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# **infoMusa**

*Banana  
commodity  
chain in  
Madagascar*

*Eradicating  
black Sigatoka  
in Australia*

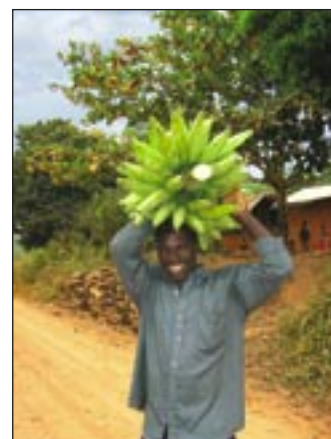
*Genetic  
diversity of  
Mycosphaerella  
in Colombia*

*Effect of  
planting hole  
depth*

*Safeguarding  
banana  
diversity*

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## Contents

<b>Economics of the Madagascan commodity chain</b> <i>L. Temple, A.H.J. Rakotomalala and T. Lescot</i>	2
<b>Eradication of black leaf streak disease from banana-growing areas in Australia</b> <i>R. Peterson, K. Grice and R. Goebel</i>	7
<b>Field evaluation of strobilurins, triazoles and acibenzolar to control Sigatoka disease in Australia</b> <i>L.L. Vawdrey and K. Grice</i>	11
<b>Fulvic acid applications for the management of diseases caused by <i>Mycosphaerella</i> spp.</b> <i>J. Hernando Escobar Vélez and J. Castaño Zapata</i>	15
<b>Genetic diversity of Colombian isolates of <i>Mycosphaerella fijensis</i> Morelet based on microsatellite markers</b> <i>I. Perea, E. Rodríguez Arango, E. Márquez and R. Arango</i>	18
<b>Estimation of the size of the root system using core samples</b> <i>H.H. Mukasa, D. Ocan, P.R. Rubaihayo and G. Blomme</i>	21
<b>The effect of planting hole depth on <i>Musa</i> spp. shoot and root development</b> <i>G. Sebuwufu, P.R. Rubaihayo and G. Blomme</i>	24
<b>Evaluation of a method to simultaneously screen <i>Musa</i> germplasm against multiple nematode species</b> <i>D.L. Coyne and A. Tenkouano</i>	27
<b>The effect of oxidative stress on 'Berangan' and 'Mas' cultivars</b> <i>C. Tsun-Thai, N.A.M. Fadzillah, M. Kusnan and M. Mahmood</i>	32
<b>Focus on Asia region</b>	36
<b>Focus on <i>Musa</i> conservation</b>	37
<b>Theses</b>	40
<b><i>Musa</i>News</b>	44

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One of the reasons is that the reach of INFOMUSA is much greater as a paper publication than an electronic one (nearly 60% of INFOMUSA subscribers have not registered an e-mail address with us). So for the time being we don't intend to stop producing paper copies of INFOMUSA, but financial constraints – namely the reduced amount of discretionary funding available for publishing INFOMUSA – might force us to reduce the number of issues printed in a year.

Producing an electronic version only of INFOMUSA would certainly reduce costs, even when taking into account the cost of producing HTML pages (at present INFOMUSA is only available electronically as a PDF of its two-column layout, which doesn't lend itself to reading on the screen and shifts the burden of printing to our readers). We are considering developing this service as part of our strategy to improve the access to our information products through our website.

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*The editors*

## Economics of the banana commodity chain in Madagascar

*L. Temple, A.H.J. Rakotomalala and T. Lescot*

**T**he demographic growth of the Malagasy population is creating a favourable environment for an increase in banana production which represents nearly 20% of the fruit supply of the population. The dessert bananas, locally called Batavia or Bitavia, mainly belong to the Cavendish varietal subgroup and represent more than 75% of the national banana production. Bananas are also eaten cooked, mainly within the production areas. This consumption has recently increased, replacing rice during the season of shortage on the east coast of the island.

The production of bananas in Madagascar grew rapidly until 1975 when it reached its highest level (about 400 000 tonnes) boosted by technical assistance societies to encourage exports<sup>1</sup> (to France between 1961 and 1971). Production then fell, stabilizing in 1979 (Figure 1). Since 1964, its growth has been steady but slow, levelling out at 290 000 tonnes after 2002 (FAO-STAT). This increase is not enough in view of the population growth. The annual availability of bananas per person fell from 60 kg in 1974 to 18 in 2002 (Figure 2). With the objective of returning to a level of availability of 26 kg (the average availability calculated over 20 years) and by taking account of the current population increase (2.8% per year), it would be necessary to almost double the production of bananas in Madagascar in less than five years, i.e. to produce more than 230 000 extra tonnes. The object of this article is to consider the conditions of production and marketing which determine the capacity of this industry to adapt to the quantitative challenges presented by the food security of the country.

### Materials and methods

As to methodology, this article makes use of the data collected from a panel of about twenty experts consulted as part of a study made to relaunch the Madagascan banana

industry (Scanagri 2003) and the results of a survey of a sample of growers and merchants in the industry done as part of a university thesis (Rakotomala 2003).

The methodological principles used are those of an a commodity chain approach. They lead successively to locate the main production centres, to study how prices are arrived at and to describe the operation of the marketing system so as to analyse its efficiency.

## Results and discussion

### Production conditions

Apart from several commercial plantations, bananas are grown mostly on small family farms with an average area of 0.3 ha, or 500 – 700 plants per grower (Bé 2003, Randrianavoson 2002). These farms, because of their structure and financial resources, are barely able to pay for their inputs such as fertilizers and pesticides.

With an average of 6 tonnes of bananas per hectare, the yields are very low compared with the potential obtainable on a research station, which can reach 100 tonnes/ha in certain very intensive production systems. The available data (FAOSTAT) show a fall in yields between 1983 and 2004 (Figure 1). These averaged data do not reveal yield trends in particular geographical locations or with particular production systems. The available work underlines the growing importance of pests and diseases, mainly black leaf streak disease, weevil and other diseases still being identified, but also a lack of good husbandry, notably plant nutrition (especially nitrogen and potassium).

Between 1976 and 1986 the province of Toamasina in the east contributed on average 51% of the national supply. In 1999, with 61 108 tonnes, it provided no more than 36% of the country's production (Rakotomalala 2003). This decline in production is mainly due to a rapid increase in pest and disease problems (Scanagri 2003). Meanwhile, banana production has developed in the south-east of the island.

<sup>1</sup> Exports reached at their maximum 33 000 tonnes. They collapsed in 1970.

In 1999, the province of Fianarantsoa provided 42% of the country's supply, or 71 285 tonnes.

The town of Antananarivo, the country's capital, with about 1.4 million inhabitants, is the main market for Madagascan consumers. From an average available supply of 18 kg per inhabitant and taking account of a lower banana consumption in urban as opposed to rural areas, one can estimate the size of this market at between 17 000 and 25 000 tonnes per year. Of the

supply to Antananarivo, 60% comes from the south-east, 30% from the east and 10% from the west (Rakotomalala 2003).

### Marketing conditions

Price recording of agricultural products is carried out mainly by the National Institute of Statistics (INSTAT) for retail prices, and (recently) by the Ministry of Agriculture within the production zones. In both cases the prices are expressed per kilogramme of ripe bananas, i.e. as sold to the consumer.

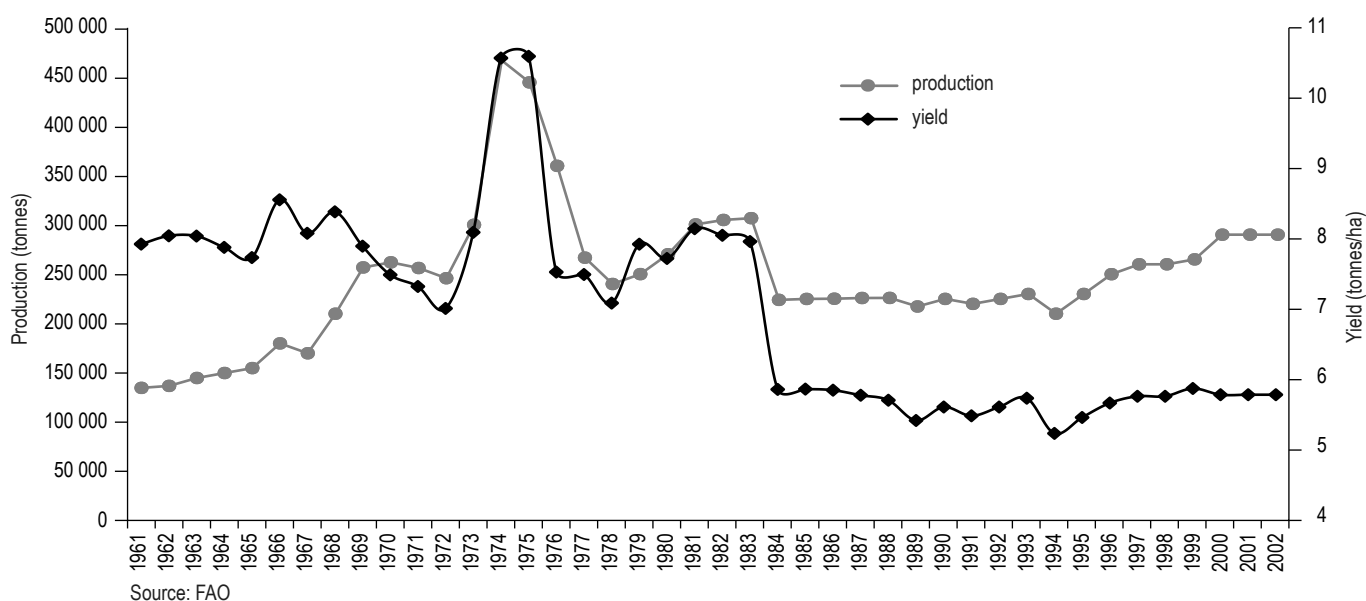


Figure 1. Evolution of banana production and banana yield in Madagascar since 1961.

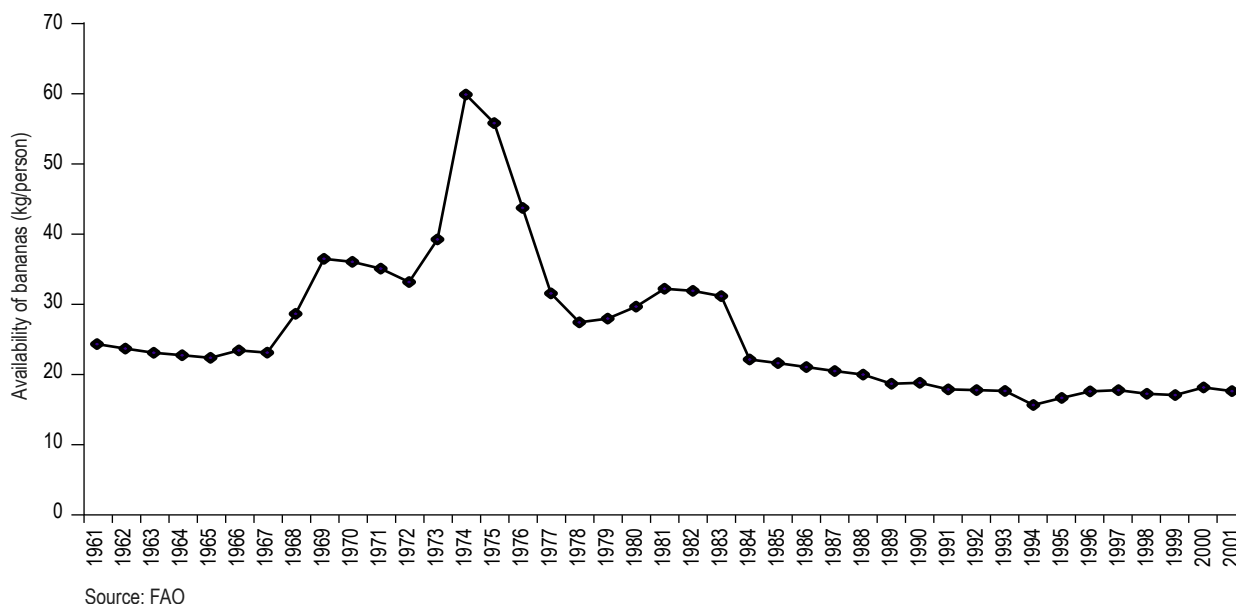
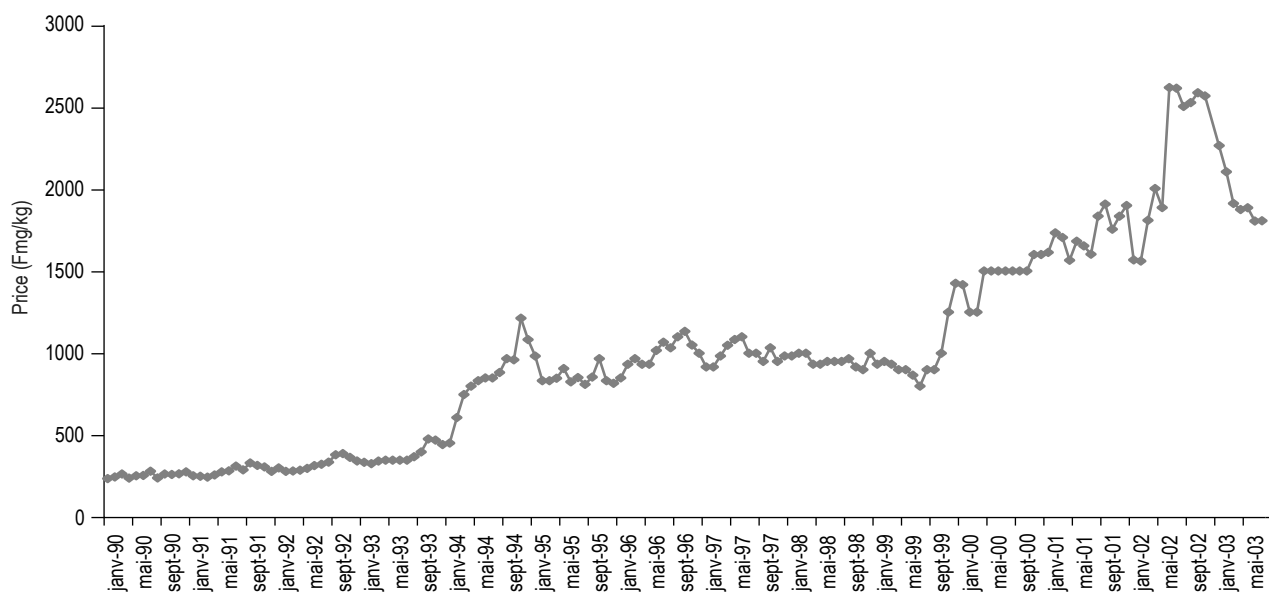


Figure 2. Evolution of banana availability per person in Madagascar.



Source: INSTAT

Figure 3. Evolution of the price of ripe bananas at Antananarivo.

The prices (INSTAT 2000) come from an average calculated for three or four markets in Antananarivo (Figure 3). The trend has been one of increase until 1994, followed by stabilisation at around 1000 Fmg\*/kg until 1999. From then on, prices increase very rapidly and become more and more unstable : year-to-year variation is of increasing amplitude. Seasonality (within-year variation) is relatively small. For a better understanding of the potential determinants of this instability, it seems necessary to characterize the structure of the marketing system so as to analyse its efficiency (Figure 4).

There is no wholesale market as such. Each market acts as both a wholesale and retail market, but the dominant function varies with the time of day or even the day or week. Several main kinds of operator can be distinguished : wholesale transporters, wholesale ripeners, clients and retailers.

- The **wholesale transporters** have their own lorry and obtain their supplies mainly from wholesale collectors at the places where rafts are unloaded. They do not possess storage depots within the town. The bananas are sold immediately on their arrival to "clients". A 10-tonne lorry load will be sold to 8–12 clients, or an average of about a tonne per client. The physical post-

harvest losses are related to the duration of storage in the lorry. Between loading and unloading (2 days) one can lose 700 kilos of fruit, but beyond 3 days the losses can reach 2–3 tonnes. These losses are mainly linked to the evapotranspiration of the bunches and their crushing in the lorries, which sometimes varies with the harvest stage.

- The **wholesale ripeners** or "clients" fix the buying price of the bananas with the wholesale transporters in the urban areas. The wholesale ripeners are in contact mainly with the wholesale transporters in a given geographical zone. They obtain their supplies with difficulty from different zones. According to our observations, the wholesale ripeners are partially specialized in a given variety. Each possesses his own ripening shed made up of a rectangular earth oven (8 m x 5 m x 3 m), with a maximum capacity of three tonnes, heated by a mixture of dry wood and sawdust. The job of the ripener involves mastering the techniques of building the ovens and of obtaining undeveloped spaces near markets, bearing in mind the high price of land. The know-how and capital has sometimes been passed on for more than 300 years.

The wholesale ripener regulates the supply to the market, as he can store the bananas "green". Nearly all the production

\* 1 € = about 11 000 Malgasy Francs (Fmg) and 2600 Ariary (new Malgasy money)

consumed in Antananarivo, or about 25 000 tonnes, passes through these traditional ripening sheds. With an average annual capacity of about 300 tonnes per ripening unit, one can estimate their number at between 80 and 100. These ripening units are built close to a banana storage warehouse. Each ripener ripens his own merchandise. Practically no wholesaler will contract out another wholesaler to do the work.

Green bananas will keep for 15–30 days, but once ripe, the keeping period without cooling is only 2–3 days. The ripeners' main function therefore is to regulate the supply of ripe bananas to the retail markets from the green stocks.

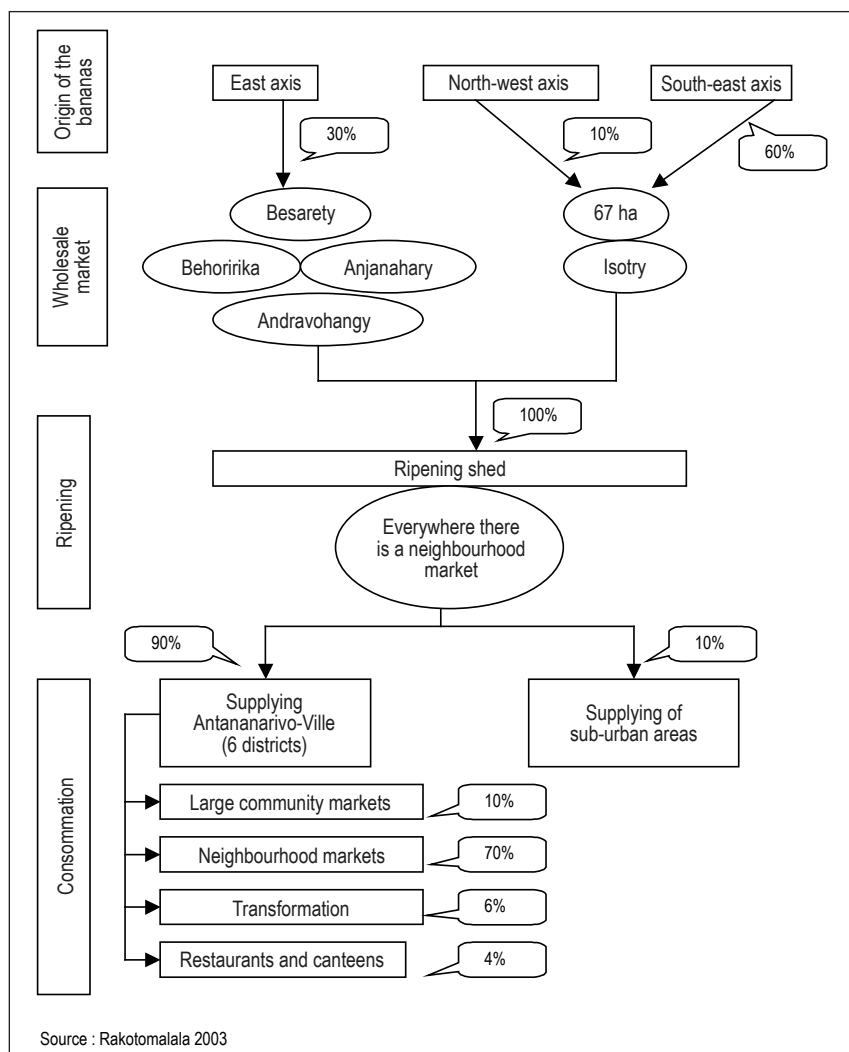
The loss of tonnage suffered by the wholesale ripener from the quantity purchased (cutting the bunch into hands, loss of weight during passage through the ripening shed, which dries out the bananas) is about 25–30% for the bananas coming from Brickaville and 20% for the bananas from Mananjary (a variety with a thicker skin which reduces evaporation in the ripening shed, and with a lower initial water content).

