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Research

Aerial Mapping of the Peruvian Chira Valley Banana Production System to Monitor the Expansion of Fusarium Wilt Caused by Tropical Race 4

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Abstract

The recent incursion of Fusarium wilt of banana (FWB) caused by TR4 threatens banana production in the Chira Valley of Peru. To develop a management strategy, we mapped the entire production area from the air. During 12 flights at an altitude of 610 m (2,000 ft), we gathered 133,700 images in a timeframe of 2 weeks and constructed an orthomosaic map of 73,000 ha. This unveiled the complex logistic network across the banana-producing region, comprising 150 km of primary roads, as well as numerous secondary and tertiary unpaved paths through and bordering banana plantations. Moreover, the Poechos reservoir—with a total length of 450 km—feeds the entire Chira Valley irrigation system, which could further exacerbate TR4 expansion. Using georeferenced landscape details (digital terrain model), we determined areas prone to flooding, which is also relevant for disseminating TR4. Analyses of the maps resulted in the identification of many suspect areas for direct sampling and subsequent analyses to study the expansion of TR4. At four locations, we confirmed two cases, and by June 8, 2023, a total of 207 cases were reported. We conclude that TR4 is a serious threat to the entire region, which exports approximately 25% of the global organic bananas.

Keywords: banana, epidemiology, Fusarium wilt, Peru, Tropical Race 4

The dissemination of plant diseases creates global concerns and is possibly related to extreme weather and climate change (Ristaino et al. 2021). Recent examples include the “quick declining symptoms in olive plants” (CoDiRO) affecting centuries-old olive orchards in North Africa and Italy due to the bacterium *Xylella fastidiosa* and the demise of millions of coast live oak and tan oak trees over the past quarter century by *Phytophthora ramorum*, which significantly affected California’s coastal forest ecosystem (Grünwald et al. 2019). These are perennial crops where disease control is complicated and resistant substituents are usually not immediately available. Plant breeding in these crops takes decades (Lebedev et al. 2020). In annual crops, fungicides with various modes of action can cope with fungal diseases, but these come with a price, as the trend of reduced sensitivity is difficult to change because not many modes of action are available (Fisher et al. 2018).

The frequent use of fungicides to control plant diseases is common practice in banana production. The black leaf streak disease (BLSD)—or black Sigatoka—caused by *Pseudocercospora fijiensis* is exclusively controlled with cocktails of different fungicides to maintain green foliage, which is essential for the export of Cavendish bananas. In many production areas, these fungicide inputs have reached a frequency of weekly applications throughout the year (Chong et al. 2021). Hence, BLSD is a critical factor to improve the sustainability of the sector (Wielemaker 2018). This can be achieved by alternative control methods (Noar et al. 2022), developing new resistant banana varieties, or cultivating bananas in areas with lower precipitation that are less prone to the disease. For example, organic banana production, thus produced without using fungicides, is located in these drier areas, such as Peru and the Dominican Republic. Peru supplies approximately 25% of the global organic banana market (Willer et al. 2021). Since the conversion from conventional to organic banana production in the late 1990s, more than 80% of the national production of bananas for export, hence devoted to Cavendish bananas, is concentrated on an estimated 10,000 ha. divided over approximately 7,000 smallholders and two dozen larger plantations in the Chira Valley in Piura (FAO 2017).

Another reason for banana production in the Chira Valley is the low investment in irrigation. Farms are simply inundated every 3 to 4 weeks using a canal system from the Poechos Reservoir (Fig. 1). However, in April 2021, Fusarium wilt of banana (FWB)—caused by the so-called Tropical Race 4 (TR4) of *Fusarium odoratissimum* (previously known as *F. oxysporum* f. sp. *cubense*) (Maryani et al. 2019; van Westerhoven et al. 2024)—was detected in the Chira Valley just South of Ecuador (Acuña et al. 2022). Given that water is a prime factor in the epidemiology of the pathogen (GLOBALG.A.P. 2017; Pegg et al. 2019; Stover 1962) and taking the aforementioned generic irrigation system into account, it is not surprising that until June 2023, 207 new cases were officially confirmed within a 25 km distance of the original location (Fig. 1; Supplementary Fig. S1) by the National Agricultural Health Service of Peru (SENASA) (Castillo 2023). This underscores the apparent difficulties of implementing effective management strategies in the complex multistakeholder environment of Peruvian banana production. Unfortunately, this is a recurrent experience in FWB management (Ploetz 2015; van Westerhoven et al. 2023). One of the reasons for TR4 expansion is that plants exhibiting chlorosis remain unnoticed or are considered to suffer from other biotic agents or abiotic conditions, and hence, management options are lagging and mostly too limited. Nevertheless, even the restrictive quarantine legislation and practices in Australia do not stop TR4 spread (Australian Banana Growers' Council 2023; Conde 2001; Cook et al. 2015;

