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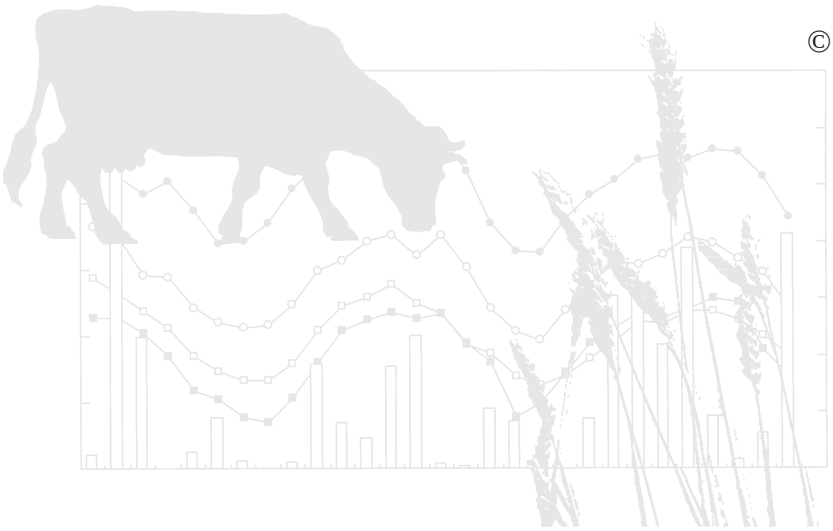

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Characterisation and early detection of an offtype from micropropagated Lady Finger bananas

M. K. Smith[AD], *S. D. Hamill*[A], *V. J. Doogan*[B] *and J. W. Daniells*[C]

[A] Queensland Horticulture Institute, Queensland Department of Primary Industries, Maroochy Research Station, PO Box 5083, Sunshine Coast Mail Centre, Nambour, Qld 4560, Australia.
[B] QHI, QDPI, Biometry, Locked Bag No. 4, Moorooka, Qld 4105, Australia.
[C] QHI, QDPI, South Johnstone Research Station, PO Box 20, South Johnstone, Qld 4859, Australia.
[D] Author for correspondence; e-mail: smithmk@dpi.qld.gov.au

Summary. An offtype has been identified from micropropagated Lady Finger bananas (*Musa* spp., AAB group, Pome subgroup) that is characterised by its slow growth and poor bunch size. Bunch weights were approximately 25% those of normal Lady Finger plants and all of the fruit produced was unmarketable. This particular offtype is the most commonly encountered from micropropagated Lady Finger plants and, in 2 instances, blocks of 3000 and 1500 plants were entirely comprised of this single offtype.

Detection of offtype plants was possible during establishment and growth of plants in the glasshouse by the presence of chlorotic streaks in the leaves. In more severe cases the streaks coalesced into chlorotic patches that developed thin, necrotic areas that eventually produced holes or splits in the leaves. Symptom expression was not ameliorated by the addition of fertiliser and even though symptoms were similar to severe Ca and B deficiency, both normal and offtype plants had similar levels of these elements in the leaves. The offtype plants were also slow growing in the glasshouse and produced significantly (P<0.05) smaller pseudostems and leaves than normal plants. Offtype plants could be readily detected after 4 weeks deflasking using the presence of chlorotic streaks in the leaves as the main selection criterion. Maximum discrimination was possible between weeks 5–7 and at the 6-leaf stage when all of the offtypes could be detected.

Introduction

Lady Finger bananas (*Musa* spp., AAB group, Pome subgroup) have a well established niche market in Australia, comprising about 5% of Australia's banana production valued at $16.85 million in 1998. A grower preference for Lady Finger bananas in many cooler, drier parts of Australia, combined with an identified segment of consumer preference within the market, has driven demand for clean planting material. This need for clean planting material is particularly important for Lady Finger growers as it is very susceptible to Fusarium wilt, the principal constraint to Lady Finger production in Australia (Pegg *et al*. 1996).

