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**DATES:**

**Received:** 08 July 2020  
**Revised:** 28 Aug. 2020  
**Accepted:** 31 Aug. 2020  
**Published:** 26 Nov. 2020

# Occurrence and spread of the banana fungus *Fusarium oxysporum* f. sp. *cabense* TR4 in Mozambique

Fusarium wilt, caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cabense* (Foc), poses a major threat to banana production globally. A variant of Foc that originated in Southeast Asia, called tropical race 4 (TR4), was detected on a Cavendish banana export plantation (Metoheria) in northern Mozambique in 2013. Foc TR4 was rapidly disseminated on the farm, and affected approximately half a million plants within 3 years. The fungus was also detected on a second commercial property approximately 200 km away (Lurio farm) a year later, and on a small-grower's property near Metoheria farm in 2015. Surveys in Mozambique showed that non-Cavendish banana varieties were only affected by Foc race 1 and race 2 strains. The testing of Cavendish banana somaclones in northern Mozambique revealed that GCTCV-119 was most resistant to Foc TR4, but that GCTCV-218 produced better bunches. The occurrence of Foc TR4 in northern Mozambique poses a potential threat to food security on the African continent, where banana is considered a staple food and source of income to millions of people. Cavendish somaclones can be used, in combination with integrated disease management practices, to replace susceptible Cavendish cultivars in southern Africa. The comprehensive testing of African cooking bananas for resistance to Foc TR4 is required, along with the improvement of biosecurity and preparedness of growers on the African continent.

**Significance:**

- This paper presents the first official report of the invasive pest Foc TR4 in Africa.
- The spread of Foc TR4 on Cavendish banana farms in Mozambique was documented.
- Banana varieties that could replace susceptible Cavendish bananas were identified.

**Introduction**

Fusarium wilt of banana was first observed in 1874<sup>1</sup>, but gained prominence when it severely affected the Gros Michel based international banana export industry in Latin America in the 1900s<sup>2</sup>. Despite a plethora of control measures tried and tested, the disease could never be brought under control. In the end, the export banana industry was forced to replace Gros Michel (AAA) bananas with a resistant variety that satisfied the international consumer market, the Cavendish (AAA) banana. Cavendish bananas soon became popular, and today constitute almost 45% of bananas grown worldwide.<sup>3</sup>

Cavendish bananas did not entirely escape Fusarium wilt, which is caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cabense* (Foc). Reports of Fusarium wilt of Cavendish bananas were first received from the Canary Islands in the 1920s, followed by losses of Cavendish banana in South Africa, Australia and Taiwan.<sup>4,5</sup> Yet, Cavendish bananas did not succumb to Fusarium wilt in severely infested fields in Latin America where Gros Michel was previously planted. This resulted in the designation of races in Foc, of which Foc race 1 affects Gros Michel and dessert banana varieties such as Pisang Awak (ABB) and Silk (AAB), race 2 affects Bluggoe (ABB), and race 4 affects Cavendish bananas, initially in the sub-tropics only.<sup>6</sup>

In the 1990s, reports were received of an Asian Foc strain that severely affected newly planted commercial Cavendish plantations in Indonesia and Malaysia.<sup>6</sup> This strain, commonly referred to as Foc TR4 (abbreviation for 'tropical race 4'), soon became the most devastating of all Foc strains, as it not only affected Cavendish bananas in the tropics and sub-tropics, but also many banana varieties susceptible to Foc races 1 and 2. For years, Foc TR4 was restricted to five Asian countries (Malaysia, Indonesia, Philippines, mainland China and Taiwan) and the Northern Territory state of Australia, but in 2011 it was detected outside Asia for the first time when it was identified in the Sultanate of Oman (Al-Kaabi S 2019, written communication, September 18).

Banana Fusarium wilt is difficult to control. Prevention of introduction is thus important to sustain the production of susceptible varieties.<sup>7</sup> Once Foc is introduced into a plantation the fungus can survive in soil for decades by producing survival structures called chlamydospores.<sup>1</sup> Chlamydospores are difficult to target with fungicides, while soil disinfection techniques such as fumigation and flood fallowing have only been marginally successful.<sup>2,8</sup> Replacing susceptible with resistant varieties thus remains the only option for growers to continue growing banana in infested fields. However, the replacement of Cavendish bananas as a popular fresh fruit has significant challenges. Cavendish bananas are difficult to breed, and most export markets do not accept genetically modified food.<sup>9</sup> Mutation breeding by the prolonged multiplication of plants in tissue culture has successfully produced Cavendish clones with improved Foc TR4 resistance.<sup>5</sup> These somaclones may not be well adapted to new environments, and thus require further selection to improve their production traits.<sup>7</sup>

Banana Fusarium wilt was first detected in Africa when it was reported in West Africa in 1924.<sup>2</sup> This introduction most likely resulted from contaminated Gros Michel plants brought to the continent from Latin America.<sup>10</sup> A second introduction occurred when Indian workers brought sweet dessert bananas to East Africa.<sup>10</sup> While the Foc strains

**HOW TO CITE:**

Viljoen A, Mostert D, Chiconela T, Beukes I, Fraser C, Dwyer J, et al. Occurrence and spread of the banana fungus *Fusarium oxysporum* f. sp. *cabense* TR4 in Mozambique. *S Afr J Sci.* 2020;116(11/12), Art. #8608, 11 pages. <https://doi.org/10.17159/sajs.2020/8608>

**ARTICLE INCLUDES:**

- Peer review
- Supplementary material

**DATA AVAILABILITY:**

- Open data set
- All data included
- On request from author(s)
- Not available
- Not applicable

**EDITORS:**

Teresa Coutinho   
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**KEYWORDS:**

Fusarium wilt, Foc TR4, Africa, plant resistance, disease spread

**FUNDING:**

Stellenbosch University, Eduardo Mondlane University, Matanuska Mozambique Ltd, the Food and Agricultural Organization of the United Nations, RUFORM

introduced into West and East Africa had not been identified at the time, it can be accepted that they belonged to Foc races 1 and 2. Surveys in East and Central Africa have since confirmed the dominance of vegetative compatibility groups (VCGs) in Foc races 1 and 2.<sup>11</sup> The most popular bananas on the continent, the East African Highland bananas (EAHBs) and African plantains, are not affected by Foc races 1 and 2. Cavendish bananas are also not affected by Foc races 1 and 2, but are seriously affected by a 'sub-tropical' strain of Foc race 4 (VCG 0120), referred to as Foc sub-tropical race 4 (STR4), in South Africa.<sup>12</sup>

