Gathering efficacy data to indentify the most effective chemicals for controlling myrtle rust (*Uredo rangelii*)

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Contents

Abstract

Fifteen fungicides were screened for control of myrtle rust under glasshouse and field conditions. Fungicides were applied at varying rates 24h before spray inoculation of *Syzygium jambos, Rhodamnia rubescens, Backhousia citriodora*, *Gossia inophloia* and *Melaleuca alternifolia* with urediniospore suspensions. Disease development was assessed by estimating the per cent leaf area covered by rust pustules after 21 days.

Amistar (azoxystrobin), Amistar Xtra (azoxystrobin + cyproconazole), Bayfidan (triadimenol), Folicur (tebuconazole), Prosaro (prothioconazole + tebuconazole), Saprol (triforine), Tilt (propiconazole), Tilt Xtra (propiconazole + cyproconazole) and Scorpio (tebuconazole + trifloxystrobin) showed good protectant activity, while copper oxychloride provided the least protection from myrtle rust.

Amistar Xtra (azoxystrobin + cyproconazole), Bayfidan (triadimenol), Folicur (tebuconazole), Opus (epoxiconazole), Prosaro (prothioconazole + tebuconazole), Scorpio (tebuconazole + trifloxystrobin), and Tilt Xtra (propiconazole + cyproconazole) gave the best eradicant activity. Copper oxychloride, mancozeb, Plantvax (oxycarboxin) and Saprol (triforine) were the least effective eradicants.

Amistar (azoxystrobin), Amistar Xtra (azoxystrobin + cyproconazole), Bayfidan (triadimenol) and Scorpio (tebuconazole + trifloxystrobin) and Score (difenconazole) effectively decreased field disease expression in lemon myrtle. Residues of all fungicides were found in leaves 1 month after treatment but had dropped considerably by 2 months after treatment.

Introduction

Myrtle rust (*Uredo rangelii*), a taxon allied with *Puccinia psidii* (guava rust) (Carnegie *et al*. 2010), was detected in Australia in 2010 in a commercial *Agonis flexuosa* plantation on the NSW Central Coast (Carnegie *et al*. 2010). Effective chemical treatments were needed for control efforts but there were no products registered for myrtle rust. Due to the urgency of the situation, there was insufficient time for fungicide screening specifically for myrtle rust. Chemicals that could be used to control *P. psidii* in the event of an outbreak in Australia had been identified in a contingency plan for managing an incursion of guava rust (Office of the Chief Plant Protection Officer 2007) and in a review of the threat posed to Australia by *P. psidii* (Glen *et al.* 2007). These included azoxystrobin, copper oxychloride, mancozeb, oxycarboxin, propiconazole, triadimenol and triforine. Within weeks of the myrtle rust outbreak emergency, permits for these fungicides were issued by the Agricultural Pesticides and Veterinary Medicines Authority (APVMA).

Myrtle rust is now established in Australia and the quarantine emergency has passed. Continued reliance on fungicides approved under the permit system is neither justifiable nor desirable. Registrations based on efficacy data produced in Australia are required. Myrtle rust has been recognised as distinct from the taxon described as *P. psidii* (Chief Plant Protection Officer 2010). The wisdom of relying on fungicides tested on *P. psidii* is questionable because the fungicidal activity spectrum may be different for myrtle rust. In addition, efficacy screening for *P. psidii* has been done mainly in South America (e.g. Ruiz 1991 *et al.*, Alfenas *et al.* 1993, Martins *et al.* 2011) and chemical activity may be influenced by the different host range and climate in Australia. Another reason to undertake further testing is to take advantage of the range of fungicides that is available in Australia, including multi-active ingredient formulations. These chemicals offer enhanced efficacy combined with improved worker and environmental safety and in-built resistance management.

This paper reports on investigations on chemical control of myrtle rust under glasshouse and field conditions.

