






Suppressiveness or conduciveness to *Fusarium* wilt of bananas differs between key Australian soils

Alison Bowen ^A, Ryan Orr ^A, Anna V. McBeath ^B, Anthony Pattison ^B,
and Paul N. Nelson ^{A,C}

^AJames Cook University, Cairns, Qld 4870, Australia.

^BDepartment of Agriculture and Fisheries, South Johnstone, Qld 4859, Australia.

^CCorresponding author. Email: paul.nelson@jcu.edu.au

Abstract. Soils are known to differ in suppressiveness to soil-borne diseases, but the suppressiveness or otherwise to *Fusarium* wilt of Australian soils used to grow bananas is unknown. In this work we tested the relative suppressiveness of six key soil types. Banana (*Musa* (AAB group) ‘Pome’, cultivar ‘Lady Finger’) was grown in pots of the soils inoculated or not with *Fusarium oxysporum* f.sp. *ubense* (*Foc*) ‘Race 1’. Sixteen weeks after inoculation the plants were harvested and disease severity was assessed by measuring discoloration within the rhizome. In the inoculated pots, disease severity was greatest in the alluvial Liverpool and Virgil soils and least in the basaltic origin Tolga soil. No disease was detected in the non-inoculated pots. Soils with the lowest disease severity had the highest root mass, irrespective of inoculation, and the largest (negative) effect of inoculation on root dry mass. Disease severity in inoculated pots was negatively correlated with soil clay content and β -glucosidase activity. The results indicate that the risk of *Fusarium* wilt negatively impacting banana growth differs between soils of the main Australian banana-growing region.

Additional keywords: clay, crop disease, microbial diversity, soil fungi, tropical soils.

Received 7 June 2018, accepted 13 December 2018, published online 7 February 2019

Introduction

Soils suppressive to soil-borne diseases, recognised for over a century, are defined as those in which disease development is minimal despite the presence of both a virulent pathogen and a susceptible plant host (Mazzola 2002). An active and functionally diverse soil microbial community, with which pathogens must compete for nutrients and habitat, is widely accepted as the basis of disease-suppressive soils (Höper and Alabouvette 1996; Brady and Weil 2008; Cha *et al.* 2016). The key role soil microorganisms perform in the development of disease suppression was supported by the observation that suppression could be eliminated through soil pasteurisation (Mazzola 2002; Adiobo *et al.* 2007), and may be restored by mixing a small quantity of suppressive soil into a conducive soil (Alabouvette 1986; Alabouvette 1999; Siegel-Hertz *et al.* 2018). However, soil physical and chemical properties may influence disease severity via their influence on microbial activity, or the health and defence capabilities of the host plant (Mazzola 2002). Suppressiveness of soils to soil-borne diseases has been reported for many diseases, including *Fusarium* wilt, an important disease of many plant species (Höper and Alabouvette 1996; Orr and Nelson 2018).

Fusarium wilt of banana (*Musa* spp.), caused by *Fusarium oxysporum* f.sp. *ubense* (*Foc*), threatens banana production throughout the world. *Foc* has traditionally been subdivided into four ‘races’ based on the banana cultivars affected

(Ploetz 2015). Race 1 was responsible for the end of large-scale commercial production of the once economically important Gros Michel cultivar. It also affects other cultivars from the ‘Pome’ subgroup. Race 2 affects cooking bananas such as Bluggoe, and Race 3 affects Heliconias. Race 4 affects Cavendish (*Musa* AAA, Cavendish subgroup) as well as cultivars susceptible to Races 1 and 2. Race 4 is subdivided into Subtropical Race 4, which affects susceptible cultivars under suboptimal growing conditions, and Tropical Race 4 (TR4), which affects susceptible cultivars in all conditions (Ploetz 2015). *Foc* TR4 is spreading rapidly throughout the world (Mostert *et al.* 2017). It reached the main banana-growing area of Australia in Far North Queensland in 2015 (O’Neill *et al.* 2016; Queensland Government 2017). Far North Queensland supplies more than 90% of Australia’s bananas (Queensland Government 2016), and Cook *et al.* (2015) predicted losses to Australia’s banana industry caused by TR4 to exceed AU\$138 million per year, despite a slow rate of disease spread.

Microbially mediated suppression to *Fusarium* wilt has been related to numerous soil biotic and abiotic properties. Soil abiotic properties have direct and indirect roles, through their influence on soil microorganisms and plant defence capabilities (Orr and Nelson 2018). However, little is known about the relative suppressiveness of Australian soils to *Foc*. Knowing the variation in suppressiveness to *Foc* among the

main Australian soil types used to grow bananas would inform decisions about banana cropping and soil management, and planning of biosecurity measures. This study aimed to determine whether the main soil types used to grow bananas in Far North Queensland differ in their suppressiveness to Fusarium wilt, and to identify soil characteristics that may be associated with suppression among these soils.

Materials and methods

Six soils with a wide range of characteristics were assayed for their suppressiveness in a greenhouse pot experiment. The experiment was a factorial design with randomised blocks. The treatments were inoculation (inoculated with *Foc* versus non-inoculated) and soil (six soils), with six replicates, giving 72 pots in total.

The soil was taken between 28 February and 9 March 2017 from locations and soil series representative of the Far North Queensland banana-growing region, from fields growing *Musa* AAA group, Cavendish subgroup bananas for at least 2 years. We understood from the growers that the sampled fields were free from previous *Foc* infection, and none of the fields had been used to grow cultivars susceptible to *Foc* Race 1. The soils, which have been described by Murtha (1986), Cannon *et al.* (1992) and Enderlin *et al.* (1997), comprised four well-drained uniform or gradational textured soils formed on alluvium: Innisfail series, a Brown Dermosol (17.485122°S, 145.859047°E), Liverpool series, an Orthic Tenosol (17.454604°S, 145.864289°E), Tully series, a Brown Dermosol (17.481890°S, 145.858678°E) and Virgil series, a Red Kandosol (17.898027°S, 145.832213°E), and two red gradational textured soils formed on basalt, both Red Ferrosols: Pin Gin series (17.592257°S, 145.833088°E) and Tolga series (17.010557°S, 145.527065°E). Soil was collected from 0–0.25 m depth, 0.4 m from the base of mature, but not bunching, plants. Rocks, large roots and debris were removed, and samples were homogenised and left in the shade until pot filling.