In Mozambique, bananas are grown by commercial and small-scale farmers on approximately 68 000 ha.<sup>13</sup> In 2017, the country produced almost 580 000 tons of bananas, of which 75% were produced by smallholder farming households.<sup>14</sup> Banana varieties produced in Mozambique mostly include Cavendish cultivars such as Williams, Grand Naine and Chinese Cavendish, although cooking and dessert bananas can be found.<sup>15</sup> Most (85%) of the bananas grown are intended for trade in local markets, and the remainder is exported to neighbouring African countries like South Africa, as well as Eastern Europe and the Middle East.<sup>16,17</sup> Commercial plantations in the Boane District in southern Mozambique, western Manica Province and northern Mozambique almost exclusively produce Cavendish bananas. The international banana export industry, therefore, has increased earnings from USD4.5 million in 2008 to USD37.7 million in 2013.<sup>16</sup>

In February 2013, conspicuous yellowing and wilting of Cavendish banana plants were observed at a commercial export plantation in northern Mozambique. The plantation was established merely 4 years earlier and had experienced a number of production challenges. These challenges included excessive climatic fluctuations under the semi-tropical conditions, heavy clay soils that made fertilisation difficult, and the absence of proper draining systems that led to compaction problems and the salinisation of soils. The summer of 2013 was also preceded by an extreme drought, followed by flooding during the rainy months of January and February. The appearance of yellow and wilted plants, initially, did not appear out of the ordinary. When the symptoms continued after the heavy rains, however, production managers suspected that the cause of the yellowing was banana Fusarium wilt.

In recent years, papers have reported on the presence of Foc TR4 in Mozambique, but without scientific evidence.<sup>18</sup> The current paper, therefore, for the first time provides scientific proof that Foc TR4 was detected in northern Mozambique. It further reports on the spread of the fungus at the affected farms and into Mozambique between 2013 and 2018, and investigates Cavendish somaclones and mutants as potential replacement varieties for susceptible Cavendish cultivars.

## Materials and methods

### *Identification of the Fusarium wilt fungus*

#### Collection of isolates and primary isolation

In March 2013, pseudostem samples collected from symptomatic Cavendish cv Williams banana plants at Metocheria farm near Namialo in Northern Mozambique (14°41'45.29"S; 40°04'05.46"E) were sent to Eduardo Mondlane University in Maputo. The affected plants all showed progressive yellowing of the older leaves and, when the pseudostems were split open, a reddish-brown discolouration of the vascular tissue (Figure 1). A second sample was sent to Eduardo Mondlane University in April 2013, and three samples to Stellenbosch University in June 2013. In November 2013, samples were collected from eight more banana plants at Metocheria farm and sent to Stellenbosch University, and samples from a commercial Cavendish banana farm called Lurio farm, approximately 200 km from Metocheria farm, were sent in May and July 2014. Lurio farm is situated next to the Lurio River that separates the Nampula and Cabo Delgado Provinces (13°42'43.56"; 39°41'44.23"). The pseudostem samples were all placed in sterile paper towels and sent to Maputo and Stellenbosch in parcels with information about the collection sites and dates.



**Figure 1:** Symptoms of banana Fusarium wilt at Metocheria farm, northern Mozambique: (a) external symptoms of leaf yellowing, progressing from the older to younger leaves, and (b) internal symptoms of reddish-brown discoloration of vascular tissue in the pseudostem.

For primary isolation of the causal agent, pseudostem samples from each plant were cut into 5-mm pieces, surface disinfected and plated onto potato dextrose agar (PDA) amended or not with 0.4% streptomycin. The plates were then incubated for 4–5 days at room temperature, and the developing fungal colonies purified and single-spored.<sup>19</sup> Bacteria that were isolated on nutrient agar were common contaminants (data not presented), and were excluded from further analysis. At Stellenbosch University, the isolates of *Fusarium* were stored at -80 °C in 30% glycerol at the facilities of the Department of Plant Pathology.

### Morphological and VCG identification

For morphological identifications, single-spore isolates were plated out on PDA and carnation leaf agar (CLA), and incubated at 25 °C for 14 days. Isolates of *F. oxysporum* were then identified based on their colony colour and morphological characteristics.<sup>20</sup> From each of the *F. oxysporum* isolates, *nit*-mutants were generated, and the mutants typed according to the methodology described by Leslie and Summerell<sup>19</sup>. *Nit*-1 mutants of isolates collected in Mozambique were thereafter paired on minimal media with *nit*-M testers of Foc available in the *Fusarium* culture collection at Stellenbosch University. The VCG group of the unknown isolates was identified if a heterokaryon was formed with a known tester strain.

### Molecular identification

DNA was extracted from the mycelia of Foc strains collected in Mozambique using the protocol provided by Wizard SV Genomic DNA Purification System Kit (Promega, Madison, USA). The DNA was then amplified by PCR using Foc TR4-specific primers developed by Dita et al.<sup>21</sup> and Li et al.<sup>22</sup> An isolate of VCG 01213/16 obtained from Queensland Department of Agriculture and Forestry in Brisbane, Australia, was used as a positive control, and nuclease-free water served as the negative control.

### Pathogenicity testing

Two-month-old Cavendish bananas were inoculated with two representative Foc isolates collected in Mozambique, using the method described by Viljoen et al.<sup>23</sup> The positive control included in the experiment was the Foc TR4 isolate from Australia, whereas the negative control was inoculated with sterile millet seeds. After inoculation, the plants were kept in a quarantine greenhouse in Stellenbosch at a 25 °C daytime and 20 °C night-time temperature, until symptoms developed. The plants were inspected for external and internal symptoms after 6 weeks, and the inoculated organism re-isolated to complete Koch's postulates.

### Spread of Foc TR4 in Mozambique

#### On-farm disease development

Cavendish banana plants with Fusarium wilt symptoms were identified at Metocheria farm between April 2013 and October 2015. The number of plants affected were recorded weekly for each plantation block. The total number of cases per plantation were then accumulated every 3 months. The counting of plants was terminated in October 2015 when the number of dead plants became too many to accurately ascertain infections of new plants. At Lurio farm, diseased plants were also counted on a weekly basis from May 2014 to December 2016.

#### National surveillance

Surveys were conducted in Mozambique in April and October 2015. In April, banana plantings were visited in the vicinity of Metocheria and Lurio farms in the Nampula Province, whereas samples were collected from plantings in northern (Nampula Province), central (Manica Province) and southern (Maputo Province) Mozambique in October. Manica, Nampula and Maputo are the highest banana-producing provinces in Mozambique. During each visit, pseudostem strands were collected from plants showing Fusarium wilt-like symptoms, and information on the location, variety and planting history was obtained.

