**Evaluation of chlorothalonil and paraffinic oil alone and in combinations
with registered fungicides for the control of yellow Sigatoka
(*Mycosphaerella musicola*) of bananas in northern Queensland, Australia**

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Abstract

The efficacy of chlorothalonil and paraffinic oil alone and in combinations with the registered fungicides propiconazole, tebuconazole, difenoconazole, epoxiconazole and pyrimethanil was evaluated in a field experiment over two cropping cycles in 2013 and 2014 in northern Queensland, Australia, for control of yellow Sigatoka (caused by *Mycosphaerella musicola*) of banana. The predominantly applied by the banana industry treatment mancozeb with paraffinic oil was included for comparison. The results from the two cropping cycles suggested that all chemicals used with paraffinic oil were as effective or more effective than when applied with chlorothalonil, and chlorothalonil alone. Difenoconazole and epoxiconazole with paraffinic oil followed by propiconazole with paraffinic oil were the most effective treatments. Pyrimethanil and tebuconazole plus chlorothalonil were the least effective treatments. None of the chemical treatments was phytotoxic or reduced yield.

Keywords: small molecule chemicals, chlorothalonil, triazoles, Biopest oil[®], yield

1. Introduction

Yellow Sigatoka (*Mycosphaerella musicola* Leach.) is considered the most serious foliar disease for the northern Queensland banana industry, which depends exclusively on the highly susceptible banana cultivars in the Cavendish subgroup [Musa (AAA; Cavendish subgroup)] (Jones 2000). Foliar necrotic lesions and reduced photosynthesis caused by yellow Sigatoka result in a reduction in bunch size, fruit number per bunch, and premature ripening in the field and post-harvest (Abadie et al. 2008; Chillet et al. 2009; Daniells et al. 1987; Marín et al. 2003). Control is largely dependent on fungicide applications supported by cultural practices (such as the removal of diseased leaves). Removing infected leaves helps reduce inoculum levels but does not provide satisfactory control and therefore chemical control is also required.

Currently, chemical control of yellow Sigatoka is achieved using spray programs consisting of protectant fungicides such as mancozeb, chlorothalonil, and pyrimethanil, and the systemic fungicides propiconazole, tebuconazole, expoxiconazole and difenoconazole (Marín et al. 2003; Vawdrey et al. 2005). It is also recommended that a petroleum-derived spray oil be used with all of these chemicals except chlorothalonil which is incompatible with paraffinic oil (Beattie et al. 2002). Oil has been shown to delay initial fungal infection and development as well as enhancing the performance of fungicides (Vawdrey et al. 2004; Vawdrey et al. 2005). However, mineral oil formulations with unsulfonated residues of less than 92% are known to photodegrade to form phytotoxic products (Ploetz 2000), which has raised a concern that the accumulation of oil might cause phytotoxicity and subsequent

reduction in fruit yield (Israeli et al. 1993). Even though the possibility of photodegradation has been significantly reduced with the current highly refined oils (e.g. Biopest oil[®] with unsulfonated residues of no less than 98%), the large number of oil sprays needed to control leaf spot may result in flecking and eventual bronzing of older leaves (Beattie et al. 2002).

A recent survey of growers in north Queensland confirmed that there has been an increased use of chlorothalonil as a replacement for mancozeb and oil and interest in combining chlorothalonil with systemic fungicides to control yellow Sigatoka (Samuelian, 2014). This change has been largely due to the rising cost of paraffinic oil, anecdotal evidence regarding the effectiveness of oil-based programs compared to chlorothalonil and chlorothalonil-based programs, and concerns that oil-based spray programs reduce yield. The aim of this study was to compare the efficacy of chlorothalonil and a paraffinic oil alone and in combinations with propiconazole, tebuconazole, epoxiconazole, difenoconazole, and pyrimethanil in controlling yellow Sigatoka in a field experiment over two cropping cycles. The phytotoxicity of the chemical treatments and possible effect of these treatments on yield, as time of bunching, average number of fruit, number of hands, and total bunch weight, and leaf number were also assessed.

