered to define the host range, and we anticipated that presence-absence profiles of effector genes would distinguish strains with a distinct pathogenic phenotype. Oxford Nanopore DNA sequencing of one strain *F. purpurascens* (Race 1) and two *F. tardichlamydosporum* (Race 2) strains, resulted in (near) chromosome-level genome assemblies of 50.6, 51.2 and 52.6Mbp with 14, 16 and 18 contigs, respectively. Whole-genome comparisons revealed that Race 2 strains share two accessory chromosomes that could not be identified in the reference genome assemblies of the other races included in the study. We then determined the presence of *SIX* genes in 175 isolates, and putative effector genes were identified by selecting genes encoding small secreted proteins, in proximity to a *mimp* transposable element, in a subset of 31 isolates. We show that predicted effector profiles do not correlate well with the pathogenic phenotype of the strains, which was underscored during comprehensive greenhouse experiments where nine banana cultivars were challenged with seven strains of *Fusarium* spp., comprising the three races. Our results underscore that the current race concept is inadequate and requires functional analyses of predicted effector genes along with genetic analyses of resistance in the host. Taken together, we conclude that accessory regions of *Fusarium* spp. causing FWB are highly variable and encode diverse effector gene repertories.

INTRODUCTION

The origin of taxonomy is in the morphological diversification of species, including microbes. However, the differentiation of species in microbial organisms has been much more challenging than for instance for plant species. Particularly, when such microbial species are in the same genus and closely resemble each other. Genomics has significantly contributed to the species concepts in e.g. fungal complexes. One of these is the *Fusarium oxysporum* species complex (FOSC) which includes species clustered in sub-taxonomic groups pathogenic on a particular host called a *forma specialis* (plural ff. spp.) (Baayen *et al.*, 2000), such as *F. oxysporum* f. sp. *lycopersoci* (Fol) or *F. oxysporum* f. sp. *melonis* for strains that are pathogenic on tomato or melon, respectively. Genetic diversity within these subgroups is further specified by pathogenicity profiles on cultivars which are called races (Correll, 1991). However, the FOSC also contains species that are apparently non-pathogenic, or more precisely species for which a host is currently unknown. Collectively, the FOSC contains isolates that are ubiquitously present in soils, host plants or non-host plants (Chapter 4), which cannot be differentiated morphologically (Leslie & Summerell, 2006). One of the contemporary challenges of this complexity is to align genomes, with predicted and proven pathogenicity in the context of dynamic species concepts.

The plant pathogens of the FOSC are responsible for destructive vascular wilt diseases in a wide range of crops (Dean *et al.*, 2012), and Fusarium wilt of banana (FWB) is one of the most well-known examples (Kema *et al.*, 2021). The disease annihilated the global banana production based on the Gros Michel cultivar during the early twentieth century (Ploetz, 2000). Currently, FWB threatens the world's banana production due to the spread of strains that are highly pathogenic on Cavendish bananas that represent more than 50% of the global production and dominate the export trade (Lescot, 2020), as well as on local germplasm important for domestic markets and food security (Staver *et al.*, 2020; Kema *et al.*, 2021). Originally, the causal agent of FWB was known as *Fusarium oxysporum* f sp. *cubense* (Foc), but recent studies based on multilocus genotyping and genome analyses revealed that Foc is a complex of eight different *Fusarium* species (Maryani *et al.*, 2019; van Westerhoven *et al.*, 2023), which comprise three races (Pérez-Vicente, 2004; Ploetz, 2015). Race 1 affects bananas of dessert types, of the Gros Michel, Silk, Pome, and Pisang Awak subgroups, and is avirulent to Cavendish under normal conditions. Race 2 affects cooking bananas such as those of the Bluggoe subgroup. Race 4 is divided into Tropical Race 4 (TR4) which affects Cavendish and cultivars susceptible to Race 1 and Race 2, and subtropical Race 4 (STR4) comprising Race 1 strains that are normally not pathogenic on Cavendish but cause FWB under abiotic stress (Ploetz, 2015).

Pathogenic fungi have evolved complex mechanisms to overcome plant immune systems. One of the ways is to secrete small cysteine-rich proteins, called effectors, into the host cells to manipulate the plant defense response (Rodriguez-Moreno *et al.*, 2018). In FOSC, a family of effector genes termed *secreted in xylem* (SIX) genes has been identified (Rep *et al.*, 2004; Takken & Rep, 2010). Although *SIX* genes were initially described and accumulated to 14 genes in the tomato pathogen Fol, homologs have been identified across other species of the FOSC

(Thatcher *et al.*, 2012; Laurence *et al.*, 2015; Niu *et al.*, 2016; Taylor *et al.*, 2016; Batson *et al.*, 2021), usually limited to unique combinations of these effectors (van Dam *et al.*, 2016). For instance, in FWB-causing strains various *SIX* genes have been identified but only *SIX1* and *SIX9* were present in all isolates studied and *SIX8* was only identified in Race 4 isolates (Fraser-Smith *et al.*, 2014; Czislowski *et al.*, 2018; Widinugraheni *et al.*, 2018; An *et al.*, 2019; Leiva *et al.*, 2022). Therefore, it was hypothesized that specific combinations of homologs of the *SIX* genes determine the different races of FWB-causing strains (Fraser-Smith *et al.*, 2014; Czislowski *et al.*, 2018). However, thus far only the involvement of the *SIX1* and *SIX8* homologs in banana pathogens was experimentally demonstrated (Widinugraheni *et al.*, 2018; An *et al.*, 2019). Recently, other candidate effectors have also been identified, based on their small size, predicted secretion signal, and proximity to a "*miniature impala*" (*mimp*) transposable element (Chang *et al.*, 2020). However, there is a lack of understanding of effector presence and its repercussion on the pathogenicity of *Fusarium* strains causing FWB.

The genome of FOSC pathogens is typically divided into a core genome which encodes housekeeping genes vital for survival and growth of the fungus, and an accessory genome encoding pathogenicity-associated genes (Ma *et al.*, 2010; Croll & McDonald, 2012; Van Dam *et al.*, 2017), like the *SIX* genes. The accessory genome, also termed as lineage-specific or adaptive genome, can be part of core chromosomes or can encompass entire accessory chromosomes (Henry *et al.*, 2021; van Westerhoven *et al.*, 2023). These accessory chromosomes are known to be required for pathogenicity towards tomato and multiple cucurbit species (Vlaardingerbroek *et al.*, 2016; Van Dam *et al.*, 2017), but little is known about the accessory regions in *Fusarium* spp. causing FWB, other than *F. odoratissimum*.

By analyzing the genome composition of *Fusarium* strains of different races, comparing their candidate effector repertoire, and characterizing their pathogenicity, we sought to better understand host-pathogen interactions in the *Fusarium*-banana pathosystem.

MATERIALS AND METHODS

DNA extraction, whole-genome sequencing, and genome assembly

To generate fungal biomass for the extraction of high molecular weight (HMW) DNA, conidia from monosporic cultures of strains cub0012, cub0034, and cub0088 were harvested from potato dextrose agar (PDA, Thermo Scientific™ Oxoid™, Landsmeer, the Netherlands) plates, transferred to potato dextrose broth (1/5 strength) and grown for three days (25°C, 150 rpm). Subsequently, fungal mycelia were collected on Miracloth, freeze-dried overnight, and ground to powder with mortar and pestle. High molecular weight (HMW) DNAs were extracted following the protocol described by Chavarro-Carrero *et al.* (2021). Genomic DNA size, concentration, and integrity were assessed by gel electrophoresis, Nanodrop, and Qubit analysis (Qubit™ Flex Fluorometer, Thermo Fisher Scientific, Switzerland).

Strains were sequenced using Oxford Nanopore Technologies (Oxford, UK). For each HMW DNA sample, a R9.4.1 flow cell was loaded and run for 24h. Then, base calling was performed using Guppy (version 3.1.5; Oxford Nanopore Technologies, Oxford, UK) with the high-accuracy base-calling algorithm. Resulting in chromosome-level *de novo* genome assemblies for each of the three sequenced strains. Adapter sequences were removed with Porechop (version 0.2.4, default settings; https://github.com/rrwick/Porechop/tree/master) and reads were self-corrected, trimmed, and assembled using Canu, version 1.8 (Koren *et al*., 2017). Genome assemblies were polished using ONT raw reads with Racon, version 1.5.0 (Vaser *et al*., 2017), and with high-quality Illumina short read using two rounds of Pilon, version 1.24 (Walker *et al*., 2014). Contigs were scaffolded, if needed, using LongStitch, version 1.0.2 (Coombe *et al*., 2021).

Determining effector profile in *Fusarium* strains

The presence of *SIX* genes was screened following the PCR protocol described by Czislowski *et al.* (2021), in the collection of 170 Cuban strains causing FWB (Chapter 2), supplemented with a panel of five *Fusarium* spp. reference strains (Supplementary Table S1). Single-spore colony of each strain were grown on PDA plates for seven days at 28°C. Then, conidiospores were collected and transferred to a flask with 100 mL potato dextrose broth (PDB, 34g/L) (Thermo Scientific™ Oxoid™, Landsmeer, the Netherlands, Landsmeer, the Netherlands) and incubated by shaking at 150 rpm and 25°C, for 5 days. The mycelium was obtained by filtering the inoculum through two layers of sterile Miracloth and washed at least twice with sterile MQ water. Subsequently, the mycelium was freeze-dried overnight in a 2 mL Eppendorf tube and ground in a mortar with a pestle using liquid nitrogen. Genomic DNA of each isolate was extracted using the MasterPure™ Yeast DNA Purification Kit (LGC Biosearch Technologies, Halle-Zoersei, Belgium), according to the manufacturer´s protocol.

A set of 15 primer combinations (Supplementary Table S2) was used to detect the *SIX* genes presence in each strain. All PCRs were conducted in 25 µL reactions consisting of 0.2 µL of GoTaq® G2 DNA Polymerase, 5 µL of GoTaq® Reaction Buffer (Promega Benelux BV, Leiden, the Netherlands), 0.35 µL of dNTPs (10 mM), 10 µM of each primer, 10–20 ng of DNA, and 17.45 µL sterile deionized water. PCR amplification reactions were conducted using a ProFlex thermocycler (Applied Biosystems by ThermoFisher Scientific, Landsmeer, the Netherlands). The thermocycling conditions were as described Czislowski *et al*. (2021). The amplicons were resolved by electrophoresis using 1.5% agarose gel, stained with ethidium bromide, visualized and photographed using the ChemidocTM MP image system (Bio-Rad Laboratories, Veenendaal, the Netherlands). Amplicon sizes were estimated using a 100-bp ladder as reference.

To expand the search for candidate effectors we selected 31 strains, comprising a subset of 21 re-sequenced strains representing the genetic diversity of FWB-causing *Fusarium* strains in Cuba (Chapter 2), along with 10 strains (Supplementary Table S1) representing different races or species from other countries. Effector candidates were predicted based on their association with *mimp*'s as described by van Dam *et al.* (2016). Briefly, ORFs were predicted 5 kb up- and downstream to *mimp*'s ('AGT[GA][GA]G[GAT][TGC]GCAA[TAG]AA') and these were refined

using AUGUSTUS, version 3.3.3 (Stanke & Morgenstern, 2005), with the *Fusarium* species specified. In the set of predicted genes, secreted proteins were predicted using SignalP (version 5) and effectors were predicted using EffectorP (version 3.0).

Plant Materials

Tissue culture plants of the cultivars Grand Naine, Gros Michel, Silk, Pisang Ceylan, Pisang Awak, Kluai namwa khom, Burro CEMSA, and FHIA-01 (Table 1) were obtained from the *Musa* Germplasm Transit Centre of Bioversity International (Leuven, Belgium); and multiplied at Iribov (Heerhugowaard, The Netherlands). They represent six of the most widely grown banana cultivars (Table 1), and Grand Naine (Cavendish subgroup), Gros Michel (Gros Michel subgroup), and Burro CEMSA (Bluggoe subgroup) are the differential cultivars to discriminate Race 4, Race 1, and Race 2, respectively. The remaining cultivars were selected based on their differential susceptibility to FWB. FHIA-01 is a hybrid resistant to Race 1 and less susceptible to TR4 than another banana germplasm (Smith *et al*., 2014; Chen *et al*., 2019; Martínez-de la Parte *et al*., 2023). Kluai namwa khom is a dwarf variant of Pisang Awak which was found to be less susceptible to Race 1 and Race 2 strains under field conditions than other cultivars of this subgroup (Ploetz *et al*., 1999). Similarly, Pisang Ceylan (Mysore subgroup) has proved to be less susceptible to TR4 (Zuo *et al*., 2018; Zhan *et al*., 2022) and to Race 1 than Gros Michel, Silk and Pisang Awak (Ploetz *et al*., 1999; Martínez-de la Parte *et al*., 2023). Finally, Silk and Pisang Awak were selected for their known susceptibility to Race 1 strains. *Musa balbisiana* Colla was selected as a diploid representative of the BB genome.

