ResearchOnline@JCU

JAMES COOK UNIVERSITY AUSTRALIA

This is the author-created version of the following work:

Bowen, Alison, Orr, Ryan, McBeath, Anna V., Pattison, Anthony, and Nelson, Paul N. (2019) ***Suppressiveness or conduciveness to Fusarium wilt of bananas differs between key Australian soils.*** **Soil Research, 57 (2) pp. 158-165.**

Access to this file is available from:

https://researchonline.jcu.edu.au/57354/

Journal compilation © CSIRO 2019. AAM may be made open access in an Institutional Repository without embargo.

Please refer to the original source for the final version of this work:

https://doi.org/10.1071/SR18159

1 Title:

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

3

4 Running head:

5 Suppressiveness of soils to banana Fusarium wilt

6

7 Authors:

8 Alison Bowen[1]

9 Ryan Orr [1]

10 Anna McBeath[2]

11 Anthony Pattison[2]

12 Paul N. Nelson (corresponding author)[1] paul.nelson@jcu.edu.au

13

14 [1]James Cook University

15 Cairns, Qld 4870

16 Australia

17

18 [2]Department of Agriculture and Fisheries

19 South Johnstone, Qld 4859

20 Australia

21

22 Summary text

23 Fusarium wilt of bananas, or Panama disease, threatens banana production globally, and a virulent
24 form of the pathogen has recently been detected in Queensland, Australia. This work showed that
25 severity of Panama disease differs between key soils used to grow bananas in Queensland. The
26 results will be useful for minimising impacts of the disease, if it is to spread beyond quarantine
27 measures, and there is not yet a resistant and commercially acceptable banana cultivar.

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

29

30

31 # Suppressiveness or conduciveness to Fusarium wilt of bananas differs between key Australian soils.

33 Alison Bowen, Ryan Orr, Anna McBeath, Anthony Pattison, Paul N. Nelson

34 ## Abstract

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

47 ## Introduction

48 Soils suppressive to soil-borne diseases, recognised for over a century, are defined as those
49 in which disease development is minimal despite the presence of both a virulent pathogen and a
50 susceptible plant host (Mazzola 2002). An active and functionally diverse soil microbial community,
51 with which pathogens must compete for nutrients and habitat, is widely accepted as the basis of
52 disease suppressive soils (Höper and Alabouvette 1996; Brady and Weil 2008; Cha *et al*. 2016). The
53 key role soil microorganisms perform in the development of disease suppression was supported by
54 the observation that suppression could be eliminated through soil pasteurisation (Mazzola 2002;
55 Adiobo *et al*. 2007), and may be restored by mixing a small quantity of suppressive soil into a

56 conducive soil (Alabouvette 1986; Alabouvette 1999; Siegel-Hertz *et al*. 2018). However, soil physical
57 and chemical properties may influence disease severity via their influence on microbial activity, or
58 the health and defence capabilities of the host plant (Mazzola 2002). Suppressiveness of soils to soil-
59 borne diseases has been reported for many diseases, including Fusarium wilt, an important disease
60 of many plant species (Höper and Alabouvette 1996; Orr and Nelson (in press).

61 Fusarium wilt of banana (*Musa* spp.), caused by *Fusarium oxysporum* f.sp. *cubense* (*Foc*),
62 threatens banana production throughout the world. *Foc* has traditionally been subdivided into four
63 'races' based on the banana cultivars affected (Ploetz 2015). Race 1 was responsible for the end of
64 large-scale commercial production of the once economically important Gros Michel cultivar. It also
65 affects other cultivars from the 'Pome' subgroup. Race 2 affects cooking bananas such as Bluggoe,
66 and Race 3 affects Heliconias. Race 4 affects Cavendish (*Musa* AAA, Cavendish subgroup) as well as
67 cultivars susceptible to Races 1 and 2. Race 4 is subdivided into Subtropical Race 4, which affects
68 susceptible cultivars under sub-optimal growing conditions, and Tropical Race 4 (TR4), which affects
69 susceptible cultivars in all conditions (Ploetz 2015). *Foc* TR4 is spreading rapidly throughout the world
70 (Mostert *et al*. 2017). It reached the main banana-growing area of Australia in Far North Queensland
71 in 2015 (O'Neil *et al*. 2016; Queensland Government 2017). Far North Queensland supplies more
72 than 90% of Australia's bananas (Queensland Government 2016), and Cook *et al*. (2015) predicted
73 losses to Australia's banana industry caused by TR4 to exceed $138 million per year, despite a slow
74 rate of disease spread.

75 Microbially mediated suppression to Fusarium wilt has been related to numerous soil biotic
76 and abiotic properties. Soil abiotic properties have direct and indirect roles, through their influence
77 on soil microorganisms and plant defence capabilities (Orr and Nelson, in press). However, little is
78 known about the relative suppressiveness of Australian soils to *Foc*. Knowing the variation in
79 suppressiveness to *Foc* among the main Australian soil types used to grow bananas would inform
80 decisions about banana cropping and soil management, and planning of biosecurity measures. This

81 study aimed to determine whether the main soil types used to grow bananas in Far North
82 Queensland differ in their suppressiveness to Fusarium wilt, and to identify soil characteristics that
83 may be associated with suppression among those soils.

