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Micropropagated bananas are more susceptible to fusarium wilt than plants grown from conventional material

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Abstract. The reaction of field-grown micropropagated bananas, *Musa* cv. Williams (AAA, Cavendish subgroup) and cv. Goldfinger (AAAB, FHIA-01), to subtropical race 4 *Fusarium oxysporum* f. sp. *cubense* (*Foc*) was compared with the reaction of plants grown from conventional planting material (sections of the rhizome, termed bits). Leaf gas exchange of plants was determined, and growth and dry matter accumulation were measured. Comparisons were made among these parameters from shortly after planting, throughout winter, and into spring when a high percentage of the plants started to show external symptoms of fusarium wilt. Micropropagated bananas were significantly more susceptible to race 4 *Foc* than plants derived from bits. This was irrespective of planting times, cultivars used, or whether the bits had first been established in containers in the glasshouse (as for micropropagated plants) or been planted directly in the field. This greater susceptibility does not appear to be a consequence of differences in maximum photoassimilation rates, greater photoassimilate demand, or lack of carbohydrate reserves once plants became established.

Additional keywords: *Musa*, micropropagation, tissue culture, *Fusarium oxysporum* f. sp. *cubense*.

Introduction

There are many advantages to using micropropagated bananas as a source of planting material (Israeli *et al.* 1995; Robinson 1996), not the least of which is their freedom from pests and diseases at planting. However, despite the widespread use of micropropagated plants, little is known about how these plants respond to pests and diseases in the field. Fusarium wilt, caused by the soil-borne pathogen *Fusarium oxysporum* f. sp. *cubense* (*Foc*), is of particular concern because of the lack of chemical control measures and the long-term viability of chlamydospores, some of which may still be capable of infection decades later (Pegg *et al.* 1996). *Foc* also has the ability to survive as a parasite on the roots of weed species (Waite and Dunlap 1953; Pedrosa 1995). In Australia, once a commercial block of bananas has been infested it is either abandoned or replanted with resistant varieties.

The increased susceptibility of Cavendish cultivars in the subtropics is thought to result from exposure to low winter temperatures (Pegg *et al.* 1996). Moore *et al.* (1993) suggested that the sensitivity of bananas to stress induced by cold temperatures, and the associated disruption of the photoassimilate mechanisms, contributed to susceptibility of Cavendish to race 4 *Foc* in the subtropics. *Foc* is able to penetrate and colonise vascular elements within the root. It is known that host defence mechanisms (i.e. formation of gels, tyloses, and phenolic infusions), which restrict invasion by the pathogen (Beckman 1990), are primarily driven by photoassimilates, either from storage or current photosynthesis. Therefore, a decrease in the carbon assimilation capacity of banana plants may reduce their ability to restrict root invasion by the pathogen.

Micropropagated banana plants, unlike conventional planting material derived from a section of the rhizome

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containing an axillary bud (bits), have no significant source of stored carbohydrate. Eckstein and Robinson (1995) reported higher CO_2 assimilation (A) rates from micropropagated plants and suggested that this may be a compensatory mechanism to offset the high assimilate demand of vigorously growing plants which lack a secondary source of photoassimilates. Micropropagated plants have also been reported to produce suckers earlier and in larger numbers than plants from conventional propagation (Israeli *et al.* 1995) and thus possess a larger sink which may stimulate CO_2 assimilation (Schaffer *et al.* 1987, 1996; Whiley *et al.* 1998).

In the present study, we wanted to determine whether micropropagated bananas were more susceptible to fusarium wilt than plants grown from conventional material. If they were found to be more susceptible, is the increased susceptibility of micropropagated plants related to the carbon assimilation capacity of plants? This information would be important to an understanding of the role of photoassimilates in the ability of *Foc* to colonise micropropagated banana plants.