The wholesale ripener then sells his ripe bananas to retailers. They are sold by the kilo. All the varieties and qualities are more or less mixed. The wholesaler packs his bananas in *garrabes* (baskets with an average capacity of 20 kg), but the unit of transaction and negotiation remains the price per kilo. One tonne supplies five or six retailers situated in different markets in the town. The retailers sell to the consumers at the markets or at roadside stalls.

The chain of operators allows us to identify the selling circuits which supply the town (Figure 4). Without going into the complexity of this system, which is largely due to the versatility of the operators at different periods, it is necessary, judging from the data collected, to question its efficiency.

### The efficiency of the marketing system

The breakdown of the costs (Table 1) enables the profit margins per kilo of each type of operator to be calculated, and those who make the biggest profit margins to be identified.



The margins of the wholesale collectors and wholesale transporters (about 7 Fmg/kg) do not appear to be very large compared with the monthly volumes involved, on the one hand, and the risks incurred and the capital investment needed on the other. The margins of the wholesale ripeners (315 Fmg/kg), however, call strongly for justification. The exercise could not be done with the final operator in the chain, the market retailer.

The diagnosis of the marketing system results in two convergent observations. The surveys of operators have revealed that the controlling price in the chain is determined by the prices which are agreed between the wholesale ripener and the wholesale transporters. The analyses of the margins draw attention to the large profits of the wholesale ripeners for large volume transactions.

The profit margins are squeezed at the level of the ripeners. In theory, this

Figure 4. Schematic movements of banana supplies to Antananarivo.

**Table 1. Price structure of bananas to the Antananarivo consumer (the cost structure between the retailers and consumers was not studied).**

<b>Brickville supply zone</b>	<b>Fmg*/kg</b>	<b>Source</b>
Purchase price to the producer/collector	425	Direct survey <sup>†</sup>
Transport by boat to storage depot	7	Direct survey
Loading onto lorry after weighing	4	Direct survey
Taxes	20	Direct survey
Estimation of transport (driver, depreciation on lorry etc.)	110	Estimate
3 days' work for 10 tonnes at 20 000 Fmg/day	6	Estimate
7% weight loss during transport (700 kilos)	30	Authors' calculation
<b>Cost/kilo to the wholesale transporter</b>	<b>601</b>	<b>Authors' calculation</b>
Sale price to the wholesale ripener	608	Direct survey
Margin/kilo	7	Authors' calculation
Transport to the ripening shed	25	Rakotomalala 2003
Cutting into hands	10	Rakotomalala 2003
Loading and unloading	20	Rakotomalala 2003
Depreciation on investments in ovens, balances etc.	7	Rakotomalala 2003
Fuel	6	Rakotomalala 2003
27% weight loss	164	Authors' calculation
Specialists' salaries (3 days @ 20 000 Fmg/day)	60	Estimate
Specialists' salaries (3 days @ 20 000 Fmg/day)	60	Estimate
<b>Cost/kilo to the wholesale ripener</b>	<b>960</b>	<b>Authors' calculation</b>
Sale price from the wholesalers to the retailers	1275	Direct survey
Net margin of wholesale ripener	315	Authors' calculation
<b>Selling price to consumers</b>	<b>2250</b>	<b>Direct survey</b>

\*1€ = approx. 11 000 Madagascan Francs (Fmg)

<sup>†</sup>Survey: L. Temple, T. Lescot – September 2003

squeezing could simultaneously increase the price paid to the producers (which would favour increased production) and reduce the price to the consumers (which would favour increased consumption). This squeezing could come about by innovations such as circulating information about the prices; the emergence of marketing organisations upstream and downstream from the ripeners; and the improvement of the present storage and ripening techniques.

## Conclusion

The production of bananas in Madagascar responds to an internal rapidly growing demand both in the towns for the consumption of fruit and, recently, in rural areas for consumption as a vegetable during the period of shortage. It is localised mainly in the south-east and east of the island, mainly on small family farms with very low yields due largely to the increasing phytosanitary and nutritional problems. Although the analysis of marketing reveals some positive features, unsatisfactory aspects are found mainly at the interface between wholesalers and wholesale ripeners. This diagnosis reveals major possibilities for improving production conditions and the efficiency

of the marketing system. Realizing these possibilities involves the teaching of techniques suited to the socio-economic conditions of production (integrated pest control, propagation techniques etc.) and optimization of the present system of market price information. The setting and publicising of a price at the green stage (before the ripening process) in the production zones and on delivery to the Antananarivo wholesale markets would probably increase the transparency of the market and create economic conditions favouring an increase in production.

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# Eradication of black leaf streak disease from banana-growing areas in Australia

R. Peterson, K. Grice and R. Goebel

In Australia, bananas are mainly grown in north Queensland along the wet tropical coast, centred on the towns of Tully and Innisfail (Anon. 2002). The area is relatively wet (3000 mm to 5000 mm of rain a year) and during the wet season (November to May), conditions are very conducive to leaf spot diseases, especially Sigatoka disease, which is caused by *Mycosphaerella musicola* Leach. The remainder of the year is either cool or generally dry.

Black leaf streak disease (BLSD), which is caused by *Mycosphaerella fijiensis* Morelet, is the major banana disease worldwide. It is endemic in Papua New Guinea and the Torres Strait islands. It was first detected on the Australian mainland in 1981 in the dry Cape York area, adjacent to the Torres Strait (Jones and Alcorn 1982). Between 1981 and 2000, it was recorded at six other locations in the Cape York area. These infestations most likely resulted from one or two introductions of infected plant material from the Torres Strait area. BLSD was eradicated from each site by destroying all leaf material and replanting the sites with resistant cultivars.

In April 2001, BLSD was detected in the Tully area of north Queensland and an eradication programme was initiated after the extent of the infestation was established.

## Materials and methods

### Delimiting surveys

The extent of the infestation was delimited through surveys of all banana areas in north Queensland, including residential areas. Diseased leaf samples were forwarded to the Department of Primary Industries and Fisheries laboratory at Mareeba. Identification of all suspicious samples was confirmed by the polymerase chain reaction test (PCR) (Henderson *et al.* 2002).

Following the delimitation of the infestation, the Tully Banana Production Area (TBPA) was legislated as a quarantine

area. The TBPA covered 4400 km<sup>2</sup> and included 4500 hectares of banana plants surrounding the townships of Tully and Mission Beach. All the bananas in the TBPA had to have a 'zero detectable disease' level. There were penalties for non-compliance. All the diseased leaf tissues on all the banana plants had to be removed and placed on the ground to decompose.

### Eradication programme

The aim of the eradication programme (Peterson 2002) was to remove all BLSD inoculum from all the plants in the area and apply an intense spraying programme to prevent the establishment of new infections. As the incidence of BLSD was relatively low in comparison to the one of Sigatoka disease, reducing the inoculum of the latter to extremely low levels would ensure that all the BLSD inoculum had been eradicated.

All the land parcels listed on cadastral maps of the TBPA were visited to destroy the inoculum on all the non-commercial banana plants. Owners who wanted to keep their banana plants were required to maintain them at a 'zero detectable disease' level by deleafing, with or without spraying. Non-compliant landowners risked having their plants destroyed and having to pay for it. All the unwanted banana plants, including the ones that had no owner (feral plants), were sampled and destroyed.

Fungicides were applied weekly from August 2001 to February 2002. They included the protectant fungicide mancozeb, and the systemic fungicides propiconazole, difenoconazole, tebuconazole and trifloxystrobin. Mineral oil at 4 to 5 L/ha was added to all sprays. From February 2002 to May 2003, a less intensive spray programme was implemented consisting of fortnightly applications of mancozeb plus oil, except when a propiconazole spray was applied in April and in May 2002. Organic growers applied copper based fungicides plus vegetable oil

in rotation with mineral oil alone at 5 L/ha from December 2001 to March 2002.

Trained monitors visited all the commercial plantations every four to six weeks from September 2001 to May 2002 and inspected all the plants for the presence of the disease. After the first two inspection rounds, all the growers with disease on their properties were regarded as non-compliant and penalties (no movement of fruit) were imposed until the 'zero detectable disease' level was achieved. All the detected diseased tissues were sampled and the causal agent identified.

### Verification programme

The outcome of the eradication programme was verified by monitoring the re-appearance of both Sigatoka disease and BLSD over a 12-month period, from May 2002 to May 2003, in plantations submitted to a less intense spraying programme, on non-commercial banana plants and on sentinel (trap) plants (Peterson 2003). Weather data were recorded and the eradication programme of feral plants audited.

Legislation was modified and the 'permitted' disease level in the TBPA was raised from 'zero detectable disease' to a maximum of 5% of diseased tissue on any leaf. The verification programme (Anon. 2003) consisted of six two-month-long rounds of surveillance. In each round, all the plantations were inspected and all the non-commercial banana plants on residential properties were visited bi-monthly. Sentinel plants (blocks of 5 to 10 unsprayed 'Williams' banana plants) were established at 138 sites on 1 to 10 km-long transects around all the sites where

BLSD had been detected. Sentinel plants were planted at more than 25 m from commercial bananas, to avoid exposure to fungicides, and over 10 m from sugarcane, to avoid exposure to herbicides. Sentinel plants were not planted in areas used for grazing or unsuitable for growing bananas, such as swamps, rainforest and public parks. All the sentinel plants were thoroughly inspected once a month and all the diseased tissue sampled.

Temperature and rainfall were recorded at three sites throughout the TBPA. A period of at least 3 consecutive wet days (>1 mm of rain) with minimum temperatures above 18°C, was considered an 'infection period'. The number of infection periods and the cumulative number of wet days from infection periods during the verification phase of the programme were compared to the previous 10-year average.

During the early part of the eradication programme, all the sites where banana plants had been destroyed during the feral eradication programme were re-visited to ensure eradication had been successful. The feral eradication programme was audited towards the end of the verification programme, with revisits to more than 10% of the high-risk land parcels, around sites where BLSD had been detected, to ensure that no banana plants were missed in the original programme.

### Results

BLSD was confirmed on 20 of the 2657 banana leaf samples collected during the delimiting surveys of the TBPA (Table 1). It was not detected outside the TBPA in the nearby banana growing areas of Innisfail or Kennedy. BLSD was detected on an additional five samples collected between

**Table 1. Banana leaf samples that tested positive (+ve) for *Mycosphaerella* leaf spot diseases between April 2001 and April 2002.**

Area	Number of samples	Samples +ve for black leaf streak disease	Samples +ve for Sigatoka disease
<b>Delimiting surveys (April to August 2001)</b>			
Tully	2657	20*	2271
Innisfail	1564	0	1310
Kennedy	244	0	228
Other areas	13	0	12
<b>Eradication programme (September 2001 to April 2002)</b>			
Tully	1787	5*	740
Innisfail	2483	0	2124
Kennedy	135	0	104
Other areas	57	0	36

\* BLSD was last recorded in a plantation in August 2001 and on non-commercial plants in November 2001.

August and November 2001. The last sample positive for BLSD was collected on a commercial plantation on 13 August 2001 and on a non-commercial plant on 25 November 2001. BLSD was detected on 13 commercial properties and on 12 non-commercial blocks of plants, indicating a recent introduction. BLSD was not detected in samples collected between April 2001 and April 2002 in other north Queensland banana areas.

### Eradication programme

The inoculum eradication exercise started in September 2001 and substantially reduced inoculum levels in all the plantations. In the first round (September to October 2001), only 11% of the properties had a 'zero detectable disease' level, whereas by the fifth round (February to April 2002), 70% of the properties had achieved a 'zero detectable disease' level. On 26% of the properties, the level was extremely low and all the diseased tissue were removed. On the remaining 4% of the properties, the 'zero detectable disease' level was achieved within seven days of inspection (Table 2). The 'zero detectable disease' and extremely low levels, where all the diseased samples were negative for BLSD in the laboratory, demonstrate that BLSD was not present in these plantations. All

the samples collected from the remaining 4% of the properties were also negative for BLSD.

A low level of *M. musicola* ascospores was observed in 27% of the samples coming from leaf material collected on the ground of 48 plantations between September and November 2001. Further ascospore assessment was not possible as sufficient quantities of intact leaf material with distinguishable lesions could not be located.

Between August 2001 and February 2002, the commercial banana plants were sprayed once a week (27 times) with systemic fungicides rotated with a protectant fungicide. The types of systemic fungicides were also rotated, based on their modes of action and on known cross-resistance issues. The spraying programme, especially the application of trifloxystrobin (Tega 1.2 L with oil 4-5 L/ha) during the hot and dry season (October to December 2001), caused considerable damage to the uncovered bunches.

A total of 7629 land parcels were visited and all the non-commercial plants were sampled for the disease. A total of 23 857 motherplants and 19 980 suckers of unwanted plants were destroyed.

**Table 2. Levels of *Mycosphaerella* leaf spot diseases in the plantations of the Tully banana production area at the end of each of the five inspection rounds conducted during the eradication programme.**

	Zero detectable disease		Extremely low disease level*		Disease present	
	Proportion of properties**	Proportion of area**	Proportion of properties	Proportion of area	Proportion of properties	Proportion of area
1. Sept-Oct 2001	11%	4%	--	89%	96%	
2. Oct-Nov 2001	51%	27%	--	49%	63%	
3. Nov-Dec 2001	32%	20%	51%	56%	16%	24%
4. Jan-Feb 2002	58%	46%	36%	44%	7%	9%
5. Feb-April 2002	70%	66%	26%	30%	4%	4%

\* Disease level so low that all the diseased tissues were removed during sampling (15-20 leaf pieces/ block).

\*\* 157-162 properties and 4400-4520 ha

**Table 3. Levels of *Mycosphaerella* leaf spot diseases at each of the six inspection rounds conducted during the verification programme.**

	Disease present		Number of samples**	Proportion of samples	
	Proportion of properties *	Proportion of area *		+ve for Sigatoka disease	+ve for black leaf streak
1. May-July 02	42%	63%	174	43%	0
2. Aug-Sept 02	55%	69%	166	55%	0
3. Oct-Nov 02	35%	45%	172	32%	0
4. Dec 02- Jan 03	40%	62%	755	17%	0
5. Feb-Mar 03	45%	63%	783	20%	0
6. April-May 03	53%	72%	786	28%	0

\* 157-161 properties and 4480-4713 ha

\*\* In rounds 1 to 3, only leaves with marks, plus a 'clean' sample from blocks with no disease, or a 'zero detectable disease' level were sampled. In Rounds 4 to 6, a preset sampling schedule based on property size was used.

## Verification programme

The incidence of Sigatoka disease increased throughout the TBPA during the 12-month-long verification programme. Sigatoka disease was detected on 53% of the properties and on 72% of the area in April and May 2003 (Table 3), compared to only 30% of the properties (4% with disease and 26% with extremely low levels) in March and April 2002 (Table 2). BLS D was not detected in the 2836 samples collected in commercial plantations, while Sigatoka disease was detected in 28% of the samples and at 51% of the sentinel sites.

A total of 302 samples were collected from sites where unwanted banana plants had previously been destroyed. BLS D was not detected and Sigatoka disease was identified on less than 10% of the samples. The audit of the eradication programme, during which 11.4% (869) of land parcels had been revisited, did not detect any banana plant that had been missed.

Weather data from the three sites indicate that there have been one to three infection periods every month between November 2002 and May 2003, which represent 86% to 106% of the 10-year average. The cumulative number of wet days from infection periods represent 77% to 87% of the 10-year average. Six disease cycles would have been completed from March 2002 (end of intense eradication programme) to June 2003.

A statistical model, developed to simulate the multiplication and spread of BLS D, was used to test the likelihood that the disease had survived undetected.

## Discussion

*M. fijiensis* is more vigorous than *M. musicola*, producing four times as many ascospores in the same period (Stover 1980). Therefore, the increase in Sigatoka disease in plantations and sentinel plants and the absence of BLS D during the verification period is a strong indication that BLS D is no longer present in the area and that the eradication programme has been successful. In addition, BLS D has not been detected in the TBPA in the less intense follow-up surveys conducted over the 17 months following May 2003. In November 2004, it had been 39 months since BLS D had last been detected in

a plantation and 36 months since it had last been observed on a non-commercial banana plant.

The eradication programme was successful in part because the disease was detected early, when its distribution was still limited. The window of opportunity provided by the approaching dry season and the biology of the fungus also contributed to the success of the programme. On the plant, ascospores can survive about 20 weeks in the leaf material but once it has fallen to the ground they survive only 6-8 weeks in the leaf tissue, according to Peterson *et al.* (2000), and as little as three weeks, according to Gauhl (1994). The fungi have no alternate hosts (Calpouzou 1955, Meredith 1970) or structures that allow them to survive for longer periods.

Based on the results of the verification programme, the statistical model suggests, with a very high level of confidence, that the Tully district is free of BLS D.

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# Field evaluation of strobilurins, triazoles and acibenzolar to control Sigatoka disease in Australia

L. L. Vawdrey and K. Grice

Since the early 1980s, the northern Queensland banana industry, which accounts for 80% of Australia's production, has relied on the use of the protectant fungicide mancozeb or the systemic triazole fungicides propiconazole or tebuconazole with mineral oil to control Sigatoka disease (caused by *Mycosphaerella musicola*), with as many as 20 to 25 applications a year (Kernot 1998). Other chemicals, however, have shown efficacy in the control of a number of foliar diseases (Hewitt 1998) and some, such as the triazole fungicides JAU 6475 and epoxiconazole, are seen as possible alternatives.

The strobilurin fungicides are synthetic analogues of naturally occurring fungitoxic metabolites produced by the woodland basidiomycete *Stobilurus tenacellus* (Ypema and Gold 1999). Unfortunately, the highly specific mode of action of the strobilurins increases the potential for the development of resistant individuals (Ypema and Gold 1999). However anti-resistance strategies based on the recommendations of the Fungicide Resistance Action Committee (FRAC) should help prevent the development of resistant strains (Gouot 1998). The plant activator acibenzolar is a functional analogue of salicylic acid shown to accumulate in plants challenged with a pathogen (Sticher *et al.* 1997). Salicylic acid plays an important signaling role in the activation of plant defense responses to pathogen attack (Sticher *et al.* 1997).