2. Materials and methods

2.1. Site description and experimental design

The experiment was conducted at the Centre for Wet Tropics Agriculture (CWTA), South Johnstone, near the town of Innisfail (17°30'S, 146°00'E) (climatic data for South Johnstone: mean temperature 23.7°C, 3337.3 mm year⁻¹ rainfall, 136 days of rain $\geq$ 1 mm; soil: reddish brown light clay (Heiner and Smith, 1987); 17.3 m elevation). The experimental site was designed as a randomised complete block with four replications on bananas Musa (AAA,

Cavendish subgroup) cv. 'Williams' irrigated by mini-sprinklers. The fertiliser program for each experiment consisted of applications of potassium nitrate (19.3% N, 0% P and 28.4% K) every two weeks at the rate of 35.7 kg/ha and urea (15.7 kg/ha) through the mini-sprinkler irrigation system. Datum plots contained a single row of 5 plants with 2 non-datum 'guard' plants separating each plot. Datum rows were separated by single 'guard' rows of unsprayed plants, which were defoliated every 4 to 6 weeks. Treatments commenced when plants had four to five fully expanded leaves with no visible Sigatoka lesions observed at this stage.

2.2. Treatment application

Eight chemicals were used in this study (Table 1). The systemic fungicides and pyrimethanil were mixed either with chlorothalonil or with paraffinic oil and these treatments were compared with mancozeb plus paraffinic oil, chlorothalonil alone, and paraffinic oil alone. Chemicals were delivered with a modified Jen-ell orchard sprayer (Silvan Pumps & Sprayers (Aust.) Pty. Ltd.) with spray volume calibrated by spraying 10 plants and adjusted to 250-285 L/ha. Chemicals were applied every two weeks during the wetter months (February- June), and every three weeks during the drier months from middle of June until December. For the first trial chemical sprays commenced on 30 January 2013 and a total of 10 treatment applications were made before disease assessment, which was conducted when 90% of the trees had bunched. The second trial commenced on 26 March 2014 and a total of 12 treatments were applied before disease assessment. Chemical applications continued until bunch measurements (average number of fruit, number of hands, and total bunch weight) were completed.

2.3. Disease assessment

Disease severity ratings were assessed on five fruiting plants using the rating scale of Stover (1971) when 90% of the trees had bunched. Rating grades were 0, leaves with no visible disease symptoms; 1, leaves with <1% of the leaf area covered with disease symptoms (10 spots per leaf); 2, 1-5% of leaf area were spotted; 3, 6-15%; 4, 16-33%; 5, 34-50%; and 6, >50% of leaf area spotted. An average leaf spot rating (ALR) was calculated for each plant as the sum of the disease severity ratings for each leaf divided by the number of leaves. A disease severity index (DSI) = $[(\sum nb)/(N-1) \times T]$, was calculated, where n = number of leaves in each grade, b = grade, N = number of grades used (total of 7), and T = total number of leaves graded on each plant. The DSI takes into account the location of yellow Sigatoka lesions on the plant, which is important when assessing the overall disease intensity (Stover and Dickson, 1970).

The total number of leaves per plant was also assessed.

2.4. Determining date of bunching, number of hands, fruit (finger) numbers/bunch, and bunch weight

Date of bunching was recorded at the ‘first week of bunch emergence or week ‘1’ when the bunch could be seen in a vertical position emerging from the top of the plant. Following established industry practices (Daniells 1984) bunches were trimmed by removing hermaphrodite type flowers and the lower one or two hands, with two fingers left on the bottom hand. The male bud was broken from the stalk and the bunch bagged within one month of bunch emergence. Number of hands, fruit (finger) numbers on bunches were determined soon after bunching according to Turner et al. (1988) by counting the number of fruit on the third hand from the proximal (top) end (f_3) and the second hand from the distal (bottom) end (f_{n-1}) of the bunch. Total number of fruit was calculated as $(F_b) = n (f_3 + f_{n-1})/2$,

where n was the total number of hands on the bunch. Any hand with less than 6 fingers was not taken into account.

Three to five bunches/plot were harvested from the second spray trial when two fingers in the middle of the outside whorl of the third hand from the proximal end of the bunch was ≥ 34.5 mm in diameter. Bunch weight was measured with a 300 kg hanging digital scale (Wedderburn).

2.5. Data analyses

All statistical analyses were performed with the GenStat 14th Edition software package (VSN International, 2011). Analysis of variance (ANOVA) was used to analyse the data from each year separately. Treatment contrasts were performed to compare chlorothalonil treatments with the paraffinic oil treatments including mancozeb+oil. The 95% Fishers protected least significant difference (LSD) was used to make pairwise comparisons. The two cropping seasons/years data were also combined into a meta-analysis using residual maximum likelihood (REML) which allows different residual terms to be fitted for each year. Year was fitted as a random term, which allows the treatment term to be compared to its interaction with year. This gives a more stringent test and a significant treatment effect suggests the effect is consistent and large compared to its variation across years. All statistical tests were performed at the 0.05 level of significance.