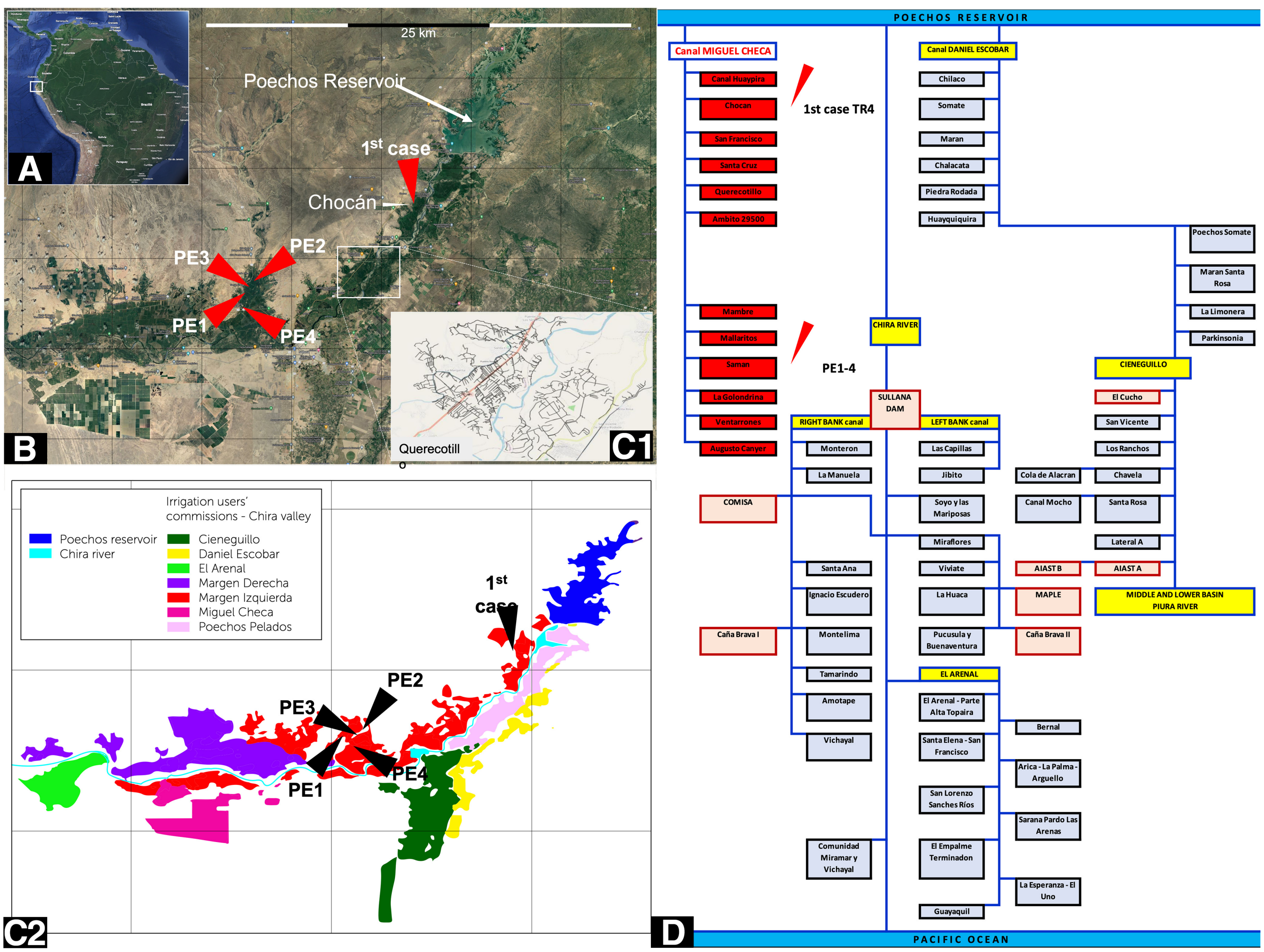


FIGURE 1

A, Google Earth location and **B,** map of the Chira Valley Peruvian banana production zone. **C1,** The detailed channel structure of irrigation canals and **C2,** irrigation commissions are shown across this region, as well as in a **D,** scheme. The initial TR4 incursion and the sampled locations (PE2-4) are indicated by arrowheads. Since then, TR4 disseminated across the "Canal Miguel Checa," indicated in red in panels C and D.

O'Neill et al. 2016), underscoring the complexity of FWB control.

A major limitation of early warning is the vast area that must be inspected. Aerial mapping counteracts the lack of personnel to manually inspect farms at a nationwide level, which is a true bottleneck, even with drones. We, therefore, initiated aerial mapping of the main banana export zones in the Piura Department (Fig. 1). The purpose was to develop a territorial approach for risk analysis and the implementation of biosecurity measures. Here we report a unique orthomosaic map of the entire Chira Valley banana-producing region, which facilitated the identification of new areas infested with TR4. We then identified areas of interest (AOI) in plantations—either with plants showing FWB symptoms (Fig. 2) or open areas—and subsequent on-farm inspections resulted in samples that were analyzed to diagnose TR4. This resulted in two new confirmed TR4 cases 35 km away from the original incursion, and whole-genome analyses indicate that these isolates had substantial variation compared with the isolates from Colombia, suggesting that the South American TR4 isolates originate from multiple independent incursions.

Materials and Methods

Aerial mapping

Mapping technology. Data were gathered with Cessna 172 or Cessna 152 airplanes, with a mobile pod mount under the plane containing a Nikon d810 35mp, a Trimble R4 RTK GPS, and a battery pack with the operator in the plane using a laptop with an Opus Insight-designed pilot interface. Images were captured at an altitude of 610 m (2,000 ft) with an interval of one per second and an overlap of 70%. Flights were undertaken from a private airfield in Chulucanas, located about 50 km from Piura, and were always planned in two sessions during mornings and afternoons to avoid delays due to weather conditions or fuel limitations.

Image processing. All imaging data were captured using a web-based platform with tools to map altitude differences (digital terrain model, DTM) and contour lines to understand the watershed and potential water accumulation. Every flight session resulted in several thousands of images that were sent to the Opus Insights headquarters in the Hague, the Netherlands, for processing and digital map creation which resulted in one full orthomosaic map with a resolution of 5 to 7 cm, in RGB colors, that was delivered to the Opus Insights service platform. AOI were identified based on color differentiation (chlorotic vs. green) of the canopy or because of open spots in plantations. These areas were visited for inspection by local crews.

Sample taking and processing

Symptomatic plants (*Musa* spp., Cavendish, AAA) were sampled, and discolored vascular strands were collected at four different locations (PE1 to PE4; Figs. 1 and 2; Table 1) by cutting a ~10 × 10 cm wound in the pseudostems of standing plants at approximately 1.5 m above soil level (Fig. 2). The samples were placed in a paper bag, and the wound was sealed with the removed pseudostem parts and closed with sticky tape. Visited AOI were delineated with red/white colored tape for follow-up quarantine activities upon TR4 confirmation. The collected samples were airdried at room temperature and sent to Wageningen University and Research (WUR) for further analyses. Upon arrival, the strands were sectioned into pieces using sterile scalpel blades

FIGURE 2

A, Banana plant affected by Fusarium wilt of banana (FWB) and **B,** internal symptoms showing discolored vascular strands, red arrowheads.

and forceps and surface sterilized with 1% NaClO and then rinsed with distilled water. Subsequently, they were blotted to remove the excess of water and placed on filter paper to be later transferred to Komada medium (Komada 1975), commonly used to isolate *Fusarium* spp. from infected plant material and infested soil (Leslie and Summerell 2008). The samples were incubated for 5 days at 26°C. After the incubation period, the colonies that resembled *Fusarium* spp. were transferred to potato dextrose agar (PDA) plates. Two monosporic isolates from two independent colonies per sampling site were obtained, which were used in multiplex diagnostic polymerase chain reaction (PCR) experiments for TR4 (Table 1) according to Dita et al. (2010), as well as in loop-mediated isothermal amplification assay (LAMP) kits developed by Ordóñez et al. (2019), with the controls water, *F. odoratissimum* II-5 TR4, and Race 1 *F. phialophorum* isolate CR.1A.