In 1998, 1999 and 2001, we conducted field experiments to evaluate the strobilurin

fungicides trifloxystrobin, azoxystrobin and pyraclostrobin, the triazoles JAU 6475 and epoxiconazole, and the plant activator acibenzolar against Sigatoka disease.

## Materials and methods

Three field experiments were conducted at the Centre for Wet Tropics Agriculture, South Johnstone, Australia. The experimental design was a randomized complete block with 3 replications. Each plot contained a single row of 10 plants of the cultivar 'Williams' (AAA) irrigated by mini-sprinklers. The treatments were separated by a single row of unsprayed plants to ensure the uniform development of disease throughout the experiment and prevent drift during applications. The planting materials (one per hole) were tissue culture plantlets (1998 evaluation) and suckers of similar size and age chosen from suckers from the previous crop (1999 and 2001 evaluations). The products described in Table 1 were applied when plants had 4-5 fully expanded leaves. There was no visible symptom of Sigatoka disease in any of the experimental plots at this stage. Treatments were applied fortnightly with a backpack mister (Efco®) during the warm and wet months (February-May) and every 3 weeks during the cool dry months from June until harvest in October or November. Spray volume was calibrated by spraying 10 plants in the guard row and varied between 107 and 353 L/ha as the plants grew.

Table 1. Name and formulation of the fungicides used to control Sigatoka disease in the 1998, 1999 and 2001 field evaluations.

Common name	Product name	Formulation (g/L)	Supplier
Trifloxystrobin	Flint/Tega 75 EC	75	Novartis/Bayer Cropsciences
Azoxystrobin	Amistar WG	500	Crop Care Australasia
Pyraclostrobin	Cabrio EC	250	BASF
Acibenzolar	Bion WG	500	Novartis
Propiconazole	Tilt EC	250	Novartis
Epoxiconazole	Opus 75 EC	75	BASF
	JAU 6476 EC	250	Bayer Cropsciences
Mancozeb	Dithane OC	125	Rohm and Haas
Mancozeb	Dithane DF	750	Dow Agrosciences
Mancozeb	Dithane M45	800	Rohm and Haas

### Disease assessment

Disease development and the efficacy of each treatment were assessed at flowering on 5 plants of similar maturity per plot using the youngest leaf spotted (YLS) method (Stover and Dickson 1970). The YLS was determined by counting from the most recent fully expanded leaf to the first leaf with  $\geq 10$  fully developed spots. Within 2 weeks of harvest, the total number of leaves per plant and the disease severity index were assessed on 5 banana plants of similar maturity per plot using Gauhl's modification of Stover's severity scoring system (Gauhl *et al.* 1993). The proportion of the leaf area showing symptoms was scored on a scale of 0 to 6 as follows:

- 0 = no disease symptom
- 1 = <1% showing symptoms
- 2 = 1-5%
- 3 = 6-15%
- 4 = 16-33%
- 5 = 34-50%
- 6 = >50%

A disease severity index (DSI) was calculated as follows:

$$\sum nb / [(N-1) \times T]$$

where n = number of leaves in each grade, b = grade, N = number of grades used (7), and T = total number of leaves graded on each plant. The DSI takes into account the age of the spotted leaves on the plant, which is important in evaluating overall disease intensity (Stover and Dickson 1970). The total number of leaves per plant was also assessed.

### 1998 field evaluation

This experiment was conducted on a crop planted with tissue-culture plantlets on 11 December 1997. Spraying of trifloxystrobin at 90 and 112.5 g a.i./ha, azoxystrobin at 100 g a.i./ha and acibenzolar at 40 g a.i./ha, which was sprayed with 1000 g a.i./ha Dithane OC every 28 days, started on 15 April 1998 and a total of nine applications were made over the course of the experiment (see table 2 for more information on the treatments). The fungicides were mixed with paraffinic oil (BP Miscible Banana Misting Oil<sup>®</sup>) at the rate of 5 L/ha, except for the mancozeb (Dithane OC<sup>®</sup>) control, which contained 412 g/L of petroleum oil. Treatments were compared

with the industry standards propiconazole and Dithane OC<sup>®</sup>.

### 1999 field evaluation

This experiment was conducted on the 1<sup>st</sup> ratoon crop. Spraying of trifloxystrobin at 75 and 112.5 g a.i./ha and acibenzolar at 40 g a.i./ha, which was sprayed with 1000 g a.i./ha Dithane OC every 14 days, started on 2 March 1999 and a total of 12 applications were made (see table 3 for more information on the treatments). The fungicides were mixed with paraffinic oil (BP Miscible Banana Misting Oil<sup>®</sup>) at the rate of 5 L/ha, except for the mancozeb (Dithane OC<sup>®</sup>) control, which contained 412 g/L of petroleum oil. Treatments were compared with the industry standards propiconazole and mancozeb as Dithane OC<sup>®</sup> and Dithane DF<sup>®</sup>.

### 2001 field evaluation

This experiment was conducted on the 3<sup>rd</sup> ratoon crop. Spraying of trifloxystrobin at 75 g a.i./ha (alone and with mancozeb), pyraclostrobin at 100 g a.i./ha (alone and with mancozeb), azoxystrobin at 100 g a.i./ha (alone and with acibenzolar), JAU 6475 at 50 g a.i./ha, epoxiconazole at 75 g a.i./ha and acibenzolar at 20 g a.i./ha started on 4 March 2001 and a total of 10 applications were made (see table 4 for more information on the treatments). All treatments were mixed with paraffinic oil as BP Miscible Banana Misting Oil<sup>®</sup> at the rate of 5 L/ha. Treatments were compared with the industry standards propiconazole and mancozeb as Dithane M45<sup>®</sup>.

### Data analysis

An ANOVA was used to analyse the YLS, the total number of leaves and the DSI. Pair-wise testing between means was done using the least significance difference (LSD) procedure at P=0.05.

## Results

### 1998 field evaluation

The YLS assessed at flowering, after 8 spray applications, shows that trifloxystrobin, followed by azoxystrobin, were significantly more effective than all other treatments (Table 2). The trifloxystrobin-treated plots were significantly less affected by Sigatoka disease than the azoxystrobin-treated plots. The DSI recorded two weeks before harvest confirmed most of the results from the YLS assessment (Table 2). The DSI shows that

**Table 2. 1998 field evaluation of chemicals for the control of Sigatoka disease following assessments of the youngest leaf spotted (YLS) at flowering, and of the total number of leaves per plant and disease severity index two weeks before harvest (n=15).**

Treatment	Rate (g a.i./ha)	YLS	Number of leaves per plant	Disease severity index
Trifloxystrobin (Flint)*	90	12.1 a	12.3 a	1.6 a
Trifloxystrobin (Flint)*	112.5	12.3 a	12.4 a	0.9 a
Azoxystrobin (Amistar)*	100	9.3 b	12.2 a	14.0 b
Acibenzolar (Bion)/ mancozeb (Dithane OC) † programme‡	40/1000	6.9 c	9.4 b	20.0 bc
Propiconazole (Tilt)*	100	5.5 c	12.4 a	21.3 c
Mancozeb (Dithane OC)†	1000	5.7 c	12.7 a	32.3 d
Least significant difference		1.5	1.0	6.16

\*Fungicide mixed with BP Banana Misting Oil at the rate of 5 L/ha.

†Contains 412 g/L petroleum oil

‡Dithane OC every 14 days and in combination with acibenzolar every 28 days.

Means in the same column followed by the same letter are not significantly different at P>0.05.

trifloxystrobin, followed by azoxystrobin, were significantly more effective than all other treatments, except the acibenzolar/mancozeb spray programme. Acibenzolar in a spray programme with mancozeb (Dithane OC®) significantly improved the control of Sigatoka disease compared to Dithane OC® alone. However, there was phytotoxicity (orange discolouration) of leaves in the acibenzolar/mancozeb-treated plots and a significant reduction in the number of leaves compared to all other treatments.

### 1999 field evaluation

The YLS assessment, after 11 spray applications, shows that trifloxystrobin at 75 and 112.5 g a.i./ha more effectively controlled leaf spot than all other treatments (Table 3). The DSI also shows that trifloxystrobin was more effective at controlling Sigatoka disease than the industry standards propiconazole, and mancozeb (Dithane DF® and OC®) (Table 3). The addition of mancozeb (Dithane OC®) to acibenzolar every 28 and 42 days reduced the severity of the disease compared to Dithane OC® alone. There was no significant difference in

disease control between to two acibenzolar treatments. In the acibenzolar/mancozeb-treated plots there was a significant reduction in the number of leaves compared to all the other treatments.

### 2001 field evaluation

The YLS assessment, after 12 spray applications, shows that the trifloxystrobin (alone and with mancozeb) and pyraclostrobin were performed better than propiconazole, and mancozeb (Dithane M45®) (Table 4). JAU 6476 was more effective at controlling Sigatoka disease than Dithane M45®. The DSI confirmed most of the results from the YLS assessment (Table 4). The DSI also showed that all the treatments, except acibenzolar, had significantly less disease than the Dithane M45® one. There were fewer leaves in the plots treated with JAU 6475, acibenzolar alone and acibenzolar with azoxystrobin than in the plots treated with propiconazole.

### Discussion

Disease levels were relatively uniform in all three experiments, with moderate

**Table 3. 1999 field evaluation of chemicals for the control of Sigatoka disease following assessments of the youngest leaf spotted (YLS) at flowering, and of the total number of leaves per plant and disease severity index two weeks before harvest (n=15).**

Treatment	Rate (g a.i./ha)	YLS	Number of leaves per plant	Disease severity index
Trifloxystrobin (Flint)*	75	14.0 a	12.0 a	0.7 a
Trifloxystrobin (Flint)*	112.5	13.7 a	12.1 a	0.2 a
Acibenzolar (Bion)* / mancozeb (Dithane OC)† programme‡	40/1000	11.9 b	10.0 b	5.3 ab
Acibenzolar (Bion)* / mancozeb (Dithane OC)† programme§	40/1000	11.0 b	9.9 b	9.1 bc
Propiconazole (Tilt)*	100	10.5 b	12.1 a	12.2 bcd
Mancozeb (Dithane DF)†	750	11.1 b	12.1 a	14.4 cd
Mancozeb (Dithane OC)†	1000	11.2 b	12.1 a	19.9 d
Least significant difference		1.8	1.0	8.2

\*Fungicide mixed with BP Banana Misting Oil at the rate of 5 L/ha.

†Contains 412 g/L petroleum oil

‡Dithane OC every 14 days and in combination with acibenzolar every 28 days.

§Dithane OC every 14 days and in combination with acibenzolar every 42 days.

Means in the same column followed by the same letter are not significantly different at P>0.05.

**Table 4. 2001 field evaluation of chemicals for the control of Sigatoka disease following assessments of the youngest leaf spotted at flowering, and of the total number of leaves per plant and disease severity index two weeks before harvest (n=15).**

Treatment	Rate (g a.i./ha)	Youngest leaf spotted	Number of leaves per plant	Disease severity index
Trifloxystrobin (Tega)*	75	13.5 a	10.9 cd	1.4 a
Trifloxystrobin spray programme†	75	12.0 ab	11.5 abc	8.7 bc
Pyraclostrobin (Cabrio)*	100	12.3 ab	12.4 a	6.0 ab
Pyraclostrobin spray programme‡	100	9.8 bcd	11.9 ab	10.5 bc
Azoxystrobin (Amistar)*	100	12.2 ab	12.2 a	4.9 ab
Azoxystrobin/acibenzolar spray programme§	100/20	11.0 abc	10.3 d	6.1 ab
JAU 6475*	50	11.1 abc	11.1 bcd	5.0 ab
Acibenzolar*	20	5.3 e	11.1 bcd	39.9 e
Epoxiconazole (Opus 75)*	75	9.7 bcd	11.8 ab	12.8 c
Propiconazole*	100	8.7 cd	12.1 a	7.3 abc
Mancozeb (Dithane M45)*	1760	7.5 de	11.2 bcd	21.0 d
Least significant difference	2.6	0.9	6.3	

\*All fungicides, except acibenzolar which was mixed with water, were mixed with paraffinic oil at the rate of 5 L/ha.

†2 sprays of trifloxystrobin followed by 2 sprays of mancozeb for a maximum of 6 sprays of trifloxystrobin.

‡2 sprays of pyraclostrobin followed by 2 sprays of mancozeb for a maximum of 8 sprays of pyraclostrobin.

§azoxystrobin at 14-21 day intervals plus acibenzolar at 42 day intervals.

Means in the same column followed by the same letter are not significantly different at P>0.05.

to severe leaf damage occurring in the guard rows. The strobilurin fungicides trifloxystrobin, pyraclostrobin and azoxystrobin proved more effective than the industry standards propiconazole and mancozeb at controlling Sigatoka disease. Trifloxystrobin and pyraclostrobin, in particular, produced a level of control never before seen in field evaluations on bananas in Australia. A similar level of effectiveness has been demonstrated against black leaf streak disease (caused by *Mycosphaerella fijiensis*) in field experiments conducted in Central America (Perez *et al.* 2002). Our results also suggest that the efficacy of the strobilurins will not be compromised when they are used in spray programmes with the protectant and industry standard mancozeb. Such spray programmes are an integral part of strategies designed to prolong the useful life of modern fungicides (Gouot 1998).

An interesting aspect of this study was the increased control achieved when the plant activator acibenzolar was used with mancozeb. Our findings also show that acibenzolar with mancozeb can be phytotoxic to leaves and significantly reduce the number of leaves. Researchers in Costa Rica obtained similar control of black leaf streak disease when acibenzolar was applied with banana spray oil (Madrigal 1998). As us, they reported phytotoxicity on the older leaves of the plants and concluded that acibenzolar used with spray oil at rates

greater than 5 L/ha could result in leaf damage. However in our study, we used oil at the rate of 3.6 L/ha, which suggests that phytotoxicity was due to something else.

The triazole fungicides JAU 6475 and epoxiconazole provided a level of control similar to the industry standard propiconazole. In 2004, epoxiconazole (Opus 75®), trifloxystrobin (Flint®) and pyraclostrobin (Cabrio®) were registered for the control of Sigatoka disease on bananaa.

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## Fulvic acid applications for the management of diseases caused by *Mycosphaerella* spp.

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**B**lack leaf streak disease (caused by *Mycosphaerella fijiensis*) and Sibatoka disease (caused by *Mycosphaerella musicola*) are among the diseases that affect banana crops most significantly, for they increase production costs, decrease production areas and reduce farmers' incomes. Chemical products are one of the ways used to control them but they increase production costs, the incidence of health problems among workers and the risk that the fungicides will select for resistant plants, as well as contaminate the fruit and the environment.

Natural compounds obtained from microorganisms have the advantage of being less harmful to the ecosystem, and of being biodegraded *in situ* by the microflora and converted into non-toxic compounds (Sanchez Rodriguez *et al.* 2002.) The search for new naturally-derived and environmentally friendly products to control diseases is an important part of sustainable agriculture (Sanchez Rodriguez *et al.* 2002).

Fulvic acids extracted from the rachis of banana plants contain high concentrations of potassium, which tends to induce resistance to some diseases (Alvarez *et al.* 2002). Studies conducted by Stindt and Weltein (1990), Weltzein (1992), and Yohalem *et al.* (1994), and cited by Alvarez *et al.* (2002), indicate that these lixivates have been used for many years in foliar sprays to control fungal plant diseases. Also, studies published by Alvarez *et al.* (2002) state that the application of 5% fulvic acids extracted from banana lixivate reduces the severity of powdery mildew in roses.

The purpose of this research was to evaluate the use of fulvic acids extracted from banana rachis as an effective, low cost alternative that helps control leaf spot diseases caused by *Mycosphaerella* spp. and does not contaminate the fruit and the environment.

### Materials and methods

The study was conducted between June 2002 and July 2003 at the Montelindo farm of Caldas University located in the Santagueda region, Palestina municipality (Caldas), 5° 05' north latitude and 75° 40' west longitude, at 1050 m above sea level, with 22.5° mean temperature, 76% relative humidity, 2100 mm annual rainfall, and 2010 hours of sunshine yearly.

A randomized complete block design with six treatments, four replications and nine plants per replication was used. The trial was established on 8 May 2002 using corms of approximately 500 g. The 180 plants covered an area of 2160 m<sup>2</sup>, with 2 m between plants and 3 m between rows. The cultivar 'Dominico harton' was used because of its high susceptibility to black leaf streak and Sigatoka diseases. To ensure adequate disease pressure, the experimental plots were established around a banana crop that had not been treated against fungi. Agronomic management was carried out following practices recommended for banana crops in the region, including fertilization, desuckering, removal of dried leaves and bracts and weeding. The study lasted 14 months, from planting to harvest.

The evaluated treatments were: 1) 0.5% fulvic acids; 2) 100% fulvic acids; 3) 1.75 L/ha Mancozeb; 4) 0.4 L/ha Propiconazole, and 5) no application (control). The 100% fulvic acid treatment was applied to the foliage undiluted, and water was added to obtain a 0.5% solution.

Fulvic acids are lixiviates produced by the biodegradation of the raquis of the cultivar 'Dominico harton'. The electric conductivity of fulvic acids is 24.75 mmho/cm, its pH 3.95, and their composition includes 260 ppm phosphorus, 155 ppm potassium, 49.74 ppm calcium, 32.36 ppm magnesium, 9.94 ppm N-NH<sub>4</sub>, 6.49 ppm sodium, 0.33 ppm iron, and 0.28 ppm manganese. The biodegrader used to obtain fulvic acids was built on the Montelindo farm.

The manganese-zinc ethylene bis (dithiocarbamate) fungicide Mancozeb is a protective fungicide that inhibits fungal respiration. Propiconazole is a systemic fungicide that acts by blocking ergosterol biosynthesis and inhibiting steroid demethylation.

Fulvic acids and Mancozeb were applied every 7 days, while Propiconazole was applied every 14 days. All treatments were applied to the leaves. According to studies conducted by Alvarez *et al.* (2002), lixiviates of banana rachis at a 5% concentration reduce powdery mildew on roses, but cause toxicity to leaves at a 50% concentration. Based on these results, and considering banana's larger and thicker leaf surface, fulvic acid treatments at 0.5 and 100% were applied.

Evaluations were conducted weekly from the first month after planting until harvest. The disease severity index (SI) was based on Gauhl's modification of Stover's scoring system (Gauhl *et al.* 1995), which used a scale between 1 to 6, according to the following formula:

$$SI = \frac{\sum nb}{(N-1)T} \times 100$$

where  $n$  = the number of leaves in each grade,  $b$  = grade (0 = no symptoms; 1 = symptoms on less than 1% of leaf blade; 2 = symptoms on 1-5% of leaf blade; 3 = symptoms on 6-15% of leaf blade; 4 = symptoms on 16-33% of leaf blade; 5 = symptoms on 34-50% of leaf blade;

and 6 = symptoms on >51% of leaf blade),  $N$  = number of grades used in the scale (7) and  $T$  = total number of evaluated leaves.

The disease development rate ( $r$ ) was also determined using the equation:

$$r = \left( \frac{1}{t_1 - t_0} \text{Log}_e \frac{X_1}{1 - X_1} - \text{Log}_e \frac{X_0}{1 - X_0} \right)$$

where  $t_1$  = final time,  $t_0$  = initial time,  $X_1$  = final severity index and  $X_0$  = initial severity index (Castaño Zapata 2002).