84 Materials and methods

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

89 The soil was taken between 28 February and 9 March 2017 from locations and soil series
90 representative of the Far North Queensland banana-growing region, from fields growing *Musa* AAA
91 group, Cavendish subgroup bananas for at least 2 years. We understood from the growers that the
92 sampled fields were free from previous *Foc* infection. Furthermore, none of the fields had been used
93 to grow cultivars susceptible to *Foc* Race 1. The soils, which have been described by Murtha (1986),
94 Cannon *et al*. (1992) and Enderlin *et al*. (1997), comprised four well drained uniform or gradational
95 textured soils formed on alluvium: Innisfail series, a Brown Dermosol (17.485122°S, 145.859047°E),
96 Liverpool series, an Orthic Tenosol (17.454604°S, 145.864289°E), Tully series, a Brown Dermosol
97 (17.481890°S, 145.858678°E) and Virgil series, a Red Kandosol (17.898027°S, 145.832213°E), and two
98 red gradational textured soils formed on basalt, both Red Ferrosols: Pin Gin series (17.592257°S,
99 145.833088°E) and Tolga series (17.010557°S, 145.527065°E). Soil was collected from 0-0.25 m
100 depth, 0.4 m from the base of mature, but not bunching, plants. Rocks, large roots and debris were
101 removed, and samples were homogenised and left in the shade until pot filling.

102 Samples of the soil were analysed for several physicochemical properties. They were
103 analysed by Nutrient Advantage Laboratory (www.nutrientadvantage.com.au), using methods
104 described in Rayment and Lyons (2011), for: particle size distribution, pH and electrical conductivity
105 (1 : 5 soil : water), total C (Dumas combustion), total metals (acid digest), DTPA- extractible metals,

106 Colwell P, MCP-extractible S, $CaCl_2$-extractible Si, hot $CaCl_2$-extractible B, and exchangeable cations
107 ($BaCl_2/NH_4Cl$ and ammonium acetate).The water holding characteristics of the soils were determined
108 by measuring water content at -10 kPa using a ceramic plate and hanging water column, and at -200
109 and -1500 kPa using a WP4 Dew Point Potentiometer. Mineralogical analysis was performed by
110 CSIRO laboratories, South Australia using X-ray diffraction. Soil mineral contents were expressed as a
111 percent of the sum of identified mineral contents, ignoring any amorphous or otherwise unidentified
112 materials. The soils ranged from sandy clay loam to clay in texture and had pH ranging from 5.6 to
113 7.1 (Table 1). Clay mineralogy was dominated by kaolin, with some mica and haematite in some soils
114 (Table 1).

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

125 The greenhouse growing conditions and fertilisation routine were adapted during the
126 experiment in response to plant requirements, but all plants were treated the same. Plants were
127 fertilised fortnightly with 83 mg urea (Richgro® fertilisers), 10 mg P as 'Mega Booster' guano
128 (Richgro® fertilisers), and 124 mg KCl, according to the rates recommended by Weinert and Simpson
129 (2016). Around 4 weeks post-inoculation, most plants appeared to be suffering some nutrient
130 deficiency symptoms, most likely Zn, Ca and B. Consequently 39 mg Ca as gypsum was added

131 fortnightly from then on, and Yates 'health tonic trace elements chelates' foliar spray (% w/v: S 0.53,
132 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.
133 Nine weeks after planting, plants were subjected to a water deficit for 3 weeks by reducing watering
134 from every day to every third day. Over the course of most of the experiment the average
135 temperature was 25.3°C in the air (12.5°C minimum to 36.9°C maximum) and 24.0°C in the soil
136 (14.5°C minimum to 33.4°C maximum), apart from several unusually hot days at the beginning of the
137 experiment, when the temperature reached a maximum of 48.1°C in the air and 35.9°C in the soil.

138 Several plant measurements were taken over the course of the experiment, once per week:
139 number of living, fully unfurled leaves; pseudostem girth at 5 cm from the soil surface; area of the
140 third most recently fully unfurled leaf (Turner and Lahav, 1983); chlorophyll content of the same leaf
141 (SPAD 502 Plus Chlorophyll Meter, Spectrum Technologies Inc.) and external disease severity score.
142 External disease severity was evaluated using a scale adapted from Nasir *et al.* (2003) and symptoms
143 described by Orjeda (1998) and Queensland Government (2017): number of yellowing leaves;
144 splitting of pseudostem; and changes to leaves such as narrowing, irregular pale margin, lamina
145 ripping, more erect habit, petiole collapse, or wilting. Scale values ranged from 0 for no symptoms to
146 3 for severe symptoms. For each plant, all 10 scores taken over the course of the experiment were
147 added together to obtain the 'area under the disease progression curve' (AUDPC).