Micropropagated plants have long been recommended as the best source of disease and pest-free planting material (Smith and Drew 1990). Commercial laboratories have been producing micropropagated Lady Finger plants for the industry for over a decade but demand has been constrained because of growers' perceptions of unsatisfactorily high levels of offtypes. These perceptions are not entirely unfounded as a survey of offtypes in tissue culture plantings in north Queensland in 1991 and 1992 revealed that of 3000 and 1500 Lady Finger plants inspected, respectively, all failed to produce normal plants (Daniells and Williams 1991; Daniells and Bryde 1993). The offtype was characterised by its slow growth, pale green leaves and pseudostem and very poor bunch yield and fruit size.

Our paper compares this low vigour offtype with normal Lady Finger plants and characterises the differences. We initiated cultures of these plants and have followed their development from deflasking through to bunch harvest. We were particularly interested in developing selection criteria that could be used in the nursery so that offtype plants could be recognised and removed by the nursery operator before supply of plants to growers. In this way confidence can be established in the use of micropropagated Lady Finger planting material.

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Materials and methods

Experimental procedures and design

Both normal and offtype Lady Finger (*Musa* spp., AAB group, Pome subgroup) suckers were selected from plants that had been grown and characterised in the field at South Johnstone Research Station. These 2 accessions were part of a much larger varietal observation block. Lady Finger was established from suckers and the Lady Finger offtype from micropropagated plants on 7 November 1990. There were 4 plants each of the 2 accessions and the planting arrangement was in single rows at a density of 1333 plants/ha. The trial block received normal industry management practices (Daniells 1984).

Plants were established in culture and grown in the glasshouse using the procedures described by Hamill *et al.* (1993) and Smith and Hamill (1993). Plantlets were deflasked in a sheltered area near the glasshouse. Roots were gently washed free of nutrient agar and planted in seedling trays (28 by 35 cm) containing steam-pasteurised potting mix. The sand-peat (2 : 1) mixture contained the following nutrients (g/m^3) with 3.6 kg/m^3 of dolomite: ammonium sulfate (544), superphosphate (184), potassium sulfate (7.2), zinc sulfate (9.6) and iron sulfate (7.2). For experiment 2, sufficient dolomite was added to raise the pH to 5.5 from an initial level of 4.3 (Handreck and Black 1984).

The plants were watered and enclosed in a plastic tent to maintain high humidity. After 1 week, the plastic was gradually opened and by the end of the second week the plants were transferred to 2.5-L planter bags. They were watered as required and for the first 4 weeks were given a fortnightly application of Aquasol at the recommended rate. Osmocote was added to the bags at weeks 4 and 11. In experiment 2, calcium nitrate was applied at 10 g/L with the Osmocote and borax (40 mg) added to each bag at week 4.

The experimental design for glasshouse trials was a randomised block with 5 blocks corresponding to rows and 10 plants in each row. The 10 plants consisted of 2 treatments replicated 5 times and randomly allocated to the 10 positions within each row. The treatments were normal and offtype plants. To minimise edge effects, guard plants were placed in a row around the entire block.

Experiment 1 took place over a 13-week period in the glasshouse before a representative sample of 5 normal and 5 offtype plants were planted in the field at Maroochy Research Station and grown to bunch harvest. This was necessary to verify trueness to type and to eliminate the possibility that further genetic change had occurred during micropropagation. Experiment 2 took place over an 18-week period in the glasshouse. Plants were grown in a glasshouse with fan-forced heaters and evaporative coolers, with daily temperature range 20–32°C.

Measurements

The following measurements were taken from the plants established at South Johnstone Research Station: date of bunch emergence (BE) and harvest (BH), bunch weight, pseudostem height, finger number per bunch, finger diameter for hand 3, finger length for hand 2, total number of leaves and the length: breadth ratio of leaf 5. The measurements were made for both the plant crop and first ratoon.