3. Results

3.1. Disease assessment

The results of the individual year analyses are presented in Table 2. The results show that significant differences ($P < 0.001$) between the treatments were found for the disease severity

index and average leaf rating in 2013 and 2014. The overall mean disease severity and overall mean leaf rating for treatments with paraffinic oil were significantly higher compared to the treatments with chlorothalonil for both assessed cropping cycles (2013: treatments chlorothalonil vs. oil, $p=0.004$; 2014: $p<0.001$).

In 2013, mancozeb+oil provided a degree of leaf spot control very similar to chlorothalonil alone with disease severity index (DSI) and average leaf rating (ALR) levels of approximately 24% lower than observed when plants were treated with paraffinic oil alone. From all treatments with paraffinic oil, difenoconazole+oil, epoxiconazole+oil and propiconazole+oil were the most effective treatments and were significantly more effective than tebuconazole+oil, pyrimethanil+oil, and oil alone. Furthermore, difenoconazole+oil and epoxiconazole+oil treated plots were significantly less affected by leaf spot than mancozeb+oil- and chlorothalonil alone-treated plots reducing disease rates by approximately 60% and 30%, respectively. DSI and ALR assessment showed that pyrimethanil+chlorothalonil was the least effective treatment for controlling yellow Sigatoka with disease levels significantly higher than observed for all treatments except tebuconazole+chlorothalonil and pyrimethanil+oil. The systemic fungicides applied in combination with chlorothalonil provided a level of leaf spot control comparable to the protectant chemicals chlorothalonil and mancozeb+oil. There were no significant differences between the mean total number of leaves for each treatment, which ranged between 12.75 for paraffinic oil treated plants to 14.50 for difenoconazole+oil treated plants.

Disease levels observed in 2014 confirmed most of the results from the 2013 assessments with epoxiconazole+oil being the most effective treatment at controlling yellow Sigatoka, followed by difenoconazole+oil and propiconazole+oil. Interestingly, mancozeb+oil was significantly more effective at controlling leaf spot than chlorothalonil alone. With the exception of epoxiconazole there was no significant difference between

treatments where systemic fungicides were applied in combination with chlorothalonil and the protectant chemical chlorothalonil delivered alone. Pyrimethanil+chlorothalonil least effectively controlled leaf spot. Within the paraffinic oil treatments, the level of disease pressure observed on the trees treated with paraffinic oil alone were significantly higher than on the trees treated with propiconazole+oil, difenoconazole+oil, and epoxiconazole+oil. Treatments with chlorothalonil had a significantly higher mean number of leaves than the paraffinic oil treatments ($p=0.018$). Paraffinic oil alone had a significantly lower mean number of leaves compared to chlorothalonil and mancozeb+oil. Within the chlorothalonil treatments, difenoconazole+chlorothalonil a significantly lower number of leaves per plant was observed than for all chlorothalonil treatments except tebuconazole+chlorothalonil; within the paraffinic oil treatments, pyrimethanil+oil treated plants had the lowest number of leaves, while mancozeb+oil treated plants had the highest.

The results of the meta-analysis of two years data suggested that significant treatment effects were evident for the disease severity and leaf ratings, but were not significant for the mean number of leaves (Fig. 1). Similar to the individual year analyses, DSI and ALR recorded showed that all chemicals when applied with oil were significantly more effective than when applied with chlorothalonil. Trees treated with epoxiconazole+oil and difenoconazole+oil were least affected by leaf spot with disease levels almost half compared to mancozeb+oil. Trees treated with pyrimethanil+chlorothalonil were most affected with disease levels approximately 38% higher than observed in plots treated with mancozeb+oil. No significant differences were found between chlorothalonil, mancozeb+oil, and paraffinic oil alone.

3.2. Determining number of hands, number of fingers, date of bunching, and bunch weight

The results from the individual year analyses and the single analysis of the two years showed that there were no significant differences ($p>0.001$) between the treatments for the number of hands, number of fingers and the bunching week (Supplementary Tables 1 and 2). The results from these analyses found no significant interactions of cropping cycle and treatment for any of the variables ($p>0.05$). No significant differences were found for bunch weight between any of the treatments with yield ranging between 28.73 kg for plants treated with difenoconazole+oil to 32.97 kg for plants treated with mancozeb+oil (Supplementary Table 3).