Materials and methods

The experimental site was part of a commercial Cavendish plantation at Wamuran in subtropical Queensland (27°S, 153°E) that was abandoned because of losses to Fusarium wilt caused by *Foc*. The soil is classified as a yellow ferrosol (gleyed podzolic soil) and is a heavy clay–clay loam of pH 5·5–6·0. The field was uniformly infested with 3 subtropical race 4 vegetative compatability groups (VCGs): 0120, 0129, and 01211 (Pegg *et al.* 1996).

Banana plants, *Musa* cv. Williams (AAA, Cavendish subgroup) and cv. Goldfinger (AAAB, FHIA-01), were initiated *in vitro,* micropropagated, established *ex vitro*, and grown under glasshouse conditions as described by Smith and Hamill (1993). The sand-peat (2 : 1) potting mix was steam-pasteurised before use. Cv. Williams is susceptible to subtropical race 4, whereas Goldfinger is resistant (Pegg *et al.* 1996). Bits of Williams and Goldfinger were obtained from field-grown plants free of *Foc*, pared to an average weight of 730 g (fresh weight), and dried at ambient temperature for 1 week before planting either in the field or in containers in a glasshouse. Three separate experiments were conducted from 1993 to 1995. For the first 2 experiments, micropropagated plants were grown in 2·5-L containers to a height of 30 cm before field-planting and bits were established directly in the field. In the third experiment, micropropagated plants and bits were grown in 10-L containers randomly arranged on benches in a glasshouse. When about 50 cm high, they were field-planted. All plants were grown in the field by standard commercial cultural practices (Gall and Vock 1994), except that plants were not desuckered.

Experiment 1

Expt 1 consisted of 2 treatments: Williams plants either produced from micropropagation or established from bits. The bits were field-planted in spring on 7 November 1993, and the micropropagated plants were planted 3 weeks later on 25 November 1993. Plants were destructively harvested on 12 May 1994 after approximately 6 months growth and when 18% of the micropropagated plants had external symptoms of fusarium wilt.

Experiment 2

Expt 2 consisted of race 4-resistant Goldfinger plants which were either micropropagated or grown from bits. The bits were field-planted on 7 November 1994, and the micropropagated plants were planted 4 weeks later on 5 December 1994. Plants were destructively harvested on 25 October 1995, after approximately 11 months growth and when 6% of the micropropagated plants had external symptoms of fusarium wilt.

A staggered planting date was used in Expts 1 and 2 in an attempt to synchronise the development of plants between the 2 treatments. However, this was only partially successful as most of the plants from micropropagation were taller than those from bits for the duration of the experiment.

Experiment 3

Expt 3 consisted of 3 treatments: Williams plants produced from micropropagation and established in 10-L containers, bits established in 10-L containers, and field-planted bits. Synchronisation of plant development was achieved by first planting bits into 10-L containers in a glasshouse. When shoot emergence from the bits was observed, acclimatised micropropagated plants about 10 cm tall were transplanted from seedling trays to 10-L containers. During the establishment phase, both sets of plants were graded for uniformity and size. Micropropagated plants and container-grown bits were planted on 10 January 1994, and field-planted bits were grown on 12 January 1994. Plants were destructively harvested on 24 October 1995 after approximately 10 months growth when 45% of the micropropagated plants had external symptoms of fusarium wilt.

Experimental design and statistical analysis

For fusarium wilt assessments, each experiment used a randomised complete block design with 6 plants per block. Within each block, treatments were randomly allocated to the 6 plants. In addition, for the growth and physiological measurements in Expt 3, there were 4 replications (blocks) per treatment per harvest time, except for the spring harvest which consisted of 8 replicates. Data were analysed by ANOVA and treatment means were separated by Tukey's multiple range test ($P \leq 0{\cdot}05$).