Greenhouse pathogenicity assays were performed with one of the TR4-positive monosporic isolates per location (Table 1) on 10-week-old Cavendish (Grand Naine) banana plants, following the protocol of García-Bastidas et al. (2019). The reference *F. odoratissimum* strain II-5 was used as a positive control, along with a negative water control. Re-isolations from all plants were executed to complete Koch's postulates.

Eventually, we performed Illumina whole-genome sequencing through the Beijing Genome Institute (Tai Po, Hong Kong) using one TR4-positive monosporic isolate per location (Table 1). The genome sequence was used to further characterize the isolates and compare the genomes to other identified TR4 strains (Supplementary Table S1). The sequencing reads were aligned against the TR4 *F. odoratissimum* strain II5 reference genome using BWA-mem (Burrows-Wheeler alignment) (v. 0.7.17) (Li 2013). After the alignment, variants were called using GATK4 (Genome Analysis Toolkit 4) (v. 4.2). To obtain high-quality single-nucleotide polymorphisms (SNPs), we first filtered the SNPs based on GATK best practices; additionally, we filtered the SNPs on chromosome 12 and the first 1.8 Mbp of chromosome 1. These regions are known to be variable and exhibit structural variants that hamper accurate SNP calling. Beside these regions we also observed some missing regions on the other chromosomes, all SNPs in these missing regions were excluded from further analysis, which resulted in 1,271 high-quality SNPs. To visualize the relationship between the TR4 isolates, we performed a principal component analysis (PCA).

Results

An orthomosaic map of the Chira Valley banana belt

Irrigation. The maps were created from 133,700 images that were captured during 12 flights of 3 to 4 h. More than 35 different production areas were identified in the region, and in total, over 100,000 ha were mapped during the surveys that took 2 weeks in 2021 and 2022. The mapped length of the irrigation system in the Chira Valley is 450 km with a vast coverage of main, secondary, and tertiary canals covering more than 150,000 ha of different crops that are divided by several commissions named after the locations (Fig. 1).

Roads. The total production area is served by a network of roads totaling to 150 km of primary main roads, connected to long secondary roads. In addition to these paved roads, there is a huge network of unpaved dirt roads or tracks, close to housing and used by motorcycles or moto taxis for local transportation. In some cases, these roads are bordering infested areas without fencing, which significantly complicates the implementation of biosecurity measures (Fig. 3).

Waterlogging. We used the DTM to determine critical areas of any field or geographic area that is prone to waterlogging to predict the potential occurrence of TR4 after precipitation, irrigation, or flooding. Indeed, we determined an association between such critical areas and recently identified TR4-positive fields (Fig. 4).

Sampling sites. During the aerial mapping and processing of the data, we detected several locations in plantations that were covered with blue plastic—after treatment with urea—as a means to stop and kill *Fusarium* inoculum (Fig. 5). In addition, we identified AOI in plantations, where banana plants had been removed, likely to stop further dissemination of TR4 (PE1 to PE4; Fig. 1; Table 1). After data analyses, we confirmed that positive cases for TR4 coincided with AOI that we determined on the maps (Fig. 5).

Expansion of TR4 in the Chira Valley banana belt

Sample analyses. Samples from PE1 to PE4 (Fig. 1) were processed, and all pieces of the discolored vascular strands developed aerial mycelium, which was taken and placed on new PDA plates. Eventually, two monosporic isolates were obtained from two independent colonies per sampling site (Table 1). After PCR, all isolates showed the EF1α amplicon (650 bp) for the internal control, indicating a successful PCR reaction. All isolates from sites PE1 and PE2 were positive for TR4 (diagnostic band of 463 bp), and the controls displayed the anticipated results—no diagnostic bands for water and Race 1 (Fig. 6)—thereby confirming the presence of *F. odoratissimum* TR4. The isolates from sampling sites PE3 and PE4 tested negative for TR4. All isolates were also subjected to the LAMP TR4 diagnostic (Ordóñez et al. 2019), which confirmed TR4 at sites PE1 and PE2 and its absence in the samples from PE3 and PE4 (data not shown).

Phenotyping. Banana plants infected with the positive TR4 *F. odoratissimum* II5 control and the four isolates from PE1

TABLE 1

The geographical coordinates of sites where banana plants with Fusarium wilt of banana (FWB) were sampled[a]

Site	Isolates	Location	GPS	TR4 diagnostic	Phenotyping	Sequencing
PE1	PE1-A	Samán Grande	4°49′25.5″S; 80°44′37.3″W	√	√	√
	PE1-B			√	X	X
PE2	PE2-A	Samán Grande	4°49′21.53208″S; 80°44′36.066″W	√	√	√
	PE2-B			√	X	X
PE3	PE3-A	Samán Grande	4°49′25.1364″S; 80°44′52.8468″W	√	√	X
	PE3-B			√	X	X
PE4	PE4-A	Samán Grande	4°50′45.895621″S; 80°45′14.286958″W	√	√	X
	PE4-B			√	X	X
First incursion[b]	–	Chocán (Querecotillo)	4°43′54.84″S; 80°33′45.00″W	√	√	√

[a] Derived monosporic isolates from these samples were characterized by molecular diagnostics, phenotyping greenhouse assays, and genome sequencing. √ = characterized by the indicated method; X = not characterized by the indicated method.
[b] Acuña et al. (2022).

and PE2 showed typical FWB symptoms 6 weeks after inoculation, whereas the water controls, as well as the plants that were inoculated with isolates from PE3 and PE4, remained healthy (Fig. 6; Supplementary Fig. S2). Subsequent re-isolations from all symptomatic plants (PE1-A and PE2-A) were confirmed as TR4 by molecular diagnostics using the aforementioned protocol (Dita et al. 2010), thereby fulfilling Koch's postulates.

Sequence analysis. To gain insight into the origin and genetic variation of TR4 in Peru, the genome sequences of the two identified TR4 isolates from PE1 and PE2 were compared with those of four previously published Peruvian TR4 isolates and 28 TR4 isolates from other geographical origins (Supplementary Table S1). We identified SNPs against the TR4 *F. odoratissimum* II5 reference genome among these 34 TR4 isolates; this resulted in a set of 1,271 high-quality SNPs. The PCA showed little genetic variation among the Peruvian isolates, indicating their close relatedness. The Peruvian isolates clearly clustered separately from other sequenced TR4 isolates (Fig. 7), including those from Colombia.