Upon transport and arrival at Wageningen University & Research (WUR, The Netherlands), plants were retrieved from the plastic containers and transferred to individual 1L pots containing a standard soil (Swedish sphagnum peat 20%, Baltic peat 30%, garden peat 30%, beam structure 20%, grinding clay granules 40.6 Kg/m^3, Lime + MgO 2.5 Kg/m^3, PG-Mix-15-10-20 0.8 Kg/m^3) from the WUR-Unifarm greenhouse facility. The potted plants were acclimatized under plastic for two weeks to maintain high humidity conditions in an environmentally controlled greenhouse compartment (28±2°C, 16h light, and ~85% relativity humidity) and were thereafter grown for ~ 2.5 months prior to inoculation.

TABLE 1. Banana germplasm used in the phenotyping experiments.

Accession name	Accession code[a]	Subgroup	Ploidy	Genome composition
Grand Naine	ITC0180	Cavendish	3x	AAA
Gros Michel	ITC1122	Gros Michel	3x	AAA
Silk	ITC0348	Silk	3x	AAB
Pisang Ceylan	ITC1441	Mysore	3x	AAB
Pisang Awak	ITC0213	Pisang Awak	3x	ABB
Kluai namwa khom	ITC1304	Pisang Awak	3x	ABB
Burro CEMSA	ITC1259	Bluggoe	3x	ABB
FHIA-01	ITC0504	FHIA hybrid	4x	AAAB
Musa balbisiana	NA	Unknown	2x	BB

[a]ITC = International *Musa* germplasm Transit Center. NA = not assigned.

Seeds of *M. balbisiana*, obtained from a plantation in Costa Rica, were surface sterilized (70% ethanol solution for five minutes), rinsed and sowed in 1L pots containing the aforementioned standard soil from the WUR-Unifarm greenhouse facility. Plants were maintained, in an environmentally controlled greenhouse compartment (28±2°C, 16h light, and ~85% relativity humidity), for three to four weeks before inoculation.

Phenotyping trials

The inoculum of each strain (Table 2) was prepared using mung bean broth (2 g of mung beans per 500 ml of water), filtered after five days of incubation (25°C, 150 rpm), and the conidial concentration was adjusted to 1.10^6 conidia/mL. Inoculations (Figure S1) were performed by first wounding the roots of the banana plants using a soil scoop on two opposite sides of the plant inside the pot, followed by drenching the soil with 200 mL of inoculum per liter of soil (García-Bastidas *et al.*, 2019). All experiments were designed following a randomized complete block design with two independent replicates. Experimental units comprised between 6 to 22 plants of each cultivar per inoculated strain (Supplementary S3) and for controls (Mock), plants of the different cultivars were root wounded and drenched with 200 ml of water. During the experiment, plants were watered daily and fertilized (NH_4^+-1.2 mM/L, K^+-7.2 mM/L, Ca^{2+}-4 mM/L, Mg^{2+}-1.82 mM/L, NO^{3-}-12.4 mM/L, SO_4^{2-}-3.32 mM/L, $H_2PO_4^-$-1.1 mM/L, Mn^{2+}-10 µMol/L, Zn^{2+}-5 µMol/L, B-30 µMol/L, Cu^{2+}-0.75 µMol/L, Mo-0.5 µMol/L, Fe/DTPA-50/3%, Fe-EDDHSA-50/3%, pH=5.8) three times per week. Ten weeks after inoculation, plants were uprooted, longitudinally sectioned, and photographed. We evaluated disease severity by quantifying the Rhizome Discoloured Area (RDA) with ImageJ 1.52r software (National Institutes of Health, Bethesda, MD, USA). Individual RDA-values for different *Fusarium* strains per banana cultivar were plotted using the web-tool BoxPlotR (Spitzer *et al.*, 2014), and compared with the Kruskal-Wallis nonparametric analysis followed by Dunn's multiple comparisons test ($p < 0.05$).

TABLE 2. *Fusarium* strains characterized under greenhouse phenotyping experiments.

Strains	Species	Clade[a]	Race	Country
II5	*F. odoratissimum*	1	TR4	Indonesia
CR1.1A	*F. phialophorum*	1	R1	Costa Rica
CNPMF 000-8-01-R1	*F. oxysporum*	3	R1	Brazil
cub0140	*F. purpurascens*	1	R1	Cuba
cub0043	*F. tardichlamydosporum*	2	R1	Cuba
cub0135	*F. tardichlamydosporum*	2	R2	Cuba
cub0034	*F. tardichlamydosporum*	2	R2	Cuba

[a]-Clades according to hierarchical clustering based on DArT presence/absence markers (Chapter 2).

RESULTS

The genomes of the races of the FWB pathogens carry a unique set of lineage-specific chromosomes

To characterize the genome structure of *Fusarium* spp. causing FWB in Cuba, we used Oxford Nanopore DNA sequencing and generated a (near) chromosome-level assemblies of 50.6, 51.2 and 52.6Mbp, corresponding respectively with one strain of *F. purpurascens* (Race 1, strain Cub0012) and two strains of *F. tardichlamydosporum* (Race 2, strains Cub0034, Cub0088). Whole-genome comparisons of Cuban strains with the *F. odoratissimum* (TR4) reference strain II5, revealed that Cuban strains also contain 11 core chromosomes but their accessory regions are composed of two or three larger regions, comprising 4.1, 4.7, and 5.8Mbp, respectively (Figure 1). In *F. purpurascens*, the lineage-specific or accessory regions comprise two entire chromosomes (chromosomes 12 and 13), whereas in *F. tardichlamydosporum* these regions extend to at least three chromosomes (chromosomes 12, 13, and 14, contigs 15 to 22).

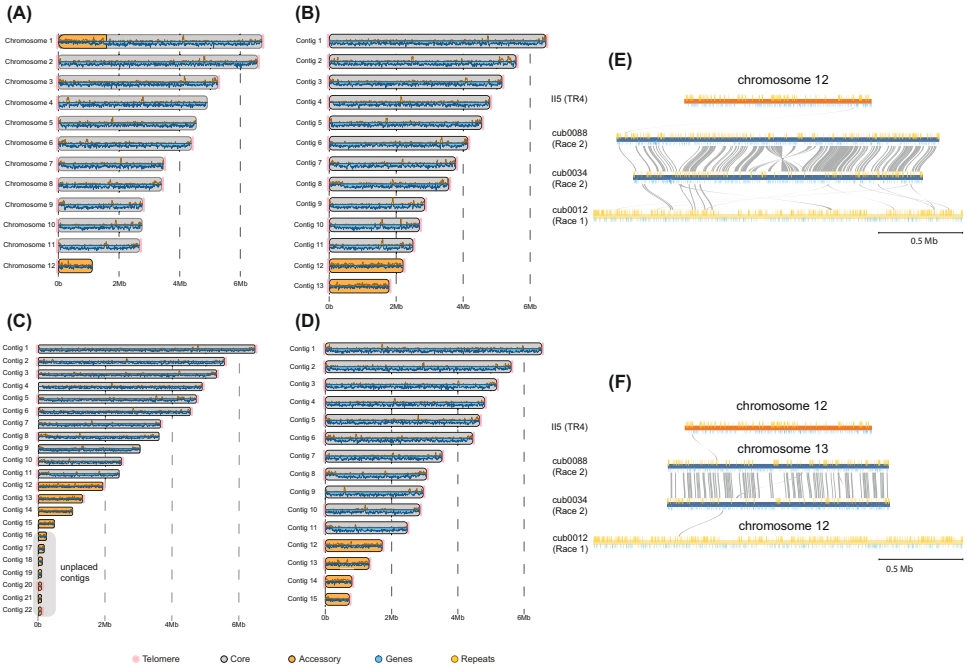


FIGURE 1. The genomes of *Fusarium* spp. infecting banana in Cuba carry two or more accessory chromosomes. (A) The TR4 reference strain II5 contains eleven core chromosomes (chromosomes 1-11) as well as two accessory regions that are highlighted in orange (van Westerhoven *et al.*, 2023). Similar to II5, strains of Race 1 (B) and Race 2 (C and D) also contain 11 core chromosomes but their accessory regions are bigger, encompassing at least three chromosomes. (E) and (F) Both Race 2 strains shared two accessory chromosomes (chromosomes 12 and 13) which show little similarity with Race 1 or TR4 strains.

We compared the accessory regions of the Cuban strains with those from three chromosome-level assemblies of reference strains (van Westerhoven *et al.*, 2023), to characterize the similarity of accessory regions between different species of the causal agent of FWB. The accessory regions of the Race 1 strain of *F. purpurascens* are highly rearranged between the representatives of the different races and show little similarity to *F. phialophorum*, the other Race 1 representative (Supplementary Figure S1). Notably, Race 2 strains shared chromosomes 12 and 13 with extensive similarity between both strains (Figure 1, Supplementary Figure S1). However, these accessory chromosomes of Race 2 strains show little similarity with Race 1 or TR4 strains (Figure 1), suggesting that these accessory regions are specific for Race 2.

Race 1 and Race 2 strains of *Fusarium* spp. infecting bananas contain a variable effector repertoire

We hypothesized that accessory regions of strains of different races contain a distinctive effector repertoire. To test this, we first examined the presence of *SIX* genes, using a PCR diagnostic protocol (Czislowski *et al.*, 2021), in a collection of 170 Cuban strains, together with five strains representative of the different races and *Fusarium* species infecting bananas (Supplementary Table S3).

Our analysis revealed that all Cuban strains examined possess *SIX1, SIX 4, SIX 9,* and *SIX13* genes (Figure 2). Additionally, *SIX6* was detected in 96% of the Cuban strains, while *SIX2* and *SIX8* were present in 27% and 30% of the analysed strains. Notably, *SIX7* was only present in four Cuban strains. The presence of *SIX* genes in Cuban strains was confirmed in a subset of whole-genome sequences of 21 strains (Supplementary Figure S2), which represents the genetic variability found among Cuban strains of *Fusarium* spp. (Chapter 2). Hierarchical clustering based on presence/absence of *SIX* genes did not group strains by race nor by *Fusarium* species (Figure S2).

To further investigate the potential effector repertoire of *Fusarium* spp. causing FWB in Cuba, we used the whole-genome sequences (Supplementary Table S1) of the above-mentioned set of 21 Cuban strains, together with the sequences from 10 other strains representing the different races and *Fusarium* species infecting bananas (van Westerhoven *et al.*, 2023). Based on the proximity of *mimp´*s known to co-localize with certain effector genes in FOSC members (van Dam *et al.*, 2016; Brenes Guallar *et al.*, 2022), we identified 88 candidate effector genes. When we performed hierarchical clustering based on the presence of these candidate effectors, the strains were mainly grouped by species. Notably, *F. tardichlamydosporum* strains grouped in two separate clusters. However, similar to what was described above, the predicted effector repertoires did not clearly separate strains according to the known race phenotypes (Figure 2). Contrary to what has been described for TR4 strains, which have very similar effector profiles (van Westerhoven *et al.*, 2023), different strains of Race 1 or Race 2 showed dissimilarity in their candidate effector profiles, suggesting that these strains can have a more diverse pathogenic phenotype than suggested by the current race concept.

Pathogenicity of *Fusarium* spp. on a banana panel

To investigate whether strains with different effector profiles have a distinct pathogenic phenotype, we challenged nine banana cultivars with seven *Fusarium* strains representative of the different species and races of the FWB pathogen in two independent greenhouse experiments, which took eight months to collect all the data. Phenotypes were assessed quantitively by scoring rhizome discolored area (RDA) ten weeks post-inoculation. The reference strain II5 (*F. odoratissimum*) was the only strain that caused symptoms in all banana cultivars (Figures 4 and 5), which agrees with the described pathogenicity for TR4 strains (Ploetz, 2015). Only three *Fusarium* strains caused RDA median scores higher than 18% on FHIA-01 plants, which confirms the reported level of resistance to FWB of this hybrid (Smith *et al*., 2014; Chen *et al*., 2019; Martínez-de la Parte *et al*., 2023). Overall, strain cub0034 (*F. tardichlamydosporum* Race 2) showed the lowest RDA values when inoculated on the different banana cultivars, which was quite different from the other Race 2 strain cub0088 (*F. tardichlamydosporum*) which has a very similar effector profile (Figure 3). Both Race 2 strains, as expected, showed higher RDA scores on Burro CEMSA, but cub0088 also caused symptoms in Silk, Pisang Ceylan, and Pisang Awak plants (Figures 4 and 5), which are considered to be only susceptible to Race 1 strains (Ploetz, 2015). Similarly, strain cub0043 which represents Race 1 of *F. tardichlamydosporum* was also pathogenic to Burro CEMSA (Figure 4) with a maximum RDA score of 37% (Figure 5), despite its different predicted effector profile compared to the aforementioned Race 2 strains.

Contrary to what was observed for the Race 2 strains, the Race 1 strains R1B (*F. oxysporum*) and cub0043 (*F. tardichlamydosporum*) showed a very similar level of pathogenicity to Race 1 susceptible cultivars despite their different effector profile. Similarly, the other Race 1 strains CR1.1A (*F. phialophorum*) and cub0140 (*F. purpurascens*), which also differ in their effector repertoire, showed the same level of pathogenicity to most of the banana cultivars tested (Figure 5). Thus, the effector repertoire of Race 1 and Race 2 strains does not correlate with their pathogenic phenotype, suggesting that the species comprising these races use different molecular mechanisms to infect banana.