Assessment of fusarium wilt infection

At final harvest for each experiment, plants were removed from the soil and a transverse cut made about one-quarter of the way from the base of the rhizome. The cut surface of the rhizome was rated for discoloration on a scale of 1–6 developed by the International Network for the Improvement of Bananas and Plantains (Jones 1994): 1, no vascular tissue discoloration; 2, isolated points of vascular tissue discoloration; 3, ≤one-third of the vascular tissue discoloured; 4, one- to two-thirds of the vascular tissue discoloured; 5, >two-thirds of the vascular tissue discoloured; and 6, total vascular tissue discoloration and/or discoloration of leaf bases. In Expt 3, visual symptoms of fusarium wilt in the aerial portions of the plants were recorded as the symptoms developed. Plants were judged to have external symptoms of fusarium wilt if they showed any signs of wilting, yellowing of foliage, petiole buckling, or splitting of the pseudostem base (Jones 1994).

Growth and physiological measurements

Detailed growth and physiological measurements were recorded on plants in Expt 3. From March 1995 until the completion of the experiment (October 1995), mean plant height and leaf numbers were determined monthly for each treatment. Destructive harvests of randomly selected sets of plants were made in late summer (30 March 1995), early winter (8 June 1995), late winter (29 August 1995), and spring (24 October 1995). At each harvest, the mother plant and suckers (when present) were separated and the leaf and sucker number, and fresh weight of the pseudostem, rhizome, and leaf (lamina and petiole) were recorded. Fresh weights of subsamples of the various organs were taken and these were then dried to a constant weight at 60°C and reweighed. The data were used to calculate total plant dry weight and dry matter partitioning. When dry, a sample of rhizome tissue was ground at 100 mesh in a Udy Mill (Udy Corporation, USA) and used to calculate starch content by a 2-stage enzymatic hydrolysis of starch to glucose (Rasmussen and Henry 1990).

CO_2 assimilation (A) and chlorophyll fluorescence measurements were made on micropropagated plants and container-grown bits in the glasshouse when they were about 20 cm tall. Measurements were repeated in the field in late summer (30 March 1995), late winter (23 August 1995), and spring (24 October 1995), about 3, 8, and 10 months after field planting, respectively. Plants in the final set of 8 replicates for each treatment harvested in spring 1995 were those selected for the ongoing A and chlorophyll fluorescence measurements. Chlorophyll fluorescence, which has been used as a rapid and non-destructive method to determine the extent of chilling or heat injury of plants (Smillie *et al*. 1983), was measured as the ratio variable fluorescence (F_v) : maximal fluorescence yield (F_m).

Leaf gas exchange measurements were made with a CIRAS-1 photosynthetic meter (PP Systems, Hitchin Herts, UK) at a photosynthetic photon flux >1200 μmol quanta/$m^2 \cdot s$, which is the light saturation point of banana for A (A. W. Whiley and C. Searle, unpubl. data). The experimental field site was irrigated the day before taking measurements to ensure that plants were not affected by an internal water deficit and a maximum CO_2 assimilation rate (A_{max}) was determined. Measurements were taken from the mid-point of the lamina of the third youngest leaf on 8 plants from each treatment and were carried out between 08 00 and 10 00 hours before a significant increase in vapour pressure deficits occurred. Chlorophyll fluorescence was measured with a BioMonitor Stress Meter (BioMonitor SCI, Umeå, Sweden) as described for bananas by Schaffer *et al*. (1996) on the same leaves used for leaf gas exchange measurements. The ratio of F_v/F_m was calculated to indicate photoinhibitory damage to PS II (Björkman 1987; Demmig and Björkman 1987).

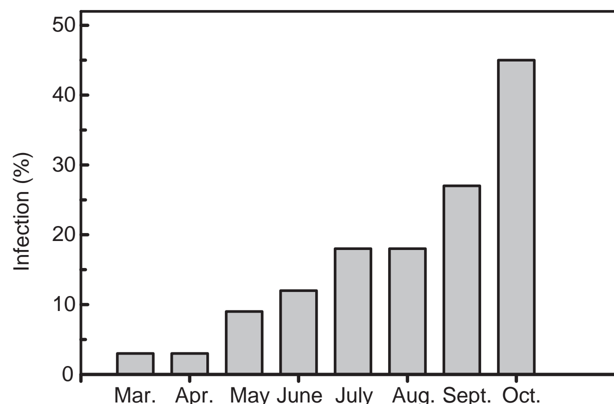

Fig. 1. Disease progress on micropropagated Williams plants as determined by the development of external symptoms during Expt 3. Plants derived from micropropagation were the only plants to express external symptoms of fusarium wilt.