Materials and methods

Plants

Glasshouse experiments were carried out from August 2012 to January 2013. Potted *Syzygium jambos* and *Rhodamnia rubescens* were used in the glasshouse experiment 1 (Fig. 1) and *Backhousia citriodora*, *Gossia inophloia* and *Melaleuca alternifolia* were used in glasshouse experiment 2. Plants were raised in a glasshouse in West Pennant Hills, New South Wales (NSW), and were frequently watered but other environmental conditions were uncontrolled. Young plants below a height of approximately 50 cm with abundant new growth were used in tests. Before use *S. jambos* plants were pruned and used when 6-8 juvenile (i.e. susceptible) leaves had re-grown.

The field trial was conducted in April 2013 in a rust-infested lemon myrtle (*B. citriodora*) plantation near Lismore, NSW (Fig. 4). Heavy rainfall on the north coast prevented the trial from starting earlier in the growing season. Trees were 2-3 m high and were closely planted in rows approximately 3 m apart.

Fig. 1. The plant species used in glasshouse experiment 1, *Syzygium jambos* (left) and *Rhodamnia rubescens* (right).

Pathogen

Urediniospores from *Uredo rangelii* isolate 115012 (collected from infected garden-grown *S. jambos* at Leonay, NSW) were cultured on glasshouse-grown *S. jambos.*

Fungicides

The fungicides screened are listed in Table 1. All fungicides except Score were screened in glasshouse experiment 1 using *S. jambos* and *R. rubescens* as hosts. Amistar, Amistar Xtra, Bayfidan, Scorpio and Saprol were screened in glasshouse experiment 2 using the host plants *B. citriodora*, *G. inophloia* and *M. alternifolia*.

The fungicides were mixed with tap water and applied to upper and lower leaf surfaces to the point-of-runoff with a hand-held atomiser. Controls were sprayed with tap water. Spray residues were allowed to dry for 24 h before plants were inoculated.

To test for protectant activity plants were sprayed prior to inoculation. To test for eradicant activity plants were sprayed 5 days after inoculation.

Table 1 Fungicides screened for efficacy against myrtle rust

Trade name	Manufacturer	Active ingredient(s)	Mode of action group(s)	Full-label rate (mg a.i./L)*
Amistar	Syngenta	azoxystrobin 250 g/L	QoI**	300
Amistar Xtra	Syngenta	azoxystrobin 200 g/L	QoI	200
		cyproconazole 80 g/L	DMI***	80
Bayfidan	Bayer	triadimenol 250 g/L	DMI	100
Copper oxychloride	Barmac	copper oxychloride 500 g/kg	multi-site activity	2000
Folicur	Bayer	tebuconazole 430 g/L	DMI	129
Mancozeb	Barmac	mancozeb 750 g/kg	multi-site activity	1500
Opus	Nufarm	epoxiconazole 125 g/L	DMI	63
Plantvax	Chemtura	oxycarboxin 750 g/kg	carboxamide	975
Prosaro	Bayer	prothioconazole 210 g/kg	DMI	63
		tebuconazole 210 g/kg	DMI	63
Saprol	Sipcam	triforine 190 g/L	DMI	285
Score	Syngenta	difenconazole 250 g/L	DMI	125
Scorpio	Bayer	tebuconazole 200 g/L	DMI	300
		trifloxystrobin 100 g/L	QoI	150
Systhane	Dow Agrosciences	myclobutanil 400 g/L	DMI	48
Tilt	Syngenta	propiconazole 250 g/L	DMI	80
Tilt Xtra	Syngenta	propiconazole 250 g/L	DMI	80
		cyproconazole 80 g/L	DMI	26

* Scorpio was the only product registered for myrtle rust control. Full-label rates for the other products corresponded to the highest permissible application rate on the product label.
* quinone outside inhibitors
** demethylation inhibitors

Plant inoculation

Plants were inoculated by lightly spraying with *U. rangelii* urediniospores in a mineral oil suspension (Isopar L) using a compressed air-powered airbrush (Fig. 2). Inoculated plants were incubated in a humidity chamber in darkness at 20°C for 24 h.

Fig. 2. Air-brush Inoculation and the humidity chambers used to promote sporulation and infection.