A total of seven districts in Nampula (Murrupula, Ribaue, Mecuburi, Rapale, Muecate, Erati and Monapo) was visited in April, and 24 samples were collected. In October, 56 samples were collected, of which 23 samples came from Nampula Province, 21 from Manica Province,

and 12 from Maputo Province. Three districts were selected per province, with two administrative posts per district and five plantations per administrative posts. In Nampula Province, the districts included Monapo, Muecate and Erati, in Manica Province it included Sussudenga, Manica and Macate Districts, and in Maputo Province it included Boane, Manhica and Moamba Districts. In Nampula Province, some samples were also collected from the Rapale and Mecuburi Districts.

Both commercial and small-scale farms were included in the survey, following a zig-zag transect strategy. GPS coordinates for each plant sample were collected. Sample collections and identifications were undertaken as described earlier. In addition to Foc TR4-specific primer sets, Foc Lineage VI-specific primers<sup>24</sup> were used for the molecular identification of collected strains. Foc Lineage VI<sup>25</sup> includes all the Foc races 1 and 2 isolates found in East and Central Africa<sup>11</sup>.

### Evaluation of Cavendish somaclones in northern Mozambique

Five Cavendish banana clones were evaluated for resistance to Foc TR4 and production traits at Metocheria and Lurio farm in northern Mozambique, respectively. These clones included four Giant Cavendish somaclones developed by the Taiwan Banana Research Institute (TBRI) (GCTCV-106, GCTCV-119, GCTCV-218 and GCTCV-247) and DPM-25, a Dwarf Parfit (Cavendish) variety mutated by gamma-ray irradiation in Australia. The GCTCV somaclones were sourced from Bioversity International's International Transit Centre in Leuven, Belgium. The Cavendish banana cultivar Nandi, an elite selection made from Grande Nain by DuRoi Laboratory in South Africa, was used as the susceptible control. All clones were multiplied by DuRoi Laboratory, and were planted in a randomised complete block design with 40 plants per block and five replications of each clone. Fertilisers and irrigation were applied according to standard operational practices. Trials were conducted over two seasons, and experimental data were collected monthly for Fusarium wilt resistance and when required for production traits.

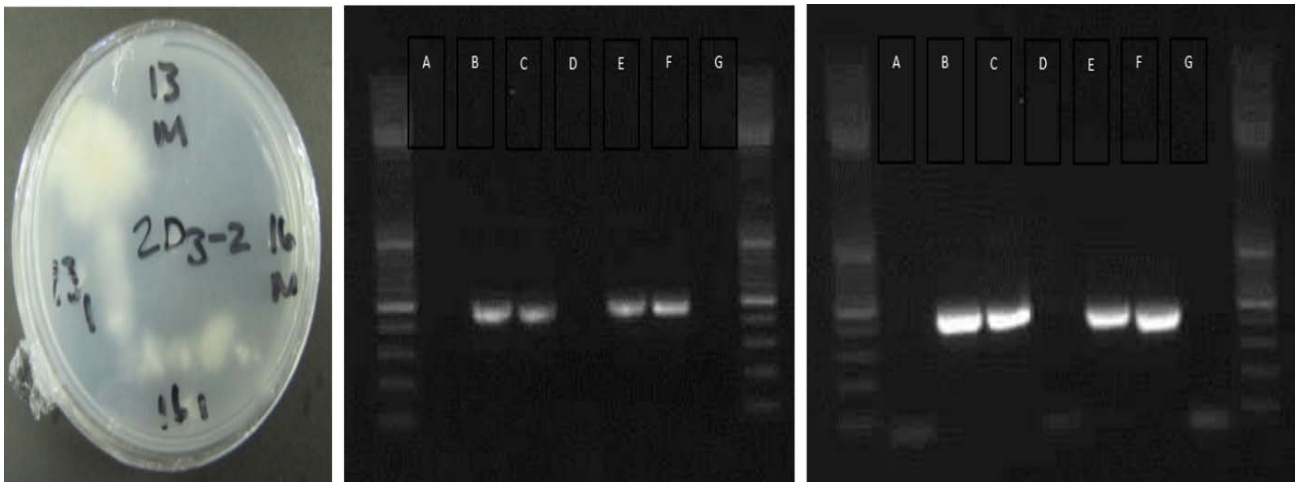
Field evaluation of Fusarium wilt severity was based on external leaf symptom development, measured on a scale of 1–5, with 1 = no symptoms and 5 = plant dead.<sup>23</sup> Susceptibility of the clones to Foc TR4 was determined by comparing the area under the disease progress curve.<sup>26</sup> For production traits, data were collected for the number of weeks from planting to flowering and harvest: plant height and pseudostem diameter at flowering, the number of leaves at flowering, gross bunch weight and number of hands at harvest, and finger length of the lower and upper hand at harvest. After data collection, the plot means were calculated and analysed at a 95% confidence level using analyses of variance (ANOVA). Pairwise comparisons were made using Fisher's protected 95% least significant difference. All analyses were conducted in XLSTAT-Premium (2018) for Windows.

## Results

### Identification of the Fusarium wilt fungus

The fungal isolates obtained from samples collected at Metocheria and Lurio farms in northern Mozambique were morphologically identified as *F. oxysporum*. They produced white to creamy aerial mycelia on PDA, with a tinge of purple in the colony centres. On CLA, microconidia were produced abundantly in false-heads on short monophialides, and macroconidia were produced sparsely in sporodochia. Single or pairs of chlamydospores were formed inside hyphae or macroconidia after 10–14 days.

VCG tests showed that the isolates from the two commercial Cavendish farms paired with tester strains belonging to Foc VCGs 01213/16 (Figure 2). This VCG is commonly known to be a member of Foc TR4. The primer set of Dita et al.<sup>21</sup> produced a 463-bp amplicon, and the one of Li et al.<sup>22</sup> a 455-bp fragment, thereby confirming that the Mozambican isolates indeed belonged to Foc TR4 (Figure 2). All subsequent collections from Metocheria and Lurio farms also showed that the Foc strains causing disease to Cavendish bananas on commercial farms in northern Mozambique were Foc TR4 (VCG 01213/16).