Results and discussion

Results from the 3 experiments demonstrated that micropropagated bananas were significantly more susceptible to race 4 *Foc* than plants derived from bits. The increase in frequency of fusarium wilt of micropropagated Williams plants, judged from the development of external symptoms during Expt 3, is illustrated in Fig. 1. Plants derived from micropropagation were the only plants to express external fusarium wilt symptoms over the duration of the experiments. The micropropagated plants had increased susceptibility to fusarium wilt, irrespective of planting times, cultivars, or whether the bits had first been established in containers or planted

Table 1. Internal disease symptom assessment of plants derived from micropropagation, field-grown bits, and container-grown bits following growth in soil infested with subtropical race 4 *Fusarium oxysporum* f. sp. *cubense* (*Foc*)

Severity ratings on a scale of 1–6: 1, no symptoms; 6, total vascular tissue discoloration of rhizome. Rating values within rows followed by different letters are significantly different at $P = 0 \cdot 05$

Cultivar	Planting material					
	Micropropagated		Field-grown bits		Container-grown bits	
	Incidence	Severity	Incidence	Severity	Incidence	Severity
Williams[A]	57·8%	2·02a	2·4%	1·02b	—	—
Goldfinger[B]	9·4%	1·22a	0·0%	1·00b	—	—
Williams[C]	54·5%	2·67a	17·9%	1·21b	9·1%	1·12b

[A]Expt 1. Data are means of 41–45 plants where bits were field-planted on 4.xi.93 and micropropagated plants on 25.xi.93. Plants were destructively harvested on 12.v.94 when 18% of micropropagated plants had external symptoms of fusarium wilt.
[B]Expt 2. Data are means of 32 plants where bits were field-planted on 7.xi.94 and micropropagated plants on 5.xii.94. Plants were destructively harvested on 25.x.95 when 6% of micropropagated plants had external symptoms.
[C]Expt 3. Data are means of 28–33 plants where micropropagated and container-grown bits were planted on 10.i.95 and field-grown bits on 12.i.95. Plants were destructively harvested on 24.x.95 when 45% of micropropagated plants had external symptoms.