Glasshouse experiment 1

To assess protectant activity, chemicals were applied to *S. jambos* and *R. rubescens* at quarter-, half- and full-label rates (Table 1). All chemicals with the exception of copper oxychloride, Opus, mancozeb, Systhane and Plantvax were also applied to *S. jambos* at eighth-label rate. For assessment of eradicant activity fungicides were applied to *S. jambos* at full-label rate.

Three replicate plants were used in trials with *S. jambos* whereas with *R. rubescens* 2, 1 and 2 replicates were assessed at quarter-, half- and full-label rate, respectively.

Disease severity was assessed using an area diagram key (Fig. 3). Up to 8 leaves were assessed from each replicate. Mean leaf area coverage figures for each treatment were calculated. Only leaves with disease were used in the analysis.

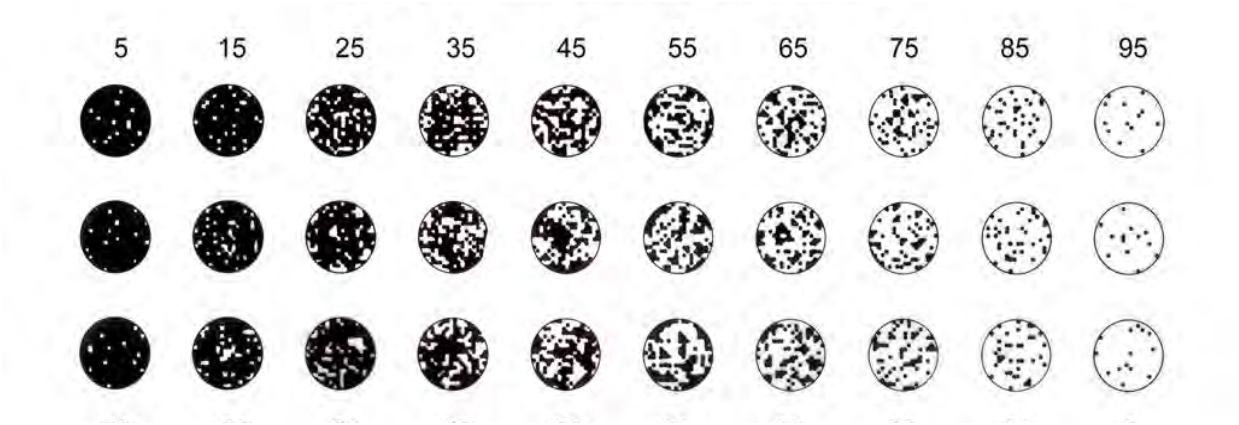

Fig. 3. Diagrammatic key for assessment of myrtle rust disease on *Syzygium jambos* and *Rhodamnia rubescens*. Disease rating scales are based on the per cent leaf area covered with pustules observed 21 days after inoculation. Three different examples of pustule distribution (pictured as white on a black background) are shown (adapted from U.S. Forest Service http://www.srs4702.forprod.vt.edu/urbantree/crown/training07.htm).

Glasshouse experiment 2

Five replicate plants were used per treatment. Protectant activity was assessed and fungicides were applied to plants at the full-label rate.

Each replicate was ranked for myrtle rust according to the following disease index (Karanjeet Sandhu *pers comm.*):

Category	Description
1	Mild hypersensitivity/dark flecks.
2	Restricted pustule/dark gray surrounding/necrosis.
3	Small pustules without chlorosis/necrosis and low in frequency.
4	Fully developed pustules on leaves, low to high in frequency.
5	Fully developed pustules in abundance (on leaves, twigs and buds).

Scores were allocated according the worst affected leaf on the plant.

Field trial

Treatments (Amistar, Amistar Xtra, Bayfidan, Mancozeb, Score, Scorpio and Saprol and a water-only control) were arranged in a randomised complete block design with 10 replicates per treatment. Diluted fungicides were mixed with Agral Spray Adjuvant (600 g/L nonyl phenol ethylene oxide condensate non-ionic organic surfactant) and applied at a rate of approximately 200 L/ha using a knapsack sprayer.