Lane A: *Fusarium inquisitii-incarnatum*; Lane B: Unknown plant sample 1; Lane C: Unknown plant sample 2; Lane D: *F. inquisitii-incarnatum*; Lane E: Unknown plant sample 3; Lane F: Foc TR4 control; Lane G: water control

**Figure 2:** Identification of isolates collected from a commercial banana plantation in northern Mozambique as *Fusarium oxysporum* f. sp. *cabense* (Foc) TR4. (a) Heterokaryon (barrier) formation between *nit-1* mutants of a Foc isolate collected at Metocheria farms and *nit-M* mutants of Foc TR4 (VCG 01213/16). (b) PCR amplification using Foc TR4-specific primer set developed by Dita et al.<sup>21</sup> (c) PCR amplification using a Foc TR4-specific primer set developed by Li et al.<sup>22</sup>

The Cavendish banana plantlets inoculated with *F. oxysporum* isolates from both Metocheria and Lurio farms developed yellow leaves within 4 weeks, which became more pronounced in the following 2 weeks until the plants wilted and died. When the pseudostems were cut open, the inner rhizomes were dark brown, and the inoculated fungus could be re-isolated from the edges of the necrotic tissue to confirm Koch's postulates. The symptoms caused by the Mozambican isolates were similar to those developing on plants inoculated with an isolate of Foc TR4 from Australia. Plants inoculated with pathogen-free millet seed did not develop any internal or external symptoms.

### Spread of Foc TR4 in Mozambique

#### On-farm disease development

After the first symptoms of Fusarium wilt were observed in February 2013, and before the identity of the fungus was confirmed in July 2013, the management at Metocheria farm started to notice more symptomatic plants. Initially, diseased plants were observed in Plantation 2 on an area of approximately 265 ha (Table 1). The worst affected blocks of 50 ha were removed by August that same year, and the area was fenced in an effort to contain the disease (Figure 3). None of the plants in the other plantations developed any symptoms until August 2013, when a few plants next to farm roads turned yellow and died.

By July 2014, the disease incidence increased almost three-fold in all plantations. By then the disease was present in all Plantation 1 and 2 blocks (data not presented), and containment became difficult, forcing a strategy to fence the entire Plantation 2. Fusarium wilt cases continued to increase, and by April 2015 the number of diseased plants in the six plantations increased between 7 and 20 times (Table 1). By the end of October 2015, more than 500 000 plants were killed by Fusarium wilt TR4, which meant that almost 20% of all plants at Metocheria farm were affected. Containment, at this stage, was no longer possible.

Fusarium wilt TR4 was first observed at Jacaranda Company's Lurio farm in April 2014. The first symptoms developed at the edge of the plantation, followed by new outbreaks next to the road 2 months later (Figure 4). Due to an early and active scouting and containment strategy, the disease was slowed down compared to that observed at Metocheria. Counting was discontinued in December 2016 when 42 of the 50 blocks were affected by Fusarium wilt TR4.

#### National surveillance

Samples collected in the Nampula and Capo Delgado Provinces in April 2015 resulted in the identification of five isolates belonging to Foc Lineage VI (VCG complex 0124/5/8/22, and VCGs 0124, 0128, 01220), and three belonging to Foc TR4 (VCG 01213/16). Of the Foc TR4 isolates, two were collected at Lurio farm and one isolate was obtained from a Cavendish banana planted on a small-grower's homestead near Metocheria farm. The plant was destroyed and the other banana plants at the property removed. All Foc Lineage VI VCGs were obtained from 'Macua' (Bluggoe) bananas.

Of the 56 samples collected in October 2015, 23 were morphologically identified as *F. oxysporum*. Of these, 13 were either Foc Lineage VI or Foc TR4. Four of the Foc Lineage VI isolates were collected from 'Apple' (Silk) and 'Macua' bananas in Nampula Province, and three from 'Apple' banana in Maputo Province (Figure 5). Foc TR4 was associated with Cavendish banana cultivars at Metocheria and Lurio farms only. VCG testing revealed that the Foc TR4 isolates belonged to VCG 01213/16, and the Foc races 1 and 2 isolates collected in the Nampula and Maputo Provinces belonged to VCGs 0124, 0125 and 01220.

#### Evaluation of Cavendish somaclones in northern Mozambique

Field evaluation of Cavendish somaclones and DPM-25 showed that the Taiwanese somaclones were significantly more resistant to Foc TR4 than the susceptible Cavendish control and DPM-25 (Figure 6). There was no significant difference in the area under the disease progress curve among the four GCTCV somaclones tested. The most resistant of the TBRI somaclones was GCTCV-119, which developed symptoms with an average disease rating of less than 2 over two disease cycles. GCTCV-218 was second most resistant with an average disease rating of less than 2 in the plant crop, and less than 2.5 in the ratoon crop (Figure 6). Disease development was also faster in Nandi and DPM-25 than in the somaclones. For instance, disease severity often progressed from Level 2 to Level 5 in less than a month, whereas the disease developed much slower in the somaclones. Nandi and DPM-25 showed symptoms 2–4 months after planting, whereas the disease in the somaclones developed from 6 months after planting until flowering (Figure 6).



**Figure 3:** Metocheria farm consisted of five plantations. More than 50 hectares of banana was killed after the discovery of Fusarium wilt in 2013 (inside red square), and the area has been replanted with Formosana somaclones from Taiwan since 2016.