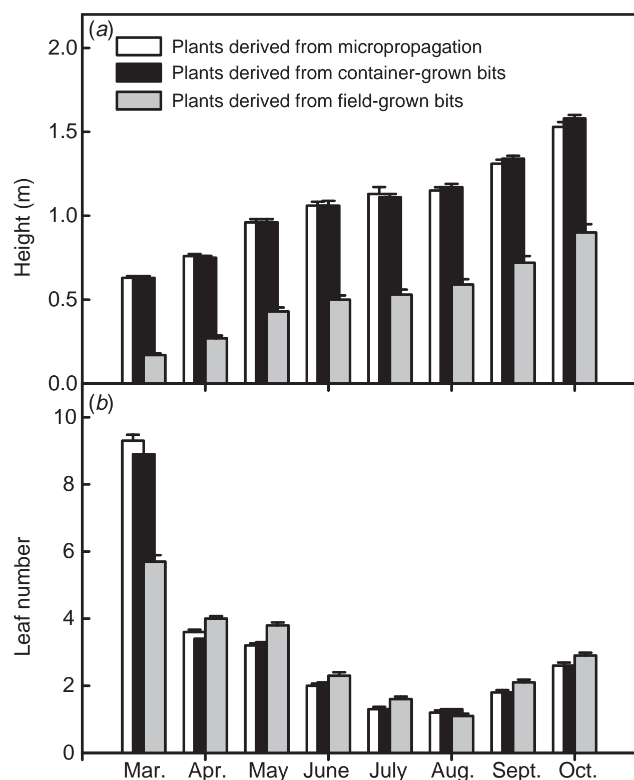

Fig. 2. Growth of plants established from micropropagation, container-grown bits, or field-grown bits over the duration of Expt 3 where (*a*) is the height of the pseudostem, and (*b*) is the mean number of new leaves per plant each month, except for March where total leaf numbers per plant are presented. Data are mean values of 35–53 plants ± s.e.

directly in the field (Table 1). Despite similarities in growth and photoassimilation of micropropagated plants and container-grown bits, a higher percentage of micropropagated plants was infected with *Foc* and had a significantly greater severity of internal symptoms.

There was no significant difference for height and leaf number between plants derived from micropropagation or container-grown bits (Fig. 2). However, plants derived from field-grown bits remained significantly shorter than those derived from the other 2 sources. In March and August, plants from field-grown bits had significantly fewer leaves than those from the other 2 treatments but for the other months of the study they had either higher leaf emergence rates or were not significantly different.

The progressive accumulation of dry matter during Expt 3 was very similar for plants derived from micropropagation and container-grown bits; however, plants from field-planted bits accumulated significantly less dry matter (Table 2). Distribution of dry matter was similar for plants derived from micropropagated and container-grown bits, except that there were significant differences in rhizome and sucker leaf dry mass between plants from micropropagation and container-grown bits. Three months after field planting, container-grown bits had accumulated significantly greater dry mass in the rhizome whereas 6 months after field planting the suckers on plants derived from micropropagation had greater leaf dry mass (Table 2).

Starch content of rhizomes from container-grown bits was also significantly higher than the other 2 treatments

Table 2. Progressive dry matter accumulation and distribution in plants derived from micropropagation, container-grown bits, and field-grown bits during the first 10 months after field-planting

Data are expressed as g dry weight and are mean values of 4–8 plants (Expt 3). Values within columns for each assessment date followed by the same letter are not significantly different at $P = 0{\cdot}05$

Treatment	Mother plant					Suckers				Total plant		
	Leaf	Pseudo-stem	Bit	Rhizome	Root	Leaf	Pseudo-stem	Rhizome	Root	Mother plant	Sucker(s)	Whole plant
Late summer (30.iii.95)												
Micropropagated	123·6a	88·2a	0·0b	64·4b	30·6a	1·7 a	12·1a	38·0a	11·6a	306·8a	63·4a	370·2a
Container bits	149·6a	95·2a	0·0b	87·0a	33·9a	0·04a	7·3b	37·5a	8·7a	365·7a	53·5a	419·2a
Field bits	120·0b	10·0b	34·3a	7·2c	2·6b	0·0 b	0·0b	0·0b	0·0b	68·7b	0·0b	68·7b
Early winter (8.vi.95)												
Micropropagated	351·2a	267·5a	0·0b	277·2a	38·6b	12·1a	55·9a	176·7a	28·2a	934·5a	272·9a	1207·5a
Container bits	340·0a	337·6a	0·0b	218·0a	42·3b	2·4b	43·1a	196·1a	26·6a	937·9a	268·2a	1206·3a
Field bits	68·9b	39·5b	25·4a	47·7b	12·7b	0·0b	0·0b	0·0b	0·0b	194·2b	0·0b	194·2b
Late winter (29.viii.95)												
Micropropagated	354·8ab	409·9ab	—	366·9a	—	11·0a	111·8a	305·4a	—	1131·5ab	428·2a	1559·8a
Container bits	435·3a	531·9a	—	437·4a	—	2·8ab	102·2a	389·6a	—	1404·7a	494·6a	1899·2a
Field bits	71·3b	77·8b	—	93·4b	—	0·0b	2·7b	21·2b	—	242·5b	23·9b	266·4b
Spring (24.x.95)												
Micropropagated	694·1a	738·7a	—	635·9ab	—	27·9a	166·1a	527·2a	—	2068·6a	721·2a	2789·8a
Container bits	774·9a	845·7a	—	863·3a	—	13·2ab	171·8a	726·9a	—	2483·8a	912·0a	3395·8a
Field bits	324·3b	293·1b	—	418·7b	—	0·0b	35·6b	160·7b	—	1036·1b	196·3b	1232·5b