148 The plants were harvested 19 weeks after planting and analysed for internal disease severity,
149 fresh mass and dry mass. Every plant was evaluated for internal disease severity by measuring
150 discoloration of transverse sections of the rhizome and pseudostem, using a modified version of the
151 disease severity methodology and scale for Fusarium wilt of banana (Carlier *et al*. 2003). The
152 pseudostem was cut transversely at the soil surface and 5 cm above it, and the rhizome was cut into
153 four equal sections. The upper surface of the two lower pseudostem sections and three lower
154 rhizome sections was photographed and the area of infected rhizome tissue was calculated using the
155 program ImageJ 1.49v (National Institute of Health, USA). The disease severity values were calculated

156 as the area of discoloured rhizome tissue as a percentage of the total rhizome tissue area. The
157 average values for the three rhizome sections was taken as the disease severity score for that plant.
158 To determine mass, each plant was subdivided into four portions (roots, rhizome, pseudostem, and
159 leaves) and the fresh mass of each portion measured. Dry mass was then determined by measuring
160 water content of a representative subsample of each portion of each plant.

161 Roots were analysed for mycorrhizae and *Foc* Race 1. Roots from three replicates were
162 analysed for abundance of vesicular-arbuscular mycorrhizae using the protocol described by Koske
163 and Gemma (1989). Roots were also analysed for the presence of *Foc* Race 1 VCG 0124, to confirm
164 success of the inoculation and check that there was no infection in the non-inoculated pots. The
165 analysis was performed on monoconidial isolates of *Foc* recovered from roots, according to the
166 technique described by Puhalla (1985). Nitrate non-utilising (nit) mutants produced from the isolates
167 were paired with tester Nit M mutants obtained from the culture collection of the Queensland Plant
168 Pathology Herbarium (BRIP). The nit mutants from the isolates anastomosed to form stable
169 heterokaryons when paired with VCG 0124 Nit M testers on minimal media, thus confirming their
170 identity as VCG 0124.

171 Soil from the harvested pots was analysed for β-glucosidase activity by measuring
172 fluorescence generated by cleavage of 4-methylumbelliferyl- β-D-glucopyranoside. Subsamples of
173 soil, shaken from the root system and homogenised, were refrigerated (<4°C) and stored for less
174 than a month between harvest and analysis. Two replicates from each treatment combination were
175 analysed, giving 24 in total. The assay protocol was adapted from Marx *et al*. (2001), Creamer *et al*.
176 (2009), and Welc *et al*. (2014).

177 All data were tested for normality and homogeneity of variance. A Levene's test for
178 homogeneity of variance showed no significant difference between the arcsine-transformed
179 variances of the disease severity means of the inoculated soils ($F_{5, 30} = 1.10$; $P = 0.382$). The effect of
180 the treatments (inoculation and soil type) on number of leaves per plant, pseudostem girth, area of

181 the third leaf and leaf chlorophyll content, external disease severity score (AUDPC), internal disease
182 severity (arcsine-transformed) and biomass was analysed by factorial analysis of variance (n=72) and
183 Tukey post-hoc analysis. The relationship between internal disease severity and soil properties was
184 examined using Spearman's rank correlation using S-PLUS 8.2 for Windows. Because there was only
185 one value of each soil property for each soil, this analysis had n=6, with the disease severity value
186 being the mean of inoculated pots in each soil type. The relationship between internal disease
187 severity and β-glucosidase activity was examined using Spearman's rank correlation using S-PLUS 8.2
188 for Windows, with n=12 (two inoculated pots from each soil type).

189

190 Results

191 Over the course of the experiment, development of disease was evident in some but not all
192 measurements. External disease severity score, as quantified by the AUDPC, was significantly
193 influenced by inoculation ($P < 0.001$) and soil type ($P = 0.013$). Of the inoculated plants, the highest
194 external disease severity score was in Liverpool soil and the lowest was in Tolga, Tully, Innisfail and
195 Pin Gin soils (Figure 1). Pseudostem girth, which increased steadily over time, was significantly
196 influenced at harvest by inoculation ($P = 0.013$) and soil type ($P = 0.014$) but not their interaction.
197 Non-inoculated plants had a mean girth of 108 mm and inoculated plants 112 mm. Pin Gin had the
198 lowest pseudostem girth (106 mm) and Virgil the highest (117 mm). Neither soil type nor inoculation
199 had a significant effect on the number of living leaves per plant, the area of the third leaf, or leaf
200 chlorophyll content. The number of leaves per plant rose to a maximum of 7.5 (grand mean, SEM
201 0.12) at 12 weeks after planting, where after it fell to 6.3 (grand mean, SEM 0.09) at harvest. The
202 area of the third leaf rose to 669.9 cm^2 (grand mean, SEM 15.9) at 14 weeks after planting, after
203 which it remained fairly constant until harvest. Leaf chlorophyll content fell steadily over time, from a
204 SPAD value of 52.5 (grand mean, SEM 0.32) at 5 weeks after planting to 37.8 (SEM 0.50) at harvest.