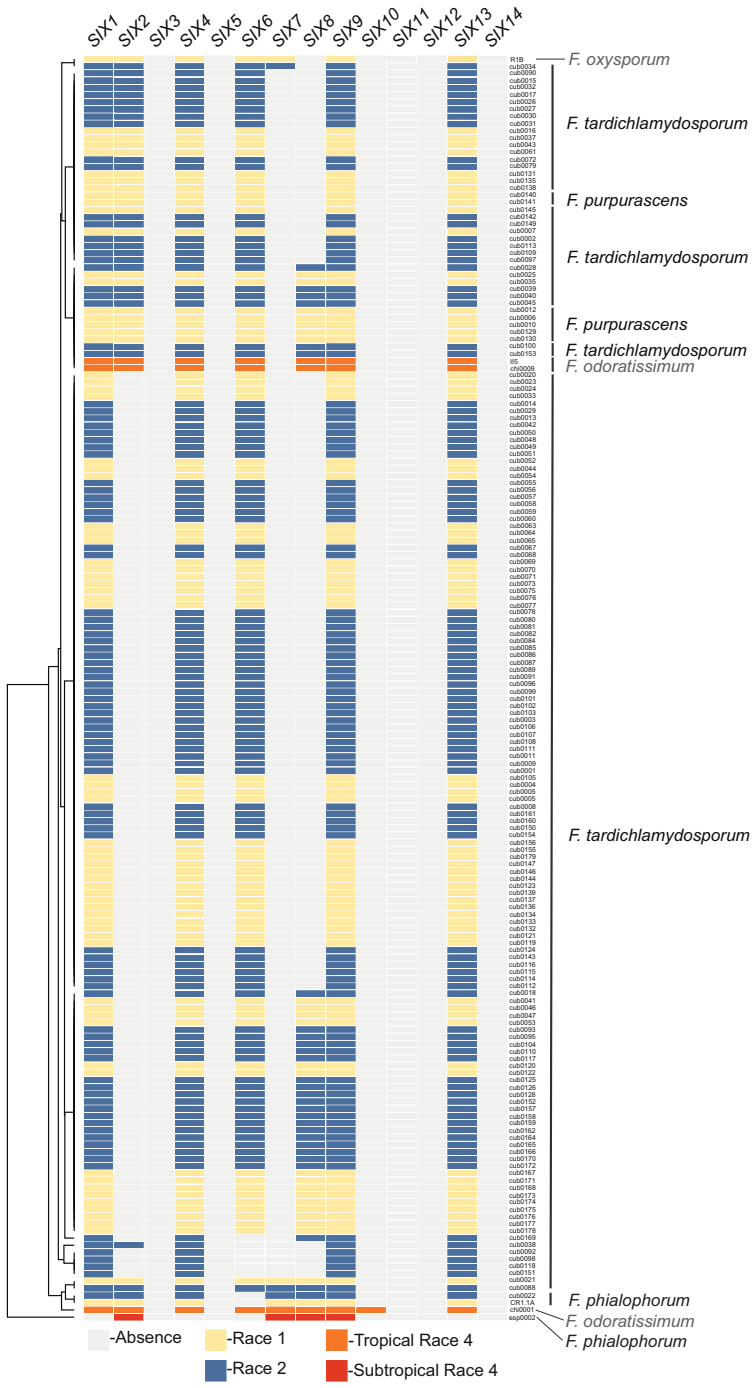


FIGURE 2. Hierarchical clustering based on presence/absence of *SIX* genes does not identify different races or species that cause Fusarium wilt of banana. *SIX* profiles were determined by PCR according to Czislowski *et al*. (2021).

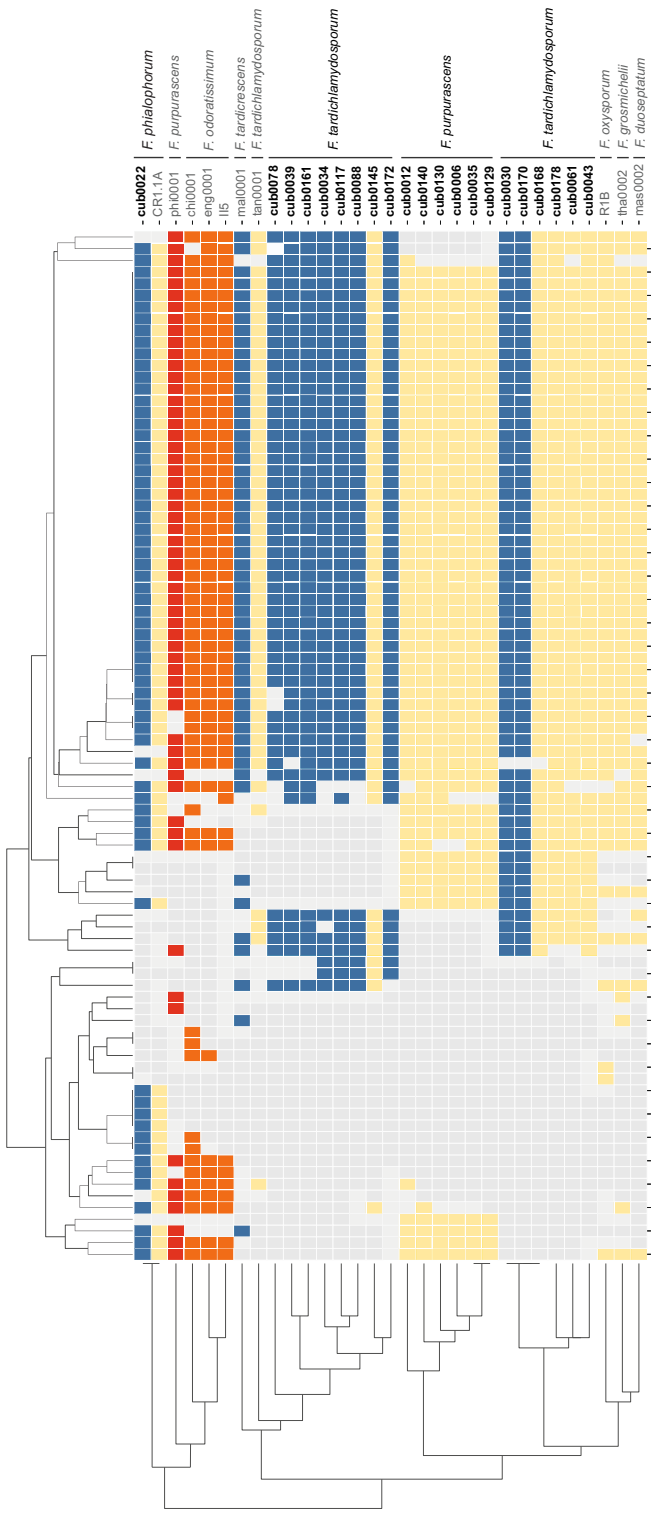


FIGURE 3. *Fusarium* spp. causing Fusarium wilt of banana harbor highly diverse effector repertoires. Based on their association with *miniature impala* (*mimp*) elements, 88 candidate effectors were identified in 32 *Fusarium* strains. The presence of the effectors is represented as blocks colored in yellow (Race 1), blue (Race 2) and orange (TR4), while their absence is represented as a gray block. Overall, hierarchical clustering differentiates the different species but not the races. The codes of Cuban strains are in black and those of other strains or species are in gray.

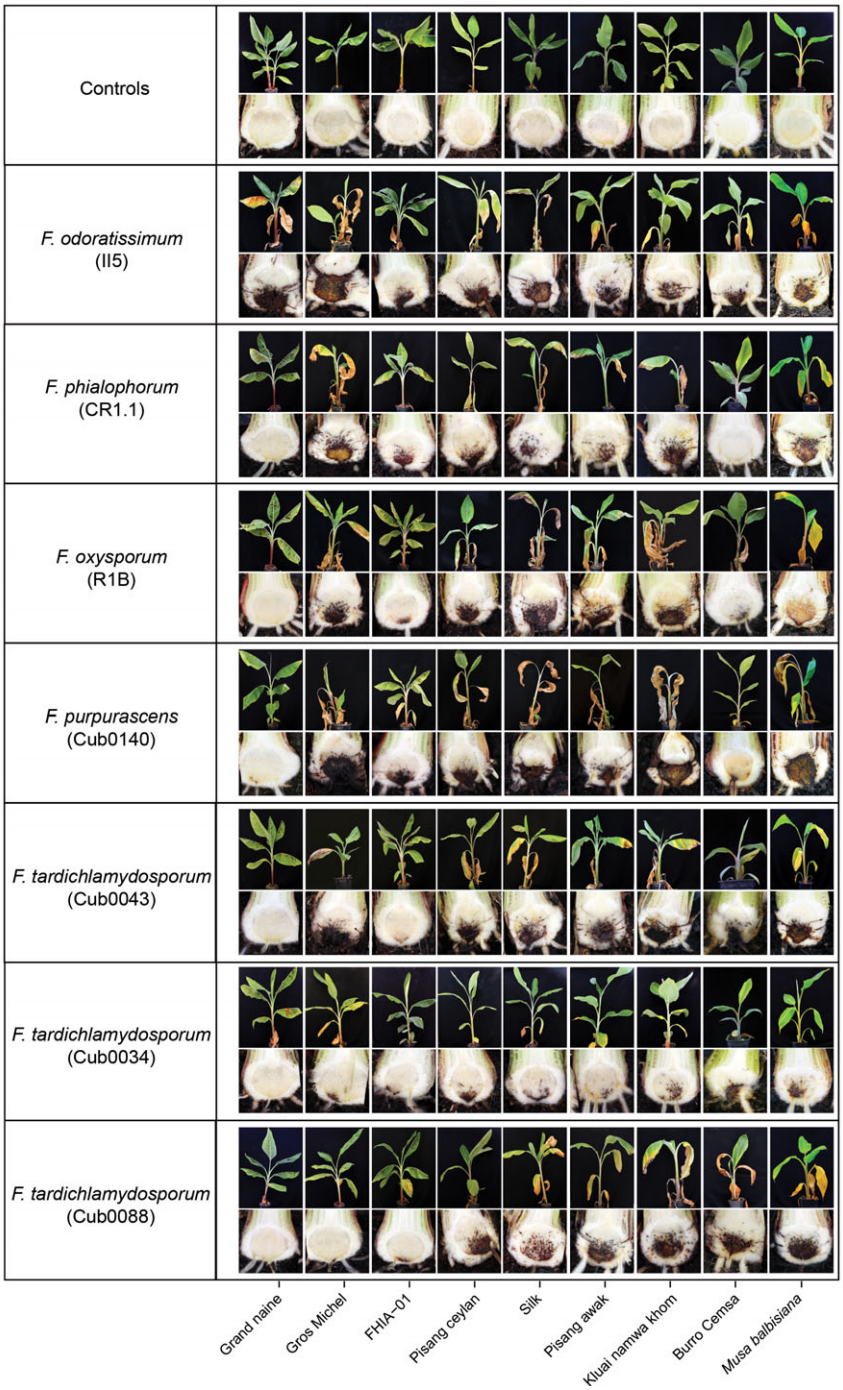
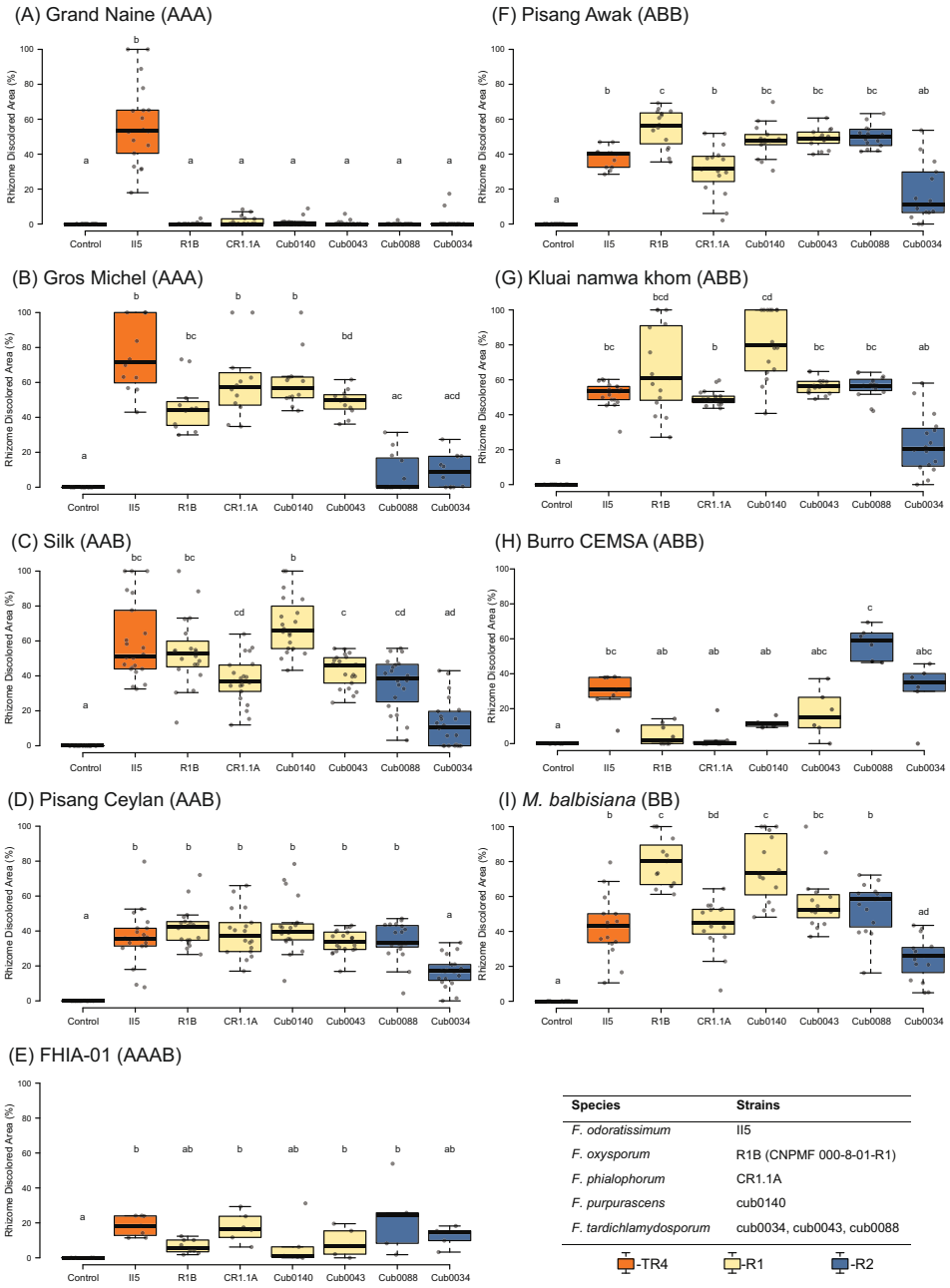


FIGURE 4. Diverse phenotypes observed after inoculating different banana cultivars with seven *Fusarium* strains. Banana plants of nine cultivars cross sectioned 10 weeks after inoculation. In each row, external wilting symptoms are shown in the upper panels, and in the lower panels depict the internal symptom of rhizome discoloration. Plants inoculated with water (controls) remained asymptomatic.