4. Discussion

Disease levels were relatively uniform in the two experiments with low to moderate leaf disease occurring in the guard rows (Average Leaf Rating of approximately 0.20 to 1.00 (Vawdrey et al. 2005)). In 2013, chlorothalonil provided levels of control similar to mancozeb plus oil while in 2014 the disease level on the plants treated with chlorothalonil alone was significantly higher than on the plants treated with mancozeb plus oil. Mancozeb plus oil performed slightly better than chlorothalonil in a study conducted by Ramsey et al. (1987). It might be speculated that with building of disease pressure mancozeb plus oil might be more effective than chlorothalonil; however, further studies are required to confirm this hypothesis. Paraffinic oil alone was the least effective chemical treatment in 2013 while pyrimethanil plus chlorothalonil was the least effective for 2014. The results from the two years showed that the most effective control of yellow Sigatoka was achieved when difenoconazole, epoxiconazole, and propiconazole were applied with oil. This was not surprising as the use of petroleum oil to enhance the effect of fungicides is well known and has been demonstrated in other studies (Ramsey et al. 1987; Vawdrey et al. 2004). However,

a similar 'synergistic' effect could not be found when these chemicals were combined with chlorothalonil. One explanation could be that as the disease levels were relatively low during our study, statistical differences between treatments were difficult to detect. One consequence of improved disease control is the elimination of premature fruit ripening, which leads to a significant fruit loss during some seasons (Ramsey et al. 1987). In addition, the ability of these fungicides to restrict fungal development is a valuable property, especially when spraying is delayed because of unfavourable weather conditions.

It is well known that the use of chlorothalonil with oil or closely following oil applications causes severe phytotoxicity but little information is available as to the phytotoxicity of chlorothalonil used alone and in tank mixes with other fungicides especially emulsifiable concentrate formulations. In this study, application of chlorothalonil with triazoles currently registered for use by the Australian banana industry did not result in phytotoxicity indicating that chlorothalonil can be used in combination with these chemicals.

Severe defoliation reduces bunch weight and premature ripening of unfilled bunches (Ramsey et al. 1987). Ramsey et al. (1987) concluded that an average leaf spot rating (ALR) of approximately 2.5 should be considered the economic threshold/grade for yellow Sigatoka. There were no significant differences between fungicides for yield and number of leaves and all the ALRs in this study were less than the threshold of 2.5, which indicated that none of the treatments used would result in loss in fruit quality.

Control of yellow Sigatoka in Australia is achieved with the alternation of protectant fungicides, such as mancozeb+oil or chlorothalonil, and systemic fungicides in conjunction with physical removal of infected tissue (generally referred to as 'deleafing'). The goal of such strategy is to delay fungicide resistance. Loss of sensitivity and appearance of resistant strains have been documented for all systemic fungicides belonging to the strobilurin (Q_oI) group (Marín et al. 2003), as well as to pyrimethanil and are no longer applied by the banana

industry (Marín et al. 2003; Samuelian 2014). Propiconazole and tebuconazole have been used for a prolonged period by the industry worldwide (Romero & Sutton 1997) as well as in Australia (Samuelian 2014). They are members of the sterol demethylation inhibitor (DMI) fungicides and possess high postinfection activity that favour long intervals between applications. However, resistance to DMIs has been reported to a number of fungi (Braun & McRae 1992; Cox et al. 2007; Eckert et al. 1994; Elad 1992; Stanis & Jones 1985) as well as for *M. fijiensis* (Smith et al. 1991; Romero & Sutton 1997). A recent study in Northern Queensland, Australia, identified moderate to severe levels of resistance to propiconazole and a serious loss of sensitivity to tebuconazole in *M. musicola* populations (Grice K, Department of Agriculture and Fisheries, Queensland, Australia, *pers. comm.*). This study also demonstrated a reduction in the efficacy of propiconazole and especially tebuconazole. The industry is currently relying predominantly on epoxiconazole and difenoconazole (Samuelian 2014) which raises a concern in regard to shift in sensitivity towards these fungicides in the near future. As a part of a resistance management strategy to avoid built up of resistance in yellow Sigatoka populations, CropLife Australia (<http://www.croplife.org.au/>) recommends use of DMI fungicides of up to 6 times per year. It has to be noted that the aim of our study was to analyse the efficacy of systemic fungicides when applied with paraffinic oil and chlorothalonil and compare them to the protectant fungicides alone and therefore the frequency of systemic fungicide delivered in this study were higher than recommended by CropLife. Monitoring of sensitivity levels in leaf spot populations and development of new and innovative integrated disease management approaches to control the pathogen should allow decrease of development and spread of resistant strains and allow the industry to make informed decisions on their practices.