The following variables were evaluated at flowering and at harvest: (1) youngest leaf diseased, i.e. the youngest leaf with streaks clearly visible from the ground (Orjeda 1998); (2) the youngest leaf spotted, which is the first totally extended leaf, counting from top to bottom, that shows 10 or more discrete necrotic and mature lesions or a large, necrotic, light-colored area (Stover and Dickson 1970); and (3) number of functional leaves.

The bunch weight, weight of the second hand, weight of the middle finger of the second hand, length of the middle finger of the second hand and diameter of the middle finger of the second hand were recorded at harvest.

It should be noted that this research was conducted over one crop cycle.

## Results and discussion

The analysis of variance of the disease severity index showed statistically significant differences between treatments and the interaction treatment\*weekly evaluation. The lowest mean severity index recorded during the trial was for the 0.5% fulvic acid treatment (42), while the highest severity was shown by the control, with (59); there were no significant differences with respect to Mancozeb and Propiconazole (Table 1).

The 0.5% fulvic acid treatment showed the highest values for youngest leaf diseased and youngest leaf spotted at flowering and at harvest, possibly due to the high potassium content in the solution. Potassium makes the leaf's cellular walls more resistant and, as a result, the germination of conidia and ascospores more difficult.

There were statistically significant differences in the number of functional leaves at flowering and at harvest (Table 1). Research conducted by Molina Tirado and Castaño

Zapata (2003) in that same region indicated that 'Dominico harton' showed no functional leaves at harvest. In this study, the control showed one functional leaf at harvest. In this sense, the evaluated treatments afforded highly effective disease control.

The disease development rates did not show significant statistical differences among treatments, but were significantly slower for the treatments compared to the control (Table 1).

As shown in Table 2, the weight, diameter and length of the middle finger of the second hand did not show statistically significant differences among treatments. As for the weight of the middle finger, it was 50 g heavier in the 0.5% fulvic acid treatment than in the control, due primarily to its controlling effect. This treatment also showed a larger area of photosynthesis and, as a result, greater accumulation of carbohydrates in the bunch, as indicated by its 4.9 kg weight advantage over the control, due primarily to the high nutrient (especially potassium) content in fulvic acids.

These results indicate that the 0.5% fulvic acid treatment is a viable, environmentally friendly alternative for managing diseases caused by *Mycosphaerella* spp. in banana.

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**Table 1. Effect of fulvic acids and two fungicides on black leaf streak disease in the banana cultivar 'Dominico harton' (n = 36).**

	SI	Youngest leaf diseased		Youngest leaf spotted		Number of functional leaves		Disease development rate
		Flowering	Harvest	Flowering	Harvest	Flowering	Harvest	
Fulvic acids (0.5%)	42 b	7 a	5 a	9 a	6 a	11 a	6 a	0.07 b
Fulvic acids (100%)	46 c	6 b	3 b	7 b	4 b	9 a	4 b	0.09 b
Mancozeb (1.75 L/ha)	47 b	6 b	2 b	7 b	3 b	10 a	4 b	0.09 b
Propiconazole (0.4 L/ha)	48 b	6 b	2 b	8 a	3 b	11 a	4 b	0.08 b
Control	59 a	4 c	1 c	5 c	1 c	6 b	1 c	0.12 a

SI = severity index.

Means followed by different letters are significantly different at 5% probability according to Tukey's test.

**Table 2. Effect of fulvic acids and two fungicides on yield and bunch quality of the banana cultivar 'Dominico harton' (n = 36).**

	Bunch weight (kg)	Number of hands/bunch	Number of fingers/hand	Weight of middle finger of second hand (g)	Diameter of middle finger of second hand (cm)	Length of middle finger of second hand (cm)
Fulvic acids (0.5%)	14.7 a	9 a	9 a	350.0 a	4.5 a	24.7 a
Fulvic acids (100%)	13.8 a	6 c	9 a	338.7 a	4.2 a	24.0 a
Mancozeb (1.75 L/ha)	13.7 a	7 b	9 a	325.0 a	4.1 a	24.1 a
Propiconazole (0.4 L/ha)	14.2 a	8 a	8 b	330.0 a	4.1 a	24.3 a
Control	9.8 b	7 b	8 b	300.0 b	3.7 c	22.1 b

Means followed by different letters are significantly different at 5% probability according to Tukey's test.

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## Genetic diversity of Colombian isolates of *Mycosphaerella fijiensis* Morelet based on microsatellite markers

I. Perea, E. Rodríguez Arango, E. Márquez and R. Arango

**B**lack leaf streak disease (BLSD), caused by the fungus *Mycosphaerella fijiensis* Morelet (Stover 1980), was first recognized on the South-eastern coast of Viti Levu in Fiji in 1963 (Rhodes 1964). Subsequently, the disease was reported in the Pacific Islands, Asia, Africa and finally in Latin America in 1972 in La Lima, Honduras (Stover and Dickson, 1976). The fungus is haploid through most of its life cycle and reproduces both asexually and sexually, via conidia and ascospores, respectively.

Several studies have described the global genetic variability of *M. fijiensis* (Carlier *et al.* 1994; 1996) based mostly on the analysis of fungal isolates from different continents using RFLPs and PCR-RFLP markers (Rivas *et al.* 2004). Conducting studies at a local level could help improve local disease management and breeding programmes for resistance to BLSD.

In Colombia, BLSD was observed for the first time in 1981 (Mourichon and Fullerton 1990) in the banana growing region of Urabá from where it probably spread to the rest of the country. The major banana production areas in Colombia are located in Urabá and Magdalena where bananas are grown at high density on large plantations and controlled by using chemical fungicides (Figure 1). Plantain production areas are mainly located in Tolima and Arauca where plantains are mainly grown on small- and medium-scale farms, in association with coffee, cacao or others, with little or no chemical control.

In the present study microsatellite markers were used to do a preliminary survey of the genetic variability of 40 isolates of *M. fijiensis* from the main local banana and plantain producing regions of Colombia.

### Materials and methods

Bananas and plantains leaves with *M. fijiensis* infection were sampled at four locations in Colombia. Of the 40 isolates collected, 11 came from Urabá, 10 from Tolima, 9 from Arauca, and 10 from Magdalena. The sampled cultivars included

'Grand naine' (AAA) in Magdalena and Urabá, and 'Dominico hartón' (AAB) in Tolima and Arauca. Each isolate was obtained from leaves of different plants collected at random. All isolates were grown from a single ascospore and maintained on PDA (potato dextrose agar) in test tubes held  $28 \pm 2^\circ\text{C}$  and incubated in darkness for 30 days.

DNA was extracted from each isolate using a CTAB based method (Weising *et al.* 1991) with minor modifications described in Romero *et al.* 1999.

Microsatellite analysis was conducted using seven primer pairs for loci *Mf* SSR 005, *Mf* SSR 025, *Mf* SSR 061, *Mf* SSR 137, *Mf* SSR 203, *Mf* SSR 194 and *Mf* SSR244 (Neu *et al.* 1999). PCR was performed in a 25 ml reaction volume of buffer containing 2 mM  $\text{MgCl}_2$ , 0.2 mM dNTPs, 12.5 pmol of each primer, 0.625 units Taq DNA polymerase (Promega, California), 1X PCR buffer (50 mM KCl, 10 mM Tris HCl, pH 8.3), and 10-50 ng template DNA. After initial



Figure 1. Main banana and plantain growing regions in Colombia.

denaturation, amplifications were performed in a thermocycler (MJ Research PT-200, Mass.) programmed for 2 min at 94°C; 30 cycles of 30 sec at 94°C, 45 sec at 55°C and 45 sec at 72°C; with a final extension of 7 min at 72°C.

To determine allele size, a volume of 2 ml of the PCR product, combined with 3 ml of loading buffer (80% (w/v) formamide, 10 mM EDTA pH (8.0), bromophenol blue 1 mg/ml and xylene cyanol FF 1mg/ml), was denatured at 98°C for 5 min. This mixture was loaded on 6% polyacrylamide denaturing gel, containing 7 mol/L urea. Electrophoresis was carried out with 1X TBE buffer in a Sequi-Gen® system (Bio-Rad). Allele sizes were estimated using the 10bp DNA ladder (Gibco BRL).

Data were considered to be haploid, as the DNA was isolated from a single ascospore culture. The number of alleles was calculated for each locus. Gene diversity was estimated using Nei's index (h) (Nei 1978) and genotypic diversity using Stoddart and Taylor's G statistic, considering each genotype as a multilocus haplotype (Hayden *et al.* 2003). To compare samples of different sizes, the value of G was divided by the sample size. Gene diversity calculations were performed using the GDA computer program (Lewis and Zaykin 2001).

Genetic differentiation was estimated by using Wright's *F<sub>st</sub>* statistic as described in Weir and Cockerham (1984), using Arlequin computer software (Schneider *et al.* 2000). The significance of the *F<sub>st</sub>* values were tested with the method described in Excoffier *et al.* (1992) using 3024 permutations.

## Results and discussion

The estimated gene diversity of the Arauca sample (0.42) was higher than the values obtained for Urabá (0.33) and Magdalena (0.36), although in the absence

of the appropriate test it is impossible to distinguish the samples (Table 1). Using nine microsatellite markers, including the seven used in our study, Molina and Kahl (2004) obtained a gene diversity of 0.13 for the Magdalena region. The difference could be due to sampling differences or to the fact that the latter authors used more markers.

The overall gene diversity (h) of our samples was 0.46 (Table 1), a value similar to the one of 0.40 reported by Neu *et al.* (1999) for *M. fijiensis* populations in Mexico and the one of 0.42 reported by Molina and Kahl (2004) for a pooled set of isolates from Costa Rica, Colombia and Venezuela.

PCR products revealed two alleles at each locus, except *Mf* SSR 194 and *Mf* SSR 244 which had three alleles per locus. On average, the population had 2.03 alleles per locus (Table 2). This value is apparently lower than the ones reported for Mexico (2.6) and Nigeria (2.7) using the same microsatellite markers (Neu *et al.* 1999), but it is not possible to say whether the difference is statistically significant.

A total of 36 distinct multilocus haplotypes were found among the 40 isolates studied. The G values calculated for each of the sampled location showed high levels of genotypic diversity (Table 2). Genotypic diversity is near the theoretical maximum as most of the haplotypes were observed only once. This is very similar to what has been described by Carlier *et al.* (1996) who observed that each isolate of *M. fijiensis* corresponded to a single multilocus haplotype across the 19 loci RFLP evaluated.

Some of the similar isolates were from distant locations, e.g. isolate 990920 from Tolima had the same haplotype as isolate 981111 from Arauca. The collection of isolates with similar haplotypes may be the

**Table 1. Nei's gene diversity estimates at seven loci of *Mycosphaerella fijiensis* isolates from four populations in Colombia.**

Locus	Arauca	Magdalena	Tolima	Urabá	Total
MfSSR 005	0.34	0.18	0.32	0.49	0.39
MfSSR 025	0.34	0.32	0.50	0.29	0.39
MfSSR 061	0.34	0.18	0.50	0.46	0.42
MfSSR 137	0.49	0.42	0.48	0.29	0.45
MfSSR 194	0.56	0.48	0.42	0.00	0.62
MfSSR 244	0.44	0.58	0.18	0.39	0.48
MfSSR 203	0.44	0.42	0.42	0.39	0.48
Mean	0.42	0.36	0.40	0.33	0.46
Standard deviation	0.08	0.15	0.11	0.16	0.07

**Table 2: Results of the analysis of four populations of *Mycosphaerella fijiensis* in Colombia using seven microsatellite markers.**

	Arauca	Magdalena	Tolima	Urabá	Total
Number of isolates	9	10	10	11	40
Number of genotypes	9	10	10	9	36
Number of alleles/locus	2.1	2.0	2.0	1.8	2.03
Gene diversity (h)	0.42	0.36	0.40	0.33	0.46
Genotypic diversity (G)	9	9.36	10	10	38.1
G/sample size	1	0.85	1	1	0.95

**Table 3. Levels of genetic differentiation (Fst) between four populations of *Mycosphaerella fijiensis* in Colombia.**

	Tolima	Urabá	Arauca	Magdalena
Tolima	0.00000			
Urabá	0.20158*	0.00000		
Arauca	0.07566	0.13086*	0.00000	
Magdalena	0.10979*	0.26992*	0.03130	0.00000

\* Denotes a p value equal or lower than 0.05. (Excoffier et al. 1992).

result of independent sampling of the most frequent combination of alleles.

The overall estimate of Fst was 0.145. As seen in table 3, Fst values ranged from 0.07566 to 0.26992 indicating some degree of differentiation, although the levels attained were not as high as those obtained by Rivas *et al.* (2004) between populations from various Latin American countries, which ranged between 0.03 and 0.58. This is not surprising since our data were collected in only one country.

Even though the number of isolates in our study is small, and further studies are necessary, our data offer a first estimate of the genetic variability of *M. fijiensis* in some regions of Colombia.

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## Estimation of the size of the root system using core samples

H.H. Mukasa, D. Ocan, P.R. Rubaihayo and G. Blomme

**E**xcauation of the root system of a field-grown banana plant is destructive and time consuming. For example, six man-hours are needed to excavate and assess the root system of a mature banana plant (Blomme 2000). Estimating the size of the mat's root system by sampling the roots would be much faster. For strawberry (*Fragaria xananassa* Duch.), Fort and Shaw (1998) demonstrated a substantial correspondence between the variability in soil core samples and the root mass of the whole plant, indicating that changes in the root system growth could be effectively estimated from soil cores. Soil core samples required at most 10% of the time needed to collect and process the entire plant's root system. Blomme (2000) assessed field-grown banana plants at the International Institute of Tropical Agriculture's high rainfall station in Nigeria and reported that the characteristics of the mat's root system could be adequately estimated from soil core samples. In addition, obtaining roots from soil core samples required only a fraction (e.g. 5% for two soil core samples) of the time needed to excavate and assess the complete root system of a mature plant.

The objective of this study was to assess whether the core sample approach would provide sufficient information to estimate the size of the mat's root system in a wide range of East African banana genotypes growing on farm.

### Materials and methods

This study was carried out on farms in Masaka and Bushenyi districts of south-western Uganda, two important banana-growing areas. The Masaka-Lwengo site

falls under the banana-coffee system, while the Bushenyi site falls under the montane system (INIBAP 2003). The altitude at the Masaka-Lwengo site varies between 1080-1330 m, while the mean annual rainfall is 1200 mm. The soils at the Masaka-Lwengo site are classified as Luvisols (FAO 1998). The altitude at the Bushenyi site varies between 1600-1800 m, while the mean annual rainfall is 1588 mm. The soils at the Bushenyi site are classified as Acrisols (FAO 1998).

At each site, eight *Musa* genotypes – the East African highland banana genotypes (AAA-EAHB) 'Mpologoma', 'Lwadungu', 'Nakitembe', 'Mbwazirume' and 'Kibuzi', the dessert banana 'Sukali Ndizi' (AAB), the plantain 'Gonja' (AAB) and the beer banana 'Kayinja' (ABB) were assessed. Twenty plants per genotype were assessed at each site with 2-5 mats per farm. The mats selected were beyond the 3<sup>rd</sup> ratoon stage and consisted of a ready-to-harvest motherplant with 2-3 suckers. Selection of the AAA-EAHB genotypes was based on clone sets in order to include a representative genotype from each of the 4 clone sets (Karamura and Pickersgill 1999).

The root system was assessed using the core sample method (Blomme 2000). Three soil core samples were taken on each mat: from the position next to the tallest sucker and at 90° and 180° clockwise from the tallest sucker. Soil cores had a diameter of 25 cm and a height of 80 cm. Cores were demarcated using a metal ring and removed using a small spade. Samples were washed to discard soil particles and data were collected for each core sample on cord root dry weight and cord root length

(Tennant 1975). Subsequently, the plants were entirely excavated and the same traits were measured on the complete mat.

Statistical analysis was performed using the Genstat statistical package (Genstat 1999). An ANOVA was carried out to determine the effects of plant and sampling location on the characteristics of the roots in the soil core sample. These root characteristics were also regressed on mat root traits.

## Results and discussion

Most of the roots were observed within a 60 cm radius from the plant and up to a depth of 50 cm. The soil core samples were thus taken from the zone with the

highest concentration of roots. Root density decreased with increasing soil depth.

The roots in the core samples represented from 5% to 8% of the mat's root system (Table 1). These figures are higher than the 1.1% to 2.7% observed in Nigeria (Blomme 2000). This could reflect differences in soil type (the sandy soils in Nigeria enabling the roots to spread out, compared to the more compact loamy soils in Uganda where roots are more concentrated around the mat) or genotype-specific responses.

Sampling location had no significant effect on cord root length and root dry weight measured in the core samples (Table 2). The interplant effect clearly exceeded the sampling location effect. Although each mat

**Table 1. Mean and coefficient of variation, in parenthesis, of the length of the cord roots and the dry weight of the roots measured in a soil core sample and on the entire banana plant.**

Genotype	Cord root length (cm)			Root dry weight (kg)		
	Whole mat	Core sample	% of whole mat	Whole mat	Core sample	% of whole mat
Mpologoma	12 277 (46)	749.1 (53)	6.3 (33)	0.39 (52)	0.02 (58)	5.2 (39)
Lwadungu	17 152 (52)	988.1 (52)	6.4 (45)	0.52 (50)	0.02 (51)	5.2 (42)
Nakitembe	14 068 (33)	989.9 (48)	7.1 (37)	0.40 (46)	0.02 (59)	5.2 (39)
Mbwazirume	18 454 (38)	1 116.7 (43)	6.5 (42)	0.56 (49)	0.03 (51)	5.2 (54)
Kibuzi	18 236 (37)	1 028.9 (50)	5.8 (38)	0.46 (38)	0.03 (58)	5.6 (50)
Sukali ndizi	20 152 (40)	1 235.5 (43)	6.5 (35)	0.97 (52)	0.05 (54)	5.5 (42)
Gonja	11 467 (48)	738.9 (52)	6.9 (49)	0.41 (55)	0.02 (57)	5.4 (52)
Kayinja	10 510 (37)	695.3 (44)	6.8 (35)	0.44 (77)	0.03 (59)	8.1 (85)

**Table 2. Mean squares and significance for 8 *Musa* genotypes assessed in already established plantations.**

Genotype	Source of variation	df	Cord root length (cm)	Root dry weight (kg)
Mplogoma	Plant	19	59 076*	0.0000504***
	Sampling location	2	3 859	0.00000913
	Residual	38	8 959	0.00000955
Lwadungu	Plant	19	102 306*	0.00005590
	Sampling location	2	35 205	0.00002705
	Residual	38	46 545	0.00003542
Nakitembe	Plant	19	79 210***	0.00004499***
	Sampling location	2	18 707	0.00002223
	Residual	38	23 401	0.00001489
Mbwazirume	Plant	19	102 915***	0.00008080***
	Sampling location	2	27 760	0.00007264
	Residual	38	30 651	0.00002431
Kibuzi	Plant	19	12 497**	0.00008818**
	Sampling location	2	97 198	0.00006036
	Residual	38	44 463	0.00003106
Sukali ndizi	Plant	19	90 493*	0.00027608**
	Sampling location	2	89 826	0.00008867
	Residual	38	45 908	0.00009381
Gonja	Plant	19	32 198**	0.00003461*
	Sampling location	2	19 336	0.00005667
	Residual	38	11 602	0.00001707
Kayinja	Plant	19	37 897***	0.00008450**
	Sampling location	2	18 562	0.00004084
	Residual	38	12 197	0.000001805

\*\*\*, \*\*, \*: Significant at P<0.001, P<0.01, P<0.05

**Table 3: R<sup>2</sup> values of regressions between cord root length (RL) or root dry weight (RW) in one, two or three soil core samples and the corresponding value obtained by excavating the mat at harvest.**

Genotype	Traits	Core sample <sup>1</sup>						
		1	2	3	1 & 2	1 & 3	2 & 3	1, 2 & 3
Mpologoma	RL	0.75	0.62	0.81	0.74	0.71	0.82	0.83
	RW	0.55	0.67	0.64	0.70	0.63	0.74	0.71
Lwadungu	RL	0.55	0.54	0.60	0.62	0.68	0.61	0.71
	RW	0.57	0.58	0.73	0.64	0.74	0.76	0.86
Nakitembe	RL	0.61	0.64	0.57	0.72	0.75	0.68	0.77
	RW	0.53	0.71	0.75	0.62	0.73	0.85	0.80
Mbwazirume	RL	0.53	0.72	0.58	0.58	0.54	0.59	0.72
	RW	0.52	0.58	0.51	0.36	0.52	0.65	0.77
Kibuzi	RL	0.48	0.57	0.40	0.65	0.53	0.54	0.71
	RW	0.63	0.68	0.64	0.79	0.81	0.73	0.83
Sukali ndizi	RL	0.59	0.54	0.49	0.68	0.72	0.45	0.79
	RW	0.63	0.53	0.54	0.57	0.67	0.49	0.71
Gonja	RL	0.78	0.54	0.87	0.72	0.91	0.83	0.86
	RW	0.72	0.59	0.57	0.64	0.74	0.58	0.68
Kayinja	RL	0.51	0.35	0.71	0.51	0.72	0.70	0.70
	RW	0.65	0.57	0.84	0.53	0.79	0.73	0.72

<sup>1</sup>Sample 1 corresponds to the site of the future axial sucker, while samples 2 and 3 were taken at 90° and 180° clockwise from the tallest sucker.

consisted of a motherplant and 2-3 suckers, large variations in plant stature were observed. These differences in plant size, and hence in the root system, would explain the high coefficient of variation values in Table 1.