Table 3. Accumulation and distribution of starch in the rhizomes of plants derived from micropropagation, container-grown bits, and field-grown bits at four different dates

Planting dates were 10 and 12 January 1995. The mean weight of starch in bits at planting was 365 g with a starch concentration of 52·7%. Where values are zero, bits had rotted away and starch concentrations could not be determined. Data are means of 4–8 plants and values within columns at each harvest date followed by the same letter are not significantly different at $P = 0 \cdot 05$

Treatment	Starch in rhizome of mother plant		Starch in bit		Starch in rhizome of sucker(s)	
	(%)	(g)	(%)	(g)	(%)	(g)
			Late summer (30.iii.95)			
Micropropagated	55·7a	35·6b	—	—	48·3a	18·8a
Container-grown bits	58·1a	50·7a	0·0	0·0	45·6a	17·2a
Field-grown bits	39·5b	2·8c	3·7	1·2	—	—
			Early winter (8.vi.95)			
Micropropagated	38·1a	104·8a	—	—	52·0a	90·9a
Container-grown bits	41·5a	90·6a	0·0	0·0	46·4a	90·7a
Field-grown bits	40·9a	18·9b	4·1	1·1	—	—
			Late winter (29.viii.95)			
Micropropagated	55·6a	200·7a	—	—	51·2a	151·0a
Container-grown bits	54·1a	234·7a	0·0	0·0	49·0a	193·3a
Field-grown bits	53·0a	50·9b	0·0	0·0	46·2a	39·2b
			Spring (24.x.95)			
Micropropagated	44·7a	292·1ab	—	—	42·2ab	222·5b
Container-grown bits	42·1a	367·4a	0·0	0·0	49·2a	364·9a
Field-grown bits	46·9a	195·1b	0·0	0·0	35·4b	56·9c

Table 4. CO_2 assimilation (A_{max}), internal CO_2 concentration (C_i), stomatal conductance (g_s), and chlorophyll fluorescence (F_v/F_m) in plants derived from micropropagation, container-grown bits, and field-grown bits at four different dates

Planting dates were 10 and 12 January 1995. Data are means of 8 plants. There were no significant differences between treatments at each recording date as tested by ANOVA

Treatment	A_{max} (μmol $CO_2/m^2 \cdot s$)	C_i (ppm)	g_s (mmol $CO_2/m^2 \cdot s$)	F_v/F_m
		Glasshouse (1.xii.94)		
Micropropagated	16·0	257·0	702·7	—
Container-grown bits	16·2	266·0	864·8	—
l.s.d. ($P = 0 \cdot 05$)	1·7	11·0	187·7	
		Late summer (30.iii.95)		
Micropropagated	21·8	259·9	1504·5	0·675
Container-grown bits	22·2	250·4	934·5	0·716
Field-grown bits	21·7	252·6	1191·5	0·672
l.s.d. ($P = 0 \cdot 05$)	3·2	27·3	739·4	0·108
		Late winter (23.viii.95)		
Micropropagated	17·7	214·1	380·6	0·726
Container-grown bits	17·0	203·5	342·5	0·732
Field-grown bits	16·4	223·0	373·1	0·700
l.s.d. ($P = 0 \cdot 05$)	1·9	29·0	108·6	0·050
		Spring (24.x.95)		
Micropropagated	20·3	239·5	496·3	0·720
Container-grown bits	20·4	237·4	494·3	0·725
Field-grown bits	20·1	231·8	468·5	0·743
l.s.d. ($P = 0 \cdot 05$)	4·6	15·1	176·6	0·064

up to the late summer harvest; however, there was no difference in rhizome size or starch content at any stage past this early point of development (Table 3). On the other hand, starch reserves from the field-grown bits were exhausted very early in the growth of the plant and they had accumulated significantly less starch than the other 2 treatments. Both treatments using bits were not as affected by *Foc* as micropropagated

plants. Our results suggest that stored carbohydrate reserves do not play a significant role in the increased susceptibility of micropropagated plants.

