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 delimitated 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^2 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
Delimiting surveys (April to August 2001)			
Tully	2657	20*	2271
Innisfail	1564	0	1310
Kennedy	244	0	228
Other areas	13	0	12
Eradication programme (September 2001 to April 2002)			
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			Proportion of samples	
	Proportion of properties *	Proportion of area *	Number of samples**	+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). BLSD 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. BLSD 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 BLSD, 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 BLSD during the verification period is a strong indication that BLSD is no longer present in the area and that the eradication programme has been successful. In addition, BLSD 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 BLSD 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 (Calpouzos 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 BLSD.

References

Anon. 2002. North Queensland Banana Production Statistics 2001. Department of Primary Industries, Bananatopics 32:20.

Anon. 2003. Agreed Protocol to demonstrate, Black Sigatoka Area Freedom for the Tully Banana Production Area. Technical Working Group to the Black Sigatoka "Area Freedom" Program, 23pp.

Calpouzos L. 1955. Studies on the Sigatoka disease of banana and its fungus pathogen. Cuba, Atkins Garden and Research Laboratory, 70pp.

Gauhl F. 1994. Epidemiology and ecology of black Sigatoka (*Mycosphaerella fijiensis* Morelet) on plantain and banana (*Musa* spp.) in Costa Rica. PdH thesis. English translation published by INIBAP, Montpellier, France, 120pp.

Henderson J., K. Grice, J.Pattemore, R. Peterson and E. Aitken. 2002. Improved PCR-based detection of Sigatoka disease and black leaf streak disease in Australian banana crops. Pp. 59-64 *in Mycosphaerella* leaf spot diseases of banana: present status and outlook. San Jose, Costa Rica 20-23 May 2002. INIBAP, Montpellier, France.

Jones D. and J. Alcorn. 1982. Freckle and black Sigatoka diseases of banana in far north Queensland. Australian Plant Pathology 11:7-9.

Meredith D. 1970. Banana leaf spot disease (Sigatoka) caused by *Mycosphaerella musicola* Leach. Phytopathological Papers, No. 11, Commonwealth Mycological Institute, Kew, Surrey, UK 147pp.

Peterson R., K. Grice and A. Wunsch. 2000. Ascospore survival in banana leaf trash. Bananatopics 28:5-6.

Peterson R. 2002. Black Sigatoka Eradication – Controlled Management Program, Tully Banana Production Area. Queensland Department Primary Industries, 51pp.

Peterson R. 2003. Black Sigatoka Area Freedom Program 2002-2003 for the Tully Banana Production Area. Queensland Department of Primary Industries, 41pp.

Stover R.H. 1980. Sigatoka leaf spots of banana and plantains. Plant Disease 64:750-756.

Ron Peterson *and* ***Kathy Grice*** *work at the Department of Primary Industries and Fisheries in Mareeba, Australia and* ***Roger Goebel*** *works at the Department of Primary Industries and Fisheries in South Johnstone, Australia.*