The root dry weight and cord root length in the core samples were regressed on their corresponding characteristics from the mat. R<sup>2</sup> values from single, double and triple core samples were obtained for the eight genotypes (Table 3). Single and double core samples gave the lowest R<sup>2</sup> values whereas a minimum R<sup>2</sup> value of 0.68 was obtained for triple core samples (Table 3). This means that at least 68% of the variation in mat root traits could be explained by assessing 3 core samples.

On-station studies carried out in Nigeria on plants in the first production cycle had indicated that at least 73% of the variation in mat root traits could be explained by taking two core samples, while 81% could be explained with three core samples (Blomme 2000). Further on-station studies on ratoon crops indicated that at least 80% of the variation in mat root traits could be explained by taking two core samples, and 85% with three core samples. These results suggest that at least 3 core samples should be taken, when assessing plants on farm, in order to explain more than 70% of the variation in mat root traits. This method is certainly attractive as the removal and assessment of 3 soil cores represents only about 8% of the time needed to excavate and measure the entire root

system of an adult *Musa* plant. In addition, the core sampling method can provide a detailed insight into root dynamics, which is critical for understanding environmental effects on root development and for investigating genetic differences in root system traits among a large number of cultivars.

## Acknowledgements

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## The effect of planting hole depth on *Musa* spp. shoot and root development

G. Sebuwufu, P.R. Rubaihayo and G. Blomme

In order to control the spread of banana pests and diseases farmers are encouraged to use *in vitro* plantlets (Robinson *et al.* 1993, Robinson 1996) or pared suckers (Nampala *et al.* 2001) as planting material. These materials are much smaller in size than the conventional unpared suckers commonly used in Uganda, where the recommended planting hole size is 60 cm x 60 cm x 60 cm. According to Swennen (1990), the minimum planting hole size for a pared banana sucker or *in vitro* plantlet should be 30 cm in diameter and 30 cm deep, especially on commercial farms where bananas are grown as an annual crop. The smaller size of the *in vitro* plantlets and pared suckers make it possible to establish a banana plantation using shallower planting holes. Reducing the size of the planting hole may accelerate the establishment of the plants given that the root-bearing zone would be placed at the level of the mineral rich topsoil layer. Swennen *et al.* (1988) reported that in bananas the root-bearing zone is geotropically negative, that is new roots are formed in the upper soil layers. The objective of this experiment was to compare the growth of plants derived from *in vitro* plantlets and from suckers after being planted in holes of different depths.

### Materials and methods

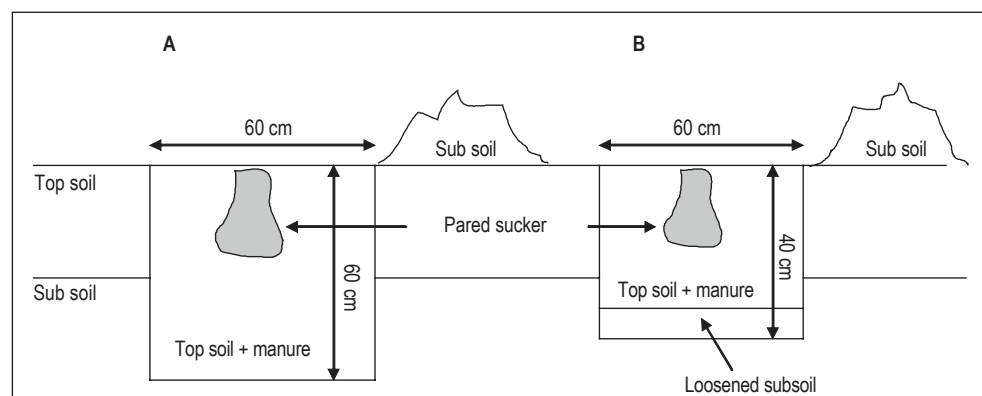
The experiment was established in March 2002 at the Makerere University Agricultural Research Institute in Kabanyolo, central Uganda. The field had previously been

under a five-year grass fallow. The soils are reddish-brown loams up to 25 cm deep and classified as Eutric Ferralsols (Yost and Estwaran 1990).

Holes of two different depths were tested. In the first method, used by farmers, a hole 60 cm in diameter and 60 cm deep was dug (Figure 1A). The topsoil from the first 30 cm was mixed with topsoil from areas surrounding the hole and 10 kg of composted cow manure. The mixture was put back in the planting hole at planting. In the second method (Figure 1B), a hole 60 cm in diameter and 40 cm deep was dug. The subsoil layer between 35 cm and 40 cm was loosened and left in the hole. A mixture of topsoil and 10 kg of composted cow manure was put on top of the loosened subsoil. Under both methods, the planting holes were filled up to 5 cm below ground level. When filling the hole, the top soil and manure mixture was gently compacted.

The planting material consisted of *in vitro* plantlets and pared sword suckers of two East African highland banana cultivars, 'Entaragaza' and 'Siira' (AAA-EAHB). The pseudostem of the sword suckers was cut 10 cm above the corm before planting. The sword suckers were homogeneous in size and when pared weighed on average 2 kg. The cut pseudostem edge of the pared sucker was positioned at soil level of the planting hole for all the genotypes and planting hole depths, thus positioning the root bearing zone of the planting material

Figure 1. Schematic representation of the methods used to dig A) a 60 cm-deep planting hole and B) a 40 cm-deep planting hole.



in the nutrient-rich top soil layer. The *in vitro* plantlets were covered with soil up to just above the collar. Twenty grams of the pesticide Furadan 3G (Carbofuran) were put in the soil-manure mixture before planting and on top of the soil 14 weeks after planting, to control nematodes and weevils. No irrigation was used.

The experimental design was a split-plot laid out as a randomized complete block with two replicates. The main plot treatment was planting hole depth, while the subplot treatment was type of planting material (i.e. *in vitro* or sucker). For each type of planting material, two varieties were assigned as sub-subplots.

Two neighbouring fields of 48 plants each were used to assess agronomic traits 24 weeks after planting (field 1) and at flower emergence (field 2). Plant spacing was 3 m x 3 m in both fields. For each planting hole depth and genotype, data were collected on 6 plants derived from *in vitro* material and 6 plants derived from suckers. Plants were completely excavated for assessment. Two fields adjacent to the previous two, and similarly laid out, were used to assess root distribution, that is cord root length as a function of soil depth, 24 weeks after planting (field 1) and at flower emergence (field 2). Each field had 24 plants. For each planting hole depth and genotype, data were collected on 3 plants derived from *in vitro* material and 3 plants derived from suckers.

The data were analysed using the mixed model procedure of SAS (Littell *et al.* 1996) in which replicates were considered random while planting hole depth and type of planting material were fixed. Because variations due to genotype were not significant, except with regards to plant height, genotype was dropped from further analyses. Means were separated using a pair-wise comparison t-test of the least square means. To establish the relationship between cord root length and planting hole depth, cord root length was plotted against soil depth for each planting material type.

## Results and discussion

Planting hole depth did not have a significant effect on the aerial, corm and root traits of the mats derived from suckers 24 weeks after planting (Table 1). In contrast, the plants derived from *in vitro* plantlets in the 60 cm-deep holes had a significantly ( $P < 0.005$ ) higher corm weight, cord root length and root dry weight compared to those planted in the 40 cm-deep holes. No significant effect of planting hole depth was observed on all growth traits assessed for both *in vitro* and sucker-derived bananas at flower emergence of the plant crop (Table 2).

The distribution of roots was similar for both planting hole depth for the plants assessed 24 weeks after flowering and those assessed at flowering (Figure 2).

**Table 1. Mean values of agronomic traits assessed 24 weeks after planting on East African highland bananas derived from two types of planting material and planted in holes of two different depths (n=12).**

Planting material	Planting hole depth (cm)	LA (m <sup>2</sup> )	NL	LW (g)	PC (cm)	PH (cm)	PW (g)	CW (g)	NR	LR (m)	RW (g)
<i>In vitro</i>	40	1.9 c	7.4 a	273 b	31 c	92 c	277 b	142 c	123 b	57 b	73 c
<i>In vitro</i>	60	2.3 bc	7.4 a	336 ab	32 bc	92 bc	349 b	285 b	126 b	91 a	111 b
Sucker	40	2.7 ab	8.2 a	411 a	37 ab	113 a	441 ab	399 a	185 a	93 a	146 ab
Sucker	60	3 a	8.1 a	444 a	38 a	112 a	503 a	490 a	161 a	98 a	160 a

LA: Leaf area, NL: Number of leaves, LW: Leaf dry weight, PC: Pseudostem circumference at soil level, PH: Plant height, PW: Pseudostem dry weight, CW: Corm dry weight, NR: Number of cord roots, LR: Cord root length, RW: Root dry weight.

In columns, means followed by the same letter are not significantly different ( $P > 0.05$ ) according to a pair wise comparison t-test of least square means.

**Table 2. Mean values of agronomic traits assessed at flowering on East African highland bananas derived from two types of planting material and planted in holes of two different depths (n=12).**

Planting material	Planting hole depth (cm)	LA (m <sup>2</sup> )	NL	LW (g)	PC (cm)	PH (cm)	PW (g)	CW (g)	NR	LR (m)	RW (g)
<i>In vitro</i>	40	5.3 a	13 ab	1060 a	51 a	191 a	2530 ab	922 b	516 a	3554 a	342 a
<i>In vitro</i>	60	4.7 a	14 a	1104 a	50 a	197 a	3060 a	1072 ab	494 a	4186 a	364 a
Sucker	40	5.7 a	10 b	1138 a	51 a	208 a	2036 b	1163 ab	520 a	4059 a	377 a
Sucker	60	5.2 a	12 ab	1059 a	48 a	200 a	2053 b	1290 a	615 a	4697 a	388 a

LA: Leaf area, NL: Number of leaves, LW: Leaf dry weight, PC: Pseudostem circumference at soil level, PH: Plant height, PW: Pseudostem dry weight, CW: Corm dry weight, NR: Number of cord roots, LR: Cord root length, RW: Root dry weight.

In columns, means followed by the same letter are not significantly different ( $P > 0.05$ ) according to a pair wise comparison t-test of least square means.

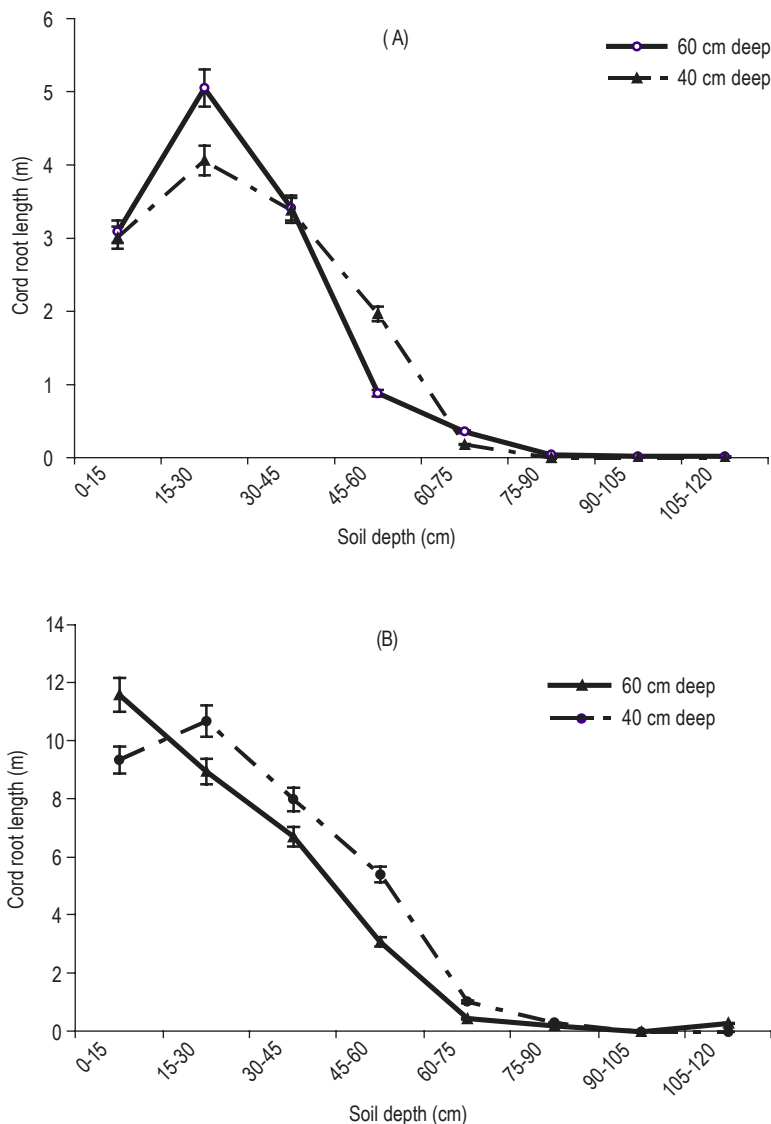


Figure 2. Cord root length as a function of soil depth of banana plants planted in holes of two different depths (40 and 60cm) and assessed A) 24 weeks after planting and B) at flowering. The values for cord root length represent the values measured on two East African highland banana cultivars (*Entaragaza* and *Siira*) planted as suckers and *in vitro* plantlets ( $n=12$  plants per planting hole depth).

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The results suggest that the shallower planting hole did not impede plant growth for both types of planting materials although further on-farm trials are needed to confirm these preliminary findings. Swennen (1990) reported that the minimum planting hole size for bananas could be as small as 30 cm x 30 cm x 30 cm. This will also ensure that the root-bearing zone of the planting material is placed in the mineral-rich top soil layer, compared to placing the corm in the subsoil. Since most banana roots grow in the upper soil layer (Araya *et al.* 1998 and Sebuwufu 2002), planting the corm at the level of the mineral rich top soil layer may result in more vigorous plant growth.

The shallower planting hole in this study would reduce labor costs per hole by as much as 50%. A hired casual field laborer receives 300-500 Ush [ $1\$=1850$  UgSh] for digging a

conventional planting hole, while only 150-250 UgSh is given for digging a shallower hole since removing the compact subsoil is more strenuous and time consuming.

Bakhiet and Elbadri (2004) planted sword suckers at various depths and reported that deep planting resulted in increased bunch weight and reduced time to flowering over successive ratoon crops. While Bakhiet and Elbadri (2004) varied planting depth of the sucker, in this study suckers and *in vitro* plants were planted at top soil level and depth of the planting hole was varied.

Further studies are however recommended in order to assess cost-benefit aspects, plant growth, yield and especially stability over subsequent ratoon cycles. Additional on-station/on-farm studies could also focus on different planting hole depths and soils types with different management history (with/without fallow, with/without soil compaction, etc). On-farm participatory technology development – or related participatory methodologies – could be used to refine planting hole depth and other related aspects under various farmers' husbandry practices and production systems. On-farm trials would generate results which are representative of, and recommendations adapted to, farmers' circumstances.

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## Evaluation of a method to simultaneously screen *Musa* germplasm against multiple nematode species

D.L. Coyne and A. Tenkouano

Evaluation method

There is no doubting the importance of nematode pests as constraints to *Musa* production (Gowen *et al.* 2005). However, emphasis on plant-parasitic nematodes affecting *Musa* has focused on the epidemiology, management and identification of resistance against *Radopholus similis* (Cobb) Thorne. Evidence is becoming increasingly clear however, that such focus should be broadened to include other nematode species, which, depending on the location and *Musa* genotype, can be of greater importance than *R. similis* (Speijer and Fogain 2000, Gowen *et al.* 2005). Nematodes such as *Helicotylenchus multicinctus* (Cobb) Golden, *Meloidogyne* spp., *Pratylenchus coffeae* (Zimmerman) Filipjev, Schuurmans and Stekhoven, for example, have been identified as primary nematode constraints on plantain in West Africa (Speijer *et al.* 2001, Brentu *et al.* 2004) and viewed as a considerable threat to *Musa* elsewhere, such as in India (Sundararaju 2001), the Pacific region (Bridge and Page 1984, Bridge 1988) and Central America (Stover 1972). Evidence is also mounting for the pathogenicity of *H. multicinctus* (Barekye *et al.* 1999, Brentu *et al.* 2004, Ssango *et al.* 2004) and *Meloidogyne* spp. (Brentu *et al.* 2004) on *Musa*.

Identifying cultivars that are resistant to pests and diseases, including nematodes, is an initial step towards the development of a management option. Much work has been undertaken on developing screening procedures to identify nematode resistance in banana (Pinochet 1996, Speijer and De Waele 1997, De Schutter *et al.* 2001,

Severn-Ellis *et al.* 2003). Using such methods, several *Musa* genotypes with resistance to *R. similis* (Pinochet 1996) have been identified. However, the process of identifying resistance remains time consuming.

The ability to rapidly screen many landraces or genotypes developed by breeders would make the process more efficient by reducing the time and space required. In this regard, De Schutter *et al.* (2001) developed a method targeting single roots and assessing nematode multiplication over an 8-week period, but it was designed for only one species, *R. similis*. Resistance-screening activities have begun to take the key nematodes into consideration (e.g. Stoffelen *et al.* 1999, Van den Bergh *et al.* 2000), although *H. multicinctus* has remained a difficult nematode to culture, impeding screening activities. With attention being on identifying resistance to nematode species other than *R. similis* and multiple-species resistance, there is a need to further develop efficient and practical screening methods. Screening large numbers of genotypes against more than one nematode species can create complications and requires more space and time. Furthermore, the availability of suckers, especially those of hybrids, may be limited. Maximum use of the available material is therefore paramount.