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1 **Table 1.** Formulation, rate of application and origin of the fungicides used in this study.

Common name	Formulation ^A	Application rate (ai/ha)	Product name	Supplier
chlorothalonil	500 g/L, s.c.	1300 g	Elect 500	Nufarm
paraffinic oil	- l.	5 L	Biopest oil [®]	Sacoa
mancozeb	750 g/L, w.p.	1650 g	Penncozeb 750 DF	Nufarm
propiconazole	500 g/L, e.c.	100 g	Throttle	Nufarm
tebuconazole	430 g/L, s.c.	99 g	Folicur 430 SC	Bayer
difenoconazole	250 g/L, e.c.	100 g	Digger	Nufarm
epoxiconazole	125 g/L, s.c.	360 g	Soprano	FarmOz
pyrimethanil	600 g/L, s.c.	396 g	Siganex	Bayer

2 ^A Type of formulation: e.c. – emulsifiable concentrate; l. – liquid; s.c. – suspension
3 concentrate; w.p. – wettable powder
4
5

Table 2. Evaluation of chemicals for the control of yellow Sigatoka of banana for two cropping cycles. Treatment means with a letter in common are not significantly different ($\alpha=0.05$). SED - standard error of the difference. LSD – least significant difference.

Year: 2013

Nr. treat	Treatment	Total leaves per plant	Disease severity index	Average leaf rating
1.	mancozeb+oil	13.25	12.57 cdef	0.75 cdef
2.	paraffinic oil	12.75	16.55 fg	0.99 fg
3.	chlorothalonil	13.85	12.63 cdef	0.76 cdef
4.	epoxiconazole+oil	14.35	7.03 ab	0.42 ab
5.	epoxiconazole+chlorothalonil	13.86	11.82 cde	0.71 cde
6.	difenoconazole+oil	14.50	5.06 a	0.30 a
7.	difenoconazole+chlorothalonil	14.13	10.63 bcd	0.64 bcd
8.	propiconazole+oil	13.75	8.80 abc	0.53 abc
9.	propiconazole+chlorothalonil	14.41	13.16 cdef	0.79 cdef
10.	tebuconazole+oil	14.05	13.32 def	0.80 def
11.	tebuconazole+chlorothalonil	12.87	15.51 efg	0.93 efg
12.	pyrimethanil+oil	13.95	13.51 defg	0.81 defg
13.	pyrimethanil+chlorothalonil	13.25	17.84 g	1.07 g
	p-value	0.064	<0.001	<0.001
	SED	0.583	2.163	0.130
	95% LSD	1.182	4.387	0.263
	Treatments chlorothalonil vs. oil	0.757	0.004	0.004

Year: 2014

Nr. treat	Treatment	Total leaves per plant	Disease severity index	Average leaf rating
1.	mancozeb+oil	14.25 e	7.53 abc	0.45 abc
2.	paraffinic oil	12.85 ab	11.45 cdef	0.69 cdef
3.	chlorothalonil	13.89 cde	13.92 def	0.84 def
4.	epoxiconazole+oil	13.70 bcde	2.64 a	0.16 a
5.	epoxiconazole+chlorothalonil	14.06 de	8.08 bc	0.48 bc
6.	difenoconazole+oil	13.50 bcde	6.04 ab	0.36 ab
7.	difenoconazole+chlorothalonil	12.91 ab	9.68 bcd	0.58 bcd
8.	propiconazole+oil	13.01 abc	6.14 ab	0.37 ab
9.	propiconazole+chlorothalonil	13.97 de	13.58 def	0.81 def
10.	tebuconazole+oil	13.20 abcd	8.05 bc	0.48 bc
11.	tebuconazole+chlorothalonil	13.75 bcde	15.13 ef	0.91 ef
12.	pyrimethanil+oil	12.54 a	10.43 bcde	0.63 bcde
13.	pyrimethanil+chlorothalonil	13.89 cde	15.64 f	0.94 f
	p-value	0.010	<0.001	<0.001
	SED	0.462	2.432	0.146
	95% LSD	0.938	4.937	0.296
	Treatments chlorothalonil vs. oil	0.018	<0.001	<0.001

Figure 1. Combined evaluation of chemicals for the control of yellow Sigatoka of banana for two cropping cycles following assessment of (a) disease severity index, (b) average leaf rating, and (c) total number of leaves per plant. Treatments with a letter in common are not significantly different ($\alpha=0.05$).

B

C