Single trees were experimental units. Leaves on 4 shoots per tree (2 on either side of a row) were monitored for rust disease during the trial. Before sprays were applied a strip of fluorescent plastic tape was attached above the 2 youngest, fully expanded leaves on the aforementioned shoots. These leaves were assessed for disease incidence and severity prior to treatment and at weekly intervals for 4 weeks after treatment. Incidence was recorded as the percentage of leaves with live (i.e. yellow) pustules and severity was ranked according to the following disease index developed for *Melaleuca quinquenervia* (Geoff Pegg *pers comm*.):

Category	Description
1	Minor infection. Lesions small in size and covering less than 10% of the total new leaf/shoot area.
2	Minor to moderate infection. Lesions still fairly small. 1-2 new leaves/shoots showing less than 25% infection.
3	Moderate infection. Lesions on 25-50% young leaves and possibly stems. Slight leaf distortion.
4	Moderate-severe infection. Lesions on 50-100% of young leaves, new shoots and/or stems. Leaves distorted.
5	Severe infection. Leaf, shoot and stem blackening, shrivelling and dieback. A mean incidence and severity measurement was calculated for each tree and used in the analysis.

The number of leaves below the marker tape was counted prior to treatment, 6 weeks after treatment and 11 weeks after treatment.

Foliage samples from sprayed trees were collected prior to treatment, 1 month after treatment and 2 months after treatment and stored in a freezer at -15^{o}C. Samples were sent to a commercial analytical laboratory (AgriSolutions Australia Pty Ltd, Deception Bay, Qld, ABN 11100118590) for residue analysis within 3 months of collection. Leaf samples were extracted with an organic solvent and quantification was by high-performance liquid chromatograph with a selective reaction monitoring and MS_2 capable mass spectrometer (AgriSolutions Australia Pty Ltd, unpublished methods of analysis ALM-13-004.02 and ALM-13-072).

Fig. 4. Lemon myrtle plantation and diseased foliage.

Statistical analysis

Glasshouse trial and field trial data were analysed with Minitab 16 statistical software by one-way analysis of variance (ANOVA) with means separated using the Fisher Method at P=0.05.

Results

Glasshouse experiment 1

Reddish marks were observed on inoculated *S. jambos* and *R. rubescens* approximately 5 days after inoculation and yellow pustules began to form 7-10 days after inoculation (Fig. 5). Disease assessments were conducted 21 days after inoculation when pustule formation had reached its full extent.

Fig. 5. Rust disease on *Syzygium jambos* (left) and *Rhodamnia rubescens* (right).

All fungicides tested at full-label rate for protectant activity significantly reduced myrtle rust pustule formation in *S. jambos* compared to controls, with the exception of copper oxychloride (Fig. 6). Very little rust development (0-3.33 % leaf area covered by pustules) was observed with applications of Amistar Xtra, Bayfidan Folicur, Prosaro, Saprol, Scorpio, Systhane and Tilt Xtra at any application rate. At half-label rate copper oxychloride, mancozeb and Plantvax were not significantly different from controls and at the at quarter-label rate copper oxychloride, Opus and Tilt were least effective. All treatments tested at eighth-label rate significantly reduced pustule formation.

