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Disease Control and Integrated Management

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. Recent outbreaks in Colombia, Perú, and Venezuela have raised 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.

Keywords: banana, *F. odoratissimum*, Fusarium wilt, plantain, variety resistance

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. After 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 has been entirely destined for the domestic market. Bananas are an important cash crop for small growers and are indispensable as a 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).

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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 (3× = AAA) and its dwarf variants, together with Manzano (Silk, 3× = AAB), Indio, Morados (red banana, 3× = AAA), and Datil (2× = AA). The second group included plantains (subgroup Plantain, 3× = AAB), represented by the main varieties Macho and Hembra (Horn and French types, respectively) and several cooking banana varieties known as Burro (subgroup Bluggoe, 3× = 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 (Maryani 2019; Ordóñez et al. 2015) and destroyed plantations of Gros Michel and Manzano. These were therefore gradually replanted since the 1950s by the resistant Cavendish varieties (3× = 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 toward BLSD-resistant banana hybrids developed by the Fundación Hondureña de Investigación Agrícola (FHIA), particularly the dessert types FHIA-23 (4× = AAAA) and FHIA-18 (4× = AAAB), the plantains FHIA-20 and 21 (4× = 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 (3× = 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 (3× = 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).

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. Although 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 (Dita et al. 2013; Maryani et al. 2019; Pérez-Vicente 2004; van Westerhoven et al. 2022a, b), and particularly for Latin America and the Caribbean region after its emergence in Colombia (García-Bastidas et al. 2020), Perú (Acuña et al. 2022), and Venezuela (IPPC 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 1-liter 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 2 weeks to maintain high humidity in an environmentally controlled greenhouse compartment (28 ± 2°C, 16 h of 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/liter, Ca^{2+}-4 mM/liter, Mg^{2+}-1.82 mM/liter, NO_3^--12.4 mM/liter, SO_4^{2-}-3.32 mM/liter, $H_2PO_4^-$-1.1 mM/liter, Mn^{2+}-10 μMol/liter, Zn^{2+}-5 μMol/liter, B-30 μMol/liter, Cu^{2+}-0.75 μMol/liter, Mo-0.5 μMol/liter, Fe/DTPA-50/3%, Fe-EDDHSA-50/3%, pH = 5.8) three times per week.

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 represents TR4. These strains have also been used in previous FWB phenotyping studies under greenhouse conditions (Dita et al. 2011; García-Bastidas et al. 2019; Rebouças et al. 2018; Ribeiro et al. 2011). Sporulation media were prepared by autoclaving 500 ml water in 1-liter Erlenmeyer flasks supplemented with 2 g of 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 5 days) and subsequently incubated in a rotary shaker at 140 rpm and 28°C for 5 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, U.S.A.) 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-to-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 × frequency)/(total number of plants × maximum class in the scale)] × 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 subtrials. This facilitates variable numbers of varieties and replicates per trial to cope with the varying success of tissue culture. Data anal-

TABLE 1. Banana germplasm phenotyped for resistance to Fusarium wilt

Name	Accession code[a]	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[b]	ITC0180	Cavendish	AAA	Dessert
Grand Naine[c]	–	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

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

ysis 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 mocks.

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 (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 and 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 (Fig. 1). Not all the tested susceptible plants showed noticeable external symptoms, but internal discoloration of their rhizomes was consistent (Figs. 1 and 2).

The response to Race 1 was diverse; median RDA values were 0 for resistant varieties CEMSA3/4, 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% (Fig. 2). Burro CEMSA is known for its susceptibility to Race 2 but also showed RDA values up to 37.6% (Fig. 2) for Race 1.

Phenotyping with TR4 showed that none of the tested varieties was resistant because 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 and 22.6%; hence, its DI was 25.9%, which classifies it as moderately susceptible (Supplementary Fig. 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).

The non-Cavendish triploid AAA varieties such as Yangambi Km5 and Gros Michel were also susceptible to TR4 (Fig. 2), with RDA values ranging from 5-54 to 41.2-100%, respectively. The