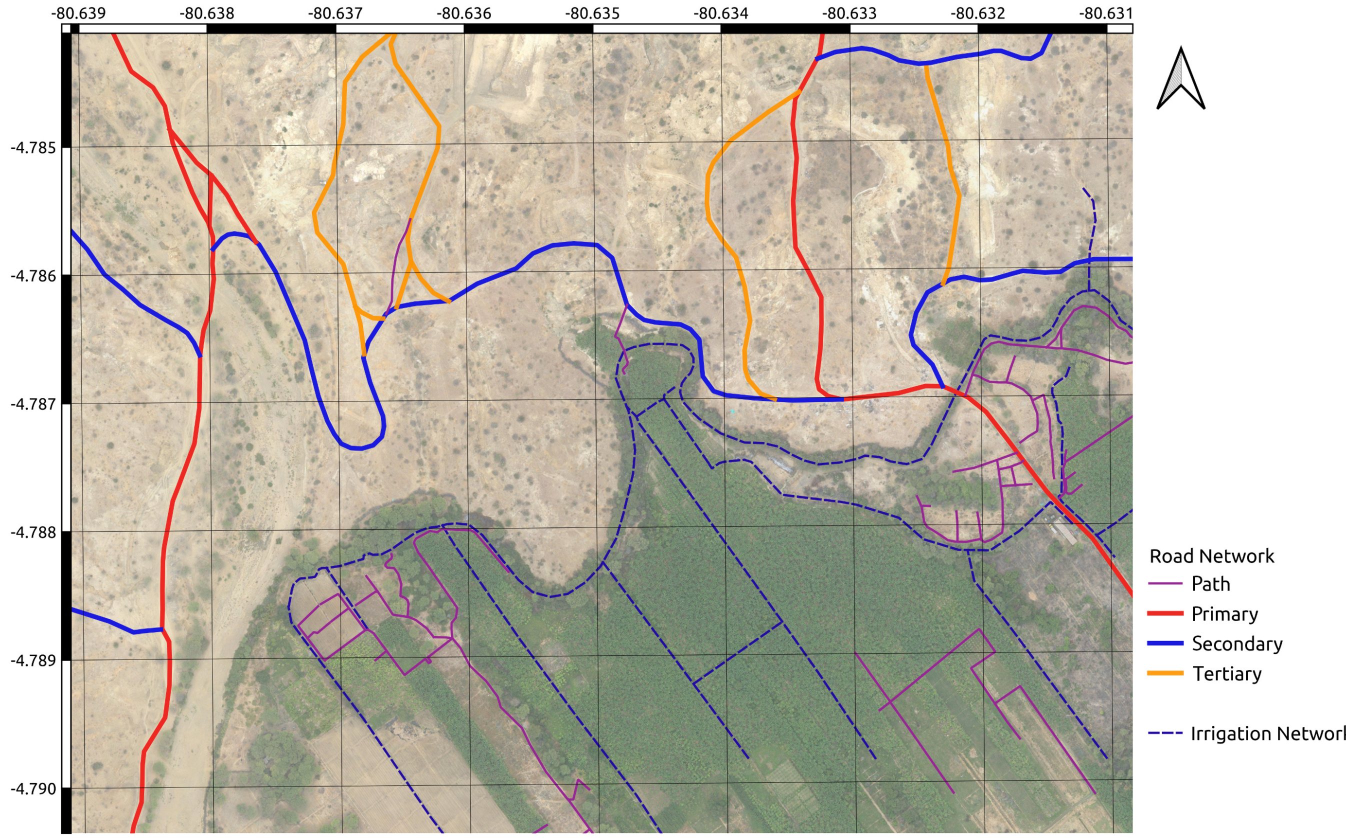


FIGURE 3
Aerial photograph of an unfenced banana plantation in the Chira Valley, transected or bordered by unpaved paths, primary, secondary, and tertiary roads. These logistic networks complicate the implementation of quarantine or biosecurity measures.

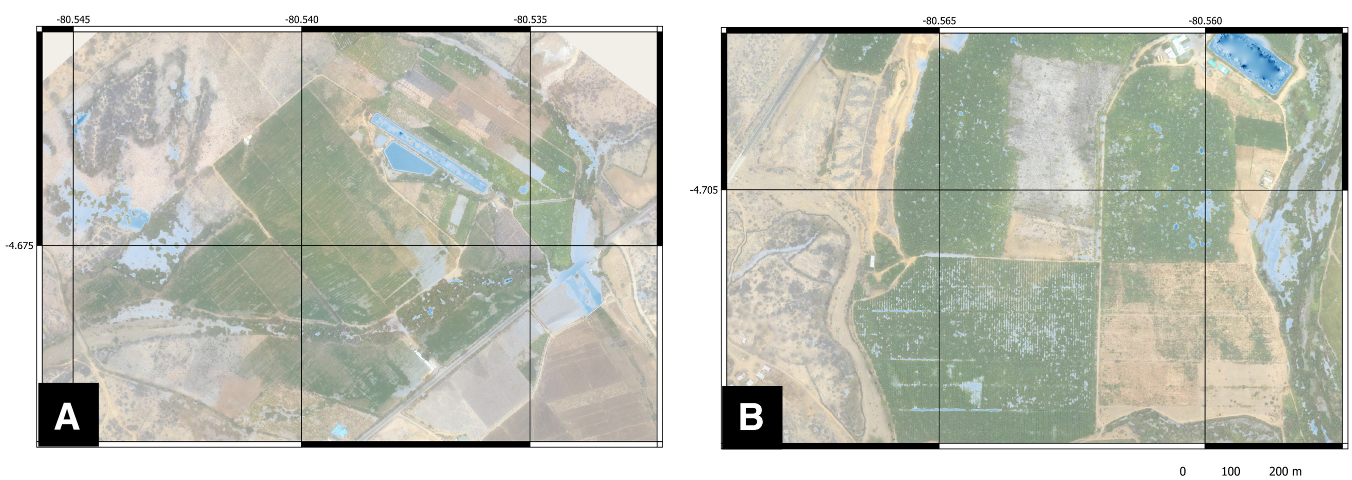


FIGURE 4
The irrigation blocks North of the Chira river are prone to floods, which increases the risks for the spread of TR4. **A and B,** Different simulated areas—by using a digital terrain model—are indicated by blue overlays (note that the photograph in panel A is the same area as in Figure 5). Flood risks were recently confirmed by floods caused by tropical cyclone Yaku (March 2023), which caused excessive precipitation across the region, including Southern Ecuador.

