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Assessment of resistance and tolerance of *in vitro*-propagated banana plants to burrowing nematode, *Radopholus similis*

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Summary. *In vitro*-propagated banana plants cv. Goldfinger were not resistant to *Radopholus similis* unless they were at least 28 weeks old at inoculation. By contrast, a Pisang jari buaya clone and SH-3142 expressed resistance when inoculated from 12 and 8 weeks of age respectively. Fewer nematodes were recovered from older plants of Goldfinger than from Cavendish 3 weeks after inoculation (i.e. before a life cycle could be completed). This suggests that the resistance mechanism of Goldfinger involves reduction in both penetration and reproduction by nematodes. Comparison of recovery of nematodes following inoculation of plants at different ages showed that resistance assays should use plants which are at least 28 weeks old. Also, plants should be at least 28 weeks old when establishing a plantation to reduce the chance of severe damage by high nematode densities. Goldfinger was more tolerant to *R. similis* than was Cavendish when supporting the same number of nematodes.

Introduction

The burrowing nematode, *Radopholus similis*, is the most important nematode pest of bananas worldwide (Stover and Simmonds 1991). It produces lesions in the root cortex thereby reducing bunch weight, increasing time between successive bunches and causing toppling of bunching pseudostems.

In commercial plantations, *R. similis* is controlled by routine use of nematicides which are expensive, toxic and subject to enhanced microbial degradation. Although some strategies, such as fallowing and nematicide application based on economic threshold, reduce the need for nematicide, resistance and/or tolerance would be a cheaper, cleaner, more reliable and a more sound strategy for controlling this pest.

Resistance to nematodes is defined as the ability of the plant to reduce reproduction of the nematode whereas tolerance is the ability of the plant to grow and yield well in the presence of nematodes. Resistance is generally more important in annual than perennial crops. Tolerance is more important in perennial crops (Roberts 1982) but is much more difficult to measure.

Musa cultivars Williams and Grand Naine (AAA genomic group, Cavendish subgroup) are the most widely cultivated genotypes in tropical Australia and are highly susceptible to *R. similis* (Gowen 1995). Lady finger cultivar (AAB) is more common in the subtropics of south-east Queensland and northern New South Wales but its resistance to *R. similis* is unknown. To date, there has been little exploitation of resistance in bananas to *R. similis*. However, strong resistance to *R. similis* has been identified in Pisang jari buaya clones (Pinochet and Rowe 1979). This source of resistance has been used in hybrid breeding in Honduras by the Fundación Hondureña de Investigación Agrícola (FHIA). One of these tetraploid hybrids, Goldfinger (AAAB, breeding line FHIA-01), was reported as resistant to *R. similis* (P. Rowe pers. comm).

As field screening of bananas is expensive and time consuming, a pot test is preferred for assessing resistance and tolerance of juvenile plants. Sarah (1993) described such a test which uses *in vitro*-propagated plants inoculated 8–12 weeks after deflasking.

This study determines the effect of plant age at inoculation on resistance, compares Sarah's (1993) pot test with field tests, and assesses the resistance and tolerance of Goldfinger to *R. similis*.

Materials and methods

In vitro-propagated (tissue-cultured) plants were deflasked into a steam-sterilised peat–sand (50:50) mix in 600 mL pots and acclimatised under plastic for 2 weeks. Plants were repotted into 1.5 and 3.5 L pots 6 and 12 weeks later, respectively. All pot tests were replicated 4 times.

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Nematodes were collected from a banana crop near Tully, North Queensland and cultured *in vitro* at 27°C in monaxenic culture on carrot pieces (Moody *et al*. 1973). Nematode inoculum was extracted from the carrot pieces by rough maceration and rinsing in water.

Field tests for resistance

Experiment 1. The cultivars Goldfinger, Cavendish cv. Williams and Lady finger were compared in the field for resistance to *R. similis*. Sixteen weeks after deflasking, plants were transplanted into *R. similis*-infested soil at Pimpama, south-east Queensland. Plants were spaced 2 m apart in single rows 2.8 m apart and managed according to commercial practice but were not irrigated. Cultivars were arranged in a randomised block design with 3 replicates. After 52 weeks, roots were collected from a cube of soil, 25 by 25 by 25 cm, next to the bunching pseudostem. Nematodes were extracted from roots in a misting chamber for 7 days.