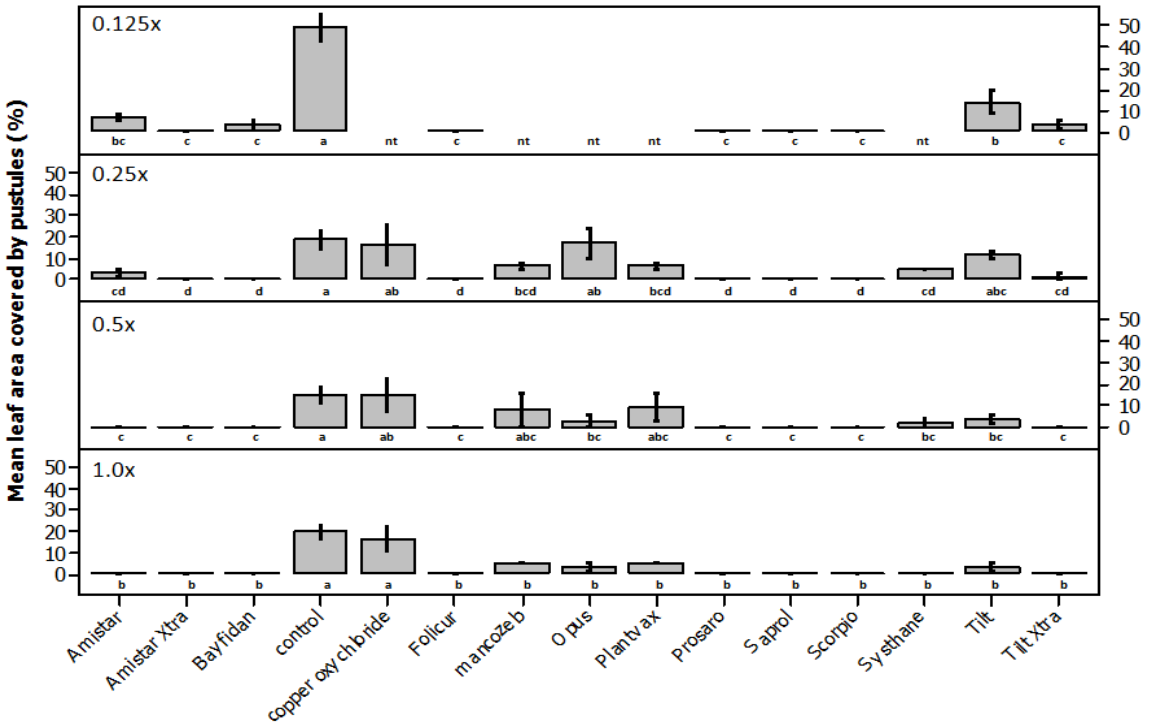

Fig. 6. Effect of pre-inoculation spraying at 0.125x, 0.25x, 0.5x and 1.0x label rate on myrtle rust infection in *Syzygium jambos.* Bars show standard error of the mean. Mean separation letters indicate significant differences at *P*=0.05 (Fisher method). nt not tested.

No rust development was observed with applications of Amistar Xtra, Bayfidan, Folicur, Opus, Prosaro, Saprol, Scorpio, Systhane, Tilt or Tilt Xtra at any application rate. Significantly more myrtle rust pustule formation in *R. rubescens* was observed with the mancozeb treatment applied at the full-label rate than on control plants (Fig. 7). At the half-label rate there were no significant differences between treatments and at the quarter-label rate copper oxychloride and mancozeb were least effective.