Samples of the soil were analysed for several physicochemical properties. They were analysed by Nutrient Advantage Laboratory (www.nutrientadvantage.com.au), using

methods described in Rayment and Lyons (2011), for particle size distribution, pH and electrical conductivity (1:5 soil:water), organic C (Walkley–Black), total metals (acid digest), DTPA-extractible metals, Colwell P, MCP-extractible S, CaCl₂-extractible Si, hot CaCl₂-extractible B, and exchangeable cations (BaCl₂/NH₄Cl and ammonium acetate). The water holding characteristics of the soils were determined by measuring water content at –10 kPa using a ceramic plate and hanging water column, and at –200 and –1500 kPa using a WP4 Dew Point Potentiometer (Decagon Devices, Pullman, WA, USA). Mineralogical analysis was performed by CSIRO laboratories, South Australia using X-ray diffraction. Soil mineral contents were expressed as a percent of the sum of identified mineral contents, ignoring any amorphous or otherwise unidentified materials. The soils ranged from sandy clay loam to clay in texture and had pH ranging from 5.6 to 7.1 (Table 1). Clay mineralogy was dominated by kaolin, with some mica and haematite in most soils (Table 1).

The soil was placed into 0.25-m diameter, 7.2-L pots to ~10 mm from the rim. Oven-dry equivalent mass and bulk density of soil ranged from 6804 g pot⁻¹ and 1.02 g cm⁻³ respectively for Pin Gin to 7615 g pot⁻¹ and 1.14 g cm⁻³ respectively for Innisfail, being uniform for each soil type. Each pot was planted on 21 March 2017 with a plantlet of tissue-culture-raised *Musa* (AAB group) ‘Pome’, cultivar ‘Lady Finger’, which is susceptible to *Foc* Race 1. After allowing 3 weeks for the plantlets to establish, the pots were inoculated with *Foc* Race 1, Vegetative Compatibility Group 0124, by mixing 10 mL of millet seed (*Pennisetum glaucum*) colonised with *Foc* Race 1 (Forsyth *et al.* 2006), supplied by the Queensland Department of Agriculture and Fisheries, into the top 2 cm of soil. Non-inoculated pots were amended in the same manner with 10 mL of autoclaved millet seed.

The greenhouse growing conditions and fertilisation routine were adapted during the experiment in response to plant requirements, but all plants were treated the same. Plants were fertilised fortnightly with 83 mg urea (Richgro[®] fertilisers), 10 mg P as ‘Mega Booster’ guano (Richgro[®] fertilisers), and 124 mg KCl, according to the rates recommended by Weinert

Table 1. Soil properties, including all those significantly correlated ($P < 0.05$) with internal disease severity in inoculated plants, showing Spearman’s rank correlation coefficient (r) and P value ($n = 6$)

FCW, field capacity water content (–10 kPa); RAW, readily available water (–10 to –200 kPa); TAW, total available water (–10 to –1500 kPa)

	Tolga	Tully	Pin Gin	Innisfail	Liverpool	Virgil	r	P
Texture	Clay	Clay loam	Clay	Clay loam	Clay loam	Sandy clay loam		
Clay (%)	56	28	39	27	26	21	–0.943	<u>0.005</u>
pH	5.6	6.6	7.1	6.7	7.1	6.8	0.667	0.148
Organic C (%)	1.7	1.0	1.5	1.5	1.0	3.2	0.147	0.781
Total Fe (g kg ⁻¹)	100	38	56	30	35	13	–0.886	<u>0.019</u>
Total Cu (mg kg ⁻¹)	110	59	89	38	35	20	–0.943	<u>0.005</u>
Total Cd (mg kg ⁻¹)	0.57	0.32	0.42	0.30	0.27	0.13	–0.943	<u>0.005</u>
Extr. B (mg kg ⁻¹)	5.9	1.4	5.1	1.2	0.8	0.8	–0.886	<u>0.019</u>
Kaolin (%)	69	23	43	24	21	20	–0.824	<u>0.042</u>
Mica (%)	<1	10	5	7	9	<1	0.058	0.913
Haematite (%)	12	1	2	2	1	2	–0.309	0.552
FCW (g g ⁻¹)	0.320	0.225	0.260	0.245	0.208	0.188	–0.899	<u>0.015</u>
RAW (g g ⁻¹)	0.103	0.123	0.091	0.108	0.093	0.069	–0.600	0.208
TAW (g g ⁻¹)	0.113	0.135	0.100	0.127	0.108	0.086	–0.600	0.208

and Simpson (2016). Around 4 weeks post-inoculation, most plants appeared to be suffering some nutrient deficiency symptoms, most likely Zn, Ca and B. Consequently 39 mg Ca as gypsum was added fortnightly from then on, and Yates 'health tonic trace elements chelates' foliar spray (% w/v: S 0.53, Fe 0.34, Mg 0.34, Zn 0.34, Mn 0.26, B 0.13, Cu 0.043, Mo 0.005) (Yates® Australia) was applied twice. Nine weeks after planting, plants were subjected to a water deficit for 3 weeks by reducing watering from every day to every third day. Over the course of most of the experiment the average temperature was 25.3°C in the air (12.5°C minimum to 36.9°C maximum) and 24.0°C in the soil (14.5°C minimum to 33.4°C maximum), apart from several unusually hot days at the beginning of the experiment, when the temperature reached a maximum of 48.1°C in the air and 35.9°C in the soil.

