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

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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
Triloxysrobin	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 ≥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:

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

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®) at the rate of 5 L/ha, except for the mancozeb (Dithane OC®) control, which contained 412 g/L of petroleum oil. Treatments were compared with the industry standards propiconazole and Dithane OC®.

1999 field evaluation

This experiment was conducted on the 1st 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®) at the rate of 5 L/ha, except for the mancozeb (Dithane OC®) control, which contained 412 g/L of petroleum oil. Treatments were compared with the industry standards propiconazole and mancozeb as Dithane OC® and Dithane DF®.

2001 field evaluation

This experiment was conducted on the 3rd 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® at the rate of 5 L/ha. Treatments were compared with the industry standards propiconazole and mancozeb as Dithane M45®.

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 (Ditane 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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Black leaf streak disease (caused by *Mycosphaerella fijiensis*) and Sikatoka 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 lixiviates 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 lixiviate 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^2, 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.