The precocity and high production of micropropagated plants compared with plants derived from conventional planting material are well documented (Israeli *et al*. 1995; Robinson 1996) and our comparative growth data between micropropagated plants and field-grown bits supports published results. However, our study also suggests that if plants from both propagation sources are established with a similar leaf area at planting, there is likely to be little difference in production. Our results demonstrated that micropropagated plants and container-grown bits grew at the same rate, produced a similar amount of dry matter, partitioned photoassimilates in a similar pattern (data not presented), and produced a similar number of suckers. Also, we were unable to demonstrate any difference between treatments in $A_{\max}$ or photoinhibitory damage determined by the F_v/F_m ratio (Table 4). The $A_{\max}$ of plants from all treatments varied between $16\cdot0$ and $22\cdot0$ μmol $CO_2/m^2\cdot s$ depending on the time of year measurements were taken, whereas the F_v/F_m ratio was approximately $0\cdot7$ irrespective of treatment and time of year when measurements were taken.

During Expt 3 we did not observe any disruption to photoassimilation which may account for a breakdown in resistance. Robinson (1996) has reported that young micropropagated plants are more sensitive to environmental stress after establishment and this may compromise the plant's resistance to fusarium wilt. In this study, CO_2 assimilation (A) was only measured after plants had been well-watered the day before in order to restore full turgor and optimise photoassimilation. Future research should investigate photoassimilation responses of plants derived from different propagation sources during soil drying cycles and determine how this may influence infection.

We believe greater attention needs to be given to the rhizosphere in investigating possible mechanisms for resistance, which should include developing a greater understanding of the root morphology and physiology of micropropagated bananas. Are roots on *in vitro* plants more susceptible to fungal colonisation? Also, it is widely known that suppressive soils exist where fusarium wilt does not reach epidemic proportions (Pegg *et al*. 1996). The existence of microbial antagonists, such as non-pathogenic isolates of *Fusarium oxysporum*, may confer resistance in some suppressive soils (Alabouvette *et al*. 1993; Larkin *et al*. 1996). Micropropagated bananas are produced in an aseptic environment. Bits dug from the field might be infected with antagonists which are not found on micropropagated plants. We intend to search for possible biocontrol isolates in some of our plantations growing in wilt-suppressive soils and to examine these in glasshouse and field tests. If effective isolates are found, an opportunity may exist to inoculate micropropagated plants with these organisms before planting, thereby protecting these plants in the field (Marois 1990).

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References

Alabouvette, C., Lemanceau, P., and Steinberg, C. (1993). Recent advances in the biological control of Fusarium wilts. *Pesticide Science* **37**, 365–73.

Beckman, C.H. (1990). Host responses to the pathogen. *In* 'Fusarium Wilt of Banana'. (Ed. R. C. Ploetz.) pp. 93–105. (American Phytopathology Society: St Paul, MN.)

Björkman, O. (1987). Low temperature chlorophyll fluorescence in leaves and its relationship to photon yield of photosynthesis and photoinhibition. *In* 'Topics in Photosynthesis. Vol. 9'. (Eds. D. J. Kyle, C. B. Osmond and C. J. Arntzen.) pp. 123–44. (Elsevier Press: Amsterdam.)

Demmig, B., and Björkman, O. (1987). Comparison of the effect of excessive light on chlorophyll fluorescence (77k) and photon yield of O_2 in leaves of higher plants. *Planta* **171**, 171–84.