Several plant measurements were taken over the course of the experiment, once per week: number of living, fully unfurled leaves; pseudostem girth at 5 cm from the soil surface; area of the third most recently fully unfurled leaf (Turner and Lahav 1983); chlorophyll content of the same leaf (SPAD 502 Plus Chlorophyll Meter, Spectrum Technologies Inc.) and external disease severity score. External disease severity was evaluated using a scale adapted from Nasir *et al.* (2003) and symptoms described by Orjeda (1998) and Queensland Government (2017): number of yellowing leaves; splitting of pseudostem; and changes to leaves such as narrower, less smooth or paler margin, lamina ripping, more erect habit, petiole collapse, or wilting. Scale values ranged from 0 for no symptoms to 3 for severe symptoms. For each plant, all 10 scores taken over the course of the experiment were added together to obtain the 'area under the disease progression curve' (AUDPC).

The plants were harvested 19 weeks after planting and analysed for internal disease severity, fresh mass and dry mass. Every plant was evaluated for internal disease severity by measuring discoloration of transverse sections of the rhizome and pseudostem, using a modified version of the disease severity methodology and scale for Fusarium wilt of banana (Carlier *et al.* 2003). The pseudostem was cut transversely at the soil surface and 5 cm above it, and the rhizome was cut into four equal sections. The upper surface of the two lower pseudostem sections and three lower rhizome sections was photographed and the area of infected rhizome tissue was calculated using the program ImageJ 1.49v (National Institute of Health, USA). The disease severity value for each plant was calculated as the area of discoloured rhizome vascular tissue as a percentage of the total rhizome vascular tissue area, averaged over the three rhizome sections. To determine mass, each plant was subdivided into four portions (roots, rhizome, pseudostem and leaves) and the fresh mass of each portion measured. Dry mass was then determined by measuring water content of a representative subsample of each portion of each plant.

Roots were analysed for mycorrhizae and *Foc* Race 1. Roots from three replicates were analysed for abundance of vesicular-arbuscular mycorrhizae using the protocol described by Koske and Gemma (1989). Roots were also analysed for the presence of *Foc* Race 1 VCG 0124, to confirm success of the inoculation and check that there was no infection in the non-inoculated pots. The analysis was performed on monoconidial isolates of *Foc* recovered from roots, according to the

technique described by Puhalla (1985). Nitrate non-utilising (nit) mutants produced from the isolates were paired with tester Nit M mutants obtained from the culture collection of the Queensland Plant Pathology Herbarium (BRIP). The nit mutants from the isolates anastomosed to form stable heterokaryons when paired with VCG 0124 Nit M testers on minimal media, thus confirming their identity as VCG 0124.

Soil from the harvested pots was analysed for β -glucosidase activity by measuring fluorescence generated by cleavage of 4-methylumbelliferyl- β -D-glucopyranoside. Subsamples of soil, shaken from the root system and homogenised, were refrigerated (<4°C) and stored for less than a month between harvest and analysis. Two replicates from each treatment combination were analysed, giving 24 samples in total. The assay protocol was adapted from Marx *et al.* (2001), Creamer *et al.* (2009), and Welc *et al.* (2014).

All data were tested for normality and homogeneity of variance. A Levene's test for homogeneity of variance showed no significant difference between the arcsine-transformed variances of the disease severity means of the inoculated soils ($F_{5, 30} = 1.10$; $P = 0.382$). The effect of the treatments (inoculation and soil type) on number of leaves per plant, pseudostem girth, area of the third leaf and leaf chlorophyll content, external disease severity score (AUDPC), internal disease severity (arcsine-transformed) and biomass was analysed by factorial analysis of variance ($n = 72$) and Tukey post-hoc analysis. The relationship between internal disease severity and soil properties was examined using Spearman's rank correlation using S-PLUS 8.2 for Windows. Because there was only one value of each soil property for each soil, this analysis had $n = 6$, with the disease severity value being the mean of inoculated pots in each soil type. The relationship between internal disease severity and β -glucosidase activity was examined using Spearman's rank correlation using S-PLUS 8.2 for Windows, with $n = 12$ (two inoculated pots from each soil type).

Results

Over the course of the experiment, development of disease was evident in some but not all measurements. External disease severity score, as quantified by the AUDPC, was significantly influenced by inoculation ($P < 0.001$) and soil type ($P = 0.013$). Of the inoculated plants, the highest external disease severity score was in Liverpool soil and the lowest was in Tolga, Tully, Innisfail and Pin Gin soils (Fig. 1). Pseudostem girth, which increased steadily over time, was significantly influenced at harvest by inoculation ($P = 0.013$) and soil type ($P = 0.014$) but not their interaction. Non-inoculated plants had a mean girth of 108 mm and inoculated plants 112 mm. Pin Gin had the lowest pseudostem girth (106 mm) and Virgil the highest (117 mm). Neither soil type nor inoculation had a significant effect on the number of living leaves per plant, the area of the third leaf, or leaf chlorophyll content. The number of leaves per plant rose to a maximum of 7.5 (grand mean, s.e.m. 0.12) at 12 weeks after planting, where after it fell to 6.3 (grand mean, s.e.m. 0.09) at harvest. The area of the third leaf rose to 669.9 cm² (grand mean, s.e.m. 15.9) at 14 weeks after planting, after which it remained fairly constant until harvest. Leaf chlorophyll content