Species	Strains
F. odoratissimum	II5
F. oxysporum	R1B (CNPMF 000-8-01-R1)
F. phialophorum	CR1.1A
F. purpurascens	cub0140
F. tardichlamydosporum	cub0034, cub0043, cub0088

-TR4 -R1 -R2

FIGURE 5. Differential pathogenicity of seven *Fusarium* strains on nine banana cultivars. Plants were observed 10 weeks after inoculation and internal symptoms were scored as rhizome discolored area (RDA) by Image J calculations. Dots correspond with individual scores of RDA, and the median values are indicated by a line across the box. Different letters indicate a significant difference (Kruskal-Wallis and Dunn's multiple comparison tests, $6 \leq N \geq 22$, $p < 0.05$).

DISCUSSION

The interaction between pathogens and their hosts creates significant bidirectional selection pressures (Torres *et al.*, 2020). Plants defend themselves by deploying a diverse range of immune responses. However, successful pathogens secrete an arsenal of effectors that manipulate the plant immune system and promote disease (Rodriguez-Moreno *et al*., 2018; Lovelace *et al*., 2023). Those effector genes are encoded in dynamic, fast-evolving genome compartments, referred to as lineage-specific or accessory regions (Dong *et al.*, 2015). Thus, a major interest in the post-genomics era has been to accurately identify the effector repertoire from a given pathogen and to understand the factors influencing the localization of effector genes in plant pathogen genomes (Torres *et al.*, 2020; Lovelace *et al.*, 2023).

Fusarium wilt of banana (FWB) is one of the most devastating plant diseases, and a major threat to food security in tropical and subtropical countries, where banana is a major staple crop (Ploetz, 2000; Staver *et al.*, 2020; Drenth & Kema, 2021). The disease is caused by a suite of soil-borne *Fusarium* species, of which *F. odoratissimum* comprises exclusively TR4 strains, while Race 2 strains belong to *F. phialophorum*, *F. tardicrescens* and *F. tardichlamydosporum* and Race 1 strains are present in at least seven species (Maryani *et al.*, 2019; Czislowski *et al.*, 2021; Santos *et al.*, 2022; Chapter 2). Race 1 strains caused epidemics in Gros Michel bananas in the 1900s and still affects regionally important banana cultivars, while TR4 strains which can affect Race 1 and Race 2 susceptible banana cultivars, cause the ongoing FWB pandemic in Cavendish plantations over the last 60 years (Ordóñez *et al*., 2015; Drenth & Kema, 2021; van Westerhoven *et al*., 2022). Despite the characterized diversity of the FWB pathogens (Ordóñez *et al*., 2015; Karangwa *et al.*, 2018; Magdama *et al*., 2020; Batista *et al.*, 2022; Mostert *et al*., 2022), little is known about the genome structure, accessory regions and their relationship to pathogenicity, with a focus on *F. odoratissimum* (Raman *et al*., 2021; van Westerhoven *et al.*, 2023).

Here we generated three (near) chromosome-level genome assemblies. One for *F. purpurascens* (Race 1) and two for *F. tardichlamydosporum* (Race 2), and compared them with reference genome assemblies of *F. odoratissimum* (TR4) and *F. phialophorum* (Race 1) to gain insights into their genomic structure and the diversity of accessory regions. Similar to other members of the FOSC (Ma *et al.*, 2010; Vlaardingerbroek *et al*., 2016; Liu *et al.*, 2019; Pontarotti *et al.*, 2023), the genomes of *F. purpurascens* (Race 1) and *F. tardichlamydosporum* (Race 2) are organized into 11 core chromosomes and accessory regions. However, these accessory regions are larger than those described for *F. odoratissimum* and *F. phialophorum* (van Westerhoven *et al*., 2023), comprising two or even three whole chromosomes. Notably, we found a remarkable similarity and specificity between the accessory regions of Race 2 strains, in contrast to those of Race 1 strains which were highly rearranged between the representatives of the different races. This led us to hypothesize that Race 2 strains possess distinct candidate effector gene profiles that define their pathogenicity towards specific banana cultivars.

However, intriguingly, during phenotyping experiments, Race 2 strains with similar effector profiles varied quantitatively in their pathogenicity to some of the banana cultivars. Moreover, cub0043 a *F. tardichlamydosporum* Race 1 strain showed similar pathogenicity to Burro CEMSA

(used as a reference for Race 2 designation), with RDA scores not significantly different from those of Race 2 strains, despite having different effector profiles. Furthermore, Race 1 strains with different effector profiles were equally pathogenic to different banana cultivars. These findings suggest that Race 1 and Race 2 strains of *F. purpurascens, F. phialophorum,* and *F. tardichlamydosporum* use varied molecular mechanisms to infect bananas.

Overall, the effector profiles determined here failed to distinguish Race 2 strains from Race 1 strains, which is consistent with the findings of Carvalhais *et al.* (2019) who also encountered challenges in distinguishing these races using *SIX* gene sequences. However, these results differ from observations in tomato, where *SIX* genes have been used to identify and distinguish races in Fol. For instance, the Fol races 2 and 3 lack the *SIX4* gene present in race 1, and are identified on the basis of variation in *SIX3* sequences (Lievens *et al.*, 2009). The difference between both pathosystems is that in Fol races are differentiated using five tomato cultivars containing race-specific resistance genes (Panthee & Chen, 2009; Biju *et al.*, 2017), whereas in FWB-causing *Fusarium* spp. races are exclusively established on the basis of the pathogenic phenotype against three banana cultivars, but the genetic basis of resistance in these cultivars is unknown. Our phenotyping experiments indicate that the current race concept is inadequate to accurately describe the variation observed in strain pathogenicity. This is, for example, demonstrated by strains cub0043 and cub0088 causing alike symptoms in cultivars that are used to differentiate Race 1 and Race 2 strains (Battle & Pérez-Vicente, 2009; Garcia *et al.*, 2018; García-Bastidas, 2019; Martínez-de la Parte *et al.*, 2023; Pant *et al.*, 2023). These data highlight the need for a more comprehensive and refined race classification system based on the genetic basis of resistance, ideally in a set of isogenic lines, along with routine functional analyses to elucidate key genes controlling interactions between plant and fungus. The presented genomic and phenotypic data further contribute to a better understanding of host-pathogen relationships in the *Fusarium*-banana pathosystem.

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SUPPLEMENTARY MATERIAL

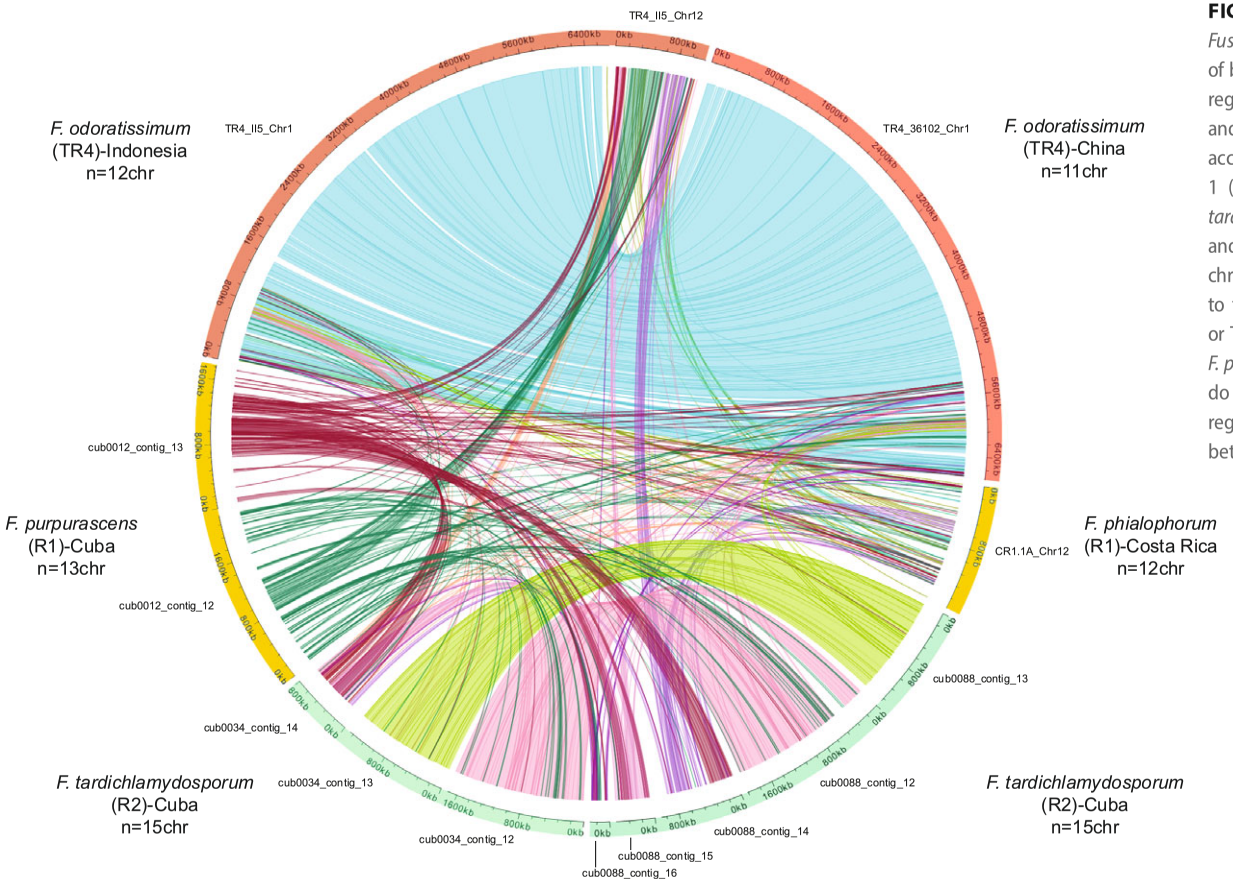

FIGURE S1. The genomes of *Fusarium* spp. causing Fusarium wilt of banana contain diverse accessory regions. *F. odoratissimum* strain II5 and 36102 (both TR4) share the accessory regions on Chromosome 1 (Chr1). Race 2 (R2) strains of *F. tardichlamydosporum* (Cub0034 and Cub0088) share two accessory chromosomes with little similarity to the accessory regions of Race 1 or TR4 strains. Race 1 (R1) strains of *F. purpurascens* and *F. phialophorum* do not share any larger accessory region, which are highly rearranged between all studied strains.