This study was carried out to adapt and expand the single-root screening method of De Schutter *et al.* (2001) to multiple species of nematodes.

## Materials and methods

The experiments were carried out in the screenhouse at the Ibadan station (7°30'N, 3°5'E) of the International Institute of Tropical Agriculture (IITA) in Nigeria.

The plantain 'Agbagba' (AAB), which is a good host for *R. similis* (Price 1994, De Schutter *et al.* 2001) was used. The suckers used as planting material from a multiplication site at the Ibadan station were selected for the absence of weevil damage. They were carefully pared to remove nematode-infected roots and corm tissue, and treated with hot water (53°C to 55°C) for 20 minutes (Colbran 1967) before planting. Six suckers per treatment and per inoculation method were planted in sawdust in wooden frames (1.0 m x 2.0 m x 0.3 m) placed on a plastic sheeting on the concrete floor of the screenhouse. Each wooden frame was divided into three equal compartments, each containing one sucker.

Single roots were inoculated according to De Schutter *et al.* (2001). Approximately four weeks after planting, primary roots at the same stage of development were selected from each sucker. Two days before inoculation, an 8 cm-long root segment at least 5 cm from the corm was carefully placed in a small plastic cup (8 cm in diameter, 5 cm high) containing steam-sterilized sandy loam soil (Figure 1). Using a pipette, each root segment was inoculated with a 1.0 ml aqueous suspension containing 50 vermiform nematodes. After inoculation each segment was carefully covered with steam-sterilized soil.

The nematode inoculum had been obtained from carrot-disc cultures (Pinochet *et al.* 1995) for *R. similis* and *P. coffeae*. The inoculum was prepared by rinsing Petri dishes containing the carrot discs with sterile distilled water and collecting the nematodes in a beaker. *Helicotylenchus multicinctus* inoculum was obtained by extracting nematodes from infected roots of 'Agbagba' plants grown in pots containing steam-sterilized soil. *Meloidogyne* spp. inoculum was obtained as for *H. multicinctus*, using *Meloidogyne* spp. isolated from plantains in Rivers State, Nigeria, and tentatively identified as a mixture of *M. incognita* and *M. javanica*. Roots were chopped into 0.5 cm long pieces and macerated in a kitchen blender for two 10 second-periods



Figure 1. The root segment to be inoculated is placed in a cup about 5 cm from the corm.

separated by an interval of 5 seconds. The nematodes were extracted using a modified Baermann funnel technique (Speijer and De Waele 1997).

The experiments were concluded ten weeks after inoculation. The plastic containers with the root segments were carefully excavated. The 8 cm-long root segments were removed, washed with tap water, dabbed dry with tissue paper, weighed, chopped into 0.5 cm long pieces and macerated in a kitchen blender for two 10 second-periods separated by an interval of 5 seconds. Vermiform nematodes (juveniles only for *Meloidogyne* spp.) were extracted over 48 h using the modified Baermann-funnel technique. Extractions were first decanted after 24 h and again 24 h later (at 48 h). At 24 h fresh distilled water was used to replace the decanted extraction. The extraction from the second 24 h, at 48 h, was then combined with the extraction removed earlier to comprise the total extraction for each root sample. For each root segment, the extraction volume was reduced to 10 ml and the nematode densities assessed from three 2 ml aliquots of the suspension.

Two methods (the single-species and the multiple-species methods), each using four nematode species – *Meloidogyne* spp., *H. multicinctus*, *P. coffeae* and *R. similis* – were compared with each other. In the single-species method, three roots per sucker were inoculated with 50 nematodes per root of one species. In the multiple-species

method, each sucker was inoculated by four nematode species, that is one species per root (50 nematodes) and three roots per species. Therefore, each sucker had 12 inoculated roots, each inoculated with one of the four nematode species. Experiments were arranged in a completely randomized design with six replications per treatment and conducted three separate times during 2004.

Nematode multiplication rate, root fresh weight and root damage was assessed from inoculated plants and data compared between nematode species and between the two treatments for each nematode species. Root necrosis index (RNI) was estimated on a scale of 0 to 20 (Speijer and De Waele 1997) for each root, by scoring each longitudinally split half root and calculating a mean value for the three roots per sucker.

Nematode population densities were  $\log_{10}(x+1)$  transformed prior to analysis (Gomez and Gomez 1984) to stabilize the variances. General linear model procedure (SAS Institute Inc. 1999) was used to compare reproduction, root fresh weight and RNI between nematode species inoculated roots. Data means between methods for each nematode species treatment were compared using Students T-test on SAS.

## Results and discussion

Of the four nematode species assessed, *Meloidogyne* spp. and *H. multincinctus* were recovered at lower densities at harvest than *R. similis* or *P. coffeae* (Table 1). Despite

the low densities, necrosis was observed in association with *Meloidogyne* spp. and *H. multincinctus*. The RNI was highest for *P. coffeae* in both the single-species and multiple-species methods.

The higher RNI associated with *P. coffeae* indicates the high damage potential of this nematode, supporting observations from microplot studies on plantain in Ghana (Brentu *et al.* 2004). Only limited densities of *Meloidogyne* spp. at the juvenile stage and *H. multincinctus* were recovered at the end of the experiment. The *Meloidogyne* spp. data are comparable to data from similar studies that lasted eight weeks (Stoffelen *et al.* 1999). To our knowledge no such similar screening studies have been undertaken for *H. multincinctus*, due largely to the difficulty of culturing this nematode. Instead, assessments of resistance against *H. multincinctus* have been derived from field studies (e.g. Speijer *et al.* 2000, J. Hartman unpublished).

No difference in nematode densities and RNI was observed between the two methods for any of the species (Table 2). The RNI for all the nematode species was relatively higher (but not significantly different) in the multiple-species method than in the single-species method. Only differences in root fresh weight were observed between methods, but not between nematode species.

Root weights were likely lower as a consequence of the higher level of nematode parasitism in the multiple-species

**Table 1. Nematode damage and density on the plantain 'Agbagba' ten weeks after inoculation with 50 nematodes per root using the single-species method (one nematode species per root and three roots per sucker) and the multiple-species method (one nematode species per root, three roots per species and four species per sucker) (n=18).**

	Fresh weight of 8 cm-long root segments (g)	Root necrosis index (RNI)	Nematode density (per 5 g roots)*
<b>Single-species method</b>			
<i>Helicotylenchus multincinctus</i>	3.63	4.52	0.98 (54)
<i>Meloidogyne</i> spp. <sup>†</sup>	3.76	4.45	0.35 (11)
<i>Pratylenchus coffeae</i>	3.70	8.50	2.34 (2495)
<i>Radopholus similis</i>	3.69	5.25	1.34 (928)
Least significant difference (P≤0.05)	ns	1.47	0.41
<b>Multiple-species method</b>			
<i>Helicotylenchus multincinctus</i>	1.21	5.33	0.70 (53)
<i>Meloidogyne</i> spp. <sup>†</sup>	1.01	5.39	0.61 (52)
<i>Pratylenchus coffeae</i>	0.91	10.44	2.03 (1423)
<i>Radopholus similis</i>	1.05	7.44	1.15 (3002)
Least significant difference (P≤0.05)	ns	3.67	1.01

\*Nematode densities were  $\log_{10}(x+1)$  transformed prior to analysis; original untransformed density means in parentheses.

<sup>†</sup>Juveniles only

ns = not significant

**Table 2. T-test values for nematode damage and density on the plantain 'Agbagba' following comparison of the single-species method (one nematode species per root and three roots per sucker) with the multiple-species method (one nematode species per root, three roots per species and four species per sucker) repeated three times (n=18).**

	Fresh weight of 8 cm-long root segments (g)	Root necrosis index (RNI)	Nematode density (per 5 g roots)*
<i>Helicotylenchus multicinctus</i>	1.11 <sup>†</sup>	2.99	0.637 (57)
<i>Meloidogyne</i> spp. <sup>‡</sup>	1.01 <sup>†</sup>	3.42	0.55 (49)
<i>Pratylenchus coffeae</i>	1.06 <sup>†</sup>	5.11	1.017 (2399)
<i>Radopholus similis</i>	1.07 <sup>†</sup>	3.44	1.035 (5310)

\*Nematode densities were log<sub>10</sub> (x+1) transformed prior to analysis; untransformed data in parentheses

<sup>†</sup>Differences between the two methods are statistically significant at P≤0.05 according to a T-test.

<sup>‡</sup>Juveniles only

method, with inoculated 12 roots with three in the single-species method. The results indicate that more than one species of parasitic nematodes can be inoculated on the same sucker, but on separate roots, to screen for resistance. This method reduces the requirements for suckers and space but inoculating 12 roots on one sucker was not entirely practical, although it was manageable by using the genotype 'Agbagba'. Arranging 12 small containers in a relatively limited area was a challenge. Genotypes that have a low root production potential may not be suitable to screen four nematode species. Fewer nematode species or fewer roots per nematode species may need to be used. Alternatively, the aeroponic system described in Severn-Ellis *et al.* (2003) could relieve some of the congestion in multiple-species inoculations. Furthermore, the multiple-species method may be problematic for genotypes that have thin roots if the inoculation of numerous roots further reduces root diameter.

The weight of the root segments from suckers inoculated with four nematode species was lower than the one of root segments from suckers inoculated with one nematode species (Table 3). Most variables measured differed between the three experiments conducted (Table 3).

It is possible that by using the multiple-species method, the resistance to one

nematode species may break down as a result of the stress incurred by inoculating many nematode species. In field situations, *Musa* plants are more often exposed to multiple nematode species, and therefore it may be beneficial to observe such resistance breakdown at an early stage. It would therefore be useful to use the multiple-species method to assess the reaction of cultivars known to be resistant to a given species of nematode. The method could also be used to assess different isolates of the same species in which pathotype variations are known to occur, such as for *R. similis* (Pinochet 1987, Dochez 2004).

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**Table 3. F values of ANOVA comparing the three repeat experiments of the single-species method (one nematode species per root and three roots per sucker) and the multiple-species method (one nematode species per root, three roots per species and four species per sucker) using the plantain 'Agbagba' (n=18).**

	Fresh weight of 8 cm-long root segments (g)	Root necrosis index (RNI)	Nematode density (per 5 g roots)	Nematode density (per 5 g roots)*
Single-species method	7.18 <sup>†</sup>	33.75 <sup>†</sup>	5.65 <sup>†</sup>	9.72 <sup>†</sup>
Multiple-species method	3.60 <sup>†</sup>	6.45 <sup>†</sup>	1.23	3.82 <sup>†</sup>

\*analysis using log<sub>10</sub> (x+1) transformed data

<sup>†</sup>Differences between the repeat experiments are statistically significant at P≤0.05

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## The effect of oxidative stress on 'Berangan' and 'Mas' cultivars

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In higher plants, excess production of reactive oxygen species (ROS), such as hydrogen peroxide and hydroxyl radicals, is an intrinsic feature of stress metabolism under various abiotic stresses. An inadequate removal of ROS often leads to oxidative stress, which is characterized by the deleterious reactions of ROS with biologically important macromolecules, such as proteins, lipids and DNA, that may lead to cell damage (Inze and Van Montagu 1995).

Studies on various crop species have revealed that stress-tolerant plants are usually endowed with efficient antioxidant defence systems (Jagtap and Bhargava 1995, Sairam *et al.* 1998). Transgenic plants overproducing antioxidant enzymes, *e. g.* superoxide dismutase and glutathione reductase, have also been associated with enhanced stress tolerance (Allen *et al.* 1997, Aono *et al.* 1995). The objective of this work was to document the tolerance of banana plants to oxidative stress, a little-studied topic.

The cultivars used were 'Berangan' (AAA) and 'Mas' (AA), two of the main banana cultivars in Malaysia. 'Mas' is the most popular dessert variety with an annual per capita consumption of 2.7 kg. 'Berangan' is the third most popular cultivar at 0.5 kg per person per year but is Malaysia's most exported dessert banana (Rohizad 1999).

### Materials and methods

Micropropagated plantlets of 'Berangan' and 'Mas' were prepared according to Novak *et al.* (1985), with minor modifications. Sword suckers were the source of shoot tips used in culture initiation. Healthy suckers were collected from a field situated approximately 600 m from the laboratory at Universiti Putra Malaysia. Collected suckers were promptly transported to the laboratory by motorcycle, a five-minute journey.

For the preparation of culture initiation media, Murashige and Skoog (1962) basal medium was supplemented with thiamine 1 mg/L, inositol 100 mg/L, sucrose 30 g/L, 10  $\mu$ M 6-benzyl aminopurine (BAP) and 5  $\mu$ M indole-3-acetic acid (IAA). The culture

medium was solidified with agar 5 g/L and the pH adjusted to 5.8 prior to autoclaving. The multiplication medium was similar to the culture initiation media except for the addition of 20  $\mu$ M of BAP and the exclusion of IAA. The rooting medium was like the culture initiation medium minus BAP. Cultures on semisolid media were grown at  $25 \pm 2^\circ\text{C}$  under a 12h:12h light/dark photoperiod and a photosynthetic photon flux density of 65  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Cultures on liquid media were placed on an orbital shaker (50 rpm) and incubated at  $25 \pm 2^\circ\text{C}$  under a 12h:12h light/dark photoperiod and a photosynthetic photon flux density of 20  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

For culture initiation, shoot tips from both cultivars were grown on the culture initiation medium for three weeks. Initiated cultures were then transferred to the multiplication medium and sub-cultured every three weeks. The shoots were then subjected to two four-week passages on the rooting medium.

To induce oxidative stress, uniform plantlets (with three fully expanded leaves and the roots trimmed off) were treated with 10 ml of a paraquat solution (methyl viologen, catalog No. M-2254, Sigma) at 10, 20 and 40  $\mu$ M. Paraquat is known to induce oxidative stress in plant cells by enhancing the production of superoxide radicals in the chloroplast (McKersie and Leshem 1994). The control was sterilized deionized water. The plantlets were kept on an orbital shaker (50 rpm) and incubated at  $25 \pm 2^\circ\text{C}$  under a 12h:12h light/dark photoperiod at a photosynthetic photon flux density of 20  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . After 24 hours, the third leaf of each plantlet was used for biochemical analyses.

Malondialdehyde (MDA) concentration and relative electrolyte leakage were measured to compare the oxidative stress tolerance of the cultivars. MDA concentration was determined as described in Chai *et al.* (1999). Relative electrolyte leakage reflects the extent of cell membrane permeability. The assumption is that the disruption and leakiness of the plasma membrane will lead to increased leakage of cytoplasmic solutes

into the aqueous medium in which a leaf tissue is immersed (Prasil and Zamecnik 1998). Relative electrolyte leakage in leaf pieces (1 cm x 0.5 cm) was determined according to Kraus and Fletcher (1994). The leaf pieces were placed in test tubes containing deionised water for 24 hours, after which conductivity ( $c_1$ ) was measured. The tubes were then placed in boiling water for 20 minutes, after which conductivity ( $c_2$ ) was measured. Relative electrolyte leakage is the proportion of  $c_1$  over  $c_2$ .

The roles of some enzymatic antioxidants known to confer tolerance to oxidative stress were also investigated. Superoxide dismutase (SOD) is a metal-containing enzyme that eliminates superoxide radicals in plant cells (Inze and Van Montagu 1995). SOD activity is a measure of the enzyme's ability to inhibit the reduction of nitro blue tetrazolium (NBT) by superoxide radicals (Beauchamp and Fridovich 1971). Leaf tissues were homogenized using 50 mM potassium phosphate buffer (pH 7.0) containing 1% polyvinylpyrrolidone and SOD activity was measured in the supernatant of the centrifuged homogenate. One unit of SOD activity is equivalent to a 50% decline in the control rate of NBT reduction. The control rate of NBT reduction was established by replacing the supernatant with an equal amount of 50 mM potassium phosphate buffer (pH 7.8). Total protein content in the supernatant was determined according to the method described in Bradford (1976).

Ascorbate peroxidase (APX) is considered the most important hydrogen peroxide scavenging enzyme in the cytosol and chloroplast of plant cells (Inze and Van Montagu 1995). APX activity is a measure of the enzyme's ability to oxidize ascorbic acid in the presence of hydrogen peroxide (Nakano and Asada 1980). Leaf tissues were homogenized using 50 mM potassium phosphate buffer (pH 7.0) containing 1% polyvinylpyrrolidone and 1 mM ascorbic acid. APX activity was measured in the supernatant of the centrifuged homogenate. The total protein content in the supernatant was determined according to the method described in Bradford (1976).

Glutathione reductase (GR) catalyses the reduction of oxidized glutathione (GSSG) to form reduced glutathione (GSH), an important cellular antioxidant (McKersie and Leshem 1994). GR activity is a measure of

the enzyme's ability to oxidize NADPH with the addition of GSSG (Hodges *et al.* 1997). Leaf tissues were homogenized using 50 mM potassium phosphate buffer (pH 7.0) containing 1% polyvinylpyrrolidone and 0.01 mM EDTA. GR activity was measured in the supernatant of the centrifuged homogenate. The total protein content in the supernatant was determined according to the method described in Bradford (1976).

Catalase (CAT) is a peroxisomal enzyme that eliminates hydrogen peroxide (Inze and Van Montagu 1995). CAT activity is a measure of the enzyme's ability to decompose hydrogen peroxide (Fadzilla *et al.* 1997). Leaf tissues were homogenized using 50 mM potassium phosphate buffer (pH 7.0). CAT activity was measured in the supernatant of the centrifuged homogenate. The total protein content in the supernatant was determined according to the method described in Bradford (1976).

The results are presented as means and standard errors of four replications and Student's *t*-test was used to evaluate differences between treatments and cultivars.

## Results and discussion

Paraquat increased the concentration of MDA in the leaf cells of 'Berangan' and 'Mas' plantlets (Table 1). MDA, a breakdown product of membrane lipid peroxidation, is considered a marker of oxidative damage (Zhang and Kirkham 1996), and its increased concentration indicates the successful induction of oxidative stress. The higher levels of MDA in 'Mas', compared to 'Berangan', also indicate that 'Berangan' is more tolerant to oxidative injury.