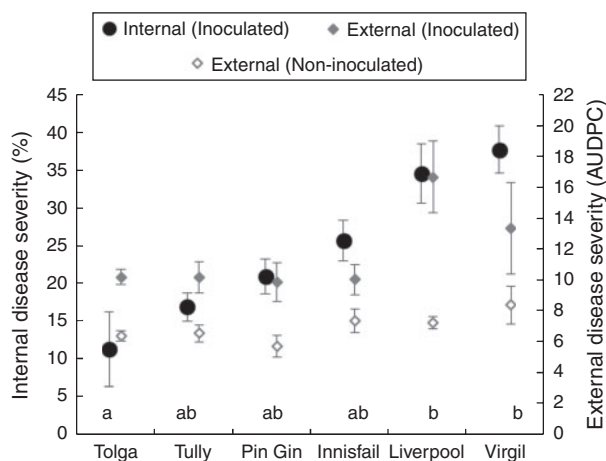


Fig. 1. Mean internal disease severity (proportion of rhizome coloured dark brown) at harvest, of plants inoculated with *Fusarium oxysporum* f.sp. *cabense* ‘Race 1’, and mean external disease severity (area under the disease progression curve, AUDPC) of inoculated and non-inoculated plants over the course of the experiment, for each of the six soils. Error bars show standard error of the mean. Mean internal disease severity values with the same letter were not significantly different at $P=0.05$. All plants in non-inoculated pots had an internal disease severity of 0% at harvest.

fell steadily over time, from a SPAD value of 52.5 (grand mean, s.e.m. 0.32) at 5 weeks after planting to 37.8 (s.e.m. 0.50) at harvest.

Internal disease severity at harvest, as determined by rhizome discoloration, was significantly influenced by soil type ($P=0.004$) and inoculation ($P<0.001$). Post-hoc analysis of the inoculated soil data demonstrated a significant difference between the mean internal disease severity of Tolga compared with Liverpool and Virgil soils ($P=0.008$, Fig. 1). No discoloration was observed in the pseudostem of inoculated plants. None of the plants grown in non-inoculated soil showed internal symptoms of *Foc* infection. Vegetative compatibility group analysis confirmed that *Foc* Race 1 was present in plants from the inoculated pots and absent in plants from the non-inoculated pots, in line with Koch’s postulate.

Several components of plant mass (fresh and dry) were significantly affected by soil type and inoculation (Table 2, Fig. 2). Tolga had the highest total fresh mass and Liverpool the lowest. The difference between soils in total plant fresh mass was mostly due to a difference in root mass. Inoculation did not affect total plant mass (fresh or dry), but it increased rhizome fresh mass and decreased root fresh mass. The increase in rhizome fresh mass was similar across soils, but the decrease in root mass (fresh and dry) was most pronounced in Tolga soil. The difference in disease severity between soils (in inoculated plants) was related to the difference in plant biomass between soils. Soils with the highest total fresh mass had the lowest disease severity and vice versa ($r = -0.886$, $P=0.041$).

Internal disease severity (rhizome discoloration) was significantly correlated with several soil properties, especially clay content and related variables (Table 1). Internal disease severity was significantly negatively correlated with soil clay content, water content at -10 kPa, total Fe, Cu and Cd content,

Table 2. P -values from analyses of variance of harvested plant biomass. Values <0.05 are underlined. Means and standard errors of variables significantly affected by the treatments are given in Fig. 2

Treatment	Leaves	Pseudostem	Rhizome	Roots	Total
Fresh weight					
Soil	<u>0.031</u>	0.540	0.415	<u><0.001</u>	<u><0.001</u>
Inoculation	0.833	0.194	<u><0.001</u>	<u>0.008</u>	0.412
Soil \times Inocul.	0.620	0.524	0.990	0.382	0.173
Dry weight					
Soil	0.093	0.967	0.397	<u>0.006</u>	0.703
Inoculation	0.178	0.424	0.364	0.174	0.388
Soil \times Inocul.	0.455	0.578	0.748	0.525	0.415

and extractable B content (Table 1), and these variables were highly correlated with each other. It was also negatively correlated with β -glucosidase activity ($r = -0.631$, $P=0.028$, $n=12$) (Fig. 3), but β -glucosidase activity was not significantly correlated with any other measured soil or plant variable. There was mycorrhizal colonisation of roots from all soils and treatments, except Pin Gin inoculated. Colonisation rating was highest in Innisfail soil (inoculated and non-inoculated). However, several samples did not stain properly and there was no significant effect of inoculation, so results are not reported further.

Discussion

The soil types of Far North Queensland examined in this study differed in their level of suppressiveness to Fusarium wilt, as determined by internal disease assessment of plants grown in *Foc*-inoculated soils. The soils in which disease severity was lowest (Tolga, Tully and Pin Gin) were characterised by a relatively high contents of clay, water at -10 kPa, total Fe, Cu and Cd and extractable B, and microbial activity, as indicated by β -glucosidase activity. Plants in these soils also had relatively high root biomass (both fresh and dry), irrespective of inoculation, suggesting that good root growth led to low disease severity. All plants in inoculated soils became infected, so suppression was related to development of the disease rather than prevention of infection.

The clay content of suppressive soils is widely credited with an indirect role in disease suppression. Sandy or sandy loam soils are often associated with high disease severity (Amir and Alabouvette 1993; Höper *et al.* 1995; Hwang and Ko 2004; Deltour *et al.* 2017). Coarser-textured soils typically have lower water- and nutrient-holding capacities, as well as organic matter content, and are thus less conducive to microbial and plant growth, relative to finer-textured soils (Amir and Alabouvette 1993; Pattison and Lindsay 2006). Clay type has also been associated with suppressiveness in previous studies. Stotzky and Martin (1963) correlated the presence of montmorillonite-type clay with wilt suppression. However, all the soils used in this study had kaolin as the predominant clay mineral, with no detectable smectite. Given that nutrients were supplied to all plants in the experiment, we speculate that the water retention properties of the soils may have been a major factor explaining the difference in plant growth and disease severity between the soils.