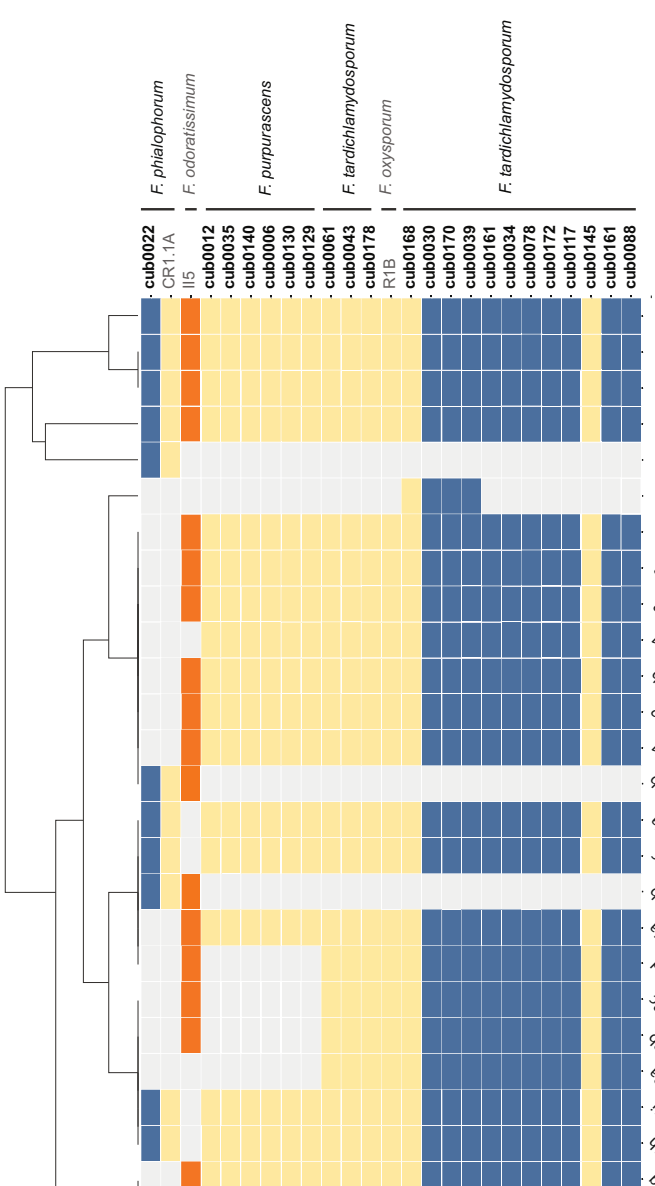

FIGURE S2. Cuban strains of the Fusarium wilt of banana pathogens harbor multiple allelic variants of *SIX* genes. Blocks colored in yellow (Race 1), blue (Race 2), and orange (TR4) indicate presence while gray blocks indicate absence of effectors. Codes of Cuban strains are in black and those from strains representing different species or regions are in gray.

TABLE S1. Codes and details of origin and references, phylogeny, and races of *Fusarium* spp. causing Fusarium banana wilt of banana whose genomes were used in this study.

Strain	Alternative Code	Country	Host	Species	clade[a]	Race[b]	Sequencing technology	Reference
bra0002	FocCNPMF-08-R1	Brazil	Silk	*F. oxysporum*	c3	R1*	Short-read (Illumina/BGISeq)	van Westerhoven et al., 2023
chi0001	NRRL36102	China	Cavendish	*F. odoratissimum*	c1	TR4*	Long-read(Nanopore)/Short-read (Illumina/BGISeq)	van Westerhoven et al., 2023
CR1.1A	-	Costa Rica	Gros Michel	*F. phialophorum*	c1	R1*	Long-read(Nanopore)/Short-read (Illumina/BGISeq)	van Westerhoven et al., 2023
Cub0006	Cub9	Cuba	Bluggoe	*F. tardichlamydosporum*	c2	R2	Short-read (Illumina/BGISeq)	Chapter 2
cub0012	Cam3	Cuba	FHIA-03	*F. purpurascens*	c1	R1*	Long-read(Nanopore)/Short-read (Illumina/BGISeq)	This study; Chapter 2
cub0022	WaHa	Cuba	Bluggoe	*F. tardichlamydosporum*	c2	R2*	Short-read (Illumina/BGISeq)	Chapter 2
Cub0030	BaHo 1	Cuba	Bluggoe	*F. tardichlamydosporum*	c2	R2	Short-read (Illumina/BGISeq)	Chapter 2
Cub0034	R-2	Cuba	Bluggoe	*F. tardichlamydosporum*	c2	R2*	Short-read (Illumina/BGISeq)	Chapter 2
Cub0035	JCien	Cuba	Pisang Awak	*F. tardichlamydosporum*	c2	R1*	Short-read (Illumina/BGISeq)	Chapter 2
Cub0039	NarBaGu	Cuba	Bluggoe	*F. tardichlamydosporum*	c2	R2	Short-read (Illumina/BGISeq)	Chapter 2
Cub0043	PunBra1	Cuba	Pisang Awak	*F. tardichlamydosporum*	c2	R1*	Short-read (Illumina/BGISeq)	Chapter 2
Cub0061	Min2Art	Cuba	Pisang Awak	*F. tardichlamydosporum*	c2	R1	Short-read (Illumina/BGISeq)	Chapter 2
Cub0078	FloCam2	Cuba	Bluggoe	*F. tardichlamydosporum*	c2	R2	Short-read (Illumina/BGISeq)	Chapter 2
Cub0088	MaHo5	Cuba	Bluggoe	*F. tardichlamydosporum*	c2	R2*	Long-read(Nanopore)/Short-read (Illumina/BGISeq)	This study; Chapter 2
Cub0117	LaMSC2.2	Cuba	Bluggoe	*F. tardichlamydosporum*	c2	R2	Short-read (Illumina/BGISeq)	Chapter 2
Cub0129	SJMay4	Cuba	Pisang Awak	*F. purpurascens*	c1	R1*	Short-read (Illumina/BGISeq)	Chapter 2
Cub0130	CabSS1	Cuba	Pisang Awak	*F. purpurascens*	c1	R1*	Short-read (Illumina/BGISeq)	Chapter 2
Cub0140	CiVC1	Cuba	Pisang Awak	*F. purpurascens*	c1	R1*	Short-read (Illumina/BGISeq)	Chapter 2
Cub0145	MAnVC1.1	Cuba	Pisang Awak	*F. tardichlamydosporum*	c2	R1*	Short-read (Illumina/BGISeq)	Chapter 2
Cub0161	PalmPR3.1	Cuba	Bluggoe	*F. tardichlamydosporum*	c2	R2	Short-read (Illumina/BGISeq)	Chapter 2
Cub0168	SanJPR3	Cuba	Pisang Awak	*F. tardichlamydosporum*	c2	R1	Short-read (Illumina/BGISeq)	Chapter 2
Cub0170	SanLPR1	Cuba	Bluggoe	*F. tardichlamydosporum*	c2	R2	Short-read (Illumina/BGISeq)	Chapter 2
Cub0172	SanLPR3	Cuba	Pisang Awak	*F. purpurascens*	c1	R1	Short-read (Illumina/BGISeq)	Chapter 2
Cub0178	Art3Art3	Cuba	Pisang Awak	*F. tardichlamydosporum*	c2	R1	Short-read (Illumina/BGISeq)	Chapter 2
eng0001	Eden	United Kingdom	NA	*F. odoratissimum*	c1	TR4	Short-read (Illumina/BGISeq)	Warmington et al. 2019
II5	-	Costa Rica	Pisang Manurung	*F. odoratissimum*	c1	TR4	Short-read (Illumina/BGISeq)	Maymon et al. 2020
mal0001	NRRL_36113	Malawi	Harare	*F. tardicrescens*	c2	R2	Short-read (Illumina/BGISeq)	van Westerhoven et al., 2023
mas0002	NRRL_36115	Malaysia	Pisang Ambon	*F. duoseptatum*	c2	R1	Short-read (Illumina/BGISeq)	van Westerhoven et al., 2023
phi0001	NRRL_36103	Philippines	Cavendish	*F. purpurascens*	c1	STR4	Short-read (Illumina/BGISeq)	van Westerhoven et al., 2023
tan0001	NRRL_36108	Tanzania	Ney Poovan	*F. tardichlamydosporum*	c2	R1	Short-read (Illumina/BGISeq)	van Westerhoven et al., 2023
tha0002	NRRL_36120	Thailand	Kluai nam wa	*F. grosmichelii*	c2	R1	Short-read (Illumina/BGISeq)	van Westerhoven et al., 2023

[a]Clade according to Chapter 2 and van Westerhoven *et al.* (2023). [b]Race determined based on the host of isolation or by pathogenicity test (*).

TABLE S2. Primer combinations for detecting *SIX* gene presence in a collection of 180 *Fusarium* strains.

Target Gen	Primer	Sequence (5´–3´)	Expected Amplicon Size (bp)	Annealing Temperature (°C)
SIX1	SIX1f	TCT CCA TTA CTT TGT CTC ACG	694-733	58
	SIX1r	CGA TTT AGG CGA TTC GGG G		
SIX2	SIX2f	GGT TCC CAT CGT TGA AGC	327-330	57
	SIX2r	TTG GTT TAA ATC TGC GTG TC		
SIX3	SIX3f	TTA CTA CGA GCT TCA GCA CC	223	60
	SIX3r	GCA TTA GGT GTT GCA ACA GG		
SIX4	SIX4f	CAG CTC AGA CAG TCA GCC	~491	58
	SIX4r	GGC CTT GAG TCG AAT GAG C		
SIX5	SIX5f	TCA TCA GTA CTG TGC TTG CC	347-354	59
	SIX5r	CAT GTT GAG TCT GCT CCT CC		
SIX6	SIX6f	CTC TCG AGA CAC SCT TCC	396-399	58
	SIX6r	GAT CCA CCA ATA CCT TCA T		
SIX7	SIX7f	GAG GTG ACA TTT GAC ATC ACC	113	60
	SIX7r	TAG TAT GCG CGC CAT TGG		
SIX8	SIX8f	CCC TAG CCG TCT CTG TGG C	163-165	64
	SIX8r	CGT TCG ACA AGG GCT CTC TCG		
SIX9 G1	SIX9G1f	TTC AAG TCG GTT GCT ACG C	118	58
	SIX9G1r	GCA TCC CAA AAT CCA AAG CG		
SIX9 G2	SIX9G2f	CCG TCT TCT CTA CCG CCG	288	58
	SIX9G2r	AGT TGA CGC AAG CAA AGT CG		
SIX10	SIX10f	TCA CGT TTC GAG TTG GTC C	202	60
	SIX10r	ACA CCA AAT CGA GTC GAT GC		
SIX11	SIX11f	GTT GCT CCT CCT TTG CTG G	163	62
	SIX11r	TAC CAC TCT GAC CAG TCA CC		
SIX12	SIX12f	CAG AAT GCT TGT GTG TGT GG	171	61
	SIX12r	ATC ACC AGA GCA TGA ACC CC		
SIX13	SIX13f	TCT GAT CAG CCT CCT AGC GT	840	60
	SIX13r	CCA CTG TAA CTC GGC ATC GA		
SIX14	SIX14f	TGT CTC AGC GTA TCC TCG GC	147-197	61
	SIX14r	ATT CAG TGA CAA CGG GAC CG		

TABLE S3. Number of plants used in the different treatments of the phenotyping experiment. Mock-plants treated with water. R1B-plants were inoculated with the *F. oxysporum* strain CNPMF 000-8-01-R1 from Brazil.

Banana cultivar	Treatment							
	Mock	II5	R1B	CR1.1A	Cub0140	Cub0043	Cub0088	Cub0034
Grand naine	16	18	18	18	18	18	18	18
Gros Michel	11	12	12	12	12	12	12	12
Silk	16	22	20	22	20	22	20	22
Pisang Ceylan	16	19	18	18	19	20	20	19
Pisang Awak	10	12	15	15	14	14	14	15
K. namwa khom	10	17	15	17	16	16	15	16
Burro CEMSA	5	7	8	9	6	6	6	6
FHIA-01	5	7	8	6	5	5	5	5
Musa balbisiana	10	15	15	15	15	15	15	15

CHAPTER 7

General discussion

INTRODUCTION

Agriculture impacts society in many ways, by supporting livelihoods through food and jobs and generating profits through trade. However, plant pathogens and abiotic threats- such as drought, floods, or hurricanes- have caused considerable losses in agriculture, with significant social impact as they threaten food security, especially when staple crops are affected (Koeppel, 2008; Bebber *et al.*, 2014; Varma & Bebber, 2019; Fones *et al.*, 2020; Turbelin *et al.*, 2023). Bananas are widely cultivated in tropical and subtropical regions around the world, where they provide an important portion of dietary diversity and income (Heslop-Harrison & Schwarzacher, 2007). Bananas are also ubiquitous in their availability in non-producing regions through international trade, which accounts for 15% of global production (FAO, 2022a). This international trade supplements nutritional diversity in non-producing countries, while making a large contribution to local and national economies in producing countries (Varma & Bebber, 2019; Bebber, 2022).

Sustainable production of bananas faces a significant threat from Fusarium wilt of banana (FWB), a devastating plant disease (Stover, 1962; Ploetz, 2015b), caused by various soil-borne *Fusarium* species (Maryani *et al.*, 2019; van Westerhoven *et al.*, 2023). Among these, *Fusarium odoratissimum* (TR4) has emerged as a major pathogen that poses a serious risk to global banana production, due to its virulence on Cavendish, the banana variety that dominates global productions (>50%) and export trade (>95%) (Lescot, 2020; FAO, 2022a). In addition to its impact on Cavendish, TR4 also affects other regionally important banana cultivars, threatening food security in those countries that heavily rely on bananas as basic staple food (Fones *et al.*, 2020; van Westerhoven *et al.*, 2022). Given the lack of effective management models, including resistant cultivars and soil management approaches, the current spread of TR4 through main banana-producing regions of the world is of great concern. To address this pressing issue, it is crucial to conduct in-depth studies of the diversity of causal fungi responsible for FWB and their range of hosts, to support breeding programs and to develop efficient management strategies.