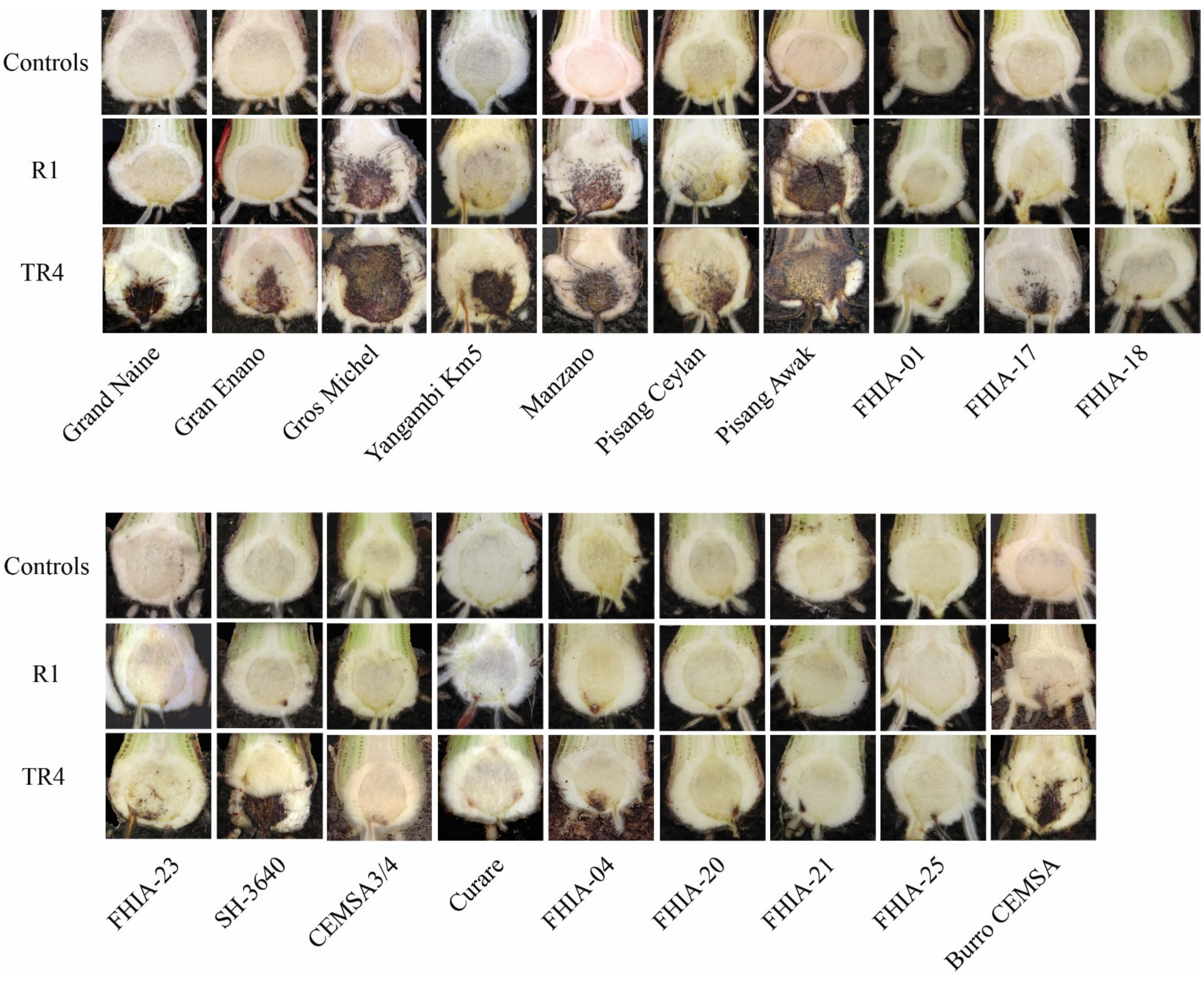

Fig. 1. Cross-sections of corms of banana accessions showing a differential response after inoculations with Race 1 (strain CNPMF 000-8-01-R1, *Fusarium oxysporum*) and TR4 (strain II-5, *F. odoratissimum*).

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 (Fig. 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 and 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 and 15.3%), whereas FHIA-17 was also