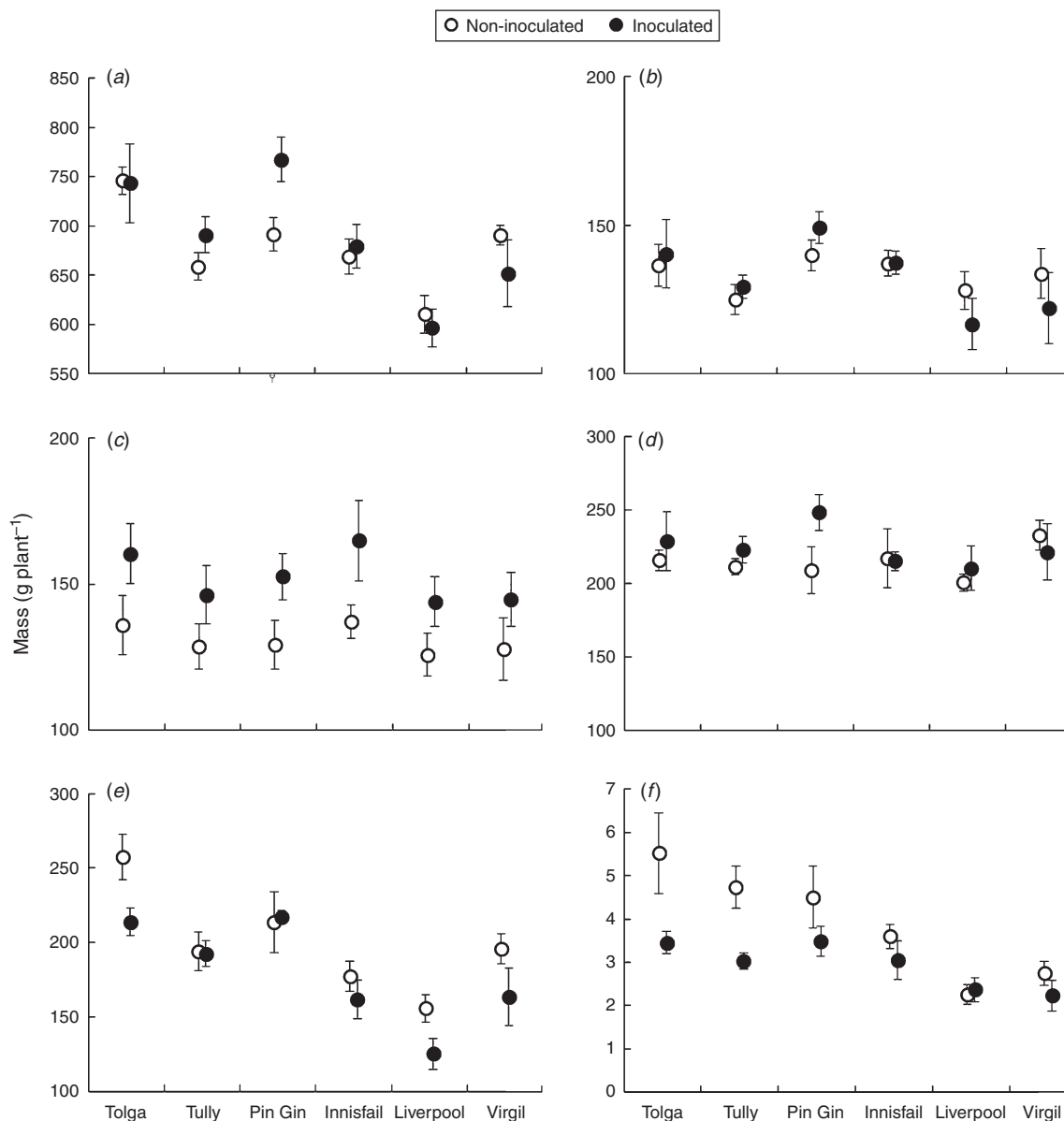


Fig. 2. Mass of plant components that were significantly affected by treatments. (a) total fresh mass, (b) leaf fresh mass, (c) rhizome fresh mass, (d) pseudostem fresh mass, (e) root fresh mass and (f) root dry mass. Significance of treatment effects is given in Table 2. Error bars show standard error of the mean.

In this study, root mass increased and disease severity decreased with increasing soil clay content. Root quantity varies considerably depending on the health of the banana plant (Stover and Simmonds 1987), and it is in the roots, as well as the vascular system, where the plant defence response determines the success or failure of plant resistance following infection by *Foc* (De Ascensao and Dubery 2000). Banana plants are sensitive to water deficit (Stover and Simmonds 1987), and a reduction of soil water content from field capacity to 40% field capacity has been reported to increase *Fusarium* wilt severity (Peng *et al.* 1999). Although there was not a significant correlation between disease severity and plant-available water holding capacity, it is likely that

plants in the Virgil soil, with the highest sand content, were subjected to a greater water deficit than plants in the other soils, contributing to higher disease severity.

Soils conducive to *Fusarium* wilt are frequently more acidic than suppressive soils (Höper *et al.* 1995; Alabouvette 1999; Shen *et al.* 2015; Deltour *et al.* 2017). However, exceptions have been found when soil pH is in the neutral to alkaline range (Peng *et al.* 1999; Domínguez-Hernández *et al.* 2008). Interactions between pH and the availability of micronutrients to the pathogen and plant are complex, and generalisations about pH and suppression in different contexts are difficult to make. In this study there was no significant association between pH and disease severity. Soil pH covered a fairly small range in

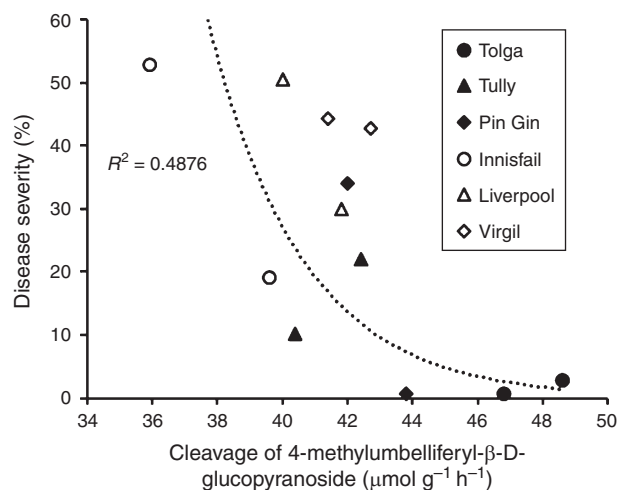


Fig. 3. Disease severity (proportion of rhizome discolouration) in relation to soil β -glucosidase activity across the inoculated pots analysed.

these soils (pH 5.6–7.1), due to regular liming of the banana fields.