Discussion

Banana is among the most important food crops (FAO 2022; Scott 2021), particularly in the least developed countries. It is also a major commodity and the first traded fruit worldwide. In 2018, the global production of fruits totaled 868 million metric tons, with the main crops banana, citrus, melons, apple, and grapes in order of importance (FAO 2020). However, FWB threatens global banana production (Kema et al. 2021). The global dissemination of TR4 is worrisome, which is underscored by the intercontinental spread into Latin America, where it not only hits another important Cavendish-based export belt but also threatens bananas and plantains for domestic consumption (Martínez de la Parte et al. 2023; van Westerhoven et al. 2022). The first incursion of TR4 in the continent was in Colombia, the department of La Guajira, in 2019 (García-Bastidas et al. 2020), followed by secondary spread into the neighboring department of Magdalena in December 2021 (Reyes-Herrera et al. 2023). In April 2021, TR4 was detected in Peru (Acuña et al. 2022), followed by the recent confirmation in Venezuela in January 2023 (Mejías Herrera et al. 2023). It seems a matter of time before TR4 occurs in other countries in the region. Taken together, the history of TR4 dissemination shows that management strategies fail and that new incursions are discovered too late to contain the disease (van Westerhoven et al. 2022, 2023).

Our survey determined many AOI and formally confirmed two new cases of TR4 in Piura. The genetic analyses showed that the strains are highly similar—consistent with other multi-strain analyses in Colombia and Mozambique (Reyes-Herrera et al. 2023; van Westerhoven et al. 2023)—which indicates rapid dissemination in Peru, likely due to the used irrigation methods and lack of adequate biosecurity measures. Hence, TR4 seems uncontained and spreads throughout the Piura region. The genetic analyses indicate that the incursions in Colombia and Peru took place independently and the Peruvian isolates are dissimilar from all other global TR4 isolates. Therefore, the origin of TR4 in Peru remains unelucidated. Taken together, we conclude that current international quarantine measures are not preventing the spread of TR4. Therefore, increased attention is required across the international banana sector.

Proactive aerial mapping facilitates regional and national surveillance to underlie frequent inspections and adequate follow-up actions by national plant protection agencies to manage FWB of banana. It is common in nature conservation and risk management of, for example, power networks in remote places (Galloway-Griesel et al. 2023; Luo et al. 2023), as well as for monitoring plant pests and diseases (Zhang et al. 2019). Recent examples include the monitoring of bark-beetle-caused tree mortality, which accumulated to 4.7% of the forest area in the Western United States (Hicke et al. 2020), as well as fine-scale