Experiment 2. To determine whether cultivar difference in resistance is due to a difference in root penetration by *R. similis* rather than rate of reproduction, a trial was established within a *R. similis*-free crop at Redlands Research Station, south-east Queensland. Goldfinger was compared with Cavendish cv. Grande Naine. Plants were transplanted in November 1994, 16 weeks after deflasking, in single rows spaced 2.8 m apart with plants in rows being 2.8 m apart and managed according to commercial practice, including irrigation. Cultivars were arranged in a randomised block design with 5 replicates. Plants were inoculated 28 weeks after deflasking with 1000 nematodes in 100 mL water poured onto the soil at one point next to the pseudostem. Three weeks later, before a complete life cycle of 27 days (Gowen and Quénéhervé 1990) could occur, roots were collected from a 25 by 25 by 25 cm soil cube centred around the point of inoculation and nematodes extracted in a misting chamber for 7 days. A pot test was undertaken concurrently with the field test by inoculating plants with 100 nematodes in 5 mL water.

Pot tests for resistance

Experiment 3. Cavendish cv. Williams (a susceptible control), Goldfinger and the resistant diploid SH-3142 (AA, FHIA breeding line) (Pinochet and Rowe 1979) were compared for resistance using a pot assay (Sarah 1993). Sixteen weeks after deflasking, plants were inoculated with 100 nematodes in 5 mL water, kept in a glasshouse and watered as required. Ten weeks after inoculation, plants were harvested. Roots and corms were weighed and nematodes were extracted in a misting chamber for 7 days.

Experiment 4. Goldfinger was compared with Cavendish cv. Grand Naine (susceptible) as above. Sixteen weeks after deflasking, plants were inoculated with 100 nematodes in 5 mL water, kept in a glasshouse and watered as required. Thirty-six weeks after inoculation, plants were harvested. Roots and corms were weighed and nematodes were extracted in a misting chamber for 7 days.

Experiment 5. The effect of plant age at inoculation on expression of resistance was tested in pots by comparing Goldfinger, Cavendish cv. Williams (susceptible control) and Pisang jari buaya (ITC.0312; resistant control). Plants were inoculated with 100 nematodes in 5 mL water 0, 4, 8, 12, 16, 20, 24, 28 or 32 weeks after deflasking, and harvested 10 weeks later.

Table 1. Effect of plant age at inoculation and harvest time after inoculation on expression of resistance in Goldfinger compared with Cavendish cultivars

Values in the same column for each experiment followed by the same letter are not significantly different at $P = 0.05$

Plant age at inoculation (weeks)	Nematode inoculum per plant	Harvest (weeks after inoculation)	Cultivar	Root weight (g)	No. of nematodes in roots	No. of nematodes per 100 g roots	Nematodes in corm	Total no. of nematodes
			Experiment 1 (field)					
16	Unknown	52	Cavendish	28	550a	2000a	n.r.	n.r
16	Unknown	52	Goldfinger	18	18b	100b	n.r	n.r
16	Unknown	52	Lady finger	18	18b	100b	n.r	n.r
			Experiment 2 (field)					
44	1000	3	Cavendish	679	1087a	283a	n.r	n.r
44	1000	3	Goldfinger	696	52b	13a	n.r	n.r
			Experiment 2 (pot)					
44	100	3	Cavendish	153	878a	1119a	1196a	2074a
44	100	3	Goldfinger	151	214b	300b	40b	255b
			Experiment 3 (pot)					
16	100	10	Cavendish	41	88a	227a	15a	103a
16	100	10	Goldfinger	43	63a	177a	21a	84a
16	100	10	SH-3142	44	13b	29b	3b	15b
			Experiment 4 (pot)					
16	100	36	Cavendish	190	177a	208a	2b	186a
16	100	36	Goldfinger	214	108b	100b	15a	109b

n.r., not recorded.

Table 2. Experiment 6. Tolerance of banana genotypes inoculated in pots with 300 *Radopholus similis* 12 weeks after deflasking and harvested 12 weeks later

Values in the same column followed by the same letter are not significantly different at $P = 0.05$

Cultivar	Inoculation	No. of nematodes in roots	No. of nematodes in corm	Root weight (g)	Corm weight (g)	Top weight (g)
Cavendish	–	0	0	61a	14a	115
Cavendish	+	405a	100a	57b	10b	122
Goldfinger	–	0	0	42b	7b	73
Goldfinger	+	330a	63a	50b	10b	96
SH-3142	–	0	0	56b	9b	78
SH-3142	+	145b	13b	58b	9b	101

Nematodes were extracted from roots and corms in a misting chamber for 7 days.

Pot tests for tolerance

Experiment 6. Goldfinger, Cavendish cv. Williams and SH-3142, were compared for tolerance using the assay developed by Sarah (1993). The method was as described above for resistance assessment in experiment 3, except that plants were inoculated with 300 nematodes in 5 mL water 12 weeks after deflasking and harvested 12 weeks later. Roots, corms and aerial parts were weighed and nematodes extracted in a misting chamber for 7 days.

Experiment 7. Sixteen weeks after deflasking, Cavendish cv. Grande Naine and Goldfinger plants were inoculated with 0, 50, 100, 500, 1000 or 2000 nematodes in 5 mL water 12 weeks after deflasking and harvested 12 weeks later. Roots, corms and aerial parts were weighed and height was recorded. Nematodes were extracted separately from corms and roots in a misting chamber for 7 days.