Soil enzyme assays are useful biological indicators due to their significance in a range of microbial processes i.e. catalysing biochemical reactions essential to nutrient cycling. Specifically, β -glucosidase activity has been demonstrated to be particularly useful for monitoring biological soil quality (Turner *et al.* 2002). Furthermore β -glucosidase activity has been previously linked to Fusarium wilt suppression (Pattison *et al.* 2018). Although not all replicates were analysed and thus a degree of caution should be applied, in this study disease severity was negatively associated with β -glucosidase activity, suggesting that greater soil biological activity aided in the disease-suppressive characteristics of some soils. The β -glucosidase activity was not significantly associated with soil clay content ($r = 0.714$, $P = 0.111$), organic carbon ($r = -0.353$, $P = 0.492$), or indeed any variable measured, therefore suggesting that microbial activity in these soils may have been driven by factors or a combination of factors not examined here, such as banana field cultivation techniques, fertiliser application and pest management methods.

Rhizome biomass and pseudostem girth at harvest were higher for inoculated plants than non-inoculated plants in this study. Contrary to our findings, previous research has found a larger pseudostem girth associated with greater plant growth and lower disease severity (Ting *et al.* 2008; Zhang *et al.* 2014). This difference may be due to our use of juvenile rather than mature plants. Banana plants are known to respond to infection by producing tyloses, gums and gels that physically block the progression of *Foc* mycelial growth within the plant tissue (Ploetz 2015). In the juvenile stage, this production of material may have caused the increase in pseudostem diameter and rhizome biomass of the inoculated plants.

The negative correlation between disease severity and soil concentrations of total Fe, Cu and Cd and extractable B may have been due to their correlation with soil clay content. High concentrations of metals and nutrients are generally associated with high clay content and particular parent materials, and they

may not have played a role in disease suppression. Cadmium is not an essential element and has been shown to reduce mycelial growth of *Fusarium* spp. (Gharieb 2001). Boron, Fe and Cu are essential micronutrients (Lahav and Israeli 2000; Marschner 2012). Copper is essential for the fungal denitrifying system in *F. oxysporum* (Matsuoka *et al.* 2017). Interestingly, neither total Fe nor DTPA-extractable Fe concentration were positively associated with disease severity, as is often the case.

Iron has previously been identified as a limiting resource for microorganisms, including *Foc*, and limiting available Fe in the soil may favour suppression of Fusarium wilt (Peng *et al.* 1999; Domínguez *et al.* 2001; Cao *et al.* 2005). Under experimental conditions, the amendment of a conducive soil with Fe-EDDHA, a strong Fe ligand, was found to induce suppression, as well as reduce *Foc* chlamyospore germination. In contrast, soil suppressiveness was nullified by the addition of Fe-EDTA, which maintains Fe in a form available to microorganisms (Peng *et al.* 1999). It is possible that the forms of Fe measured in this study did not reflect Fe bioavailability, that the range of concentrations in these soils was not in the range influencing suppression, or that the effect of Fe was influenced by interactions with other nutrients, as indicated by Dong *et al.* (2016).

To gain a better understanding of how soil characteristics influence disease severity it would be useful to evaluate a wider range of soils and management histories, and carry out manipulative experiments to determine the effect of particular variables. Also, it would be instructive to grow plants through to maturity and evaluate effects on the various *Foc* races, especially TR4.

In conclusion, this study demonstrated that key banana cropping soils of Australia differed in their suppressiveness to Fusarium wilt of banana (*Foc* Race 1). This indicates that risk of future disease impacts differs between soils. In the soils examined, disease severity was negatively correlated with soil clay content and associated variables. Further investigation of the biotic and abiotic characteristics of the soils associated with suppressiveness or conduciveness appears worthwhile. Such information could inform biosecurity measures and management practices to reduce the impact of this important disease.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

We are grateful to Célia Colin, Maya Martin, and Sharni Day for their assistance in the field and greenhouse, Tobin Northfield for his statistical help, Wayne O'Neill for supplying the inoculum and testing for inoculation success, and Tasmin Rymer for moral support. This project has been funded by Hort Innovation banana research and development levy, with co-investment from Queensland Government. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian Horticulture.

References

Adiobo A, Oumar O, Perneel M, Zok S, Höfte M (2007) Variation of *Pythium*-induced cocoyam root rot severity in response to soil type.