Despite increased levels of lipid peroxidation in the 10  $\mu$ M and 20  $\mu$ M paraquat-treated 'Berangan' plantlets and in the 10  $\mu$ M paraquat-treated 'Mas' plantlets, relative electrolyte leakage was not significantly different in these treatments (Table 1). The increased MDA concentrations observed in these plantlets may be accounted largely by enhanced lipid peroxidation inside the leaf cells. However, in the 20  $\mu$ M and 40  $\mu$ M paraquat-treated 'Mas' plantlets, in which significant increases in MDA concentrations were accompanied by significant increases in relative electrolyte leakage, the loss of integrity of the plasma membrane suggests the spread of lipid peroxidation from the

**Table 1. Concentration of malondialdehyde (MDA) and relative electrolyte leakage in the leaf cells of 'Berangan' and 'Mas' after a 24-hour exposure to different concentrations of paraquat (n= 4).**

Paraquat concentration ( $\mu\text{M}$ )	Malondialdehyde (nmole/g fresh weight)		Relative electrolyte leakage (%)	
	Berangan	Mas	Berangan	Mas
0	10.4 $\pm$ 0.8 <sup>a</sup>	16.6 $\pm$ 0.6 <sup>**a</sup>	7.0 $\pm$ 0.2 <sup>a</sup>	7.1 $\pm$ 0.1 <sup>a</sup>
10	15.7 $\pm$ 0.9 <sup>b</sup>	22.8 $\pm$ 2.2 <sup>**b</sup>	7.1 $\pm$ 0.2 <sup>a</sup>	7.1 $\pm$ 0.3 <sup>a</sup>
20	22.7 $\pm$ 1.4 <sup>c</sup>	29.4 $\pm$ 1.9 <sup>**c</sup>	7.0 $\pm$ 0.2 <sup>a</sup>	8.7 $\pm$ 0.4 <sup>**b</sup>
40	17.2 $\pm$ 0.7 <sup>b</sup>	25.6 $\pm$ 1.3 <sup>**bc</sup>	5.8 $\pm$ 0.1 <sup>b</sup>	7.9 $\pm$ 0.3 <sup>**b</sup>

In each column, means followed by the same letter are not significantly different at  $P < 0.05$  according to Student's t-test. In each row, significant differences at  $P < 0.05$  according to Student's t-test are indicated by a different number of asterisks.

cellular components, such as chloroplasts, to the plasma membrane.

The significantly lower relative electrolyte leakage measured in 'Berangan' plantlets in the 20  $\mu\text{M}$  and 40  $\mu\text{M}$  paraquat treatments, compared to the one for 'Mas' plantlets, indicates that the plasma membrane of the former was less disrupted, in keeping with the observation that 'Berangan' is more tolerant to oxidative stress.

SOD activity was significantly higher in 'Berangan' than in 'Mas' plantlets (Table 2), indicating a greater capacity of 'Berangan' to eliminate superoxide radicals. Our results agree with previous observations that enhanced SOD activity is associated with increased protection against oxidative damage (Van Camp *et al.* 1996, Sen Gupta *et al.* 1993).

The APX activity in stressed 'Berangan' plantlets was significantly higher than the one in the control group, whereas it was either unchanged or reduced in the stressed

'Mas' plantlets. (Table 2). With regards to differences between the cultivars, APX activity was higher in 'Berangan', suggesting that it was better than 'Mas' at detoxifying hydrogen peroxide.

In 'Berangan', higher APX activity was clearly associated with greater protection against oxidative injury. On the other hand, the reduced and unchanged APX activity in 20  $\mu\text{M}$  and 40  $\mu\text{M}$  paraquat-treated 'Mas' may have favoured an accumulation of hydrogen peroxide in the leaf cells, which in turn resulted in the reduced SOD activity observed at these concentrations. According to Casano *et al.* (1997), SOD activity can be inhibited by hydrogen peroxide. Effective scavenging action and conservation of SOD activity depends in part on the activity of the hydrogen peroxide removal system in plant cells.

The GR activity measured in 'Berangan' plantlets was significantly higher than the one measured in 'Mas' plantlets (Table 3).

**Table 2. Activity of superoxide dismutase (SOD) and ascorbate peroxidase (APX) in the leaf cells of 'Berangan' and 'Mas' after a 24-hour exposure to different concentrations of paraquat (n= 4).**

Paraquat concentration ( $\mu\text{M}$ )	Superoxide dismutase (unit of activity/mg of protein in an hour/mg of protein)		Ascorbate peroxidase ( $\mu\text{mole}$ of ascorbate oxidized)	
	Berangan	Mas	Berangan	Mas
0	180.4 $\pm$ 15.9 <sup>a</sup>	95.4 $\pm$ 4.4 <sup>**a</sup>	233.3 $\pm$ 4.4 <sup>a</sup>	211.36 $\pm$ 7.6 <sup>**a</sup>
10	202.6 $\pm$ 14.6 <sup>a</sup>	123.2 $\pm$ 2.0 <sup>**b</sup>	310.8 $\pm$ 24.3 <sup>b</sup>	220.1 $\pm$ 8.3 <sup>**a</sup>
20	218.7 $\pm$ 20.2 <sup>a</sup>	80.3 $\pm$ 1.8 <sup>**c</sup>	273.0 $\pm$ 14.2 <sup>b</sup>	191.0 $\pm$ 2.2 <sup>**b</sup>
40	272.5 $\pm$ 13.0 <sup>b</sup>	67.2 $\pm$ 2.1 <sup>**d</sup>	295.5 $\pm$ 21.2 <sup>b</sup>	194.8 $\pm$ 16.2 <sup>**ab</sup>

In each column, means followed by the same letter are not significantly different at  $P < 0.05$  according to Student's t-test. In each row, significant differences at  $P < 0.05$  according to Student's t-test are indicated by a different number of asterisks.

**Table 3. Activity of glutathione reductase (GR) and catalase (CAT) in the leaf cells of 'Berangan' and 'Mas' after a 24-hour exposure to different concentrations of paraquat (n= 4).**

Paraquat concentration ( $\mu\text{M}$ )	Glutathione reductase ( $\mu\text{mole}$ of NADPH oxidized in an hour/mg of protein)		Catalase ( $\mu\text{mole}$ of $\text{H}_2\text{O}_2$ consumed in a minute/mg of protein)	
	Berangan	Mas	Berangan	Mas
0	3.1 $\pm$ 0.1 <sup>a</sup>	2.6 $\pm$ 0.1 <sup>**a</sup>	28.2 $\pm$ 4.5 <sup>a</sup>	57.8 $\pm$ 4.8 <sup>**a</sup>
10	2.8 $\pm$ 0.1 <sup>a</sup>	2.1 $\pm$ 0.1 <sup>**b</sup>	25.5 $\pm$ 2.7 <sup>ab</sup>	35.0 $\pm$ 0.9 <sup>**b</sup>
20	3.8 $\pm$ 0.3 <sup>b</sup>	2.6 $\pm$ 0.1 <sup>**a</sup>	31.0 $\pm$ 4.8 <sup>a</sup>	58.2 $\pm$ 2.5 <sup>**a</sup>
40	3.9 $\pm$ 0.1 <sup>b</sup>	3.3 $\pm$ 0.1 <sup>**c</sup>	16.6 $\pm$ 0.9 <sup>b</sup>	75.6 $\pm$ 2.5 <sup>**c</sup>

In each column, means followed by the same letter are not significantly different at  $P < 0.05$  according to Student's t-test. In each row, significant differences at  $P < 0.05$  according to Student's t-test are indicated by a different number of asterisks.

In transgenic plants modified to overexpress GR, a positive correlation has been observed between increased GR activity and tolerance to paraquat-induced oxidative stress (Allen *et al.* 1997).

On the other hand, CAT activity was lower in 'Berangan' than in 'Mas' (Table 3) even though 'Berangan' was better protected against paraquat-induced oxidative stress than 'Mas'. Our results show that higher CAT activity was not associated with lower MDA concentrations or lower relative electrolyte leakage. Since paraquat initiates oxidative stress in the chloroplast (McKersie and Leshem 1994), it is possible that the compartmentalization of catalase in peroxisomes may have limited the enzyme's role in curbing hydrogen peroxide production in the stressed plants.

Our results demonstrate that 'Berangan' is more tolerant to oxidative stress than 'Mas', as reflected in the higher SOD, APX and GR activities. Further investigations in the laboratory and under field conditions are needed to confirm the contribution of these enzymes to tolerance. It would be interesting to find out whether a greater antioxidant capacity correlates with a higher survival or growth when banana plants are exposed to a stress.

Transgenic alfalfa modified to overproduce SOD was less affected by water deficit and freezing temperatures under field conditions (McKersie *et al.* 1996, McKersie *et al.* 1999). It is possible that enhancing the antioxidant defence system through genetic manipulation could produce more tolerant plants. Based on our results, we propose APX, SOD and GR as antioxidant enzymes that deserve attention in research programmes trying to engineer abiotic stress tolerance in banana cultivars.

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## Street children turned banana farmers

*I. Van den Bergh, M.A.G. Maghuyop, K.H. Borromeo, V.N. Roa and A.B. Molina*

In February 2003, the INIBAP regional office for Asia and the Pacific was approached by a Belgian volunteer working for the Virlanie Foundation, a French NGO caring for some 300 Filipino street children at 11 homes in Manila and one farm in Balayan, a two-hour drive from Manila. The Foundation was seeking INIBAP's support for its *Buhay Kalikasan* (Living with Nature) programme in which its charges in the countryside are being introduced to the basics of farming.

After visiting the farm in Balayan, INIBAP agreed to provide the budding farmers with clean plantlets of three banana hybrids (FHIA-18, FHIA-23 and FHIA-25) and two local favourites ('Lakatan' and 'Bungolan'). In addition, the project leader Telesforo J. Caminsi, the agronomist Eddie Ynion and four of the young adults attended one of INIBAP's hands-on trainings on nursery and field management of tissue-culture plantlets (Figure 1).

After the training, the place was prepared for the arrival of the tissue-culture plantlets

in May 2003. The young men converted the skeleton of an old building into a screenhouse in which to grow the small plantlets until they could safely be planted in the field in August.

Two years later, the barren patch of land had been transformed into a lush banana garden. As far as the eyes can see there are healthy banana plants bearing heavy bunches (Figure 2).

The metamorphosis has not gone unnoticed by the local farmers who, at first, were very skeptical about the project. Balayan lies in an area that was renowned for its bananas until the late 1990s, when production was abandoned because of the rapid spread of the *Banana bunchy top virus*



I. Van den Berg/INIBAP

Figure 1. Participants in the INIBAP training on the management of banana plantlets (from right to left, the Virlanie project agronomist, Eddie Ynion, the Philippines government scientist, Edna Anit, and the project leader Telesforo J. Caminsi (second from left) with former street children).



I. Van den Berg/INIBAP

Figure 2. Eddie Ynion and Maria Angeli Maghuyop of INIBAP discussing field management in the shade of 'Lakatan' banana plants.

(BBTV). The farmers had problems believing that fragile-looking plantlets would succeed where suckers, the traditional planting material, had failed. But clean planting material that had been checked free of the virus is exactly what was needed to start afresh.

The disease is still occasionally observed. When this happens, the boys immediately get rid of the diseased plants, as they were told to do, and that simple gesture helps to keep the disease under control. Having seen what good management and clean planting material can do, many farmers have expressed interest in buying tissue-culture plantlets.

The bananas are benefiting everybody at Virlanie. The homes in Manila buy their bananas from the farm in Balayan at 25% less than the market price, which is fine with the young farmers who like having a regular and reliable buyer for their bananas. In turn, the city kids get a steady supply of a healthy and nourishing food.

The 'Lakatan', with its sweet taste, is still the local favourite. The introduced FHIA-23, an hybrid related to the 'Gros Michel' banana, comes second, except among the foreign volunteers working at Virlanie who prefer it to

the other varieties. Another attractive feature of FHIA-23 is its huge bunch. FHIA-18, a cooking banana, is eaten as a snack, either boiled or fried. As part of the project, the boys will be trained by the Cavite State University on how to process FHIA-18 bananas into chips.

The project is proving a success not only with the young Virlanie farmers (Figure 3), who want to try other new hybrids, but with the local farmers who want to get back into banana production.



Figure 3. Former street children enjoying a well-deserved banana after a day's work on the Virlanie farm in Balayan.

I. Van den Berg/INBAP

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## Safeguarding banana diversity

A new effort is under way to promote the use of *Musa* diversity in the form of a global strategy to conserve banana and plantain.

Existing improvement programmes only use a fraction of the genetic diversity concealed in wild and edible *Musa* species. For instance, the ecology of various wild species suggest that sources of resistance to abiotic stresses exist in *Eumusa* along the northern periphery of its distribution, including mechanisms for tolerance to cold (*Musa sikkimensis*, *Musa basjoo*, *Musa thomsonii*), water-logging (*Musa itinerans*), and drought (*Musa balbisiana*, *Musa nagensium*). Recent collecting expeditions in northern India and Malaysia suggest that other poorly known or unexplored areas of diversity are likely to harbour other

agronomically-interesting characteristics. In addition, the development of powerful molecular tools by initiatives such as the Global *Musa* Genomics Consortium provides an unprecedented opportunity to use the diversity available in wild and cultivated *Musa*.

The vast majority of *Musa* diversity, at least in cultivated form, is being conserved in the 60 or so *Musa*-dedicated national collections. More than 6000 accessions are held in field collections and a further 3000 are kept as plantlets in test tubes (*in vitro* collections). The global collection at the INIBAP Transit Centre (ITC), which is hosted by the *Katholieke Universiteit Leuven* in Belgium, holds nearly 1200 accessions as plantlets and is in the process of rejuvenating and cryopreserving the entire collection. A

Focus on *Musa* conservation

Breeders need the genetic diversity being conserved in genebanks to develop cultivars that can withstand biotic and abiotic stresses, such as drought.



R. Markham/INBAP

new collection of lyophilised leaves is also being developed to supply samples for molecular research.

At a global level, the ITC assures cost-effective long-term conservation and provides limited samples of a large range of germplasm that are guaranteed clean of pests and diseases. However, there is no recognised network of collaborating national or regional collections. Instead there are numerous national collections, of which a number are notable for the richness of their collection or for the research, expertise, services or capacity building that they provide. Networking fora exist in the form of the regional banana and plantain networks and ProMusa, and the *Musa* Germplasm Information System (MGIS) provides a model for information exchange. Within the present system, however, the germplasm needs at a regional or national level are not being met. Numerous national collections, particularly those which are poorly-resourced in Africa and in Asia and the Pacific, are functioning less than optimally: a significant number of accessions are diseased or being lost from the collections, germplasm exchange mechanisms are inadequate and the user community is not as well served as it might be.

In a 2005 survey of 28 responding collections, 62% said that part of their collection (10-25% or more) was deteriorating because of management limitations; 69% declared that existing

skilled staff capacity was insufficient for long-term conservation needs. When asked what their additional human resource requirements were, more than half of the collections specified the need for technical support in characterization; approximately a third asked for support in the general management of their field and/or *in vitro* collections. Related to this is the problem of lack of use of the collections, exemplified by the fact that 70% of accessions in the ITC have never been requested and remain unused. Even though the entire collection has been virus-indexed, the demand for germplasm has not increased. Diversity is demanded by researchers and growers and yet many national collections and large parts of major collections are under-utilized. As long as diversity remains underused the management of and investment in the collections is likely to be compromised.

Taxonomic experts, breeders and researchers attribute a large part of the problem of under-utilization to inadequate information. Many *Musa* collections, including the ITC collection, have not been systematically documented; only limited characterization and evaluation data are available, and information may be scattered between several institutes. The IPGRI - INIBAP/CIRAD descriptors for *Musa* are often ineffectively applied where curators are working in isolation with little training. According to the survey, an average collection is 45% described using either the full or a partial

set of the descriptors for *Musa*. While the International *Musa* Testing Programme has made progress evaluating elite *Musa* varieties in multiple sites, the quality of data provided both in response to the survey and to MGIS illustrates that some characterization efforts do not meet scientific standards.

Despite the constraints identified in the survey, the *Musa* community is served by several important active collections and resources. It is on these strengths that the Global Conservation Strategy for *Musa*, is being developed under the coordination of INIBAP. The project was initiated as a response to a request from the Global Crop Diversity Trust (GCDT), a newly-launched endowment fund managed by the FAO and the Consultative Group on International Agricultural Research to support the long-term conservation of crops on Annex 1 of the International Treaty for Plant Genetic Resources for Food and Agriculture. The initiation of the strategy development process is providing the impetus to coordinate efforts and bring experts from different disciplines, including molecular biologists

and taxonomists, as well as members of regional networks to consider what are the constraints in *Musa* conservation and how we can overcome them. The current working model (Figure 1) takes into account the global collection at the ITC, and the role of several "internationally-recognised" collections that provide various services to both the ITC and the national collections.

It is also recognised that the success of the strategy depends on the genuine collaboration of a wide range of national collections; that these collections gain clear benefits from being involved, and that a parallel investment is provided at a national level. Rather than providing support to the individual needs of each collection, a global initiative provides the opportunity to use centralized resources to resolve shared problems and specific bottlenecks in the system.

Currently the draft strategy is making the rounds of the regional networks with the aim of identifying priority collections for support by the GCDT and a detailed plan of action.

For more information, contact Charlotte Lusty at [c.lusty@cgiar.org](mailto:c.lusty@cgiar.org).

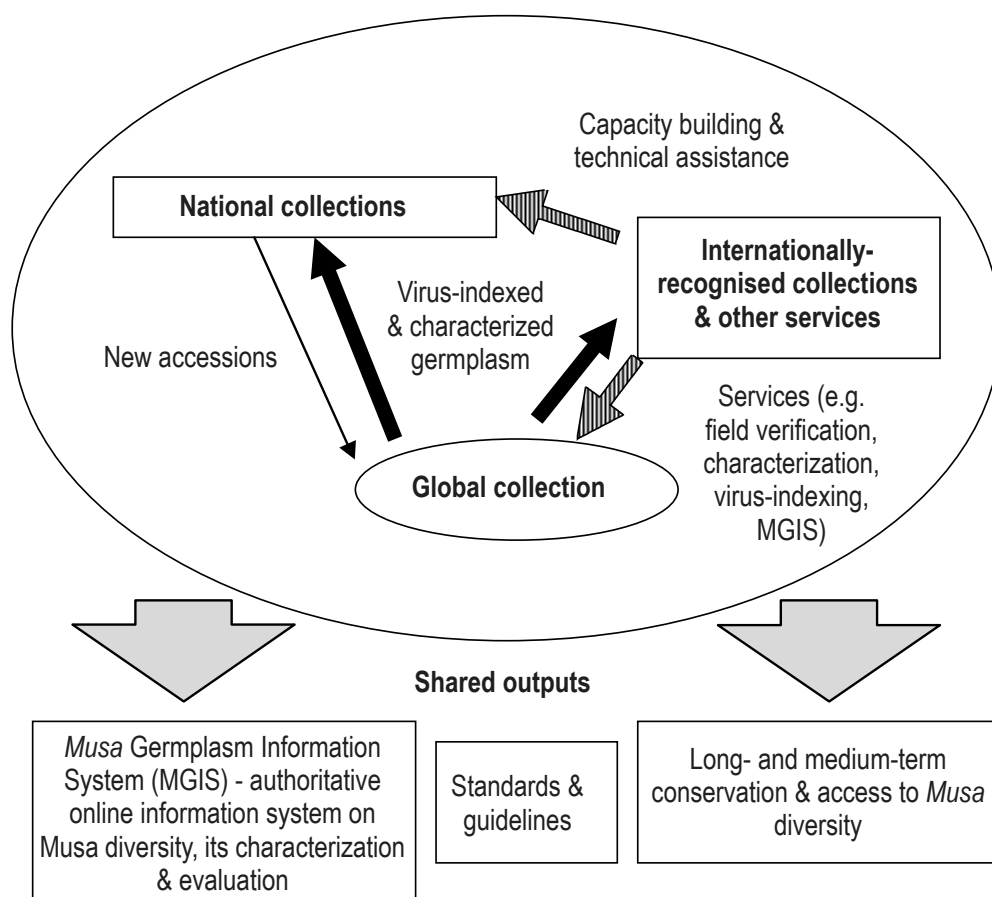


Figure 1. Schematic representation of the Global Musa Conservation Strategy.