For the glasshouse studies, measurements commenced when the plants were transferred to the 2.5-L planter bags. The first leaf that emerged after deflasking was labelled with a permanent ink marker and was designated as leaf 1. This served as a reference point for further measurements. Leaf data were obtained from fully expanded leaves only. Measurements included: height from soil level to the base of the youngest petiole, pseudostem diameter at the base of the plant; petiole length from the point where the petiole adjoins the pseudostem to the base of the lamina; lamina length and lamina width one-third of the way along the length of the lamina.

Symptom severity on leaves was rated on a scale of 1–5 : 1, large chlorotic areas with holes and splits in the leaves; 2, chlorotic patches with some thinning and necrosis; 3, chlorotic streaks; 4, chlorotic spots few in number; 5, no symptoms.

Height, pseudostem diameter, leaf number and leaf symptoms were recorded weekly. Leaf measurements and nutrient analyses were made at the end of the glasshouse evaluation.

Boron and calcium analysis

The youngest fully expanded leaf was removed from each plant and oven dried at 70°C for 48 h. After drying, the leaves were ground using a hammermill grinder fitted with a 1 mm sieve. Ground leaf subsamples weighing 0.5 g were placed into crucibles for dry ashing at 500°C for 6 h. The ashed material was then dissolved in 1 mol/L HCl and analysed for boron and calcium using an Inductively Coupled Plasma Atomic Emission Spectrophotometer (ICPAES).

The boron and calcium status of leaf samples was evaluated using the levels reported by Reuter and Robinson (1986); where <10 mg B/g was regarded as deficient, 10–20 mg B/g marginal and 20–80 mg B/g adequate; while <0.5% Ca was regarded as deficient, 0.5–0.7% Ca marginal and 0.8–1.2% Ca as adequate.

Virus indexing

Plants were indexed for banana bunchy top virus (BBTV) by triple antibody sandwich ELISA (Thomas and Dietzgen 1991) and for cucumber mosaic virus (CMV) by biotin/streptavidin indirect ELISA (Thomas 1991). Plants were inspected by a virologist and were also examined by electron microscopy of partially purified miniprep extracts designed to detect rod-shaped and isometric virus particles (adapted from Diekmann and Putter 1996).

Statistical analysis

The statistical software package GENSTAT was used for all analyses. Repeated measures analysis of variance was used to compare the 2 treatments over time for pseudostem height and diameter. All other data were analysed by analysis of variance, with the exception of the field trial where means and standard errors were recorded. Where appropriate, pair-wise testing between treatments was done using l.s.d. at $P = 0.05$ and 0.01.

Results

A Lady Finger offtype was identified from micropropagated planting material and compared with normal Lady Finger in the field at South Johnstone Research Station (Table 1). The offtypes were initially of a paler green appearance, although no obvious leaf symptoms were noted. The offtypes grew and developed at a slower rate, both a slower leaf emergence rate (data not presented) and a greater number of leaves emerged prior to bunch emergence, than normal Lady Finger plants. However, at bunch emergence the offtype and normal plants were of a similar height with leaves of similar size and appearance. Bunch emergence and

Table 1. Plant and bunch characteristics of Lady Finger and Lady Finger offtype at South Johnstone Research Station

Lady Finger was established from suckers and the Lady Finger offtype from micropropagated plants on 7 November 1990
P, planting; BE, bunch emergence; BH, bunch harvest; H3, hand 3; H2, hand 2
Values are the means of 4 plants; standard errors are in parentheses

Character	Plant crop		Ratoon 1	
	Normal	Offtype	Normal	Offtype
Bunch weight (kg)	17.0 (6.2)	3.8 (0.3)	14.5 (2.4)	4.8 (2.1)
Days (P to BE)	262 (59)	427 (124)	510 (108)	940 (110)
Days (P to BH)	446 (37)	679 (96)	689 (108)	1115 (103)
Pseudostem height (cm)	318 (18)	311 (8)	384 (6)	373 (35)
Finger number/bunch	95 (17)	88 (20)	101 (4)	87 (11)
Finger diameter H3 (cm)	4.25 (0.47)	2.78 (0.28)	3.90 (0.23)	2.89 (0.07)
Average finger weight (g)	156 (38)	40 (7)	30 (27)	47 (16)
Finger length H2 (cm)	18.3 (2.0)	9.8 (0.3)	17.7 (1.8)	10.9 (1.2)
Total leaf number	38.5 (3.4)	46.3 (4.4)	n.a.	n.a.
Leaf 5 (length : breadth)	3.4 (0.1)	3.3 (0.4)	3.8 (0.3)	3.5 (0.4)