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

213 *Insert Table 1 near here*

214 *Insert Figure 1 near here*

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

224 *Insert Table 2 near here*

225 *Insert Figure 2 near here*

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

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

236 *Insert Figure 3 near here*

237 Discussion

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

246 The clay content of suppressive soils is widely credited with an indirect role in disease
247 suppression. Sandy or sandy loam soils are often associated with high disease severity (Amir and
248 Alabouvette 1993; Höper *et al*. 1995; Hwang and Ko 2004; Deltour *et al*. 2017). Coarser-textured soils
249 typically have lower water- and nutrient-holding capacities, as well as organic matter content, and
250 are thus less conducive to microbial and plant growth, relative to finer-textured soils (Amir and
251 Alabouvette 1993; Pattison and Lindsay 2006). Clay type has also been associated with
252 suppressiveness in previous studies. Stotzky and Martin (1963) correlated the presence of
253 montmorillonite-type clay with wilt suppression. However, all the soils used in this study had kaolin

254 as the predominant clay mineral, with no detectable smectite. Given that nutrients were supplied to
255 all plants in the experiment, we speculate that the water retention properties of the soils may have
256 been a major factor explaining the difference in plant growth and disease severity between the soils.

257 In this study, root mass increased and disease severity decreased with increasing soil clay
258 content. Root quantity varies considerably depending on the health of the banana plant (Stover and
259 Simmonds 1987), and it is in the roots, as well as the vascular system, where the plant defence
260 response determines the success or failure of plant resistance following infection by *Foc* (De
261 Ascensao and Dubery 2000). Banana plants are sensitive to water deficit (Stover and Simmonds
262 1987), and a reduction of soil water content from field capacity to 40% field capacity has been
263 reported to increase Fusarium wilt severity (Peng *et al*. 1999). Although there was not a significant
264 correlation between disease severity and plant-available water holding capacity, it is likely that plants
265 in the Virgil soil, with the highest sand content, were subjected to a greater water deficit than plants
266 in the other soils, contributing to higher disease severity.

267 Soils conducive to Fusarium wilt are frequently more acidic than suppressive soils (Höper *et*
268 *al*. 1995; Alabouvette 1999; Shen *et al*. 2015; Deltour *et al*. 2017). However, exceptions have been
269 found when soil pH is in the neutral to alkaline range (Peng *et al*. 1999; Dominguez-Hernandez *et al*.
270 2008). Interactions between pH and the availability of micronutrients to the pathogen and plant are
271 complex, and generalisations about pH and suppression in different contexts are difficult to make. In
272 this study there was no significant association between pH and disease severity. Soil pH covered a
273 fairly small range in these soils (pH 5.6 – 7.1), due to regular liming of the banana fields.

274 Soil enzyme assays are useful biological indicators due to their significance in a range of
275 microbial processes i.e. catalysing biochemical reactions essential to nutrient cycling. Specifically, β-
276 glucosidase activity has been demonstrated to be particularly useful for monitoring biological soil
277 quality (Turner *et al*. 2002). Furthermore β-glucosidase activity has been previously linked to
278 Fusarium wilt suppression (Pattison *et al*. 2018). Although not all replicates were analysed and thus a

279 degree of caution should be applied, in this study disease severity was negatively associated with β-
280 glucosidase activity, suggesting that the improved biological soil health aided in the disease-
281 suppressive characteristics of some soils. The β-glucosidase activity was not significantly associated
282 with soil clay content (r = 0.714, *P* = 0.111), organic carbon (r = -0.353, *P* = 0.492), or indeed any
283 variable measured, therefore suggesting that microbial activity in these soils may have been driven
284 by factors or a combination of factors not examined here, such as banana field cultivation
285 techniques, fertiliser application and pest management methods.

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

294 The negative correlation between disease severity and soil concentrations of total Fe, Cu and
295 Cd and extractable B may have been due to their correlation with soil clay content. High
296 concentrations of metals and nutrients are generally associated with high clay content and particular
297 parent materials, and they may not have played a role in disease suppression. Cadmium is not an
298 essential element and has been shown to reduce mycelial growth of *Fusarium* spp. (Gharieb 2001).
299 Boron, Fe and Cu are essential micronutrients (Lahav and Israeli 2000; Marschner 2012). Copper is
300 essential for the fungal denitrifying system in *F. oxysporum* (Matsuoka *et al*. 2017). Interestingly,
301 neither total Fe nor DTPA-extractable Fe concentration were positively associated with disease
302 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; Dominguez *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* chlamydospore 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 valuable to carry out further research. 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.

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

328 and testing for inoculation success, and Tasmin Rymer for moral support. This project has been
329 funded by Hort Innovation banana research and development levy, with co-investment from
330 Queensland Government. Hort Innovation is the grower-owned, not-for-profit research and
331 development corporation for Australian Horticulture.

332

333 ## Conflicts of interest

334 The authors declare no conflicts of interest.

335

336 ## References

337 Adiobo A, Oumar O, Perneel M, Zok S, Höfte M (2007) Variation of Pythium-induced cocoyam
338 root rot severity in response to soil type. *Soil Biology and Biochemistry* **39**, 2915-2925.

339 Alabouvette C (1986) Fusarium-wilt suppressive soils from the Chậteaurenard region: review
340 of a 10 year study. *Agronomie* **6**, 273-284.

341 Alabouvette C (1999) Fusarium wilt suppressive soils: an example of disease-suppressive
342 soils. *Australasian Plant Pathology* **28**, 57-64.