Toward a meaningful classification of strains of the FWB pathogen

Fungal species are generally defined and recognized using various approaches, such as the morphological species concept (phenotype), the biological species concept (sexual reproduction), and the phylogenetic species concept (evolutionary relationships) (Xu, 2020). Within the *Fusarium* genus, the *F. oxysporum* species complex (FOSC) comprises pathogenic isolates and putatively non-pathogenic isolates ubiquitously present in soils, which cannot be differentiated morphologically (Leslie & Summerell, 2006; Lombard *et al.*, 2019). Although recent findings suggest that sexual reproduction in *F. oxysporum* f. sp. *ciceris* could take place (Fayyaz *et al.*, 2023), members of FOSC have no known sexual stage (Lombard *et al.*, 2019). Consequently, traditional methods like the morphological and biological species concepts remained but are inadequate for defining FOSC members. Instead, the prevalent nomenclature employed in the scientific literature for strains within FOSC is the use of *forma specialis* (f. sp.),

a grouping system based on pathogenicity towards a plant host or a group of - usually related - hosts (Snyder & Hansen, 1940; Leslie & Summerell, 2006; Lievens *et al.*, 2009a). However, it is important to note that this widely adopted classification usually does not reflect the phylogeny of strains (van Dam *et al.*, 2018; van Westerhoven *et al.*, 2023).

Nearly all *formae speciales* show a polyphyletic structure (O'Donnell *et al.*, 1998; Fourie *et al.*, 2009; Biju *et al.*, 2017; van Dam *et al.*, 2018; Batson *et al.*, 2021; Sabahi *et al.*, 2021; Mostert *et al.*, 2022; Pontarotti *et al.*, 2023). Strains with pathogenicity to bananas are grouped under f. sp. *cubense*, which is the only *forma specialis* named after the origin of the first characterized strains (Cuba) and not by the crop it affects. Further subdivisions of *forma specialis* include the use of races, vegetative compatibility groups (VCG) and phylogenetic clades/lineages. However, the relationships among these classification systems are complex in populations of the causal agent of FWB.

Physiological specialization towards particular banana cultivars resulted in the definition of races; named Race 1, Race 2 and Race 4, the latter being divided into Subtropical Race 4 (STR4) and Tropical Race 4 (TR4). Race 1 strains have been identified in no less than nine VCGs, are non-pathogenic to Cavendish under normal conditions, but can cause FWB under abiotic stress and are then categorized as STR4 (Buddenhagen, 2009; Ploetz, 2015b; Mostert *et al.*, 2017). Strains of the same race can belong to different VCGs and vice versa, illustrating that there is no strict correlation between VCGs and races in the FWB-banana pathosystem (Ordóñez *et al.*, 2015). Although the race concept is important for communicating disease outbreaks and describing banana cultivar responses (Buddenhagen, 2009; Ploetz, 2015b), it does not accurately reflect the virulence spectrum of pathogenic strains. In Chapters 2 and 6, we demonstrate that some Cuban Race 1 strains also cause disease in Bluggoe, and some Race 2 strains also cause disease symptoms in Gros Michel, Silk, and Pisang Awak plants. These findings are consistent with previous studies using strains from Brazil, East Africa, Florida, Nepal, and Puerto Rico (Ploetz & Churchill, 2011; Garcia *et al.*, 2018; García-Bastidas, 2019; Martínez-de la Parte *et al.*, 2023; Pant *et al.*, 2023), and challenge the current race classification of strains of the FWB pathogens.

Unlike in strains causing FWB, races in *F. oxysporum* f. sp. *lactucae* are differentiated by their distinct pathogenicity on six lettuce cultivars (Gilardi *et al.*, 2017). Similarly, in f. sp. *lycopersici* (Fol) races are differentiated using five tomato cultivars containing race-specific resistance (*R*) genes (Panthee & Chen, 2009; Biju *et al.*, 2017). Tomato *R* genes that confer race-specific resistance to Fol are known as immunity (*I*) genes. The three hitherto identified genes *I-1, I-2,* and *I-3* confer resistance against Fol races containing the three matching avirulence genes *AVR1, AVR2*, and *AVR3*, respectively (Rep *et al.*, 2004; Houterman *et al.*, 2008; Gawehns *et al.*, 2014; Ma *et al.*, 2015). *AVR3,* also known as *SIX1* (Secreted In Xylem), is present in all Fol strains. Race 1 strains of Fol are characterized by the presence of *AVR1* (*SIX4*) in their genomes but it is absent in race 2 and race 3 strains. The absence of *AVR1* enables strains of race 2 and race 3 to infect tomato cultivars carrying *I-1*. *AVR2* (*SIX3*) is also present in all Fol strains, albeit those of race 3 contain a point mutation leading to an amino acid change in the protein, which allow them to infect tomato carrying *I-2* gene, which differentiates them from race 2 strains (Lievens *et al.*, 2009b; Houterman *et al.*, 2009).

RGA2 from *Musa acuminata* ssp. *malaccensis* confers resistance to TR4 (Dale *et al.*, 2017), but it is the only *R* gene that has been identified in banana, while the genetic basis of the resistance in Cavendish to Race 1 and Race 2 strains is largely unknown (Ahmad *et al.*, 2020). Hence, races in FWB causing *Fusarium* spp. are established solely on the basis of the pathogenic phenotype against just three banana cultivars (Ploetz, 2015b). Indeed, in Chapter 6 we demonstrated that Race 1 and Race 2 strains of the FWB pathogens have indeed a diverse candidate effector gene repertoire, but cannot be differentiated on this basis, perhaps due to the above-mentioned inaccuracy of race classification of the current system. Thus, I argue that the race concept in the *Fusarium*-banana pathosystem is flawed because it does not usually follow pathogenicity testing with multiple banana cultivars, which is crucial for a comprehensive understanding of the pathogen's behavior and lacks a solid molecular genetic foundation, making it susceptible to misinterpretation. Considering these shortcomings, it is evident that a more refined and molecularly-informed approach is necessary to effectively address the diversity of the *Fusarium*-banana pathosystem.

Lastly, phylogenetic analyses have consistently shown that strains causing FWB are divided into three major clades, which are further subdivided into eight to 10 lineages (Bentley *et al.*, 1998; O'Donnell *et al.*, 1998; Groenewald *et al.*, 2006; Fourie *et al.*, 2009; Mostert *et al.*, 2017, 2022; Maryani *et al.*, 2019). These lineages encompassing the various physiological races were recently classified as individual phylogenetic species, with TR4 strains classified as *Fusarium odoratissimum* and strains of Race 1 comprising a suite of seven *Fusarium* spp., while Race 2 strains belong to *F. phialophorum, F. tardicrescens* and *F. tardichlamydosporum* (Maryani *et al.*, 2019; Czislowski *et al.*, 2021; Santos *et al.*, 2022). This new nomenclature of FWB causing *Fusarium* spp. has generated some controversy (Torres Bedoya *et al.*, 2021), but the data generated in Chapter 2 and by van Westerhoven *et al.* (2023) support this classification.

The integration of comprehensive pathogenicity assessments across different banana cultivars, and the incorporation of molecular genetic insights into pathogen diversity, will allow us to move towards a more accurate classification system that will facilitate improved disease management and banana crop protection.

The unstoppable expansion of the banana menace

Current agricultural systems, characterized by intensive monoculture practices, and globalized markets are driving the emergence and spread of new pathogens, with severe direct impacts on the production of staple crops and key commodity crops (Fones *et al.*, 2020; Bebber, 2022). The costs of these pathogen invasions have increased dramatically over the past 20 years, emulating the magnitude of the impact of natural hazards (Turbelin *et al.*, 2023).

The banana industry has historically relied on monocultures of nearly identical genotypes. Monocultures are easier to manage, and identical plants and fruits are easier to plant, grow, harvest, process, and transport (Bebber, 2022). However, the large-scale monoculture based on a single cultivar has resulted in an extreme level of genetic vulnerability to globally emerging diseases (Drenth & Kema, 2021). As a result, the banana industry based on

the exploitation of the Gros Michel cultivar succumbed to the first epidemic of FWB in the first half of the 20th century (Ploetz & Churchill, 2011; Ploetz *et al.*, 2015). Thus, the industry eventually moved away from Gros Michel, and replaced it with another group of genetically identical cultivars, the Cavendish. These cultivars are resistant to the Race 1 strains of various *Fusarium* spp., responsible for the annihilation of Gros Michel plantations. Since then, commercial banana production has been based on monocultures of Cavendish cultivars (Drenth & Kema, 2021; Bebber, 2022).

The replacement of Gros Michel with bananas of the Cavendish subgroup did not solve the inherent vulnerability of growing a single clone on a global scale. As a consequence, we are now facing a new FWB pandemic, caused by *F. odoratissimum* (TR4), which originates from Indonesia and disseminates across continents thereby threatening the global banana industry and food security of producing countries (See Chapter 2; Maryani *et al.*, 2019; Ordóñez *et al.*, 2015; Ploetz, 2021; Westerhoven *et al.*, 2022). The pathogen was first identified in Taiwan killing and severely damaging Cavendish bananas (Sun *et al.*, 1978), and for the next 45 years, its distribution was restricted to Southeast Asia and Northern Australia. However, since TR4 was detected in Jordan (García-Bastidas *et al.*, 2014), its spread has accelerated dramatically reaching Africa, the Indian subcontinent, the Middle East, and Latin America within a decade (García-Bastidas *et al.*, 2014; Ordóñez *et al.*, 2015; Maymon *et al.*, 2018; Thangavelu *et al.*, 2019; Aguayo *et al.*, 2020; Garcia-Bastidas *et al.*, 2020; Acuña *et al.*, 2021; van Westerhoven *et al.*, 2022a; Herrera *et al.*, 2023), with the latest expansion into the Comoros archipelago (Mmadi *et al.*, 2023). This accelerated spread can be explained by the anthropogenic movement of the pathogen; possible transmission routes include the movement of infected rhizomes, the mobility of banana stakeholders, or the transport of spores in clothing and footwear (Drenth & Guest, 2016; Fones *et al.*, 2020)

The incursions into Latin America and the Caribbean (LAC) triggered global concerns, as 75% of international export bananas are produced in the region (FAO, 2022). For Cuba, these incursions are particularly worrisome for food security because bananas are indispensable as staple food, representing nearly 30% of all fruit produced in the country (ONEI, 2022). In Chapter 2 we show that FWB is present in all banana-producing regions of Cuba, affecting many popular varieties. In addition, nearly 56% of the Cuban national acreage is planted with varieties that are susceptible or very susceptible to TR4, as highlighted in Chapter 3. In this scenario, the phytosanitary surveillance aimed at detecting an outbreak of TR4 in Cuba seems to be an extremely difficult task, that will require the processing of a huge amount of samples at a considerable cost. However, if the general strategy - driven by limiting costs – only focuses on detecting FWB cases in Cavendish or Plantains, there is a huge risk that the first case of TR4 will go unnoticed. Consequently, once the outbreak is detected, it will be too late to implement successful containment strategies, similar to all previous TR4 outbreaks (van Westerhoven *et al.*, 2022b; Nakasato Tagami *et al.*, 2023; Herrera *et al.*, 2023).

The apparently unstoppable spread of TR4 mirrors what happened with Race 1 during the Gros Michel era. Therefore, considerable efforts should be directed to proactively identify suspect cases, support breeding programs to improve the identification or development of new

resistant banana genotypes, and to find effective treatments for the disease. At the same time, it is extremely important to update and implement strict exclusion and biosecurity measures by using certified disease-free planting material and preventing the movement of contaminated soil and water. However, the level of success of these measures will heavily depend on the commitment of growers, industry, scientists, governments, and regional phytosanitary organizations for their implementation.

The implications of a pathogen expanded host range for FWB management

The use of resistant cultivars is frequently stated as the only effective measure to manage FWB (Ploetz, 2015a,b), and indeed Cavendish bananas stopped the Race 1- driven epidemic. However, at present there is no commercial banana resistant to *F. odoratissimum* (TR4). Therefore, the use of clean planting material and machinery, and the quarantining of infested farms are currently the only strategies to control its further dissemination (Ploetz, 2015a; Dita *et al.*, 2018; Viljoen *et al.*, 2020).

The ongoing global spread shows that FWB successfully disseminates despite these extensive prevention efforts. Being a soil-borne pathogen, eradication of TR4 once it enters a new area is very difficult, as illustrated by the cases of Colombia, Mozambique, and Peru, where TR4 spread to new areas despite the implementation of containment strategies (van Westerhoven *et al.*, 2022a; Reyes-Herrera *et al.*, 2022; Nakasato Tagami *et al.*, 2023). Notably, the documented spread of TR4 primarily involves commercial banana farms, and the unsuccessful management of the disease on these large farms is worrisome for small-scale farms that are prevalent in Africa and the Americas (Dita *et al.*, 2013; van Westerhoven *et al.*, 2022b; Nakasato Tagami *et al.*, 2023). Unlike large commercial farms, smallholders cannot afford the costly quarantine protocols and often have limited expertise in disease and pest management. Consequently, the occurrence of TR4 outside commercial farms goes largely unaddressed, facilitating its spread, and posing a direct threat to the livelihoods, and food security of those depending on banana cultivation (van Westerhoven *et al.*, 2022b).