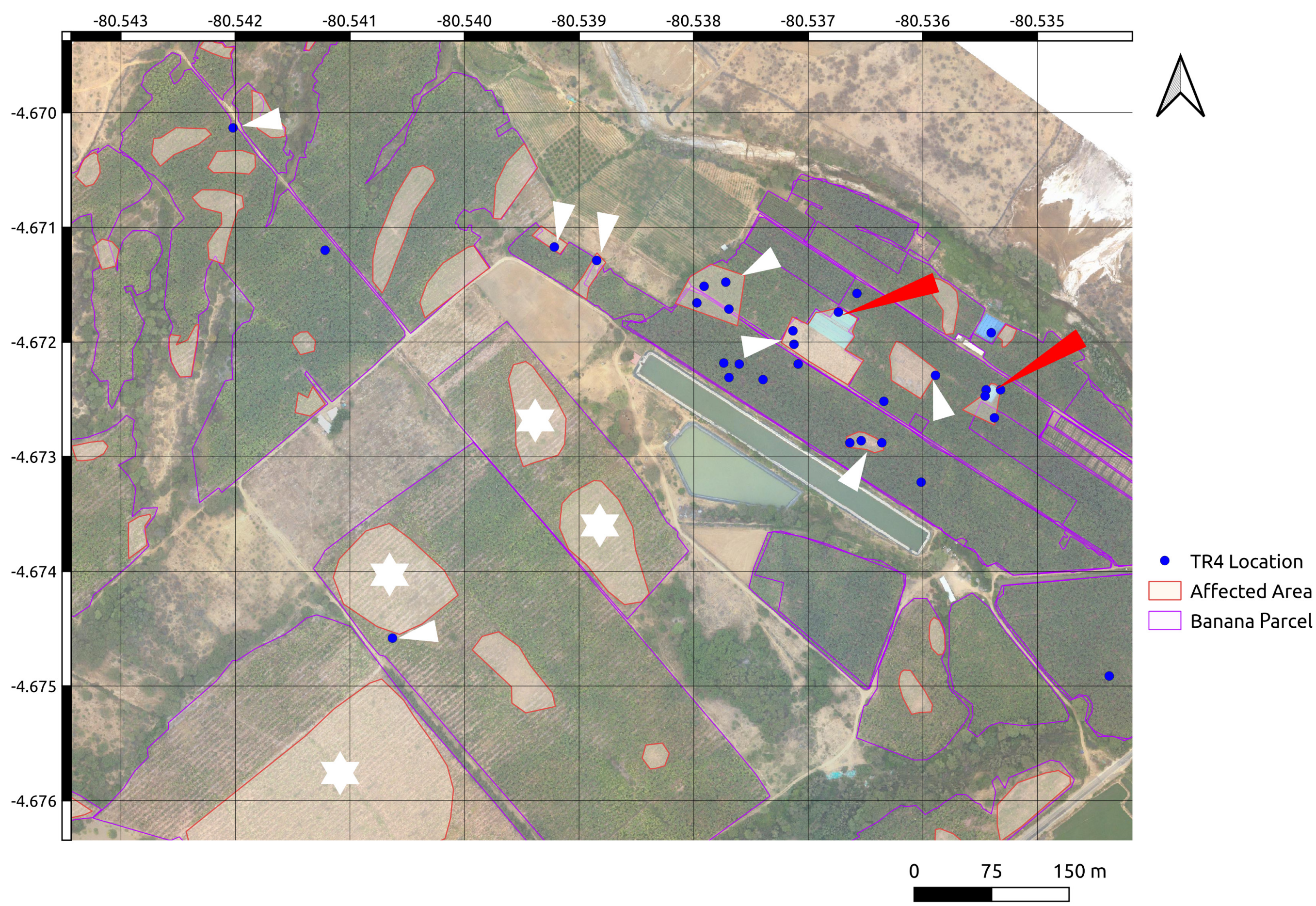


FIGURE 5

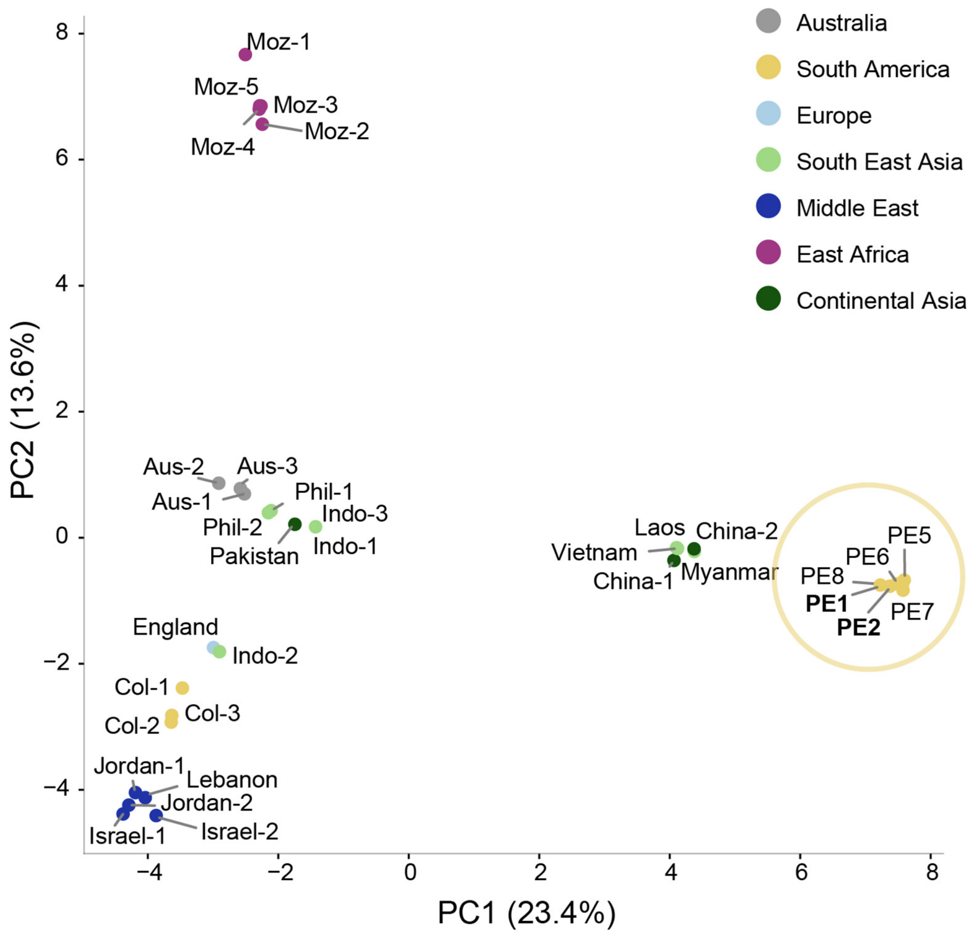
TR4 expansion in the Chira Valley. Initial infestations are covered by blue plastic (red arrowheads). Blue bullets indicate subsequent TR4 confirmations, in the majority of cases coinciding with predetermined areas of interest (white arrowheads). Multiple flights have shown that this expansion takes place in a 35 km radius of the first introduction (see Figure 1) and that earlier TR4-infested farm plots have been planted to other crops (white stars). These crops are not affected by Fusarium wilt of banana (FWB), but the soil is contaminated with TR4 and is a source for further uncontained dissemination.

forest health surveillance (Fraser and Congalton 2021). The latter study used high-resolution imagery and machine learning to classify trees as healthy, stressed, or degraded, thereby significantly improving forest health evaluations. Hence, similar strategies would support overall TR4 surveillance in addition to the identification of AOI. Here, we also mapped the watershed and logistic network in the Chira Valley, which complicate managing soilborne diseases. Pegg et al. (2019) and GLOBALG.A.P. (2017) discussed the importance of water for FWB dissemination, which is commonly observed and anecdotally reported after extreme weather events, such as after the devastating typhoon Pablo in the Philippines in 2012 and cyclone Freddy in Northern Mozambique in March 2023, which may have contributed to an even wider dissemination of TR4. Evidently, such events represent an acute challenge to effective disease management strategies. Although we lack experimental data showing waterlogging areas as risk zones for TR4, it is useful to plot those areas, particularly in the proximity of positive TR4 cases to determine the causal relationship between floods and TR4 incidence.

Clearly, aerial mapping enables territorial risk analyses beyond the boundaries of small banana plots and rapid alert and response actions. We developed a versatile data platform enabling the integration of all aerial imagery for inspection and identified small plots with quarantine measures and AOI potentially infested with TR4, 35 km away from the first reported incursion (Acuña et al. 2022). Ideally, restricted access should be implemented upon a positive diagnosis, including a substantial no-go zone to prevent further dissemination. In practice, there is a

FIGURE 6 TR4 diagnosis of Peruvian samples. **A,** Diagnostic PCR for TR4 according to Dita et al. (2010) on the eight isolates from PE1 to PE4 along with the TR4, Race 1, and water controls. The TR4 diagnostic band size is 463 bp, whereas the EF1α band size for the internal PCR control is 650 bp. **B,** The controls of the phenotyping assays showed Fusarium wilt of banana (FWB) in the TR4-inoculated Grand Naine plants and the absence of FWB symptoms in the plants that were treated with water. **C,** Inoculations with isolates obtained from sites PE1 and PE2 showed FWB symptoms in Grand Naine plants, but those inoculated with the strains from sites PE3 and PE4 remained healthy. **D,** Reisolated strains from symptomatic plants and the TR4 and Race 1 controls showed the presence of the EF1α diagnostic amplicon for a successful PCR. The strains reisolated from the plant infected with isolates from PE1 and PE2 showed the diagnostic TR4-specific amplicon (463 bp), alongside the TR4 positive control. The race 1 and water controls were negative, as well as the strains retrieved from plants infected with isolates obtained from PE3 and PE4 (not shown).

FIGURE 7 Whole-genome analysis of 1,271 single-nucleotide polymorphisms (SNPs) in 34 TR4 samples, relative to the TR4 reference isolate II5. The isolates from Peru show little genetic variation. The isolates obtained in this study (PE1, PE2) cluster together with the samples obtained from Peru by Acuña et al. (2022) (PE5 to PE8; SRR15514269 to SRR15514272). Peruvian isolates are distinct from those of all other geographical origins.



substantial lag between identifying suspect plants with FWB symptoms and formal confirmation of TR4 as the causal agent. For instance, suspect plants in the villages La Margarita, Santa Cruz, and Querecotillo—all close to the Miguel Checa canal—were sampled between March and September 2022, but new TR4 cases were only confirmed in February 2023. The many roads and paths with heavy traffic between banana plantations, packing stations, communities, and logistic centers, and the potential dissemination of TR4 by flood irrigation in this area, are likely associated with the accumulated 207 TR4 cases across the Miguel Checa canal and the worrisome first cases in another irrigation canal south of the Chira river, probably after the recent floods. Taken together, mapping AOI and actual information on positive TR4 cases obtained from SENASA showed the accuracy of identifying TR4-prone sites. It also indicates that a close collaboration among various stakeholders is likely needed for FWB management. The Chira Valley, however, is perhaps the most challenging area for FWB disease control and could be a source of secondary expansion of TR4 in the region.