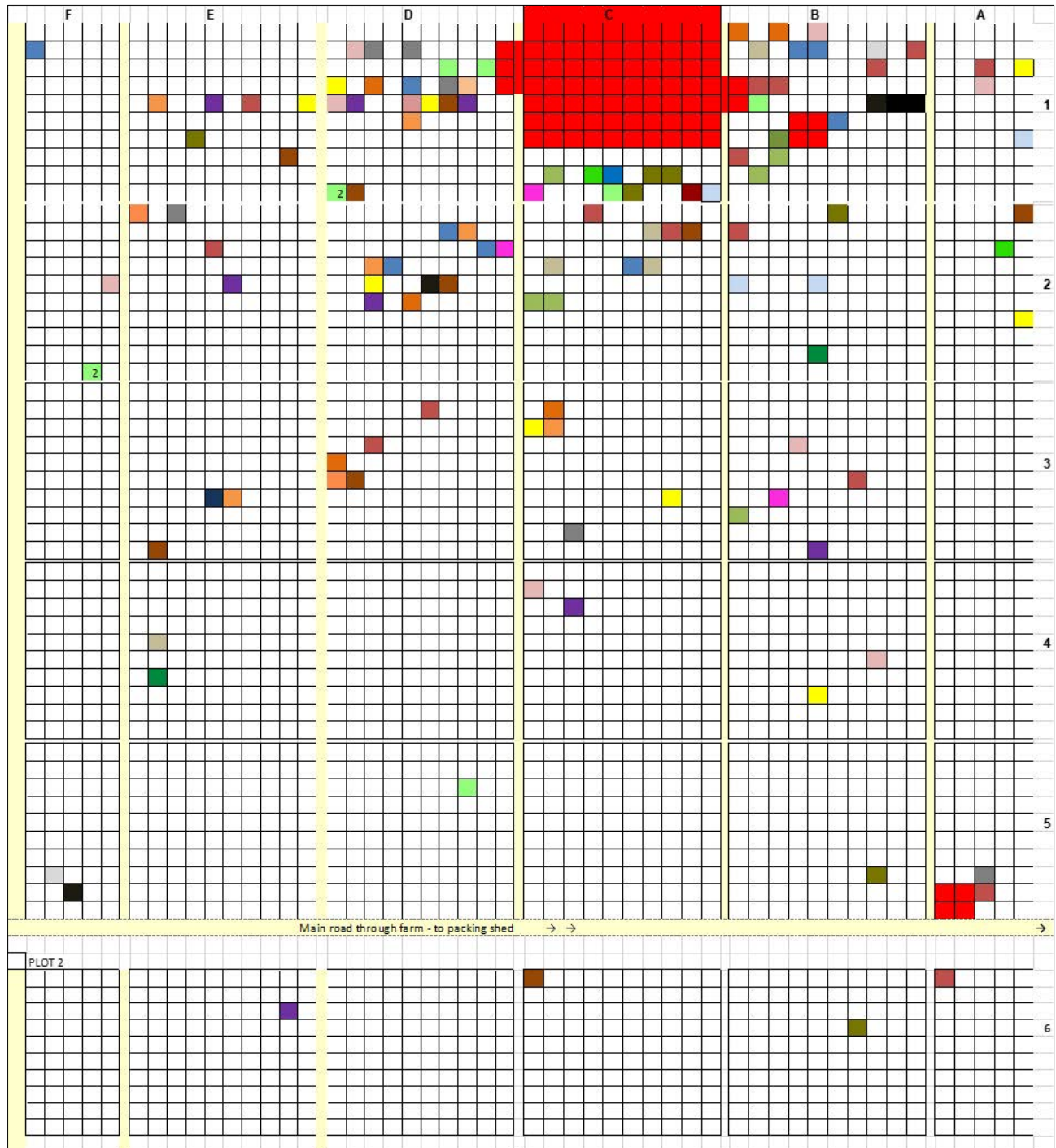
**Table 1:** The number of plants affected by banana Fusarium wilt TR4 at Metocheria farm between August 2013 and July 2016

Date	Plantation					Total
	1	2	3	4	5	
April 2013	0	780	0	0	0	780
July 2013	0	4756	0	0	0	4756
October 2013	55	5650	25	10	30	5770
January 2014	143	6187	129	46	91	6596
April 2014	1164	7888	445	215	239	9951
July 2014	4476	19 858	2736	1150	503	28 723
October 2014	7121	45 680	6315	2538	852	62 506
January 2015	11 286	87 096	18 852	7645	4539	129 418
April 2015	22 082	142 480	43 919	24 954	13 743	247 178
July 2015	56 816	198 843	88 235	49 611	25 722	419 227
October 2015	76 280	237 556	111 426	67 004	31 918	524 184

In the plant crop, more than 75% of Nandi and DPM-25 plants were infected at the end of the crop cycle (data not presented), with a disease severity of more than 4 on a rating scale of 1–5. Less than 25% of the somaclones were infected, with an average disease severity of less than 2.5 (Figure 6). In the ratoon crop, about 50% of the surviving Nandi and DPM-25 plants were infected, with a disease severity of more than 4.5 out of 5. Less than 25% of GCTCV-119 and GCTCV-218 were infected, with a disease severity of less than 2.6. About 60–70% of somaclones that showed symptoms at an early stage, recovered to produce bunches. Internal discolouration of the susceptible and the partially resistant somaclones also differed. When Nandi and DPM-25 were split longitudinally, vascular discolouration was visible to the centre of the pseudostem, whereas pseudostem discolouration in the somaclones

was limited to the outer leaf blades. More disease developed in the second than in the first crop cycle.

Nandi and DPM-25 grew significantly faster than the somaclones, with a period from planting to harvest of less than 51 days compared to most somaclones that took more than 60 days (Table 2). In the plant crop, Nandi was the fastest grower, but had a great variation in bunch weights between the 2 years. In the plant crop, the biggest bunches were produced by DPM-25 and GCTCV-218, while the largest bunches in the ratoon crop were produced by Nandi and DPM-25. In the ratoon crop, the somaclones produced smaller bunches and grew into taller plants, making harvesting more difficult to manage compared to Nandi and DPM-25 (Table 2).



**Figure 4:** An illustration of the spread of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 at Lurio farm in northern Mozambique by July 2015. Each block represents 1 ha, and each colour indicates a different week. The yellow lines are farm roads. The red blocks indicate the areas where banana Fusarium wilt TR4 was first detected, whereafter production in block C1 was terminated.