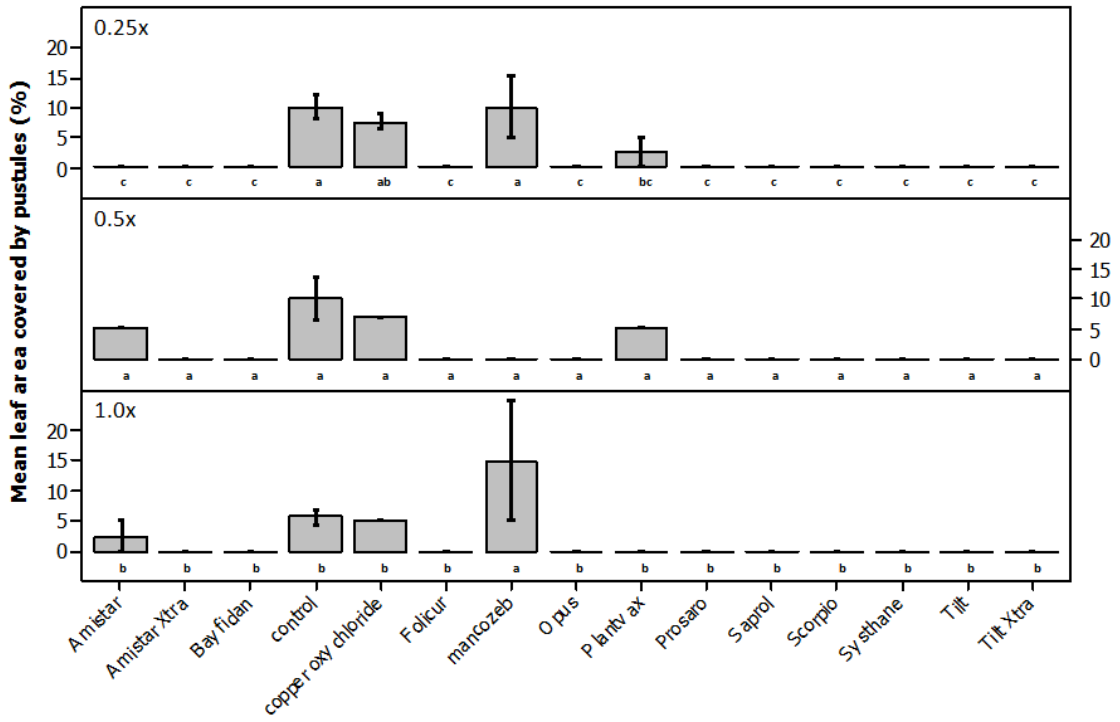

Fig. 7. Effect of pre-inoculation spraying at 0.25x, 0.5x and 1.0x label rate on myrtle rust infection in *Rhodamnia rubescens.* Bars show standard error of the mean. Mean separation letters indicate significant differences at *P*=0.05 (Fisher method).

Of the fungicides tested for eradicant activity, Amistar, Amistar Xtra, Bayfidan, Folicur, Opus, Prosaro Scorpio, Systhane, Tilt and Tilt Xtra significantly reduced myrtle rust pustule formation compared to untreated control plants (Fig. 8). Untreated plants were severely infected.