Finally, the dissemination of TR4 addresses important multidisciplinary aspects of disease control. The societal context of quarantine measures could make farmers reluctant to report suspicious plants to SENASA because these measures reduce their yields. Therefore, even after official confirmation, quarantine measures are limited to merely destroying plants with visible symptoms. This contradicts the protocol of the Regional International Organization for Plant and Animal Health (OIRSA) (Dita et al. 2017), but for small banana farmers in Peru—and many other areas—the recommended practice essentially stops their business. This underscores the huge dilemmas in FWB control around the world: balancing between the social conditions and what is required to slow down the spread of TR4 from a technical perspective. For those reasons, proactive identification of AOI, followed by rapid alert and identification actions, is relevant and necessary. Eventually, however, the development and cultivation of resistant germplasm is the best and proven method to overcome FWB devastation, as exemplified by nearly 100 years of global Cavendish cultivation in Race 1-infested soils.

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Literature Cited

Acuña, R., Rouard, M., Leiva, A. M., Marques, C., Olortegui, J. A., Ureta, C., Cabrera-Pintado, R. M., Rojas, J. C., Lopez-Alvarez, D., Cenci, A., Cuellar, W. J., and Dita, M. 2022. First report of *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 causing Fusarium wilt in Cavendish bananas in Peru. Plant Dis. 106:2268.

Australian Banana Growers' Council. 2023. Panama disease Tropical Race 4. https://www.panamatr4protect.com.au/ (accessed 15 March 2023).

Castillo, F. 2023. Reporte de Focos de FOC TR4 en Perú. https://datastudio.google.com/s/pFsfhMWcL4Q (accessed 2 June 2023).

Chong, P., Essoh, J. N., Arango Isaza, R. E., Keizer, P., Stergiopoulos, I., Seidl, M. F., Guzman, M., Sandoval, J., Verweij, P. E., Scalliet, G., Sierotzski, H., de Lapeyre de Bellaire, L., Crous, P. W., Carlier, J., Cros, S., Meijer, H. J. G., Peralta, E. L., and Kema, G. H. J. 2021. A world-wide analysis of reduced sensitivity to DMI fungicides in the banana pathogen *Pseudocercospora fijiensis*. Pest Manage. Sci. 77:3273-3288.

Conde, B. D. 2001. Discovery, identification and management of banana Fusarium wilt outbreaks in the Northern Territory of Australia. In: International Workshop on the Banana Fusarium Wilt Disease, Genting Highlands Resort (Malaysia), October 18-20, 1999.

Cook, D. C., Taylor, A. S., Meldrum, R. A., and Drenth, A. 2015. Potential economic impact of Panama disease (Tropical Race 4) on the Australian banana industry. J. Plant Dis. Prot. 122:229-237.

Dita, M. A., Echegoyén, P. E., and Pérez Vicente, L. F. 2017. Plan de contingencia ante un brote de *Fusarium oxysporum* f. sp. *cubense* en un país de la región del OIRSA. Versión 2. San Salvador, OIRSA.

Dita, M. A., Waalwijk, C., Buddenhagen, I. W., Souza, M. T., Jr., and Kema, G. H. J. 2010. A molecular diagnostic for Tropical Race 4 of the banana Fusarium wilt pathogen. Plant Pathol. 59:348-357.

FAO. 2017. Producción de banano orgánico en Perú. https://www.fao.org/world-banana-forum/projects/good-practices/organic-production-peru/es/ (accessed 10 January 2023).

FAO. 2020. Fruit and vegetables – your dietary essentials. The International Year of Fruits and Vegetables, 2021, Background paper. Rome, Italy.

FAO. 2022. Banana Market Review - Preliminary Results 2022. Rome, Italy.

Fisher, M. C., Hawkins, N. J., Sanglard, D., and Gurr, S. J. 2018. Worldwide emergence of resistance to antifungal drugs challenges human health and food security. Science 360:739-742.

Fraser, B. T., and Congalton, R. G. 2021. Monitoring fine-scale forest health using unmanned aerial systems (UAS) multispectral models. Remote Sens. 13:4873.

Galloway-Griesel, T., Roxburgh, L., Smith, T., Mccann, K., Coverdale, B., Craigie, J., Pretorius, M., Nicholson, S., Michael, M., Durgapersad, K., And Chetty, K. 2023. Evidence of the effectiveness of conservation interventions from long-term aerial monitoring of three crane species in KwaZulu-Natal, South Africa. Bird Conserv. Int. 33:e7.

García-Bastidas, F. A., Quintero-Vargas, J. C., Ayala-Vasquez, M., Schermer, T., Seidl, M. F., Santos-Paiva, M., Noguera, A. M., Aguilera-Galvez, C., Wittenberg, A., Hofstede, R., Sørensen, A., and Kema, G. H. J. 2020. First report of Fusarium wilt Tropical Race 4 in Cavendish bananas caused by *Fusarium odoratissimum* in Colombia. Plant Dis. 104:994.

García-Bastidas, F. A., Van der Veen, A. J. T., Nakasato-Tagami, G., Meijer, H. J. G., Arango-Isaza, R. E., and Kema, G. H. J. 2019. An improved phenotyping protocol for Panama disease in banana. Front. Plant Sci. 10:1006.

GLOBALG.A.P. 2017. Control Points and Compliance Criteria - TR4 Biosecurity Add-on for Bananas. Cologne, Germany. https://documents.globalgap.org/documents/170427_TR4_BioSecurity_Add-On_Bananas_CPCC_V1-0_en.pdf (accessed 15 July 2023).

Grünwald, N. J., LeBoldus, J. M., and Hamelin, R. C. 2019. Ecology and evolution of the sudden oak death pathogen *Phytophthora ramorum*. Annu. Rev. Phytopathol. 57:301-321.

Hicke, J. A., Xu, B., Meddens, A. J. H., and Egan, J. M., 2020. Characterizing recent bark beetle-caused tree mortality in the western United States from aerial surveys. Forest Ecol. Manag. 475:118402.