Figure 1. Comparison of (*a*) a normal Lady Finger bunch with (*b*) a Lady Finger offtype bunch.

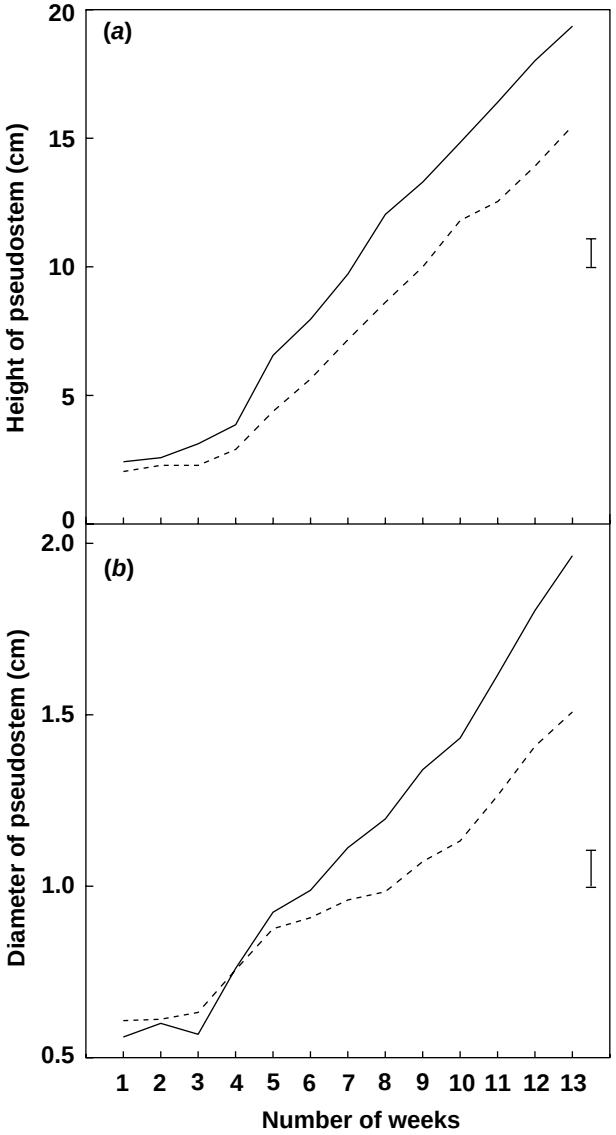

Figure 2. Comparison of (*a*) pseudostem height and (*b*) pseudostem diameter of micropropagated Lady Finger (solid line) and Lady Finger offtype (dashed line) grown under glasshouse conditions. Values are the means of 25 replicates. Vertical bars indicate l.s.d. values at $P = 0.05$.

harvest were appreciably delayed for the offtype plants and bunches were also very small with short, thin fingers (Fig. 1). Bunch weights of the offtype were only about 25% those of the normal Lady Finger plants. All of the fruit from the offtype plants were unmarketable.

Suckers from both normal and offtype plants were selected at South Johnstone Research Station and established as micropropagated plants at Maroochy Research Station. No differences were seen between offtype and normal plants when grown as *in vitro* plantlets. Even at deflasking no differences were detected. However, significant differences in height were apparent by week 5 and increased thereafter. Plant height and pseudostem diameter (Fig. 2), as well as leaf length, width and petiole length (Table 2) were significantly ($P<0.05$) lower for offtype plants. Plant height and pseudostem diameters continued to diverge and show significant differences up to planting, whereas leaf length, width and petiole length show maximum differences with leaves 6 and 7 (corresponding to weeks 7–12) and decrease thereafter.