Traditionally, after a TR4 incursion is identified based on visual wilting symptoms in Cavendish plants in combination with diagnostic protocols, the eradication of infected plants takes place and pathogen containment strategies are implemented (Dita *et al.*, 2018). However, these strategies tend to overlook the presence of asymptomatic secondary hosts in the field. In Chapter 4 we showed that TR4 can endophytically colonize 12 weed species from different botanical families, which expands previous findings reported under Australian field conditions (Hennessy *et al.*, 2005). The colonization of these weeds may play a significant role in TR4 long-term survival. Moreover, in Chapter 5 we demonstrate that several species of *Heliconia*, can be affected by TR4, with some displaying no external symptoms. This finding is particularly relevant given the vast diversity of *Heliconia* species present in LAC where TR4 is actively spreading. Overall, these findings highlight the importance of secondary hosts as potential reservoirs for the pathogen and call for a re-evaluation of quarantine regulations and disease management strategies.

Therefore, it is imperative to implement measures to address this risk effectively. Firstly, *Heliconia* plants should not be grown near or within banana plantations. Additionally, the certification of TR4-free *Heliconia* plants must be regularly and legally verified during export or import activities. Furthermore, containment protocols must include the eradication of weeds in and near TR4-infested plots. Additional research is needed to ascertain whether the use of herbicides is sufficient to eliminate TR4 inoculum in those secondary hosts.

Knowing the enemy´s strategy - a way to improve banana breeding programs

As argued above, the use of resistant cultivars is the basis for sustainable disease management (Carolan *et al.*, 2017), and the most effective means to manage FWB (Dita *et al.*, 2018). The resistance of Cavendish to Race 1 had an enormous impact on the banana industry contributing to near-complete Cavendish dominance in export trade (FAO, 2022; van Westerhoven *et al.*, 2022b). However, TR4 circumvents this resistance and next to Cavendish cultivars it also affects a wide range of banana germplasm (Zuo *et al.*, 2018; Chen *et al.*, 2019; García-Bastidas, 2019; Zhan *et al.*, 2022; Chapter 3). Thus, we are now confronted with the consequence of a huge knowledge gap in host resistance, consequently resulting in an overall absence of commercial cultivars with adequate resistance to TR4. Despite several years of research, the identification and characterization of genes conferring resistance (*R* genes) to TR4 and R1 remain scarce (Paul *et al.*, 2011; Fraser-Smith *et al.*, 2016; Dale *et al.*, 2017; Sun *et al.*, 2019; Ahmad *et al.*, 2020). In parallel to the identification of *R* genes, another breeding strategy focuses on resistance mediated by impaired susceptibility (*S*) genes, which are host factors that facilitate a compatible interaction between the host and the pathogen (Van Schie & Takken, 2014). To achieve a compatible interaction, plant pathogenic fungi secrete proteins, as well as other types of molecules (such as secondary metabolites and small RNAs) to circumvent plant immune systems, which are referred to as effectors (Yadeta & Thomma, 2013; Rodriguez-Moreno *et al.*, 2018). One of the approaches to identifying *S* genes is unveiling effector targets, which depend on the function of the effector in plant susceptibility, but also on its role in the pathogen (Van Schie & Takken, 2014). Therefore, there is an urgent need to demonstrate which effector genes are responsible for host specificity. For this purpose, knowing the genetic structure of pathogen populations, and their effector repertoires, is critical to guide cultivar deployment and breeding-strategies. Chapter 6 delves into the genomic structure and diversity of accessory regions in Race 1 and Race 2 strains of the FWB-causing *Fusarium* species. The data indicate that Race 1 and Race 2 strains have diverse candidate effector gene profiles, contributing to their pathogenicity towards banana cultivars. However, the effector repertoires showed extensive variation between Race 1 and Race 2 strains and did not correlate with their pathogenicity, which contrasts with the very similar effector profiles reported for TR4 strains (Czislowski *et al.*, 2018; van Westerhoven *et al.*, 2023). Thus, functional analyses are required to discover effector genes that confer host specificity, by directly targeting components of the banana plant immune system, such as immune receptors and downstream signaling components, but also by modulating gene transcription, RNA silencing, phytohormone biosynthesis or other diverse

plant processes. The discovery of these effector genes and their targets is crucial to support the development of resistant banana cultivars through breeding programs.

CONCLUDING REMARKS

The rapid spread of FWB caused by *F. odoratissimum* poses a significant threat to the food security of millions of people and the global banana industry. Effective management of FWB requires a comprehensive understanding of the diversity of causal fungi responsible for the disease and their host range. Here, we unveil the genetic and pathogenic diversity of *Fusarium* spp. causing FWB in Cuba and other countries in Latin America and the Caribbean region. Expanding knowledge on the genome composition and effector repertoire of these *Fusarium* spp. should bridge the gap in understanding their pathogenicity and niche adaptation, which can help in developing resistant banana cultivars through breeding programs. Therefore, studies involving high-throughput host-pathogen genomics are needed to unravel their interaction. This should include genome analysis to identify *R* genes and effector genes, as well as functional studies aimed at characterizing pathogen effectors and predicting their plant targets. In addition, recently developed technologies for determining protein-protein interactions, such as proximity labeling, are enabling the identification of those interactions in different crops (Xu *et al.*, 2023), bringing new and exciting possibilities for unraveling the targets of *Fusarium* effectors and understanding their mode of action. In addition, the functional characterization of effectors may elucidate whether FWB-causing species secrete effectors aimed to suppress other soilborne microbes as was discovered for *Verticillium dahliae* (Snelders *et al.*, 2020), which is relevant for the development of management strategies directed to engineer suppressive soils. Our data shed light on the importance of secondary hosts, such as weed and *Heliconia* species, as potential reservoirs for TR4. However, further research, with the use of quantitative molecular diagnostic protocols, is needed to understand TR4 survival in the soil in the absence of bananas and understand the relevance of these secondary hosts in disease dissemination and inoculum persistence.

Finally, multidisciplinary research is very important to develop resistant varieties that meet market preferences and to optimize current containment and management strategies, which ultimately will safeguard the future of global banana production. This will attract more attention to bananas and their phytosanitary problems, stimulate a new generation of scientists, and embed this important food crop and its problems in academia.

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Summary

Banana (*Musa* spp.), comprising cooking or dessert types, is one of the most important staple crops in tropical and subtropical regions, providing a significant source of nutrition and income for millions of people. However, the cultivation of bananas was and is currently threatened by Fusarium wilt of banana (FWB), a devastating disease caused by various soil-borne *Fusarium* species. Among these, *Fusarium odoratissimum,* also known as Tropical Race 4 (TR4), has emerged as a major pathogen, causing widespread economic losses and posing a serious risk to global banana production and food security. This thesis provides unprecedented insights into the population genetic structure and diversity of the FWB pathogens in Cuba and in the Latin American and Caribbean region (LAC). It explores the potential repercussions of a possible *F. odoratissimum* outbreak by assessing the susceptibility of cultivars commonly cultivated in banana production systems in Cuba and LAC. The research also sheds light on the urgent need for the revision and implementation of quarantine measures and management strategies, taking into account the endophytic behavior of *F. odoratissimum* during colonization of alternative hosts.

Chapter 1 is the general introduction of the thesis and encompasses the history and early domestication of banana, its production, cultivar diversity, and its importance as a fruit crop commodity. This is then connected with FWB as one of the main constraints of the crop, culminating with the importance of studying the diversity of the causal pathogens and their host range for the implementation of efficient containment and management strategies.

Chapter 2 is a comprehensive survey of symptomatic banana plants conducted across all production zones in Cuba, which resulted in a collection of 170 *Fusarium* isolates that were thoroughly characterized by genotyping-by-sequencing and whole-genome comparisons. This resulted in a suite of *Fusarium* species causing FWB in Cuba. Furthermore, the genetic diversity of Cuban FWB-causing strains was compared with *Fusarium* strains from LAC and a global panel. Taken together, this resulted in a thorough analysis of the population genetic structure of the FWB pathogens in Cuba and LAC.

Chapter 3 describes the susceptibility of 18 important Cuban banana and plantain varieties to two *Fusarium* strains, Tropical Race 4 (TR4), and Race 1. Under greenhouse conditions, the results revealed a broad range of disease responses, ranging from resistant to very susceptible against Race 1. However, concerning TR4, not a single banana variety exhibited sufficient resistance. This underscores the potential threat of TR4 to nearly 56% of the contemporary Cuban banana production area. These findings emphasize the urgent need for preemptive evaluation of new banana varieties obtained from the national breeding program and the strengthening of quarantine measures to prevent the introduction of TR4 into the country.

Chapter 4 focuses on a survey conducted in three banana farms in The Philippines to investigate the survival of *F. odoratissimum* (TR4) between crop cycles. The study revealed that TR4 can survive as an endophyte in eight weed species from different botanical families, which may play a significant role in its long-term survival. Greenhouse experiments confirmed these field data and demonstrated that TR4 can endophytically colonize four other weed species

and that its presence was not restricted to the root systems of these weeds. Subsequent pathogenicity tests demonstrated that passing TR4 through some of these plant species can affect its pathogenicity on bananas. These findings highlight the importance of weeds as potential reservoirs for the pathogen and call for reevaluating disease management strategies.

Chapter 5 explores if *Heliconia,* ornamental banana species, and abacá, can be hosts for FWB-causing *Fusarium* strains. Greenhouse assays were conducted to investigate the infection capacity of two strains, *F. phialophorum* (Race 1) and *F. odoratissimum* (TR4), on these species. The results indicated that some *Heliconia* and non-edible banana species displayed symptoms, while others were asymptomatically colonized by TR4, which urgently requires a revision of current containment protocols for FWB.

Chapter 6 delves into the relationship between effector gene repertoires and the pathogenicity of different races of *Fusarium* spp. causing FWB. Therefore, the genomes of Race 1 and Race 2 strains were sequenced to near-complete level and compared with reference strains for TR4 and Race 1, and other resequenced strains representing the Cuban *Fusarium* diversity. Genome analyses and effector profiling delivered a comprehensive data set across the various races that are recognized in the *Fusarium*-banana pathosystem. Finally, seven strains were phenotyped on nine banana accessions to verify the race determination. The results revealed that effector profiles did not predict the pathogenic phenotype of the strains. The findings suggest that various other effectors should play a role in the *Fusarium*-banana pathosystem but these await discovery and functional analyses in order to contribute to breeding for disease-resistant bananas.

Finally, in **Chapter 7** the data collected during this PhD project are summarized in an overarching discussion and placed in a forward-looking research strategy addressing unexplored areas of research towards comprehensive disease control. The relevance of the genetic diversity of FWB-causing *Fusarium* species, and their survival in alternative hosts that act as inoculum reservoirs is explored and discussed in the broader context of sustaining banana production. In the absence of fully resistant commercial cultivars, developing effective containment protocols based on the latest information is vital for ensuring food security and safeguarding the livelihoods of millions of people in tropical and subtropical regions.

Resumen

Los bananos y plátanos (*Musa* spp.), son uno de los cultivos básicos más importantes de las regiones tropicales y subtropicales, y constituyen una importante fuente de nutrición e ingresos para millones de personas. Sin embargo, su cultivo ha estado y está actualmente amenazado por la marchitez por Fusarium del banano (MFB), una enfermedad devastadora causada por varias especies de *Fusarium* transmitidas por el suelo. Entre ellas, *Fusarium odoratissimum*, también conocido como Raza Tropical 4 (TR4), ha emergido como uno de los principales patógenos, causando pérdidas económicas generalizadas y suponiendo un grave riesgo para la seguridad alimentaria y la producción mundial de bananos. Esta tesis proporciona una visión sin precedentes de la estructura genética y diversidad de las poblaciones de los patógenos agentes causales de la MFB en Cuba y en la región de América Latina y el Caribe (ALC). Explora las repercusiones potenciales de un posible brote de *F. odoratissimum* mediante la evaluación de la susceptibilidad de los cultivares comúnmente cultivados en los sistemas de producción de banano en Cuba y ALC. La investigación también pone de manifiesto la urgente necesidad de revisar e implementar medidas cuarentenarias y estrategias de manejo, teniendo en cuenta el comportamiento endofítico de *F. odoratissimum* durante la colonización de hospederos secundarios.

El **Capítulo 1** es la introducción general de la tesis y abarca la historia y domesticación temprana del banano, su producción, la diversidad de cultivares y su importancia como cultivo frutícola. A continuación, se relaciona con la MFB como una de las principales limitaciones del cultivo, culminando con la importancia de estudiar la diversidad de los patógenos agentes causales y su rango de hospederos, para la implementación de estrategias eficientes de contención y manejo.

El **Capítulo 2** describe un muestreo exhaustivo de plantas sintomáticas de banano realizado en todas las zonas de producción de Cuba, que dio lugar a una colección de 170 aislados de *Fusarium,* que fueron caracterizados a fondo mediante genotipificación por secuenciación y comparaciones del genoma completo. Se determinó que la MFB en Cuba es causada por tres especies de *Fusarium*. Además, la diversidad genética de las cepas cubanas causantes de MFB se comparó con cepas de *Fusarium* de ALC y de un panel internacional. En conjunto, esto resultó en un análisis exhaustivo de la estructura genética poblacional de los patógenos de la MFB en Cuba y ALC.