- Soil Biology & Biochemistry* **39**, 2915–2925. doi:10.1016/j.soilbio.2007.06.012
- Alabouvette C (1986) Fusarium-wilt suppressive soils from the Châteaurenard region: review of a 10-year study. *Agronomie* **6**, 273–284. doi:10.1051/agro:19860307
- Alabouvette C (1999) Fusarium wilt suppressive soils: an example of disease-suppressive soils. *Australasian Plant Pathology* **28**, 57–64. doi:10.1071/AP99008
- Amir H, Alabouvette C (1993) Involvement of soil abiotic factors in the mechanisms of soil suppressiveness to fusarium wilts. *Soil Biology & Biochemistry* **25**, 157–164. doi:10.1016/0038-0717(93)90022-4
- Brady NC, Weil RR (2008). 'The nature and properties of soils.' 14th edn. (Prentice Hall: Upper Saddle River, NJ)
- Cannon MG, Murtha GG, Smith CD (1992) 'Soils of the Cardwell-Tully area, North Queensland.' (CSIRO: Adelaide, SA)
- Cao L, Qiu Z, You J, Tan H, Zhou S (2005) Isolation and characterization of endophytic streptomycete antagonists of fusarium wilt pathogen from surface-sterile banana roots. *FEMS Microbiology Letters* **247**, 147–152. doi:10.1016/j.femsle.2005.05.006
- Carlier JD, De Waele D, Escalant JV (2003) Global evaluation of *Musa* germplasm for resistance to Fusarium wilt, *Mycosphaerella* leaf spot diseases and nematodes. Performance evaluation (Eds A Vézina, C Picq) INIBAP Technical Guidelines 7. The International Network for the Improvement of Banana and Plantain: Montpellier, France.
- Cha JY, Han S, Hong HJ, Cho H, Kim D, Kwon Y, Kwon SK, Crüsemann M, Lee YB, Kim JF, Giaever G, Nislow C, Moore BS, Thomashow LS, Weller DM, Kwak Y-S (2016) Microbial and biochemical basis of a Fusarium wilt-suppressive soil. *The ISME Journal* **10**, 119–129. doi:10.1038/ismej.2015.95
- Cook DC, Taylor AS, Meldrum RA, Drenth A (2015) Potential economic impact of Panama disease (Tropical Race 4) on the Australian banana industry. *Journal of Plant Diseases and Protection* **122**, 229–237. doi:10.1007/BF03356557
- Creamer RE, Bellamy P, Black HI, Cameron CM, Campbell CD, Chamberlain P, Harris J, Parekh N, Pawlett M, Poskitt J (2009) An inter-laboratory comparison of multi-enzyme and multiple substrate-induced respiration assays to assess method consistency in soil monitoring. *Biology and Fertility of Soils* **45**, 623–633. doi:10.1007/s00374-009-0374-y
- De Ascensao ARDCF, Dubery IA (2000) Panama disease: cell wall reinforcement in banana roots in response to elicitors from *Fusarium oxysporum* f.sp. *cubense* race four. *Biochemistry and Cell Biology* **90**, 1173–1180.
- Deltour P, França SC, Pereira OL, Cardoso I, De Neve S, Debode J, Höfte M (2017) Disease suppressiveness to Fusarium wilt of banana in an agroforestry system: influence of soil characteristics and plant community. *Agriculture, Ecosystems & Environment* **239**, 173–181. doi:10.1016/j.agee.2017.01.018
- Dominguez J, Negrin MA, Rodriguez CM (2001) Aggregate water-stability, particle-size and soil solution properties in conducive and suppressive soils to Fusarium wilt of banana from Canary Islands (Spain). *Soil Biology & Biochemistry* **33**, 449–455. doi:10.1016/S0038-0717(00)00184-X
- Dominguez-Hernández J, Negrin MA, Rodriguez CM (2008) Soil potassium indices and clay-sized particles affecting banana-wilt expression caused by soil fungus in banana plantation development on transported volcanic soils. *Communications in Soil Science and Plant Analysis* **39**, 397–412. doi:10.1080/00103620701826522
- Dong X, Wang M, Ling N, Shen Q, Guo S (2016) Effects of iron and boron combinations on the suppression of *Fusarium* wilt in banana. *Scientific Reports* **6**, 38944.
- Enderlin N, Barry E, Seonaid R (1997) 'Soils of the Mareeba-Dimbulah Irrigation Area (MDIA).' (Queensland Department of Natural Resources: Mareeba, Qld)
- Forsyth LM, Smith LJ, Aitken EAB (2006) Identification and characterization of non-pathogenic *Fusarium oxysporum* capable of increasing and decreasing Fusarium wilt severity. *Mycological Research* **110**, 929–935. doi:10.1016/j.mycres.2006.03.008
- Gharieb M (2001) Pattern of cadmium accumulation and essential cations during growth of cadmium-tolerant fungi. *Biomaterials* **14**, 143–151. doi:10.1023/A:1016658512161
- Höper H, Alabouvette C (1996) Importance of physical and chemical soil properties in the suppressiveness of soils to plant diseases. *European Journal of Soil Biology* **32**, 41–58.
- Höper H, Steinberg C, Alabouvette C (1995) Involvement of clay type and pH in the mechanisms of soil suppressiveness to Fusarium wilt of flax. *Soil Biology & Biochemistry* **27**, 955–967. doi:10.1016/0038-0717(94)00238-V
- Hwang S-C, Ko W-H (2004) Cavendish banana cultivars resistant to Fusarium wilt acquired through somaclonal variation in Taiwan. *Plant Disease* **88**, 580–588. doi:10.1094/PDIS.2004.88.6.580
- Koske RE, Gemma JN (1989) A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research* **92**, 486–488. doi:10.1016/S0953-7562(89)80195-9
- Lahav E, Israeli Y (2000) Mineral deficiencies of banana. In 'Diseases of banana, Abacá and Enset'. (Ed. DR Jones) pp. 339–350 (CABI Publishing: Wallingford, UK)
- Marschner P (2012) 'Marschner's mineral nutrition of higher plants.' 3rd edn. (Academic Press: London, UK)
- Marx MC, Wood M, Jarvis SC (2001) A microplate fluorimetric assay for the study of enzyme diversity in soils. *Soil Biology & Biochemistry* **33**, 1633–1640. doi:10.1016/S0038-0717(01)00079-7
- Matsuoka M, Kumar A, Muddassar M, Matsuyama A, Yoshida M, Zhang KYJ (2017) Discovery of fungal denitrification inhibitors by targeting copper nitrite reductase from *Fusarium oxysporum*. *Journal of Chemical Information and Modeling* **57**, 203–213. doi:10.1021/acs.jcim.6b00649
- Mazzola M (2002) Mechanisms of natural soil suppressiveness to soilborne diseases. *Antonie van Leeuwenhoek* **81**, 557–564. doi:10.1023/A:1020557523557
- Mostert D, Molina AB, Daniells J, Fourie G, Hermanto C, Chao C-P, Emily F, Sinohin VG, Masdek N, Thangavelu R, Li C, Yi G, Mostert L, Viljoen A (2017) The distribution and host range of the banana Fusarium wilt fungus, *Fusarium oxysporum* f. sp. *cubense*, in Asia. *PLoS One* **12**, e0181630. doi:10.1371/journal.pone.0181630
- Murtha GG (1986) 'Soils of the Tully-Innisfail area North Queensland.' (CSIRO: Adelaide, SA)
- Nasir N, Pittaway PA, Pegg KG, Lisle AT (2003) A foliar rating system for comparing the resistance of banana cultivars grown as tissue-cultured plantlets in the laboratory to Fusarium wilt. *Australasian Plant Pathology* **32**, 521–526. doi:10.1071/AP03065
- O'Neill WT, Henderson J, Pattermore JA, O'Dwyer C, Perry S, Beasley DR, Tan YP, Smyth AL, Goosem CH, Thomson KM, Hobbs RL, Grice KRE, Trevorrow P, Vawdrey LL, Pathania N, Shivas RG (2016) Detection of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 strain in northern Queensland. *Australasian Plant Disease Notes* **11**, 33. doi:10.1007/s13314-016-0218-1
- Orjeda G (1998) Evaluation of *Musa* germplasm for resistance to Sigatoka diseases and Fusarium wilt. INIBAP Technical Guidelines 3. International Plant Genetic Resources Institute, Rome, Italy; International Network for the Improvement of Banana and Plantain, Montpellier, France; ACP-EU Technical Centre for Agricultural and Rural Cooperation, Wageningen, The Netherlands.
- Orr R, Nelson PN (2018) Impacts of soil abiotic attributes on Fusarium wilt, focusing on bananas. *Applied Soil Ecology* **132**, 20–33. doi:10.1016/j.apsoil.2018.06.019
- Pattison T, Lindsay S (2006) 'Banana root and soil health user's manual.' (Department of Primary Industries and Fisheries: Brisbane, Qld)