Symptoms of interveinal chlorosis arose early with almost all of the offtype plants exhibiting symptoms by week 4 (92%) compared to normal plants (8%) (Fig. 3). From weeks 5–7 all of the offtype plants showed interveinal chlorosis, while between 4–12% of the normal plants had some chlorotic streaks detectable in the leaves. Symptom severity increased with the offtype plants and large chlorotic patches developed that coalesced into thin necrotic areas which collapsed to cause splits and holes in the leaves (Fig. 4). By week 9, the normal plants also began to develop chlorotic streaks in the leaves but the addition of fertiliser was able to reverse this development. At no time did the normal

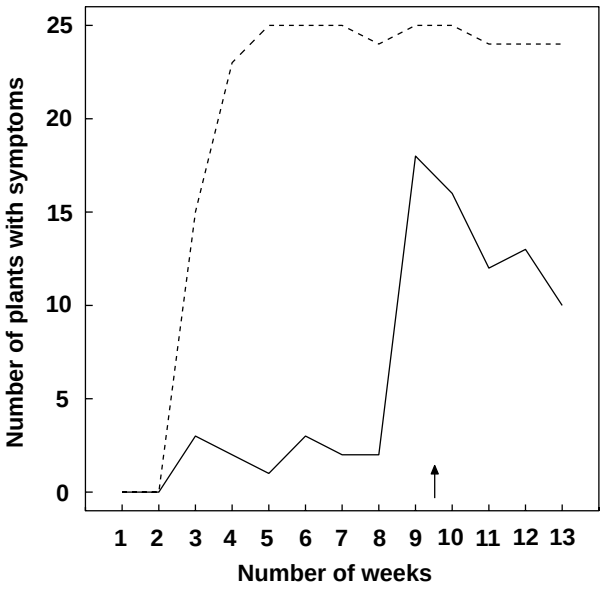

Figure 3. Comparison of leaf symptom expression of micropropagated Lady Finger (solid line) and Lady Finger offtype (dashed line) grown under glasshouse conditions. Symptoms are from the first fully expanded leaf and range from a few chlorotic spots to necrotic lesions and leaf deformation. There were a total of 25 plants for each treatment. Arrow indicates a fertiliser (Osmocote) application.

Table 2. Leaf characteristics of micropropagated Lady Finger and Lady Finger offtype grown under glasshouse conditions
Leaves were measured from 17-week-old plants from experiment 1

Values are the means of 18–25 replicates; l.s.d. values at $P = 0.05$; n.s., not significant
N, Lady Finger; O, Lady Finger offtype

Leaf number	Leaf length (cm)			Leaf width (cm)			Petiole length (cm)		
	N	O	l.s.d.	N	O	l.s.d.	N	O	l.s.d.
Leaf 6	21.4	18.6	1.13	8.3	7.1	0.62	3.9	2.5	0.34
Leaf 7	24.9	21.7	1.29	10.0	8.9	0.56	3.9	2.9	0.38
Leaf 8	28.7	26.2	1.53	11.5	10.9	0.61	4.7	3.5	0.38
Leaf 9	33.3	31.2	1.66	13.5	12.9	0.59	5.3	4.3	0.49
Leaf 10	37.0	36.5	n.s.	14.6	14.2	n.s.	6.2	5.3	0.48

Lady Finger plants exhibit the more extreme symptoms encountered in the offtype plants. These leaf symptoms were the most obvious and characteristic feature of the Lady Finger offtypes.

These symptoms were thought to be similar to Ca or B deficiency in banana, and leaf nutrient analyses were made from 17-week-old plants in pots from experiment 1. However, no significant differences were seen between normal and offtype plants, with Ca levels of 0.30% and 0.34% and B measuring 15.2 mg/g and 14.2 mg/g, respectively.