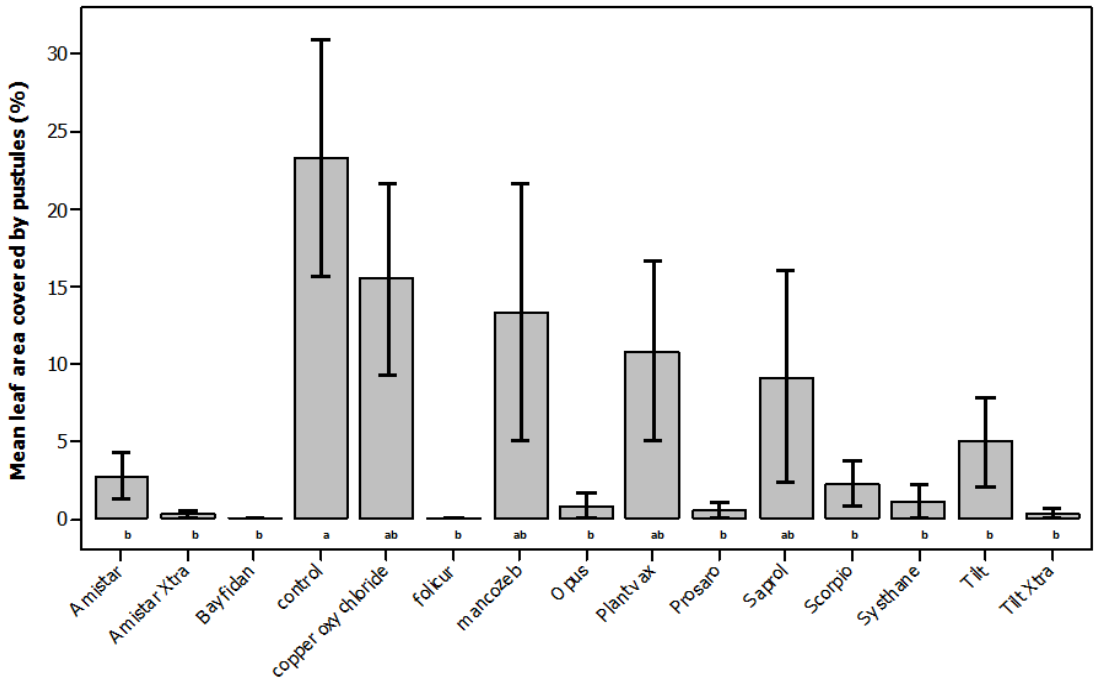

Fig. 8. Effect of post-inoculation spraying on myrtle rust infection in *Syzygium jambos.* Bars show standard error of the mean. Mean separation letters indicate significant differences at *P*=0.05 (Fisher method).

Glasshouse experiment 2

Application of all fungicides significantly reduced the level of disease on *B. citriodora* and *G. inophloia*. Only Scorpio showed that showed significant activity on all *M. alternifolia*.

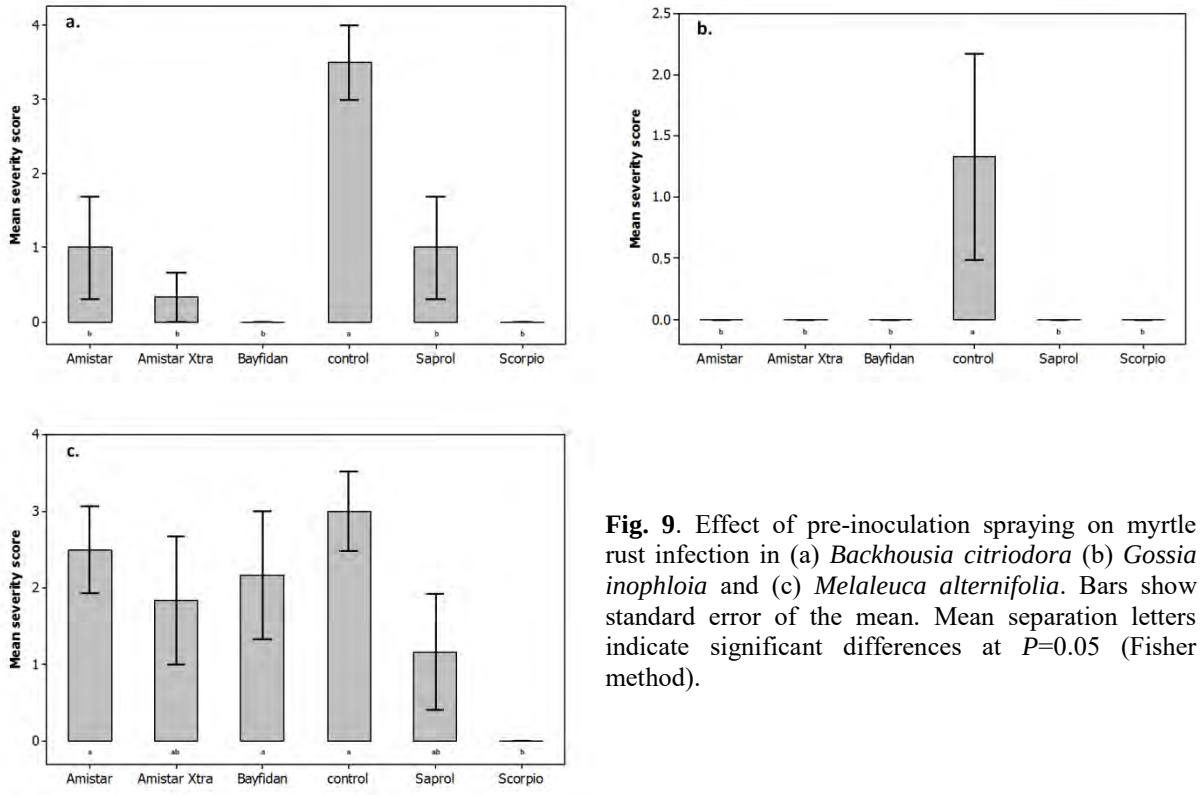

Fig. 9. Effect of pre-inoculation spraying on myrtle rust infection in (a) *Backhousia citriodora* (b) *Gossia inophloia* and (c) *Melaleuca alternifolia*. Bars show standard error of the mean. Mean separation letters indicate significant differences at *P*=0.05 (Fisher method).