- Pattison AB, Molina AB, Chao CP, Viljoen A, Lindsay SJ (2018) Integrating management practices to support banana production in the presence of Fusarium wilt. *Acta Horticulturae* **1196**, 129–136. doi:10.17660/ActaHortic.2018.1196.15
- Peng HX, Sivasithamparam K, Turner DW (1999) Chlamydospore germination and Fusarium wilt of banana plantlets in suppressive and conducive soils are affected by physical and chemical factors. *Soil Biology & Biochemistry* **31**, 1363–1374. doi:10.1016/S0038-0717(99)00045-0
- Ploetz RC (2015) Fusarium wilt of banana. *Phytopathology* **105**, 1512–1521. doi:10.1094/PHYTO-04-15-0101-RVW
- Puhalla JE (1985) Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. *Canadian Journal of Botany* **63**, 179–183. doi:10.1139/b85-020
- Queensland Government (2016) Queensland's banana industry. <https://www.business.qld.gov.au/industries/farms-fishing-forestry/agriculture/crop-growing/banana-industry>. Accessed 30 September 2017.
- Queensland Government (2017) Panama disease. <https://www.daf.qld.gov.au/plants/health-pests-diseases/a-z-significant/panama-disease2>. Accessed 30 September 2017.
- Rayment GE, Lyons DJ (2011). 'Soil chemical methods: Australasia.' (CSIRO Publishing: Collingwood, Vic.)
- Shen Z, Ruan Y, Xue C, Zhong S, Li R, Shen Q (2015) Soils naturally suppressive to banana *Fusarium* wilt disease harbor unique bacterial communities. *Plant and Soil* **393**, 21–33. doi:10.1007/s11104-015-2474-9
- Siegel-Hertz K, Edel-Hermann V, Chapelle E, Terrat S, Raaijmakers JM, Steinberg C (2018) Comparative microbiome analysis of a Fusarium wilt suppressive and a Fusarium wilt conducive soil from the Châteaurenard region. *Frontiers in Microbiology* **9**. doi:10.3389/fmicb.2018.00568
- Stotzky G, Martin RT (1963) Soil mineralogy in relation to the spread of Fusarium wilt of banana in Central America. *Plant and Soil* **18**, 317–337. doi:10.1007/BF01347232
- Stover RH, Simmonds NW (1987) 'Bananas.' 3rd edn. (Longman Scientific & Technical: New York).
- Ting ASY, Meon S, Kadir J, Radu S, Singh G (2008) Endophytic microorganisms as potential growth promoters of banana. *BioControl* **53**, 541–553. doi:10.1007/s10526-007-9093-1
- Turner DW, Lahav E (1983) The growth of banana plants in relation to temperature. *Australian Journal of Plant Physiology* **10**, 43–53.
- Turner BL, Hopkins DW, Haygarth PH, Ostle N (2002) β -Glucosidase activity in pasture soils. *Applied Soil Ecology* **20**, 157–162. doi:10.1016/S0929-1393(02)00020-3
- Weinert M, Simpson M (2016) 'Sub tropical banana nutrition – matching nutrition requirements to growth demands.' (NSW Department of Primary Industries: Wollongbar, NSW)
- Welc M, Frossard E, Egli S, Bünemann EK, Jansa J (2014) Rhizosphere fungal assemblages and soil enzymatic activities in a 110-years alpine chronosequence. *Soil Biology & Biochemistry* **74**, 21–30. doi:10.1016/j.soilbio.2014.02.014
- Zhang N, He X, Zhang J, Raza W, Xing-Ming YA, Ru Y-Z, Qi-Rong SH, Huang QW (2014) Suppression of Fusarium wilt of banana with application of bio-organic fertilizers. *Pedosphere* **24**, 613–624. doi:10.1016/S1002-0160(14)60047-3

Handling Editor: Ji-Zheng He