The experiment was repeated with additional fertiliser treatments and significant ($P<0.05$) differences were once again seen between normal and offtype plants with the offtypes being smaller, thinner and with a greater severity of symptom development than the normal plants (Table 3). No significant differences were seen with B and Ca levels in the leaves between offtype and normal plants, even with the addition of these elements to the plants. The expression of leaf symptoms was again the most useful feature for discriminating between Lady Finger offtypes and normal plants during glasshouse evaluation.

Table 3. Plant characteristics of micropropagated Lady Finger and Lady Finger offtype grown under glasshouse conditions

Leaves were measured on 18-week-old plants from experiment 2
Severity rating on the first fully expanded leaf on a scale of 1–5: 1, leaf with chlorotic patches and necrotic lesions with leaf deformation; 5, no symptoms

Character	Normal	Offtype	l.s.d. ($P = 0.05$)
Pseudostem height (cm)	45	41	3.3
Pseudostem diameter (cm)	3.1	2.7	0.24
Symptom severity	4.8	2.8	0.41
Leaf boron (mg/g)	9.32	8.97	n.s.
Leaf calcium (%)	0.31	0.30	n.s.

The leaves did not exhibit typical virus symptoms and no virus particles were detected after miniprep purification. The plants tested negative to CMV and BBTV by ELISA serology.

The normal and offtype plants grown in the field at Maroochy Research Station displayed similar characteristics to those mother plants grown at South Johnstone Research Station, confirming that no further genetic variation occurred during culture establishment and multiplication. The Lady Finger offtypes at MRS were slow growing with poor bunch characteristics.

Discussion

Lady Finger offtypes characterised by slow growth and poor yield in the field (Table 1) could be readily distinguished from normal micropropagated Lady Finger plants in the glasshouse. They also had a slower growth rate and were characterised as small, thin plants with leaves and petioles that were smaller than normal plants (Fig. 2, Table 2). However, the most characteristic feature, and the one a nursery operator could use for detecting these offtypes, was the presence of chlorotic streaks in the leaves. As symptom severity increased, these streaks coalesced to form chlorotic patches and eventually thin, necrotic areas developed to form holes and tears in the leaves (Fig. 4). The progression of these symptoms could not be alleviated by the addition of fertiliser, and Ca and B analyses of the leaves revealed no significant differences between normal and offtype plants (Table 3). The best time to select these offtypes was between 4–8 weeks after deflasking. However, maximum discrimination was possible between weeks 5 and 7 and at the 6-leaf stage. All of the offtypes could be detected at this stage while from 4 to 12% of the normal plants could also be recognised as offtypes on the basis of leaf symptoms. These chlorotic spots or flecks are present on the leaves of normal plants (even in the field)