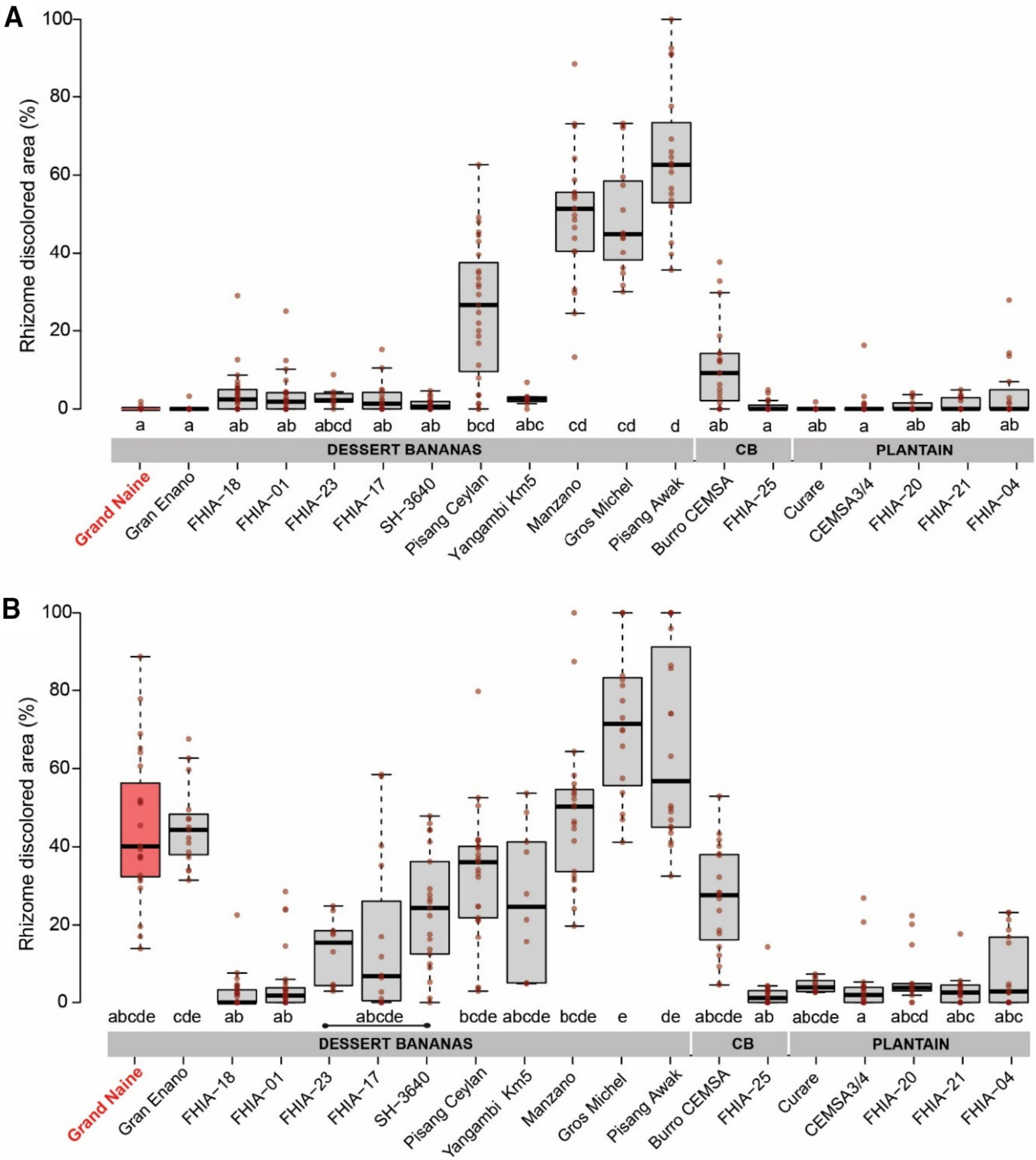

Fig. 2. Response of banana and plantain cultivars to **A,** Race 1 (strain CNPMF 000-8-01-R1, *Fusarium oxysporum*) and **B,** TR4 (strain II-5, *F. odoratissimum*) expressed as a percentage of rhizome discolored area (RDA). The reference Grand Naine is indicated in red. CB = cooking banana. Different 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.

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 with all other varieties. CEMSA3/4 and Curare were resistant to Race 1 (median RDA 0%) but moderately susceptible to TR4 (median RDA 25.2 and 3.8%, respectively) (Fig. 2; Supplementary Table S2).

The more conventional RDI showed that on the 1-to-6 scale, resistant varieties ranged between 1 and 2, whereas all others had various levels of susceptibility ranging from 2 to 5 (Supplementary Fig. S1). However, there are a few exceptions: CEMSA3/4 which was classified as resistant to Race 1 (DI 21.4%) showed an RDI of 3 in some plants (Supplementary Fig. S1). Similarly, FHIA-18 and FHIA-25, despite their RDI median values of 1, had DIs greater than 25% and hence were classified as moderately susceptible to TR4 (Supplementary Fig. 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-Bastidas (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.

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 et al. 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 (Acuña et al. 2022; García-Bastidas et al. 2020; IPPC 2023) and local expansions (Fruitrop 2022; Nakasato Tagami et al. 2023) 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 (Chen et al. 2019; García-Bastidas 2019; Rebouças et al. 2018; Zhan et al. 2022) and in the field (Orjeda 2000; Pérez-Vicente et al. 2009; Smith et al. 2018; Zhan et al. 2022; Zuo et al. 2018). Like Gros Michel and Silk, the high level of susceptibility of Pisang Awak to Race 1 is well known (Ploetz 2006; Stover 1962), 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. 2023). 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 East African countries where it is widely grown and appreciated (Chittarath et al. 2022; Maryani 2019; Ploetz and Churchill 2011).