343 Amir H, Alabouvette C (1993) Involvement of soil abiotic factors in the mechanisms of soil
344 suppressiveness to fusarium wilts. *Soil Biol. Biochem.* **25**, 157-164.

345 Brady NC, Weil RR (2008), 'The nature and properties of soils, 14th edn.' (Prentice Hall: New
346 Jersey, USA).

347 Cannon MG, Murtha GG, Smith CD (1992) 'Soils of the Cardwell-Tully area, North
348 Queensland.' (CSIRO Publishing: Glen Osmond, Australia).

349 Cao L, Qiu Z, You J, Tan H, Zhou S (2005) Isolation and characterization of endophytic
350 streptomycete antagonists of fusarium wilt pathogen from surface-sterile banana roots. *FEMS*
351 *Microbiology Letter* **247**, 147-152.

352 Carlier JD, De Waele D, Escalant JV (2003) Global evaluation of *Musa* germplasm for
353 resistance to Fusarium wilt, *Mycosphaerella* leaf spot diseases and nematodes. Performance
354 evaluation (A. Vézina and C. Picq, eds.). INIBAP Technical Guidelines 7. The International Network for
355 the Improvement of Banana and Plantain: Montpellier, France.

356 Cha JY, Han S, Hong HJ, Cho H, Kim D, Kwon Y, Kwon SK, Crüsemann M, Lee YB, Kim JF,
357 Giaever G, Nislow C, Moore BS, Thomashow LS, Weller DM, Kwak Y-S (2016) Microbial and
358 biochemical basis of a Fusarium wilt-suppressive soil. *The ISME journal* **10**,119-29.

359 Cook DC, Taylor AS, Meldrum RA, Drenth A (2015) Potential economic impact of Panama
360 disease (Tropical Race 4) on the Australian banana industry. *Journal of Plant Diseases and Protection*
361 **122**, 229-237.

362 Creamer RE, Bellamy P, Black HI, Cameron CM, Campbell CD, Chamberlain P, Harris J, Parekh
363 N, Pawlett M, Poskitt J (2009) An inter-laboratory comparison of multi-enzyme and multiple
364 substrate-induced respiration assays to assess method consistency in soil monitoring. *Biology and*
365 *Fertility of Soils* **45**, 623-633.

366 De Ascensao ARDCF, Dubery IA (2000) Panama disease: cell wall reinforcement in banana
367 roots in response to elicitors from *Fusarium oxysporum* f.sp. *cubense* race four. *Biochemistry and Cell*
368 *Biology* **90**, 1173-1180.

369 Deltour P, França SC, Pereira OL, Cardoso I, De Neve S, Debode J, Höfte M (2017) Disease
370 suppressiveness to Fusarium wilt of banana in an agroforestry system: Influence of soil
371 characteristics and plant community. *Agriculture, Ecosystems & Environment* **239**, 173-81.

372 Dominguez J, Negrin MA, Rodriguez CM (2001) Aggregate water-stability, particle-size and
373 soil solution properties in conducive and suppressive soils to Fusarium wilt of banana from Canary
374 Islands (Spain). *Soil Biology and Biochemistry* **33**, 449-455.

375 Dominguez-Hernandez J, Negrin MA, Rodriguez CM (2008) Soil potassium indices and clay-
376 sized particles affecting banana-wilt expression caused by soil fungus in banana plantation
377 development on transported volcanic soils. *Communications in Soil Science and Plant Analysis* **39**,
378 397-412.

379 Dong X, Wang M, Ling N, Shen Q, Guo S (2016) Effects of iron and boron combinations on the
380 suppression of *Fusarium* wilt in banana. *Scientific Reports* **6,**
381 doi:http://dx.doi.org.elibrary.jcu.edu.au/10.1038/srep38944

382 Enderlin N, Barry E, Seonaid R (1997) Soils of the Mareeba-Dimbulah Irrigation Area (MDIA).
383 Queensland Department of Natural Resources, Mareeba, Qld.

384 Forsyth LM, Smith LJ, Aitken EAB (2006) Identification and characterization of non-
385 pathogenic *Fusarium oxysporum* capable of increasing and decreasing Fusarium wilt severity.
386 *Mycological Research* **110**, 929-935.

387 Gharieb M (2001) Pattern of cadmium accumulation and essential cations during growth of
388 cadmium-tolerant fungi. *BioMetals* **14**, 143-151.

389 Höper H, Alabouvette C (1996) Importance of physical and chemical soil properties in the
390 suppressiveness of soils to plant diseases. *European Journal of Soil Biology* **32**, 41-58.

391 Höper H, Steinberg C, Alabouvette C (1995) Involvement of clay type and pH in the
392 mechanisms of soil suppressiveness to Fusarium wilt of flax. *Soil Biology and Biochemistry* **27**, 955-
393 967.

394 Hwang S-C, Ko W-H, 2004. Cavendish banana cultivars resistant to Fusarium wilt acquired
395 through somaclonal variation in Taiwan. *Plant Disease* **88**, 580-588.