Statistical analyses

In experiments 1–6, differences between cultivars were analysed using analysis of variance. In experiment 7, tolerance was assessed by comparing the slopes of regression lines relating inoculum density to growth measurements using GENSTAT. Less steep slopes ($P = 0.05$) indicate greater tolerance.

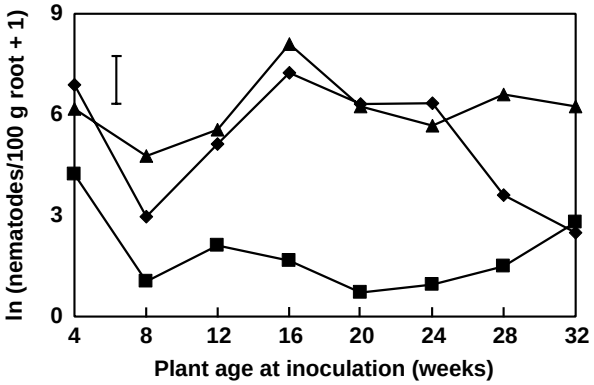

Figure 1. Experiment 5. Effect of plant age at inoculation on expression of resistance by banana cultivars Goldfinger (◆), Cavendish cv. Williams (▲) and Pisang jari buaya (■). Vertical bar shows l.s.d. ($P = 0.05$).

Results

When inoculated in pots 16 weeks after deflasking and harvested 10 weeks later (experiment 3), Goldfinger was as susceptible as Cavendish while its pollen-fertile, diploid parent, SH-3142 (Stover and Simmonds 1991), was more resistant (Table 1). However, when inoculated at the same time after deflasking but harvested 36 weeks later (experiment 4), Goldfinger was more resistant than Cavendish. This was consistent with the field test (experiment 1) where plants were transplanted into infested soil 16 weeks after deflasking and harvested 52 weeks later. In this experiment, Lady finger was as resistant as Goldfinger. When field and pot tests were compared directly (experiment 2), fewer nematodes were recovered from Goldfinger than from Cavendish in both cases.

In determining the optimum plant age for inoculation to demonstrate the field resistance of Goldfinger (experiment 5), Goldfinger was as susceptible as Cavendish when inoculated 4–24 weeks after deflasking (Fig. 1). However, when inoculated 28 or 32 weeks after deflasking, Goldfinger appeared as resistant as Pisang jari buaya.

Goldfinger was more tolerant to *R. similis* than Cavendish in both pot trials (experiments 6 and 7, Table 2, Fig. 2). There was no significant difference between the number of nematodes extracted from roots and corms of Goldfinger and Cavendish. Because of this, Cavendish and Goldfinger could be compared directly for tolerance. Tolerance of Goldfinger was demonstrated by no reduction in root and corm weight when infested by nematodes. Similarly, SH-3142 was also tolerant and had significantly fewer nematodes than Cavendish and Goldfinger and was therefore more resistant.

In experiment 7, the slopes of regression lines relating inoculum density and top weight and height were significantly different for Cavendish and Goldfinger with

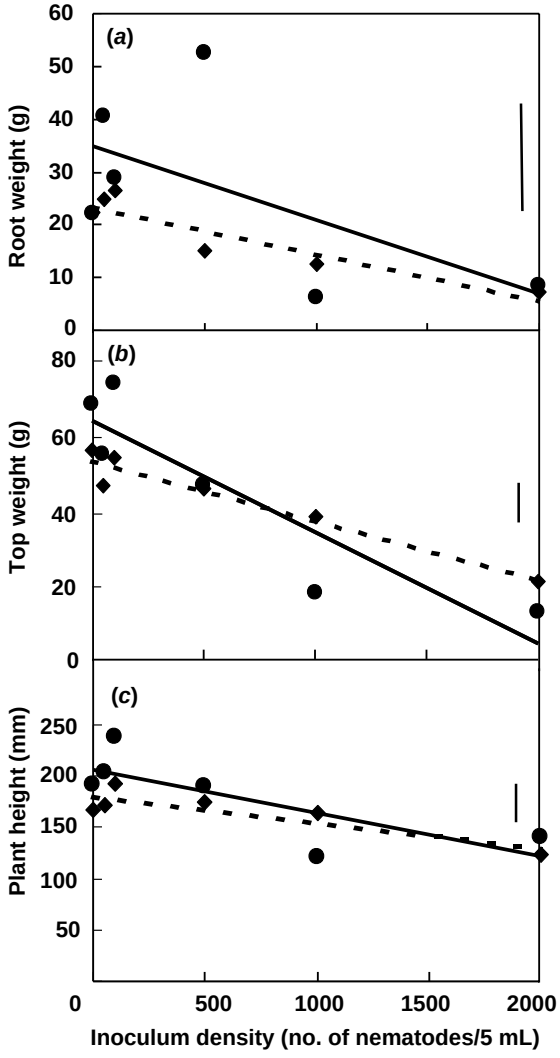

Figure 2. Experiment 7. Tolerance of banana cv. Goldfinger (◆, dashed line) to *Radopholus similis* as measured by its effect on (*a*) root weight, (*b*) top weight and (*c*) height compared with Cavendish cv. Grand Naine (●, solid line) after inoculation with various inoculum densities 16 weeks after deflasking and harvest 12 weeks later. Vertical bars show standard error. Equations of the lines are:

(*a*) Grand Naine: $y = 35 - 0.01x$ ($r^2 = 0.36$, n.s.)
Goldfinger: $y = 24 - 0.01x$ ($r^2 = 0.85$, $P = 0.05$)
(*b*) Grand Naine: $y = 63 - 0.03x$ ($r^2 = 0.82$, $P = 0.05$)
Goldfinger: $y = 53 - 0.02x$ ($r^2 = 0.94$, $P = 0.05$)
(*c*) Grand Naine: $y = 206 - 0.04x$ ($r^2 = 0.60$, $P = 0.05$)
Goldfinger: $y = 181 - 0.03x$ ($r^2 = 0.76$, $P = 0.05$)

those for Goldfinger being less steep (Fig. 2) (i.e. Goldfinger was more tolerant). The slopes of lines for root weight versus inoculum density were not significantly different.

Discussion

It has been suggested that resistance to *R. similis* of cultivars Yangambi Km 5 and Gros Michel may be related to the presence of phenolic compounds (Sarah *et al.* 1997) and lignification in the roots (Fogain and Gowen 1996). However, Pisang jari buaya had few phenolic cells but many cells with lignified walls (Fogain and Gowen 1996) suggesting a different mechanism of resistance in this cultivar. Even though the resistance of Goldfinger and SH-3142 was derived from Pisang jari buaya, resistance expression in Goldfinger was delayed while Pisang jari buaya and SH-3142 were resistant when inoculated 12 and 8 weeks after deflasking respectively. This suggests that the source of resistance in Pisang jari buaya is multigenic and that the full resistance trait of the parent was not inherited by its Goldfinger progeny and/or that Goldfinger requires a physiological maturation of the root tissues before it can express resistance.

Fewer nematodes were recovered after 3 weeks from Goldfinger roots than from Cavendish in experiment 2. Because reproduction in this experiment would have been minimal, the difference in recovery may have been due either to reduced penetration of Goldfinger roots or reduced survival or motility of those nematodes which penetrated. Therefore, resistance in Goldfinger probably involves a mechanism other than reduced rate of reproduction.

Smith *et al.* (1998) reported delayed expression of resistance to *Fusarium oxysporum* f.sp. *cubense* which causes Panama disease in banana. They showed that young, *in vitro*-propagated plants of Goldfinger and Cavendish were more susceptible than conventionally propagated plants and that this was not due to a difference in growth rate, photoassimilation rate or starch content of plants. They suggested that delayed resistance to *F. oxysporum* f.sp. *cubense* may be due to the lack of mycorrhizae, rhizosphere bacteria or endophytes in sterile, *in vitro*-cultured plants. This is not likely to be the only cause of the delay in expression of resistance by Goldfinger to *R. similis* because Pisang jari buaya and SH-3142 did not show that reaction to the nematode. Of their other possible explanations for the difference between cultivars (Smith *et al.* 1998), the one most likely to be responsible for the susceptibility of young, *in vitro*-propagated Goldfinger plants to *R. similis* is a difference in root morphology and/or physiology.

Inoculation in pots 28 or 32 weeks after deflasking was the earliest that Goldfinger expressed its resistance

if harvested 10–12 weeks later. However, when inoculated 16 weeks after deflasking and harvested 36 weeks later, Goldfinger expressed resistance. This was probably due to resistance which was expressed from about 12 to 16 weeks after inoculation onwards and masked the effect of its early susceptibility.

We have observed (J. M. Stanton and W. E. O'Donnell unpublished data) that plantations established as young, tissue-cultured plants suffer greater yield losses due to *R. similis* than those established by conventional planting material. Therefore, care must be taken when establishing a plantation using tissue-cultured plants on nematode-infested land. Ideally, they would not be transplanted until at least 28 weeks after deflasking to prevent early damage by *R. similis*.

In addition, when screening for resistance to *R. similis*, *in vitro*-propagated plants should be inoculated at least 28 weeks after deflasking. Although some forms of resistance can be detected by inoculation of younger plants (e.g. Pisang jari buaya), the resistance present in Goldfinger would have been rejected if inoculated as young plants.

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