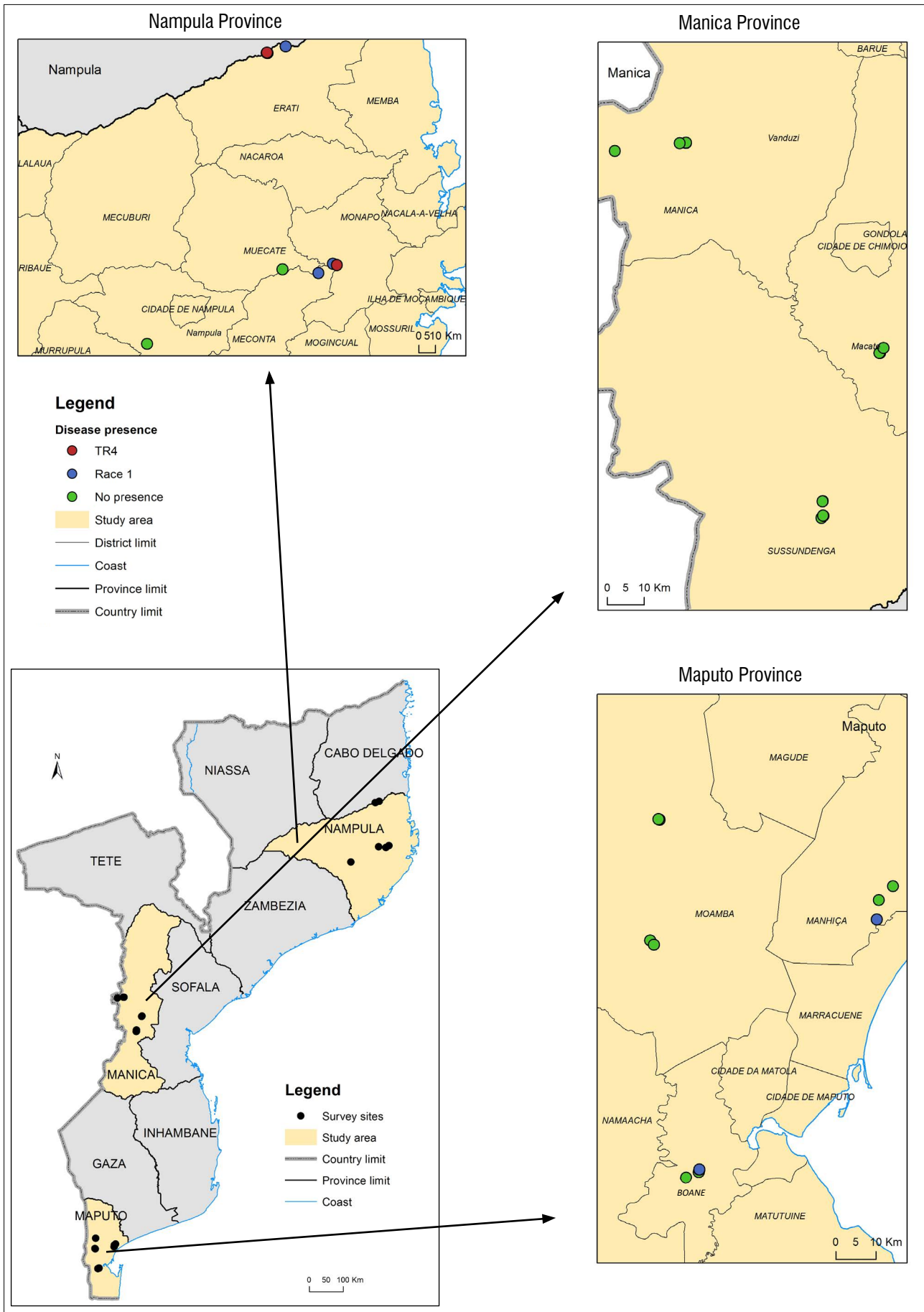
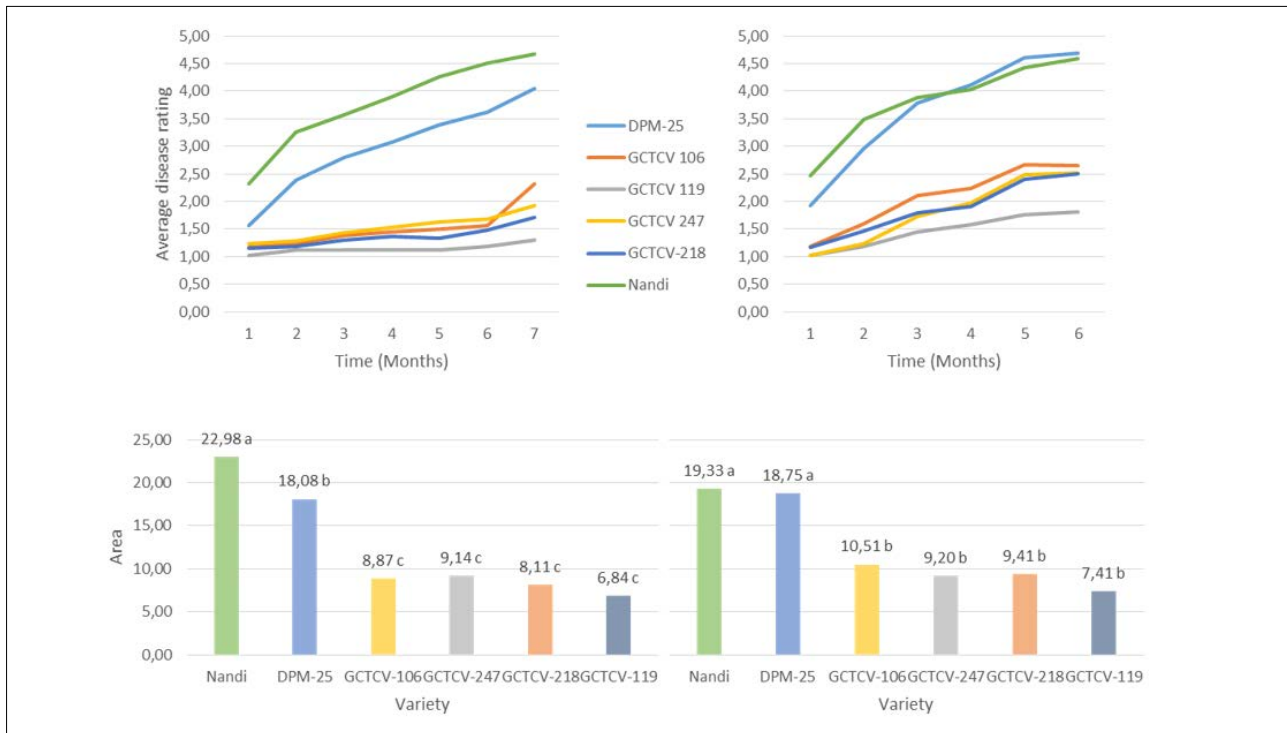


Figure 5: The distribution of *Fusarium oxysporum* f. sp. *cubense* in Mozambique.



**Figure 6:** Field evaluation of Cavendish banana somaclones for resistance to Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense* TR4, over two seasons. The figures at the top represent the average disease rating over two seasons, and the figures at the bottom represent the area under the disease progress curve. The figures on the left represent the disease development in the plant crop, and those on the right show disease development in the ratoon crop.