396 Koske RE, Gemma JN (1989) A modified procedure for staining roots to detect VA
397 mycorrhizas. *Mycological Research* **92**, 486-488.

398 Lahav E, Israeli Y (2000) Mineral deficiencies of banana. In 'Diseases of Banana, Abacá and
399 Enset'. (Ed. DR Jones) pp. 339-350 (CABI Publishing: Wallingford, UK).

400 Marschner P (2012) 'Marschner's mineral nutrition of higher plants, 3rd edn.' (Academic
401 Press: London).

402 Marx MC, Wood M, Jarvis SC (2001) A microplate fluorimetric assay for the study of enzyme
403 diversity in soils. *Soil Biology and Biochemistry* **33**, 1633-1640.

404 Matsuoka M, Kumar A, Muddassar M, Matsuyama A, Yoshida M, Zhang KYJ (2017) Discovery
405 of fungal denitrification inhibitors by targeting copper nitrite reductase from *Fusarium oxysporum*.
406 *Journal of Chemical Information and Modeling* **57**, 203-213.

407 Mazzola M (2002) Mechanisms of natural soil suppressiveness to soilborne diseases. *Antonie*
408 *van Leeuwenhoek* **81**, 557-564.

409 Mostert D, Molina AB, Daniells J, Fourie G, Hermanto C, Chao C-P, Emily F, Sinohin VG,
410 Masdek N, Thangavelu R, Li C, Yi G, Mostert L, Viljoen A (2017) The distribution and host range of the
411 banana Fusarium wilt fungus, Fusarium oxysporum f. sp. cubense, in Asia. *PLoS ONE* **12**: e0181630.
412 doi: 10.1371/journal.pone.0181630.

413 Murtha GG (1986) 'Soils of the Tully - Innisfail area North Queensland.' (CSIRO: Adelaide, SA)

414 Nasir N, Pittaway PA, Pegg KG, Lisle AT (2003) A foliar rating system for comparing the
415 resistance of banana cultivars grown as tissue-cultured plantlets in the laboratory to Fusarium wilt.
416 *Australasian Plant Pathology* **32**, 521-526.

417 O'Neill WT, Henderson J, Pattemore JA, O'Dwyer C, Perry S, Beasley DR, Tan YP, Smyth AL,
418 Goosem CH, Thomson KM, Hobbs RL, Grice KRE, Trevorrow P, Vawdrey LL, Pathania N, Shivas RG
419 (2016) Detection of Fusarium oxysporum f. sp. cubense tropical race 4 strain in northern Queensland.
420 *Australasian Plant Disease Notes* **11**. doi: 10.1007/s13314-016-0218-1.

421 Orjeda G (1998) Evaluation of Musa germplasm for resistance to Sigatoka diseases and
422 Fusarium wilt. INIBAP Technical Guidelines 3. International Plant Genetic Resources Institute, Rome,
423 Italy; International Network for the Improvement of Banana and Plantain, Montpellier, France; ACP-
424 EU Technical Centre for Agricultural and Rural Cooperation, Wageningen, The Netherlands.

425 Orr R, Nelson PN (2018) Impacts of soil abiotic attributes on Fusarium wilt, focusing on
426 bananas. *Applied Soil Ecology* **132**, 20-33.

427 Pattison T, Lindsay S (2006) 'Banana root and soil health user's manual.' (Department of
428 Primary Industries and Fisheries: Brisbane, Qld).

429 Pattison AB, Molina AB, Chao CP, Viljoen A, Lindsay SJ (2018) 'Integrating management
430 practices to support banana production in the presence of Fusarium wilt, 1196 ed'. (International
431 Society for Horticultural Science (ISHS), Leuven, Belgium).

432 Peng HX, Sivasithamparam K, Turner DW (1999) Chlamydospore germination and Fusarium
433 wilt of banana plantlets in suppressive and conducive soils are affected by physical and chemical
434 factors. *Soil Biology and Biochemistry* **31**, 1363-74.

435 Ploetz RC (2015) Fusarium wilt of banana. *Phytopathology* **105**, 1512-1521.

436 Puhalla JE (1985) Classification of strains of *Fusarium oxysporum* on the basis of vegetative
437 compatibility. *Canadian Journal of Botany* **63**, 179-83.

438 Queensland Government (2016) Queensland's banana industry.
439 https://www.business.qld.gov.au/industries/farms-fishing-forestry/agriculture/crop-
440 growing/banana-industry. Accessed 30 September 2017.

441 Queensland Government (2017) Panama disease. https://www.daf.qld.gov.au/plants/health-
442 pests-diseases/a-z-significant/panama-disease2. Accessed 30 September 2017.

443 Rayment GE, Lyons DJ (2011). ‘Soil chemical methods: Australasia’. (CSIRO Publishing:
444 Collingwood, Vic.).

445 Shen Z, Ruan Y, Xue C, Zhong S, Li R, Shen Q (2015) Soils naturally suppressive to banana
446 *Fusarium* wilt disease harbor unique bacterial communities. *Plant and Soil* **393**, 21-33.