Kema, G. H. J., Drenth, A., Dita, M., Jansen, K., Vellema, S., and Stoorvogel, J. J. 2021. Fusarium wilt of banana, a recurring threat to global banana production. Front. Plant Sci. 11:628888.

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Prot. Res. 8:114-124.

Lebedev, V. G., Lebedeva, T. N., Chernodubov, A. I., and Shestibratov, K. A. 2020. Genomic selection for forest tree improvement: Methods, achievements and perspectives. Forests 11:1190.

Leslie, J. F., and Summerell, B. A. 2008. The *Fusarium* Laboratory Manual. Blackwell Publishing, Oxford, U.K.

Li, H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv: 1303.3997.

Luo, Y., Yu, X., Yang, D., and Zhou, B. 2023. A survey of intelligent transmission line inspection based on unmanned aerial vehicle. Artif. Intell. Rev. 56:173-201.

Martínez de la Parte, E., Perez Vicente, L., García-Bastidas, F., Bermúdez-Caraballoso, I., Schnabel, S., Meijer, H. J. G., and Kema, G. H. J. 2023. The vulnerability of Cuban banana production to Fusarium wilt caused by Tropical Race 4. Phytopathology 114:111-118.

Maryani, N., Lombard, L., Poerba, Y. S., Subandiyah, S., Crous, P. W., and Kema, G. H. J. 2019. Phylogeny and genetic diversity of the banana Fusarium wilt pathogen *Fusarium oxysporum* f. sp. *cubense* in the Indonesian centre of origin. Stud. Mycol. 92:155-194.

Mejías Herrera, R., Hernández, Y., Magdama, F., Mostert, D., Bothma, S., Paredes Salgado, E. M., Terán, D., González, E., Angulo, R., Angel, L., Rodríguez, Y., Ortega, R., Viljoen, A., and Marys, E. E. 2023. First report of Fusarium wilt of Cavendish bananas caused by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 in Venezuela. Plant Dis. 107:3297.

Noar, R. D., Thomas, E., and Daub, M. E. 2022. Genetic characteristics and metabolic interactions between *Pseudocercospora fijiensis* and banana: Progress toward controlling black sigatoka. Plants 11:948.

O'Neill, W. T., Henderson, J., Pattemore, J. A., O'Dwyer, C., Perry, S., Beasley, D. R., Tan, Y. P., Smyth, A. L., Goosem, C. H., Thomson, K. M., Hobbs, R. L., Grice, K. R. E., Trevorrow, P., Vawdrey, L. L., Pathania, N., and Shivas, R. G. 2016. Detection of *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 strain in northern Queensland. Australas. Plant Dis. Notes 11:33.

Ordóñez, N., Salacinas, M., Mendes, O., Seidl, M. F., Meijer, H. J. G., Schoen, C. D., and Kema, G. H. J. 2019. A loop-mediated isothermal amplification (LAMP) assay based on unique markers derived from genotyping by sequencing data for rapid in planta diagnosis of Panama disease caused by Tropical Race 4 in banana. Plant Pathol. 68:1682-1693.

Pegg, K. G., Coates, L. M., O'Neill, W. T., and Turner, D. W. 2019. The epidemiology of Fusarium wilt of banana. Front. Plant Sci. 10:1395.

Ploetz, R. C. 2015. Management of Fusarium wilt of banana: A review with special reference to Tropical Race 4. Crop Prot. 73:7-15.

Reyes-Herrera, P. H., Torres-Bedoya, E., Lopez-Alvarez, D., Burbano-David, D., Carmona, S. L., Bebber, D. P., Studholme, D. J., Betancourt, M., and Soto-Suarez, M. 2023. Genome sequence data reveal at least two distinct incursions of the Tropical Race 4 variant of Fusarium wilt into South America. Phytopathology 113:90-97.

Ristaino, J. B., Anderson, P. K., Bebber, D. P., Brauman, K. A., Cunniffe, N. J., Fedoroff, N. V., Finegold, C., Garrett, K. A., Gilligan, C. A., Jones, C. M., Martin, M. D., MacDonald, G. K., Neenan, P., Records, A., Schmale, D. G., Tateosian, L., and Wei, Q. 2021. The persistent threat of emerging plant disease pandemics to global food security. Proc. Natl. Acad. Sci. U.S.A. 118:e2022239118.

Scott, G. J. 2021. A review of root, tuber and banana crops in developing countries: Past, present and future. Int. J. Food Sci. Technol. 56:1093-1114.

Stover, R. H. 1962. Fusarial wilt (Panama disease) of bananas and other *Musa* species. The Commonwealth Mycological Institute, Kew, U.K.

van Westerhoven, A. C., Aguilera-Galvez, C., Nakasato-Tagami, G., Shi-Kunne, X., Dijkstra, J., de la Parte, M., Chavarro Carero, E., Meijer, H. J. G., Feurtey, A., Maryani, N., Ordóñez, N., Hofstede, R., Wittenberg, A. H. J., Sørensen, E. H., García-Bastidas, F., Schneiders, H., Nijbroek, K., Stukenbrock, E. H., Kema, G. H. J., and Seidl, M. F. 2024. Segmental duplications drive the evolution of accessory regions in a major crop pathogen. New Phytol. 242:610-625.

van Westerhoven, A. C., Meijer, H. J. G., Houdijk, J., Martínez de la Parte, E., Matabuana, E. L., Seidl, M. F., and Kema, G. H. J. 2023. Dissemination of Fusarium wilt of banana in Mozambique caused by *Fusarium odoratissimum* Tropical Race 4. Plant Dis. 107:628-632.

van Westerhoven, A. C., Meijer, H. J. G., Seidl, M. F., and Kema, G. H. J. 2022. Uncontained spread of Fusarium wilt of banana threatens African food security. PLoS Pathog. 18:e1010769.

Wielemaker, F. 2018. Organic banana cultivation and sustainability. Page 357 in: Achieving Sustainable Cultivation of Bananas. G. H. Kema and A. Drenth, eds. Burleigh Dodds Science Publishing.

Willer, H., Trávníček, J., Meier, C., and Schlatter, B. 2021. The World of Organic Agriculture 2021: Statistics and Emerging Trends. Research Institute of Organic Agriculture FiBL, Frick, Switzerland.

Zhang, J., Huang, Y., Pu, R., Gonzalez-Moreno, P., Yuan, L., Wu, K., and Huang, W. 2019. Monitoring plant diseases and pests through remote sensing technology: A review. Comput. Electron. Agric. 165:104943.