**Table 2:** Agronomic properties recorded for the plant crop and first ratoon of Cavendish clones at Jacaranda’s Lurio farm, northern Mozambique

Plant crop									
Variety	Weeks from planting to flowering	Weeks from planting to harvest	Height at flowering (cm)	Pseudostem diameter at flowering (1 m)	Number of leaves at flowering	Gross bunch weight	Number of hands	Finger length: Lower hand	Finger length: Upper hand
'Nandi'	38.078 f	47.853 f	193.742 e	49.102 d	11.845 b	16.149 d	6.020 c	19.849 b	22.723 b
DPM-25	39.998 e	50.905 e	223.940 d	50.829 c	12.879 a	19.546 a	6.888 a	20.494 a	23.374 a
GCTCV106	49.283 c	60.236 c	240.120 c	49.720 d	11.778 b	17.472 c	5.904 c	18.733 d	20.965 d
GCTCV247	44.558 d	57.679 d	253.255 b	47.324 e	11.370 c	17.546 bc	5.954 c	19.821 b	22.517 b
GCTCV218	54.027 b	62.806 b	244.169 c	53.093 b	12.096 b	19.710 a	6.524 b	19.898 b	22.483 b
GCTCV119	64.899 a	72.578 a	281.198 a	54.852 a	10.837 d	18.242 b	5.151 d	19.364 c	21.379 c

First ratoon									
Variety	Weeks from planting to flowering	Weeks from planting to harvest	Height at flowering (cm)	Pseudostem diameter at flowering (1 m)	Number of leaves at flowering	Gross bunch weight	Number of hands	Finger length: Lower hand	Finger length: Upper hand
'Nandi'	73.762 e	82.867 d	220.965 d	57.475 de	12.724 a	20.220 ab	6.263 a	20.394 a	22.595 a
DPM-25	76.122 e	86.612 c	279.453 c	59.889 b	12.703 a	19.644 bc	6.096 a	19.753 ab	22.157 ab
GCTCV106	84.968 c	93.658 b	292.056 b	56.875 e	11.848 b	19.217 c	5.769 b	18.908 c	20.913 c
GCTCV247	80.673 d	91.492 b	313.267 a	58.263 cd	11.944 b	18.229 d	5.754 b	19.383 bc	21.779 b
GCTCV218	88.322 b	98.962 a	281.965 c	61.319 a	11.993 b	20.535 a	6.241 a	19.176 bc	21.694 bc
GCTCV119	91.127 a	97.940 a	308.091 a	59.350 bc	10.989 c	19.602 bc	5.170 c	19.494 bc	21.798 b

Values followed by the same number in each column do not differ significantly from each other (p<0.05).





## Discussion

The discovery of banana Fusarium wilt TR4 on a commercial Cavendish banana farm in northern Mozambique signifies the first occurrence of Foc TR4 on the African continent. It was initially believed that the disease could have been caused by Foc STR4, a strain present in South Africa.<sup>12</sup> International staff and workers employed at Metocheria farm included South Africans, and the farm was frequently visited by South African service providers. Planting material used at the farm was not believed to be the source of introduction, as these plants were obtained from reputable banana tissue culture companies in South Africa and Israel. Banana plantlets produced *in vitro* are also known to be Foc-free. The eventual identification of the causal agent as Foc TR4 came as a massive surprise. The only areas where Foc TR4 was known to occur by February 2013 were Southeast Asia, the Northern Territory of Australia, and Oman.<sup>9</sup> It is thus believed that Foc TR4 was most likely introduced into Metocheria from Asia, but it is unclear when and how this happened. Circumstantial evidence has subsequently implied that Foc TR4 might have been present at Metocheria farm as early as 2010 when several plants with leaf yellowing were noticed and destroyed. The fungus was introduced from Metocheria farm onto Lurio farm most likely by visitors or trucks visiting both farms before TR4 was known to occur at Metocheria farm.

The early outbreaks of Fusarium wilt TR4 at Metocheria farm's Plantation 2, and the progression of the epidemic, suggest that this field was the source from where Foc TR4 spread to the rest of the farm. Google Earth images also clearly show the spots where plants were first affected by the disease. The spread of Foc TR4 in this plantation, if present in 2010 already, might have been caused by the movement of plantation workers. The heavy clay soil can easily attach to shoes, plantation tools and machinery and, if infested with Foc TR4, could have moved the fungus into new areas.<sup>2,9</sup> The first cases of the disease in other plantations were initially found on road sides, and could have been spread by vehicles or the rotation of farm workers between plantations. Another possible source of dissemination could have been irrigation water, as water from infested plantations was drained into the river from where it was pumped back onto the plantations. The rapid increase of the disease by mid-2015 is most likely due to severe flooding in northern Mozambique in February 2015, which disseminated the soil-borne fungus everywhere. After this, all efforts to contain Foc TR4 on the farm became obsolete.

Fusarium wilt turned into a full-blown epidemic at Metocheria within 3 years, which prompted discussions of alternative options to deal with the losses. One option was to replace dead plants with new ones after 3–6 months, or the replanting of previously diseased areas at higher densities. The use of Cavendish somaclones and DPM-25 as potential replacement of susceptible Cavendish cultivars, and the selection of survivor plants in severely infested fields, were discussed, and trials initiated. The conversion of banana to other crops was considered, but the financial investment, logistics and market opportunities obstructed this option. Metocheria farm also invested in measures to prevent the spread of Foc TR4 off the farm. Sanitation processes and farm security were improved, standard operating procedures altered, and the movement of people and vehicles on the farm restricted.

Foc TR4 at Lurio farm was most likely introduced from Metocheria farm. In 2014, Lurio farm employed only one non-African worker who came from Latin America where Foc TR4 was not known to occur. The farm, however, was often visited by trucks transporting fruit, or by service providers and donor organisations, after they had visited Metocheria farm. Not many of the visitors drove into the plantation with their own vehicles. They did, however, visit banana fields with the owners without cleaning their shoes, as Foc TR4 had not yet been detected at Metocheria farm at the time. After the detection of Foc TR4 at Lurio farm, a scouting and containment strategy implemented at the farm significantly slowed down the spread of the disease compared to that observed at Metocheria. The property of 50 ha, also, was smaller and easier to manage. Still, the disease could not be contained, and by 2016 led to the systematic killing of many susceptible Cavendish banana plants.

The presence of Foc TR4 in northern Mozambique prompted the Department of Agriculture and Food Security to introduce quarantine measures that restricted the movement of plants and planting material off the affected farms, and the transport of fruit outside the Nampula and Cabo Delgado Provinces. Strict containment measures were introduced at both Metocheria and Lurio farms, which included gate control, the use of disinfectants and the issuing of footwear to all workers and visitors. Only farm vehicles were allowed to enter plantations. Farm workers, communities and plant protection officials were trained in symptom identification, and awareness programmes were launched. The single case of Foc TR4 affecting a Cavendish banana plant on a small-grower's homestead near Metocheria farm could be tracked back to planting material taken off the farm before Foc TR4 was discovered in northern Mozambique. Foc TR4 was not detected in local cooking bananas found in small patches everywhere in northern Mozambique, even though the variety was shown to be susceptible when tested at Metocheria farm in 2015 (Viljoen, unpublished data).