447 Siegel-Hertz K, Edel-Hermann V, Chapelle E, Terrat S, Raaijmakers JM, Steinberg C (2018)
448 Comparative microbiome analysis of a Fusarium wilt suppressive and a Fusarium wilt conducive soil
449 from the Châteaurenard region. *Frontiers in Microbiology* **9**. doi: 10.3389/fmicb.2018.00568.

450 Stotsky G, Martin RT (1963) Soil mineralogy in relation to the spread of Fusarium wilt of
451 banana in Central America. *Plant and Soil* **18**, 317-337.

452 Stover RH, Simmonds NW (1987) ‘Bananas, 3rd edn.’ (Longman Scientific & Technical: New
453 York).

454 Ting ASY, Meon S, Kadir J, Radu S, Singh G (2008) Endophytic microorganisms as potential
455 growth promoters of banana. *BioControl* **53**, 541-553.

456 Turner BL, Hopkins DW, Haygarth PH, Ostle N (2002) β-Glucosidase activity in pasture soils.
457 *Applied Soil Ecology* **20**, 157-162.

458 Turner DW, Lahav E (1983) The growth of banana plants in relation to temperature.
459 *Australian Journal of Plant Physiology* **10**, 43-53.

460 Weinert M, Simpson M (2016) ‘Sub tropical banana nutrition – matching nutrition
461 requirements to growth demands.’ (NSW Department of Primary Industries: Wollongbar, NSW).

462 Welc M, Frossard E, Egli S, Bünemann EK, Jansa J (2014) Rhizosphere fungal assemblages and
463 soil enzymatic activities in a 110-years alpine chronosequence. *Soil Biology and Biochemistry* **74**, 21-
464 30.

465 Zhang N, He X, Zhang J, Raza W, Xing-Ming YA, Yun-Ze Ru, Qi-Rong SH, Huang QW (2014)
466 Suppression of Fusarium wilt of banana with application of bio-organic fertilizers. *Pedosphere* **24**,
467 613-24.
468

469 Table 1. Soil properties, including all those significantly correlated ($p < 0.05$) with internal

470 disease severity in inoculated plants, showing Spearman's rank correlation coefficient (r) and *P* value

471 (n = 6).

	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

472 FCW, field capacity water content (-10 kPa); RAW, readily available water (-10 to -200 kPa); TAW,

473 total available water (-10 to -1500 kPa)

474

475 Table 2. *P*-values from analyses of variance of harvested plant biomass. Values <0.05 are

476 underlined. Means and standard errors of variables significantly affected by the treatments are given

477 in Figure 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 x 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 x Inocul.	0.455	0.578	0.748	0.525	0.415