Similarly, Bluggoes are very popular as a staple food and consequently provide a source of income for many farmers across Latin America and Africa (Blomme et al. 2013; Dita 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 represents almost the entire Bluggoe area (96.6%) and covers 43.8% of the national banana acreage. This dominance and the susceptibility to TR4—shown 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 one coherent and unanimous group (García-Bastidas

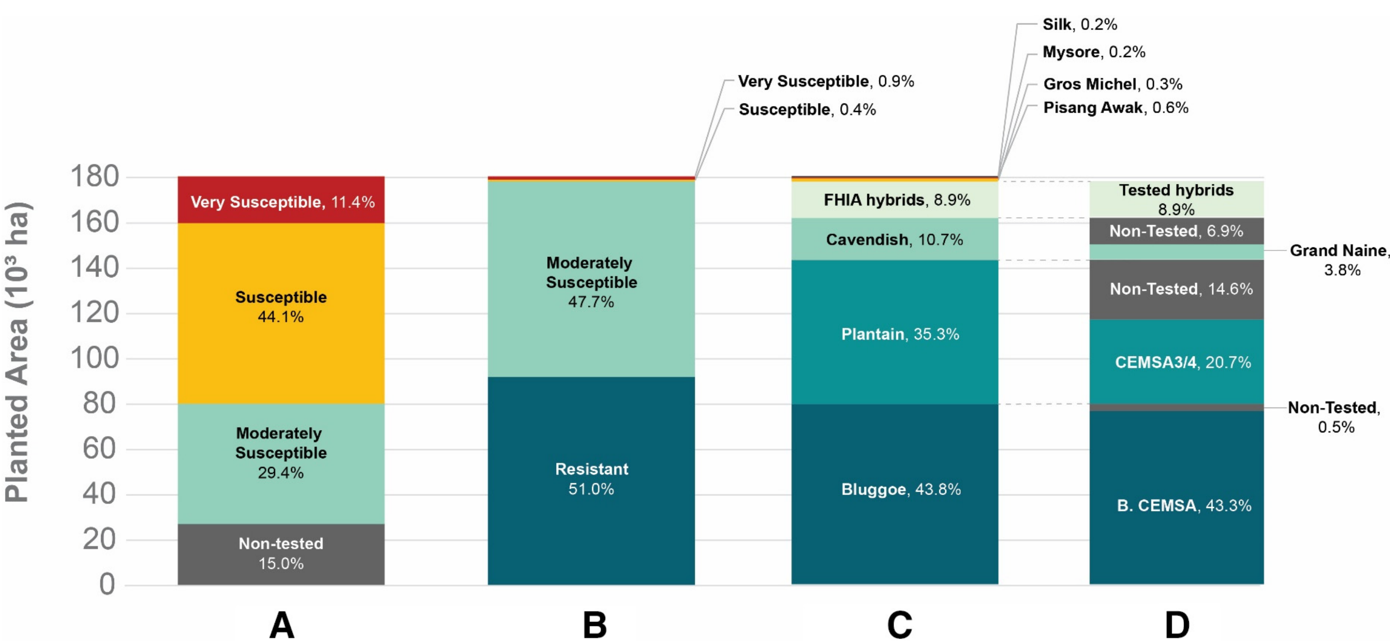

Fig. 3. The overall vulnerability of cultivated bananas in Cuba to Fusarium wilt caused by **A,** TR4 or **B,** Race 1 based on the current study together with the results from Zuo et al. (2018) and García-Bastidas (2019). **C,** Subgroup composition in relation to the total national banana acreage of Cuba, according to the last national survey (Plant Health Directorate of the Cuban Ministry of Agriculture, DSV 2014, *unpublished*) and **D,** in proportion within subgroups of the tested and non-tested cultivars of the present study.

2019; Li et al. 2020; Molina et al. 2016; Zhan et al. 2022; Zuo et al. 2018), despite the fact that only a limited number of plantain varieties have been evaluated with TR4. In our study, CEMSA3/4, 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 plantain varieties, such as Enano guantanamero and Macho 3/4, 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 (Chen et al. 2019; Mintoff et al. 2021; Walduck and Daly 2007; Zhan et al. 2022; Zuo et al. 2018). Our data, however, show that it is moderately susceptible to TR4, which agrees with García-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 was moderately susceptible to Race 1 in our experiments, but Bluggoe is, according to the current FWB race nomenclature, only susceptible to Race 2 strains (Pérez-Vicente et al. 2014; Ploetz 2006; 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 (Li et al. 2020; Zhan et al. 2022; Zuo et al. 2018). 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; Mintoff et al. 2021; Zuo et al. 2018), which in turn affect overall strategies for disease management, particularly after a TR4 outbreak. Therefore, we advocate for 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 (García-Bastidas et al. 2019; Ribeiro et al. 2018; Smith et al. 2008; Zuo et al. 2018). Moreover, hidden experimental factors, such as irregular inoculum distribution and identity (Chen et al. 2019; Mintoff et al. 2021; Ribeiro et al. 2011; Zuo et al. 2018), or possible compound effects from interactions with pests such as nematodes and banana weevils (Dita et al. 2018; Meldrum et al. 2013), 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 2023) 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. In 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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