El **Capítulo 3** describe la susceptibilidad de 18 importantes variedades cubanas de banano y plátano a dos cepas de *Fusarium*, la Raza Tropical 4 (TR4), y la Raza 1. Los resultados de los ensayos, realizados bajo condiciones de invernadero, revelaron un amplio rango de respuestas a la enfermedad, que van desde resistentes hasta muy susceptibles contra la Raza 1. Sin embargo, en lo que respecta a TR4, ni una sola variedad de banano mostró suficiente resistencia. Esto subraya la amenaza potencial que TR4 representa para casi el 56% del área de producción contemporánea de banano en Cuba. Estos resultados enfatizan la urgente necesidad de una evaluación preventiva de las nuevas variedades de banano obtenidas del programa nacional de mejoramiento genético y el fortalecimiento de las medidas cuarentenarias para prevenir la introducción de TR4 en el país.

El **Capítulo 4** se centra en un estudio realizado en tres fincas bananeras de Filipinas para investigar la supervivencia de *F. odoratissimum* (TR4) entre ciclos de cultivo. El estudio reveló que TR4 puede sobrevivir como endófito en ocho especies de malezas de diferentes familias botánicas, lo que puede desempeñar un papel importante en su supervivencia a largo plazo. Los experimentos de invernadero confirmaron estos resltados y demostraron además que TR4 puede colonizar endófitamente otras cuatro especies de malezas, y que su presencia no se limita a los sistemas radiculares de estas malezas. Pruebas posteriores de patogenicidad demostraron que el paso de TR4 a través de algunas de estas especies de plantas puede afectar a su patogenicidad en banano. Estos hallazgos destacan la importancia de las malezas como reservorios potenciales del patógeno y llaman a reevaluar las estrategias de manejo de la enfermedad.

El **Capítulo 5** explora si *Heliconia*, especies ornamentales de banano y abacá, pueden ser hospedantes de cepas de *Fusarium* causantes de MFB. Se realizaron ensayos en invernadero para investigar la capacidad de infección de dos cepas, *F. phialophorum* (Raza 1) y *F. odoratissimum* (TR4), en estas especies. Los resultados indicaron que algunas especies de *Heliconia* y de banano mostraron síntomas, mientras que otras fueron colonizadas asintomáticamente por TR4, lo que demanda urgentemente una revisión de los actuales protocolos de contención de MFB.

El **Capítulo 6** profundiza en la relación entre los repertorios de genes efectores y la patogenicidad de las diferentes razas de *Fusarium* spp. causantes de la MFB. Para ello, se secuenciaron los genomas de cepas de Raza 1 y Raza 2 hasta un nivel casi completo y se compararon con el de las cepas de referencia para TR4 y Raza 1, y otras cepas re-secuenciadas representativas de la diversidad de *Fusarium* presente en Cuba. Los análisis del genoma y los perfiles de efectores proporcionaron un amplio conjunto de datos sobre las diversas razas reconocidas en el patosistema *Fusarium*-banano. Finalmente, siete cepas fueron fenotipadas en nueve accesiones de banano para verificar la determinación de las razas. Los resultados revelaron que los perfiles de efectores no predecían el fenotipo patogénico de las cepas. Los hallazgos sugieren que otros varios efectores deben jugar un papel durante la interacción *Fusarium*-banano, pero se necesita de análisis funcionales que permitan su descubrimiento para contribuir a la mejora genética de bananos resistentes a la enfermedad.

Por último, en el **Capítulo 7**, los datos recogidos durante este proyecto de doctorado se resumen en una discusión general y se sitúan en una estrategia de investigación con visión de futuro que aborda áreas inexploradas de investigación hacia un control integral de la enfermedad. La relevancia de la diversidad genética de las especies de *Fusarium* causantes de la MFB, y su supervivencia en hospederos secundarios que actúan como reservorios de inóculo, es explorada y discutida en el contexto más amplio de una producción bananera sustentable. En ausencia de cultivares comerciales totalmente resistentes, el desarrollo de protocolos de contención eficaces basados en la información más reciente es vital para garantizar la seguridad alimentaria y salvaguardar los medios de subsistencia de millones de personas, en las regiones tropicales y subtropicales.

Nederlandse Samenvatting

Bananen (*Musa* spp.), zowel kook- als dessertbananen, zijn een van de belangrijkste basisgewassen in tropische en subtropische gebieden en vormen een belangrijke bron van voeding en inkomen voor miljoenen mensen. De bananenteelt werd en wordt momenteel echter bedreigd door Fusarium verwelkingsziekte van bananen (FWB), een verwoestende ziekte die wordt veroorzaakt door verschillende bodem gebonden *Fusarium*-soorten. Hiervan is *Fusarium odoratissimum*, ook bekend als Tropical Race 4 (TR4), een belangrijke ziekteverwekker die wijdverspreide economische verliezen veroorzaakt en een ernstig risico vormt voor de wereldwijde bananenproductie en voedselzekerheid. Dit proefschrift biedt ongekende inzichten in de genetische populatiestructuur en diversiteit van de FWB-pathogenen in Cuba en in de Latijns-Amerikaanse en Caribische regio (LAC). Het onderzoekt de mogelijke repercussies van een mogelijke uitbraak van *F. odoratissimum* door de gevoeligheid te bepalen van cultivars die vaak geteeld worden in bananenproductiesystemen in Cuba en Latijns-Amerika en het Caraïbisch gebied. Het onderzoek werpt ook licht op de dringende noodzaak van herziening en implementatie van quarantainemaatregelen en beheers strategieën, rekening houdend met het endofytische gedrag van *F. odoratissimum* tijdens kolonisatie van alternatieve gastheren.

Hoofdstuk 1 is de algemene inleiding van het proefschrift en gaat over de geschiedenis en vroege domesticatie van banaan, de productie, de diversiteit aan cultivars en het belang van banaan als fruitgewas. Dit wordt vervolgens verbonden met FWB als een van de belangrijkste beperkingen van het gewas, culminerend in het belang van het bestuderen van de diversiteit van de causale pathogenen en hun gastheerbereik voor de implementatie van efficiënte beheers- en inperkingsstrategieën.

Hoofdstuk 2 is een uitgebreid onderzoek van symptomatische bananenplanten, uitgevoerd in alle productiezones in Cuba, dat resulteerde in een verzameling van 170 *Fusarium* isolaten die grondig gekarakteriseerd werden door genotypering-door-sequencing en genoomvergelijkingen. Er werd vastgesteld dat BFM in Cuba wordt veroorzaakt door drie Fusarium-soorten. Bovendien werd de genetische diversiteit van Cubaanse FWB-veroorzakende stammen vergeleken met *Fusarium* stammen uit LAC en een wereldwijd panel. Samen resulteerde dit in een grondige analyse van de genetische populatiestructuur van de FWB-verwekkers in Cuba en Latijns-Amerika en het Caraïbisch gebied.

Hoofdstuk 3 beschrijft de gevoeligheid van 18 belangrijke Cubaanse bananen- en bakbanaan variëteiten voor twee *Fusarium* stammen, TR4 en Race 1. Onder kasomstandigheden toonden de resultaten een gevoeligheid voor de *Fusarium* stammen aan en toonden de resultaten een breed scala aan ziekteresponsen, variërend van resistent tot zeer vatbaar tegen Race 1. Wat TR4 betreft, vertoonde echter geen enkele bananenvariëteit voldoende resistentie. Dit onderstreept de potentiële bedreiging van TR4 voor bijna 56% van het huidige Cubaanse bananenareaal. Deze bevindingen benadrukken de dringende noodzaak van preventieve evaluatie van nieuwe bananenvariëteiten uit het nationale veredelingsprogramma en het versterken van quarantainemaatregelen om de introductie van TR4 in het land te voorkomen.

Hoofdstuk 4 richt zich op een onderzoek dat is uitgevoerd op drie bananenplantages in de Filippijnen om de overleving van *F. odoratissimum* (TR4) tussen teeltcycli te onderzoeken. Uit het onderzoek bleek dat TR4 als endofyt kan overleven in acht onkruidsoorten uit verschillende botanische families, wat een belangrijke rol kan spelen bij de overleving op lange termijn. Kasexperimenten bevestigden deze veldgegevens en toonden aan dat TR4 vier andere onkruidsoorten endofytisch kan koloniseren en dat zijn aanwezigheid niet beperkt was tot de wortelsystemen van deze onkruiden. Daaropvolgende pathogeniciteitstesten toonden aan dat de aanwezigheid van TR4 in sommige van deze plantensoorten de pathogeniciteit op bananen kan beïnvloeden. Deze bevindingen benadrukken het belang van onkruiden als potentiële reservoirs voor de ziekteverwekker en vragen om een heroverweging van de huidige beheersing strategieën.

Hoofdstuk 5 onderzoekt of *Heliconia*, sierbananensoorten en abacá gastheren kunnen zijn voor *Fusarium* stammen die FWB veroorzaken. Er werden tests in kassen uitgevoerd om de infectiecapaciteit van twee stammen, *F. phialophorum* (Race 1) en F. *odoratissimum* (TR4), op deze soorten te onderzoeken. De resultaten gaven aan dat sommige *Heliconia*- en niet-eetbare bananensoorten symptomen vertoonden, terwijl andere soorten asymptomatisch gekoloniseerd werden door TR4, waardoor de huidige inperkingsprotocollen voor FWB dringend herzien moeten worden.

Hoofdstuk 6 gaat in op de relatie tussen effectorgenenrepertoires en de pathogeniteit van verschillende *Fusarium* Races die FWB veroorzaken. Daarom werden de genomen van stammen van Race 1 en Race 2 (bijna volledig) gesequenced en vergeleken met referentiestammen voor TR4 en Race 1 en andere opnieuw gesquencete stammen die de Cubaanse *Fusarium* diversiteit vertegenwoordigen. Genoomanalyses en effectorprofilering leverden een uitgebreide dataset op voor de verschillende Races die voorkomen in het *Fusarium*-bananen pathosysteem. Tot slot werden zeven stammen gefenotypeerd op negen bananenrassen om de pathogeniteitspatronen te verifiëren. De resultaten toonden aan dat effectorprofielen het fenotype van de stammen niet goed voorspelden. De bevindingen suggereren dat verschillende andere effectoren een rol spelen in het *Fusarium*-bananen pathosysteem, maar deze moeten nog ontdekt en functioneel geanalyseerd worden om bij te dragen aan de veredeling van ziekteresistente bananen.

Tot slot worden in **Hoofdstuk 7** de gegevens die tijdens dit onderzoek zijn verzameld, samengevat in een overkoepelende discussie en geplaatst in een toekomstgerichte onderzoeksstrategie die zich richt op onontgonnen onderzoeksgebieden voor een allesomvattende ziektebestrijding. De relevantie van de genetische diversiteit van *Fusarium* soorten die FWB veroorzaken en hun overleving in alternatieve gastheren die als inoculumreservoirs fungeren, wordt onderzocht en besproken in de bredere context van de instandhouding van de bananenproductie. Bij gebrek aan volledig resistente commerciële cultivars is de ontwikkeling van doeltreffende beheersingsprotocollen op basis van de meest recente informatie van vitaal belang om de voedselzekerheid te garanderen en het levensonderhoud van miljoenen mensen in tropische en subtropische gebieden veilig te stellen.

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About the author

Einar Martínez de la Parte was born on September 15th, 1975, in Santa Clara, Villa Clara, Cuba. In 1994, he started his bachelor studies at the Faculty of Biology at the University of Havana, where he became passionate about fungi. Einar obtained a bachelor's degree in Microbiology (1999), with the completion of the thesis "Sampling methods and culture media for the study of airborne indoor fungi" under the supervision of Dr. Teresa I. Rojas Flores.

In 2004, Einar started working in the Tropical Fruticulture Research Institute where he was involved in an international project with the Max-Planck Institute (Köln, Germany) on the characterization of *Hirsutella thompsonii*, a biological control agent of mites. Then in 2006, he started working in the Central Plant Quarantine Laboratory (LCCV), of the National Plant Health Center. He specialized in fungal plant pathogen diagnosis and reported seven new fungal diseases in Cuba. From 2011 to 2013, while working at LCCV, he started an MSc degree in Microbial Ecology at the Faculty of Biology at the University of Havana, where he received his degree with the thesis "*Verticillium albo-atrum* and *V. dahliae* on vegetable seeds: incidence, detection, disinfection methods and *in vitro* antagonism of *Trichoderma spp*." under the supervision of Dr. Luis Pérez Vicente. After completing his MSc degree, he started working as a researcher in the Phytopathology department of the Plant Health Research Institute (INISAV), where he conducted research projects related to diagnosing, characterizing, and managing fungal plant pathogens in several horticulture and fruit crops. Since 2010, Einar has been involved in teaching, participating in several national and international courses related to the diagnosis and characterization of fungal plant pathogens. He was as instructor in seminars and workshops conducted in Costa Rica, México, and Trinidad and Tobago directed to detecting and diagnosing the causal agent of Fusarium wilt of banana. In the fall of 2017, Einar joined the group of Prof. Gert Kema at Wageningen University & Research as a PhD candidate. Throughout his doctorate research, Einar has investigated the genetic diversity and pathogenicity of the populations of the causal agents of Fusarium wilt of banana in Cuba and the extended host range of these pathogens.

List of Publications

Martinez de la Parte E*, Perez Vicente L*, Torres DE*, van Westerhoven AC, Meijer HJG, Seidl MF, & Kema GHJ. 2023. A deep genetic analysis of banana Fusarium wilt pathogens of Cuba in a Latin American and Caribbean diversity landscape. *bioRxiv*: 2023.08.29.553192. doi: 10.1101/2023.08.29.553192.

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* Authors with same contribution

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