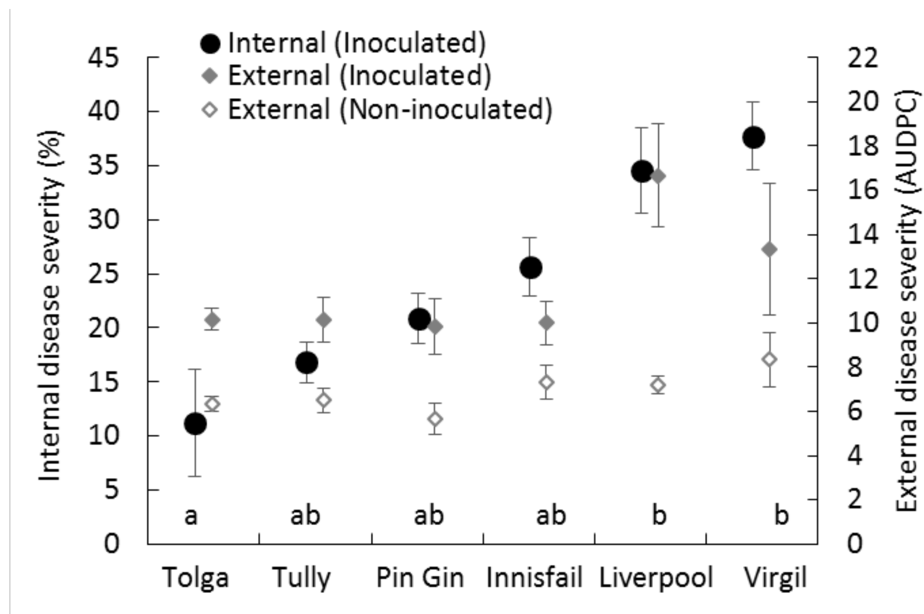
478

479

480

481

482



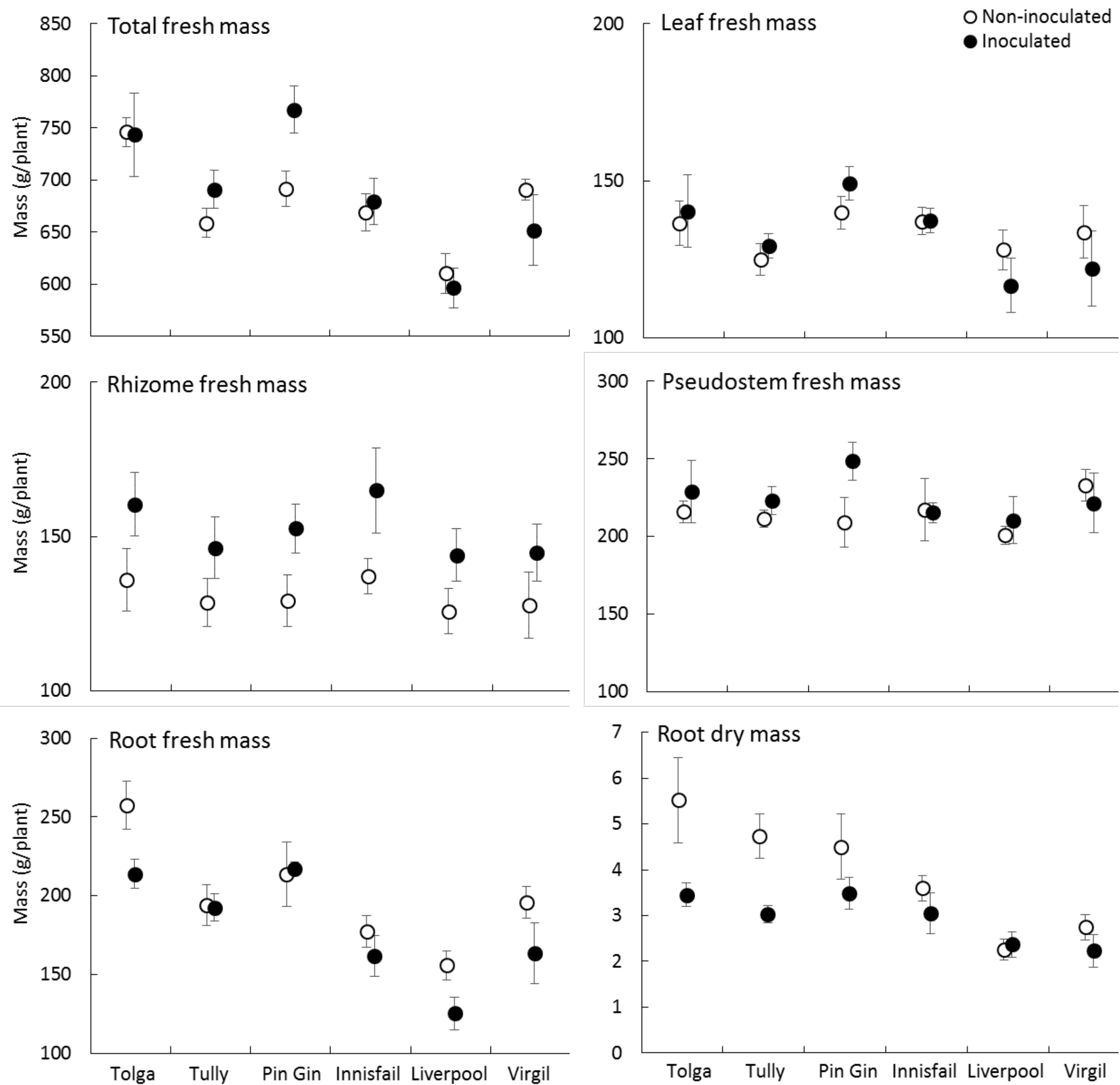
483

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

490

491

492



493

494 Figure 2. Mass of plant components that were significantly affected by treatments. (*a*) total

495 fresh mass, (*b*) leaf fresh mass, (*c*) rhizome fresh mass, (*d*) pseudostem fresh mass, (*e*) root fresh

496 mass and (*f*) root dry mass. Significance of treatment effects is given in Table 2. Bars represent the

497 s.e.m.

498

499

500

501

502

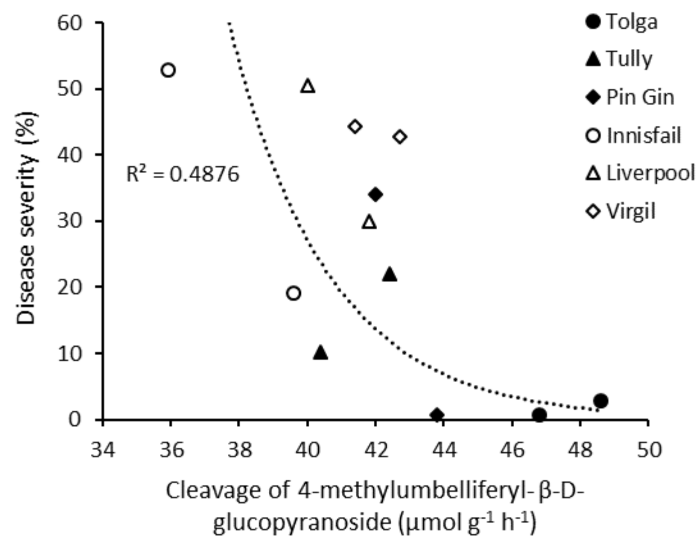
503

504

505

506

507



508

509 Figure 3. Disease severity (proportion of rhizome discolouration) in relation to soil β-

510 glucosidase activity across the inoculated pots analysed.

511