The national survey conducted in 2015 in Mozambique confirmed that Foc races 1 and 2 strains are spread across Mozambique, as previously documented.<sup>10</sup> Their presence, however, is limited to areas where farmers grow non-Cavendish local cultivars such as 'Apple' and 'Macua' bananas. The absence of Fusarium wilt in Manica Province can be attributed mainly to the widespread planting of Cavendish cultivars in the province. These Cavendish bananas are also not affected by Foc TR4 that is present in Nampula Province, or Foc STR4 that is present in South Africa.<sup>12</sup> The occurrence of Foc TR4 in Mozambique has become of great concern to growers in Mozambique, its neighbours and other African countries as the pathogen does not only affect Cavendish cultivars, but also all other cultivars susceptible to Foc races 1 and 2.<sup>6,9</sup>

The Cavendish somaclones evaluated in this study were significantly more resistant to Foc TR4 than Nandi and DPM-25 over two planting seasons, even though they were slower growing, generally taller, and produced smaller bunches in the ratoon cycle. While GCTCV-119 was most resistant to Foc TR4, it performed worst in production properties. GCTCV-218, however, was moderately resistant but produced excellent bunches. GCTCV-218 is registered as Formosana, a banana cultivar developed by TBRI in Taiwan. This cultivar is now also replacing susceptible Cavendish varieties in the Philippines. Mozambique, and southern Africa in general, have a climate different from that in Taiwan, and it is suggested that further selection be made of GCTCV-218 for higher-yielding clones adapted to local climatic conditions. The other GCTCV somaclones could also be considered for field selection of clones with superior production properties. This has been the case with the selection of GCTCV-219 from GCTCV-119 in the Philippines.<sup>27</sup>

The detection of Foc TR4 in northern Mozambique resulted in significant concerns about the threat of the fungus to bananas on the African continent, as the crop is considered a staple food in many countries. Most bananas grown in Mozambique, like the rest of southern Africa, are Cavendish bananas known to be highly susceptible to Foc TR4.<sup>9</sup> In East and Central Africa, banana provides an income to over 20 million resource-poor farmers, and is more profitable than any other crop grown in the region.<sup>28</sup> These bananas include a group of unique cooking and beer bananas, called East African Highland bananas (EAHBs-AAA). West Africa produces primarily African plantains (AAB), another type of cooking banana that evolved on the continent after being introduced almost 3000 years earlier.<sup>29</sup> West Africa also produces Cavendish bananas for export, while Cavendish plantations are being expanded in many countries on the east coast of Africa. The testing of Cavendish somaclones in this study was therefore of great significance to the replacement of susceptible Cavendish plantings if necessary. A small group of EAHBs and plantains had been evaluated in Asia for resistance to Foc TR4<sup>30,31</sup>, but the testing of a much larger set of EAHBs and plantains is required to fully understand their response to the fungus.

In May 2019, the owners of Metocheria farm filed for insolvency. The primary reason was a limitation in cash flow to deal with Foc TR4 and not because Formosana, which was widely replanted at the time, had failed as a replacement variety. Jacaranda Agricultura has since purchased Metocheria farm, and has renamed it 'Monapo River farm'.

Containment measures have been strengthened, and fields are being replanted with Formosana. The availability of this plant has changed the lives and livelihoods of thousands of Mozambicans employed at Monapo River farm and living in the surrounding communities. Surveillance efforts to establish Foc TR4 distribution in northern Mozambique and in southern Africa are continuing. Urgent priorities to deal with Foc TR4 in southern and eastern Africa were also identified at a Foc TR4 strategy meeting in Maputo in 2019. These include the testing of African banana varieties for resistance to Foc TR4 at Monapo River farm, the enhancement of Formosana's resilience to Foc TR4 with integrated disease management practices, an assessment of the impact of Foc TR4 in Mozambique, and the improvement of biosecurity and preparedness on the African continent.

The possibility of Fusarium wilt spreading from the infested properties in northern Mozambique into other parts of the country and to neighbouring countries remains. To deal with this possibility, three strategies to manage the pathogen should be employed. These include the prevention of introduction into new areas by proper awareness campaigns, farm border control, and the use of clean planting and field materials; the early detection and containment of new outbreaks, which involves routine scouting and the appropriate isolation of newly infested field sites; and the management of the disease by planting disease-resistant varieties and reducing inoculum levels through good agricultural practices, and soil and water management. Given the difficulties in managing the disease, these measures should be a collective effort involving policymakers, growers and the scientific community, and should be implemented on local and regional levels. A proper pest risk analysis for Foc TR4 in Mozambique and Africa is required in the event that the fungus spreads beyond the borders of the two affected farms. Banana cultivars and production areas that can be affected need to be identified, potential pathways of spread determined, and the risk of the fungus spreading to these areas managed. Resistant banana varieties in Mozambique and Africa still need to be identified, and the possible spread of Foc TR4 in Mozambique needs to be continuously investigated. The strict implementation and effectiveness of quarantine measures announced by the Mozambican government should also be continuously monitored.

## Acknowledgements

The research undertaken in this study was funded by Stellenbosch University, Eduardo Mondlane University, Matanuska Mozambique Ltd, the Food and Agricultural Organization of the United Nations and RUFORM. We also want to acknowledge Dr Serafina Mangana, former Head of Departamento de Sanidade Vegetal at the Ministério da Agricultura, for her invaluable contribution to activities related to mitigating Foc TR4 in Mozambique; DuRoi Laboratories for providing banana plants for the trials; and Matanuska Mozambique and Jacaranda Agricultura for performing field trials and collecting data on disease incidence.

## Competing interests

We declare that there are no competing interests.

## Authors' contributions

A.V.: Conceptualisation, methodology, data collection, validation, writing the initial draft, student supervision, project leadership, project management and funding acquisition. D.M.: Conceptualisation, methodology, sample analysis, validation, data curation, writing revisions, student supervision and project management. T.C.: Sample analysis, data analysis, writing revisions, student supervision, and funding acquisition. I.B.: Sample analysis and data analysis. C.F.: Methodology, data collection and writing revisions. J.D.; H.M.: Methodology, data collection, validation, writing revisions, project management and funding acquisition. J.A.: Methodology, data collection, validation and writing revisions. E.L.M.: Data collection and writing revisions. G.T.: Data collection, data analysis, validation and project management. O.M.A.: Methodology, data collection, data analysis, validation, data curation and writing revisions. A.M.: Methodology, data analysis, validation, student supervision and funding acquisition. A.V.: Writing revisions, project management and funding acquisition. A.P.; S.B.: Data collection and data curation. L.J.R.: Student

supervision and writing revisions. F.B.; A.B.M.; C.-P.C.: Conceptualisation and writing revisions. F.D.: Writing revisions, project management and funding acquisition.

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