Eckstein, K., and Robinson, J. C. (1995). Physiological responses of banana (*Musa* AAA; Cavendish subgroup) in the subtropics. (IV) Comparison between tissue culture and conventional planting material during the first months of development. *Journal of Horticultural Science* **70**, 549–59.

Gall, E., and Vock, N. (1994). 'The Queensland Banana Information Kit. South Queensland Edition.' (Queensland Government Printers: Brisbane.)

Israeli, Y., Lahav, E., and Reuveni, O. (1995). *In vitro* culture of bananas. *In* 'Bananas and Plantains'. (Ed. S. R. Gowen.) pp. 147–78. (Chapman and Hall: London.)

Jones, D. R. (1994). 'The Improvement and Testing of *Musa*: a Global Partnership.' (INIBAP: Montpellier.)

Larkin, R. P., Hopkins, D. L., and Martin, F. N. (1996). Suppression of Fusarium wilt of watermelon by nonpathogenic *Fusarium oxysporum* and other microorganisms recovered from a disease-suppressive soil. *Phytopathology* **86**, 812–19.

Marois, J. J. (1990). Biological control of diseases caused by *Fusarium oxysporum*. *In* 'Fusarium Wilt of Banana'. (Ed. R. C. Ploetz.) pp. 77–81. (American Phytopathology Society: St. Paul, MN.)

Moore, N. Y., Pegg, K. G., Langdon, P. W., Smith, M. K., and Whiley, A. W. (1993). Current research on Fusarium wilt of banana in Australia. *In* 'Proceedings: International Symposium on Recent Developments in Banana Cultivation Technology'. (Eds R. V. Valmayor, S. C. Hwang, R. C. Ploetz, S. W. Lee and N. V. Roa.) pp. 270–84. (INIBAP/ASPNET: Laguna, Philippines.)

Pedrosa, A. M. (1995). Incidence of Fusarium wilt in commercial Cavendish plantations in Davao, Philippines. *Southern Philippines Journal of Research and Development* **2**, 1–22.

Pegg, K. G., Moore, N. Y., and Bentley, S. (1996). Fusarium wilt of banana in Australia: a review. *Australian Journal of Agricultural Research* **47**, 637–50.

Rasmussen T. S., and Henry, R. J. (1990). Starch determination in horticultural plant material by an enzymic colorimetric procedure. *Journal of Science Food and Agriculture* **53**, 159–70.

Robinson, J. C. (1996). 'Bananas and Plantains. Crop Production Science in Horticulture, Series 5.' (CAB International: Cambridge.)

Schaffer, B., Ramos, L., and Lara, S. P. (1987). Effect of fruit removal on net gas exchange of avocado leaves. *HortScience* **22**, 925–7.

Schaffer, B., Searle, C., Whiley, A. W., and Nissen, R. J. (1996). Effects of atmospheric CO_2 enrichment and root restriction on leaf gas exchange and growth of banana (*Musa*). *Physiologia Plantarum* **97**, 685–93.

Smillie, R. M., Hetherington, S. E., Ochoa, C., and Magalamba, P. (1983). Tolerance of wild potato species from different altitude to cold and heat. *Planta* **157**, 112–18.

Smith, M. K., and Hamill, S. D. (1993). Early detection of dwarf off-types from micropropagated Cavendish bananas. *Australian Journal of Experimental Agriculture* **33**, 639–44.

Waite, B. H., and Dunlap, V. C. (1953). Preliminary host range studies with *Fusarium oxysporum* f.sp. *cubense*. *Plant Disease Reporter* **37**, 79–80.

Whiley, A. W., Searle, C., and Schaffer, B. (1997). Leaf gas exchange responses of avocado (*Persea americana* Mill.) and mango (*Mangifera indica* L.) trees to photon and CO_2 fluxes. *Journal of the American Society for Horticultural Science* (in press).

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