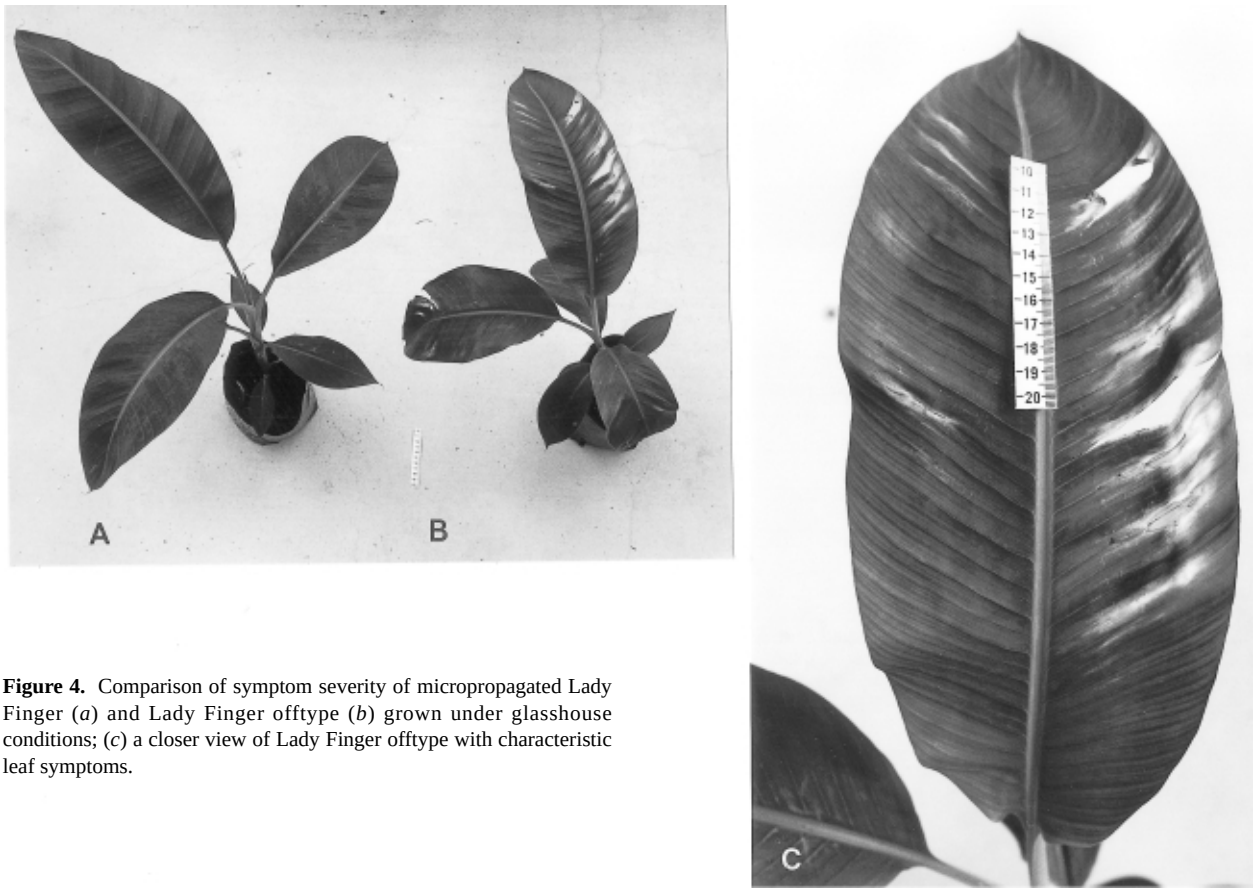

Figure 4. Comparison of symptom severity of micropropagated Lady Finger (*a*) and Lady Finger offtype (*b*) grown under glasshouse conditions; (*c*) a closer view of Lady Finger offtype with characteristic leaf symptoms.

and can add to uncertainty over identification of offtype plants; however, they need not be confused with the long chlorotic streaks and more severe symptoms associated with the offtypes.

It was interesting that leaf symptoms diminished once plants were planted in the field, and although the offtypes were generally paler green and extremely slow growing, it was not until bunch emergence that growers would see the serious consequences of this offtype. Offtypes produced very small bunches of unmarketable fruit and so it is important that these offtypes are removed before micropropagated plants are sold to growers.

There are a number of strategies that tissue culture laboratories can use to minimise the occurrence of offtypes (Israeli *et al*. 1995; Damasco *et al*. 1998). Nurseries also have strategies for minimising the impact of offtypes on the farm. For instance, nurseries will often supply growers with a cross-section of plants from a number of micropropagated lines to prevent an individual grower receiving all the plants from a single line that may have associated offtype problems. Nurseries continue to be the last line of defence when it comes to protecting growers from offtypes. They require reliable guides for the identification of some of the major offtypes they are likely to encounter.

This paper has characterised a major offtype frequently seen in micropropagated Lady Finger plants and has identified traits that can be used to detect it in the nursery. By removing offtypes before their sale to growers, nurseries can ensure that only the best quality plants are released to industry. This will help establish confidence in the use of micropropagated Lady Finger planting material.

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