## Model-assisted design of sustainable production systems: an application to banana plantations in Guadeloupe

Philippe Tixier

### PhD thesis submitted in December 2004 to Agro-Montpellier, France

Banana production in the Caribbean is currently facing problems that threaten the sustainability of the commodity chain: low yields due to diseases, leaching of pesticides and soil to surface water exacerbated by the context of a fragile island ecology, and economic problems linked to variations in the retail price of the fruit and high labour costs. In this context, innovative production systems that address environmental and economic problems must be developed. Many avenues aiming to integrate fallow periods, crop rotation or associated plants are being explored.

The design and evaluation of such innovative crop systems require the use of specific modelling tools that take into account the particular characteristics of the production system. To that end we developed a model called SIMBA. SIMBA simulates the evolution of the structure of a banana plantation over crop cycles, a key element that conditions the dynamics of the system. The parasitism component, which affects the sustainability of the plantation and

requires the use of phytosanitary products, is also taken into account. The parasitism of phytonematodes is simulated in interaction with the growth and structure of the plantation, the state of the soil and the use of nematicides. SIMBA also simulates plant growth, productivity, soil structure and cover, and water balance. Coupled to biophysical modules, the qualitative and integrated indicators developed for this system are used to evaluate environmental risks, such as water pollution by phytosanitary products and erosion. Cultural practices are taken into account through decision rules which can then be assessed. By supplying agronomic, environmental and economic outputs (gross margin), the SIMBA model, enables multicriteria evaluations of production systems from many points of view.

SIMBA was then piloted through an original two-step method (global exploration and specific optimization). The results allowed us to identify production systems that could be field-tested. This systemic and functional approach, which has resulted in advances in the modelling of banana production systems, is a powerful tool to help design sustainable production systems.

## Study on post-harvest fruit rots of banana and their control

Omar Ibn-i-Hassan

### PhD thesis submitted in 2004 to Bangladesh Agricultural University, Mymensingh, Bangladesh

Post-harvest handling of banana in Bangladesh was studied. Harvesting procedure, latex removal, transport system and ripening procedure of three varieties of banana, 'Sabri', 'Amrita sagar' and 'Chinichampa' were recorded in 49 locations of Bangladesh between July 1999 and December 2000. A total of 5 658 207 bunches of 579 566 633 fingers were examined. Faulty harvesting procedures, loading and unloading, and vibration and compression during transport caused cracks, cuts and blemishes.

*Botryodiplodia theobromae*\* was consistently isolated from the bananas affected by finger rot, stem-end rot, distal end rot and *Colletotrichum gloeosporioides* from fruits showing symptoms of anthracnose. Wounding on the fruit surface accelerated infection. Biochemical analyses indicated that the nutritional quality of the three varieties decreased due to rot diseases. Market losses attributed to injuries and fruit rots were respectively estimated at US\$ 1.5 million and US\$10.5 million. Application of Bavistin (1000 ppm) and Tilt (2000 ppm) prior to infection proved effective in preventing development of fruit rots of banana.

\*Renamed *Lasiodiplodia theobromae*

## Molecular biology and diagnosis of *Banana bunchy top virus* and its management through induced systemic resistance

S. Harish

**PhD Thesis submitted in 2004 to the Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India**

Banana bunchy top disease, caused by *Banana bunchy top virus* (BBTV), is the most important and devastating banana disease in many tropical countries. It is transmitted by the aphid *Pentalonia nigronervosa*. Once established, the disease is very difficult to eradicate. In the present study, BBTV isolates associated with 'Virupakshi' (AAB), 'Poovan' (AAB), 'Malaipoovan' (AAB) and 'Robusta' (AAA) were collected from different places in Tamil Nadu. Under greenhouse conditions, the isolates expressed various symptoms such as vein clearing, green streaks, leaf atrophy and bunchy top.

The virus was purified from infected banana plants and the virus concentration in the midrib was estimated to be 0.57 mg/kg of tissue. Polyclonal antiserum against BBTV produced in New Zealand white rabbit was used for serological detection of the virus. Western blot analysis revealed the presence of 20kDa coat protein in the infected samples. Immunocapture PCR was used for the detection of BBTV in infected samples. Restriction digestion of the replicase protein gene with *AluI* indicated that there was no

significant variation between the isolates. Amino acid and nucleotide sequences were aligned by using CLUSTAL X 1.81. The multiple sequences aligning with the sequences from GenBank revealed that some of the isolates of BBTV collected from lower Pulney hills, Western Ghats, are homologous to some Indian and Egyptian isolates.

Forty endophytic bacteria were isolated from banana corms. They were divided into two broad clusters: *Pseudomonas* and *Bacillus* spp. using phenotypic and molecular characterization. Most *Bacillus* spp. produced fingerprinting patterns different from that of *Pseudomonas* spp. and within a genus, no significant differences in the protein pattern were seen, except for the RAPD banding pattern.

The efficacy of endophytic bacteria against BBTV was tested in a pot trial using 'Robusta' (AAA) banana plants and in field trials using suckers and tissue culture plantlets of 'Virupakshi' (AAB) that had been exposed to endophytic bacteria at hardening, transplanting, and 3, 5 and 7 months after planting. Various combinations of endophytic bacteria resulted in significant reductions of BBTV.

## Molecular approaches for the management of *Banana bunchy top virus* through induced systemic resistance in banana

M. Kavino

**PhD Thesis submitted in November 2004 to the Faculty of Horticulture, Tamil Nadu Agricultural University, Coimbatore, India**

In India, the *Banana bunchy top virus* (BBTV) has been reported in all the banana-growing states, with the most severe

infestations observed in the lower Pulney hills of Dindugal district in Tamil Nadu. In this region 'Virupakshi' (AAB), a prized fruit that was once grown on more than 18 000 hectares as a rainfed perennial crop, has been devastated by bunchy top and the area reduced to 2000 hectares. The current

research was undertaken to assess the effect of induced systemic resistance against BBTv in 'Virupakshi' and 'Robusta' (AAA).

A survey was conducted in Dindugal district of Tamil Nadu to collect apparently healthy 'Virupakshi'. The collected plants were examined for the presence of the virus by using ELISA and RAPD analysis. The plants testing negative with ELISA were micropropagated. The 40 plants produced were tested for the presence of the virus. A field experiment was conducted in an area of Tamil Nadu infested with BBTv, to test the efficacy of various bioformulations against BBTv on 'Virupakshi'. The experiment was laid out in a randomized block design with eight treatments replicated thrice. In each treatment, there were 20 plants per replication spaced at 2 m x 2 m. Two strains of *Pseudomonas fluorescens* (Pf1 and CHA0) were used in this study, with or without chitin, at different stages of crop

growth: at the time of planting, and at 3, 5 and 7 months after planting. Another field trial was conducted with tissue-culture plants of 'Virupakshi' in order to test the efficacy of mixture of *Pseudomonas* strains (Pf1, CHA0 and EPB22). These strains were applied at the primary and secondary hardening stages, transplanting, and 3, 5 and 7 months after planting.

The *P. fluorescens* strain CHA0, applied with chitin at planting, and 3, 5 and 7 months after planting, increased pseudostem height, girth, number of leaves and leaf area, and reduced the incidence of BBTv in the greenhouse and in the field. Application of a mixture of strains in tissue-culture plants of 'Virupakshi' at hardening, transplanting, and 3, 5 and 7 months after planting also improved agronomic performance and reduced disease incidence in the greenhouse and in the field.

## Molecular detection and characterization of bacterial contaminants in tissue-culture banana (*Musa* spp.)

K.G. Wasmund

**BSc (Honours) thesis submitted in 2004 to the University of the Sunshine Coast, Queensland, Australia**

Bacterial contamination of *Musa* tissue culture is a significant and widespread problem. Problematic bacterial contaminants are thought to be derived from within the initiating explant. This study aimed to verify whether endophytic bacteria residing within the initiating explant can be a source of problematic bacteria, and to develop and assess a culture-independent, PCR-based method for the routine detection of these bacteria.

Bacteria were isolated and identified by partial 16S rDNA sequence analysis from 6 of the 72 explants used to initiate tissue culture. These were identified as a *Klebsiella* sp., a *Herbaspirillum* sp., an *Agrobacterium* sp., and a bacterium belonging to Enterobacteriaceae, all renowned endophytic bacteria. The *Herbaspirillum* sp. and *Agrobacterium* sp. were re-isolated from the resulting

tissue-culture plantlets, and all the cultures derived from the contaminated explants became contaminated after a period of latent growth. *Paenibacillus* sp. was also isolated from tissue-culture plantlets that became visibly contaminated after over 12 months of apparently axenic growth.

A method was successfully developed for the detection of a broad range of bacteria in *Musa* tissue that was not complicated by the co-amplification of plant-derived plastid 16S rDNA. A control detection limit of approximately  $1 \times 10^5$  *Escherichia coli* cells extracted (equivalent to approximately  $7 \times 10^3$  16S rDNA copies per PCR) was determined. PCR parameters such as the cycle number, MgCl<sub>2</sub> concentration, and the annealing temperature were optimized. The broad-range applicability of this method found contaminating bacterial DNA sourced from Taq DNA polymerase and exogenous aerosols a significant problem.

This work has provided direct evidence that endophytes residing within

initiating explants can be a source of problematic bacterial contaminants in tissue-culture *Musa* plantlets, and that a culture-independent detection method is warranted. However, in order to develop a

sensitive and reliable PCR-based method for the detection of these bacteria, the PCR should target specific groups of bacteria, as the broad-range method developed in this study is extremely prone to contamination.

## Optimization of *in vitro* multiple meristem cultures and embryogenic cell suspensions in banana (*Musa* spp.)

Hannelore Strosse

**PhD thesis submitted in May 2005 to the Faculty of Bioscience Engineering, Katholieke Universiteit Leuven, Belgium**

Bananas and plantains (*Musa* spp.) are prone to many pests and diseases causing serious yield declines. Biotechnology offers breeders important tools to accelerate the production of improved varieties. Highly regenerable and rapidly multiplying shoot tip cultures – also called multiple meristem cultures (MMC) – are extremely valuable for various biotechnological applications on bananas. The development of high quality MMC corresponds to the first crucial *in vitro* phase towards the successful establishment of embryogenic cell suspensions (ECS) via the 'scalp method'. High quality ECS are the most suitable target material for genetic engineering in banana.

In the first part of our study, a new method was developed to derive high quality MMC. Besides the variation in size of the initial explants (shoots excised from *in vitro* rooted plants), the major part of this research consisted in evaluating the most suitable combination of plant growth regulators (PGR). Reduction of the initial shoot length (from 1.5 cm to 0.5 cm) and the use of 10  $\mu$ M thidiazuron (TDZ) as PGR (selected from 242 cytokinin/auxin combinations) resulted in (i) an equal or higher proportion of meristematic tissue as opposed to more differentiated corm and leaf tissue and in (ii) a reduction of the time required to obtain MMC (from 1 to 6 months depending on the variety). This in combination with a comparative study on the *in vitro* behavior of maize and banana, contributed to our knowledge on the origin of multiple shoots in banana. While adventitious shoot formation was achieved relatively easily in many monocots, axillary

shoot proliferation in banana could not be overcome by any of the 242 different PGR treatments tested.

The second part of our study covered the induction of embryogenesis in meristematic tissue ('scalps'), the establishment of ECS and the control of quality. The embryogenic frequency increased 2- to 4-fold when cultures were grown in darkness and when TDZ was used instead of benzylaminopurine (BAP) to prepare the initial explants. Induction of embryogenesis was successfully achieved on 17 of the 22 varieties tested. The average embryogenic frequency among embryogenesis-competent banana types (plantains, Cavendish and cooking bananas) ranged from 1.9% to 18.1%. About one third of the formed embryogenic complexes gave rise to ECS that consisted of more than 75% embryogenic cell clusters. The capacity of tested ECS to form embryos ranged from 36 – 466  $\times 10^3$  embryos/ml settled cells, while the average conversion frequencies of embryos into plants ranged from 8 to 46%. On average, the quality of ECS decreased two years after initiation, even when the suspensions were subcultured regularly and non-regenerable structures were removed. In 5 of the 59 cell suspension lines analysed by flow cytometry, genomic aberrations such as mixoploidy and polyploidy were detected. These genomic aberrations were associated with a drastic reduction in regeneration capacity of cell cultures. However, somaclonal variation is still best evaluated in regenerated plants. Finally, preliminary experiments were performed to explore whether the *in vitro* response of different varieties could be linked to different concentrations of endogenous hormones.

## Early African bananas

According to recent evidence from Uganda, the banana may have arrived on the African continent 4000 years ago, some 2000 years before the accepted introduction of the fruit in the region. The findings are published in the January 2006 issue of the *Journal of Archaeological Science* (Vol. 33(1):102-113). The authors, B. Julius Lejju, Peter Robertshaw and David Taylor, base their claim on banana phytoliths that they found in sedimentary layers

estimated to be between 4000 to 4500 years old. Phytoliths are distinctive microscopic silica bodies that accumulate in plant cells.

Earlier findings in Cameroon of 2500 year-old banana phytoliths (*Vegetation History and Archeobotany* 2001, 10:1-6) had been disputed on the basis that bananas had been brought by traders to eastern Africa some 2000 years ago. The evidence from Uganda brings support to those defending an early start of banana farming on the African continent.

## Wild *Musa* of Vietnam

A recent article by Ramon Valmayor, Le Dinh Danh and Markku Hakkinen published in the June 2005 issue of the *Philippine Agricultural Scientist* (Vol. 88(2):236-244) illustrates and summarizes the distinguishing characteristics of the newly described species of *Musella* indigenous to Vietnam, three new species of wild bananas with ornamental value namely *Musa exotica*, *Musa viridis* and *Musa lutea* and the rediscovery of the very rare and nearly forgotten *Musa splendida*. The authors also describe two new travelling bananas, *Musa tonkinensis* and *Musa itinerans* ssp. *annamica*. Travelling bananas are plants whose long and extended rhizome produces suckers far from the motherplant.

In the 1990s, the Vietnam Agricultural Science Institute launched an intensive banana collection programme which covered most of the country. The banana exploration missions under Director Le Dinh Danh of Phu Ho Fruit Research Center gathered 88 banana cultivars and 19 wild species, 17 of which belong to the genus *Musa*, while the remaining two were classified under *Ensete* and *Musella*. The indigenous *Ensete* of Vietnam was identified as *Ensete glaucun* (Roxb.) Cheeseman. Detailed morphological studies of the indigenous *Musella* specimen of Vietnam showed that it is distinct from *Musella lasiocarpa* Franchet of South China and was described as a new species, *Musella splendida* R. Valmayor and L.D. Danh.

Of the 17 accessions that were identified under the genus *Musa*, 12 were classified as members of the ubiquitous *Musa balbisiana*, *Musa acuminata* and *Musa itinerans*, the remaining five had never been described before. The most attractive and very beautiful indigenous ornamental banana was the first accession formally described as *Musa exotica*. Later, after completion of

morphological studies using the IPGRI-INIBAP/CIRAD Descriptors for Banana (*Musa* spp.) and a thorough review of the scientific literature and herbaria, *M. viridis* and *M. lutea* were described as new species. The two other *Musa* species that lacked descriptions are the subject of this article.

*Musa tonkinensis* can be segregated from other rhizomatous species of *Musa* by its unique male bud. The apex of the male bud is markedly imbricated and the tips of individual bracts are neatly arranged in a beautiful spiral so different from other species of *Musa*. While the external colour of mature bracts are purple with green margins, the young, unexposed bracts are solid yellow. The exposed tips of the imbricated bracts dry up early and turn brown. These unique features serve as diagnostic characters of *M. tonkinensis*.

The morphology of *M. itinerans* ssp. *annamica* is very similar to the common *M. itinerans* but can easily be distinguished by the unique method of bract opening. The bracts twist and curl sideways instead of rolling upwards as is commonly observed in the other species of *Musa*. Other distinguishing characteristics are based on their fruits. The fruits of the subspecies *annamica* are elongated and slightly narrowing towards both ends while those of *itinerans* are short and obovoid, widest near the apex and narrowing gradually towards the pedicel. Ripe fruits of the former species turn brown with cracked peeling while those of the latter turn yellow with its pericarp remaining smooth.

The Latin term for the species *tonkinensis* and subspecies *annamica* were selected to indicate the regions where the original specimens were collected. Tonkin was an ancient empire embracing northern Vietnam and southeastern China while Annam was an old kingdom based on Central Vietnam.

## Instructions to authors

INFOMUSA is an international journal published twice a year in English, French and Spanish. Our focus is to provide an outlet for research results and reports of interest to the *Musa* community. As INFOMUSA publishes articles on any *Musa*-related issue, authors should aim for simple and clear phrases that avoid unnecessary jargon in order to make their paper accessible to readers in other disciplines.

Manuscripts should be prepared in English, French or Spanish and should not exceed 2500 words, including references. They should be double-spaced throughout. All pages (including tables figures, legends and references) should be numbered consecutively.

Include the full name of all the authors of the paper, together with the addresses of the authors at the time of the work reported in the paper. Indicate also the author nominated to receive correspondence regarding the paper.

Manuscripts can be sent as e-mail attachments or put on a 3.5-inch disk for PC-compatible machines. Please indicate the name and version of the word processing software used and the author's e-mail address. In either case, we will need to receive by mail two printed copies of the manuscript.

**Title:** The title should be as short as possible and should not have numbers, acronyms, abbreviations or punctuation.

**Abstract:** An abstract, not exceeding 200-250 words, should be provided. It should concisely summarise the basic contents and should be sent in the same language as the manuscript. Translations (including the title) into the two other languages should also be sent if this is possible.

**Key words:** Provide a maximum of six key words, in alphabetical order, below the native-language abstract.

**Introduction:** The introduction should provide the rationale for the research and any relevant background information. Since it is not meant to be an exhaustive review of the topic, the number of references should be kept to a minimum. Introductions on the importance of bananas as a staple food or a traded commodity should be avoided, unless they are absolutely necessary for the comprehension of the article.

**Materials and methods:** The authors should provide enough details of their experimental design to allow the reader to gauge the validity of the research. For commonly used materials and methods, a simple reference is sufficient.

**Results:** The unit should be separated from the number by a single space and follow SI nomenclature, or the nomenclature common to a particular field. Unusual units or abbreviations should be defined.

Present data in the text, or as a figure, or a table, but never in more than one of these ways. Avoid extensive use of graphs to present data that could be more concisely presented in the text or in a table. Limit photographs to those that are absolutely necessary to show the experimental findings.

**Discussion:** The discussion should not contain extensive repetition of the results section nor should it reiterate the introduction. It can be combined with the results section.

**References:** All references to the literature made in the text should be referred to by author(s) and year of publication (e.g.: Sarah *et al.* 1992, Rowe 1995). References to not widely circulated documents, such as annual reports, and citations of personal communications and of unpublished data should be avoided. A list of references, in alphabetical order, should be provided at the end of the text.

Please follow the style shown below:

*Periodicals:* Sarah J.L., C. Blavignac & M. Boisseau. 1992. Une méthode de laboratoire pour le criblage variétal des bananiers vis-à-vis de la résistance aux nématodes. *Fruits* 47(5):559-564.

*Books:* Stover R.H. & N.W. Simmonds. 1987. *Bananas* (3<sup>rd</sup> edition). Longman, London, United Kingdom.

*Articles (or chapters) in books:* Bakry F. & J.P. Horry. 1994. *Musa* breeding at CIRAD-FLHOR. Pp. 169-175 in *The Improvement and Testing of Musa: a Global Partnership* (D.R. Jones, ed.). INIBAP, Montpellier, France.

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**Recent publications**

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