Field trial

Temperatures during the trial ranged from 10.3°C and 27.7°C and rain fell on 11 days (total rainfall 53 mm). In the latter half of the trial conditions, especially temperature, were not conducive to rust infection and disease incidence and severity declined naturally. Nevertheless, several treatments caused significant reductions in disease levels compared with untreated controls.

Disease incidence was unaffected by fungicides for the initial 2 weeks of the trial (Table 4). By week 4, however, Bayfidan, Score and Scorpio had significantly reduced the incidence of disease compared with controls. Two weeks after spraying, disease severity was significantly reduced by all fungicides, except mancozeb. After 4 weeks the greatest reductions in disease severity were caused by Amistar Xtra, Bayfidan, Score and Scorpio. Amistar Xtra produced significantly higher foliation compared to mancozeb but none of the treatments significantly improved foliation compared with the control. It was observed that there was considerable defoliation in plots treated with mancozeb.

Table 4. Effect of selected fungicides on disease incidence, disease severity and foliation in lemon myrtle trees infested with myrtle rust. Means values for weeks post-treatment are shown. Mean separation letters indicate significant differences at *P*=0.05 (Fisher method).

	Disease incidence (%)			Disease severity (1-5)			No. of leaves		
Product	0	2	4	0	2	4	0	6	11
Amistar	98.8a	91.2a	63.1ab	1.95ab	1.80bc	1.38ab	2.00a	4.22a	5.38ab
Amistar Xtra	98.8a	90.0a	67.5a	2.25a	1.70bc	1.15b	2.00a	4.15a	5.45a
Bayfidan	100a	93.8a	51.2b	2.10ab	1.78bc	1.15b	2.00a	3.92a	5.28ab
Control	95.0a	95.0a	72.5a	1.95ab	2.15a	1.55a	2.00a	3.82a	4.90ab
Mancozeb	100a	94.4a	61.2ab	2.00ab	2.00ab	1.24ab	2.00a	2.88b	4.55b
Saprol	98.8a	96.2a	68.1a	2.00ab	1.78bc	1.32ab	2.00a	4.18a	5.30ab
Score	95.0a	87.5a	51.2b	1.72b	1.60c	1.12b	2.00a	3.92a	5.30ab
Scorpio	98.8a	93.8a	50.6b	2.00ab	1.78bc	1.10b	2.00a	4.00a	5.25ab

All product active ingredients were detected in lemon myrtle foliage 1 month after application (Table 5). By 2 months after application, however, residue concentrations had dropped considerably.

Table 5. Fungicide residues in lemon myrtle leaves before and after treatment.

Fungicide	Active ingredient(s)	Residue (mg/kg)*		
		Pre-treatment	1 month	2 months
Amistar	azoxystrobin	<0.01	0.10	0.02
Amistar Xtra	azoxystrobin	<0.01	0.07	0.01
	cyproconazole	<0.01	0.04	<0.01
Bayfidan	triadimenol	<0.01	0.02	<0.01
Mancozeb	mancozeb	<0.01	0.52	0.07
Saprol	triforine	<0.01	0.05	<0.01
Score	difenconazole	<0.01	0.13	0.02
Scorpio	tebuconazole	<0.01	0.08	<0.01
	trifloxystrobin	<0.01	0.03	<0.01

* Limit of quantitation for all target analytes = 0.01 mg/kg.

Discussion

The need for locally generated data specifically relating to myrtle rust combined with the availability of a wide range of available fungicides prompted this investigation.

With the exception of copper oxychloride and mancozeb, all of the fungicides on APVMA permits for the control of myrtle rust reduced disease development in most glasshouse trials, but not equally. The single-active ingredient fungicides that consistently prevented rust development were the demethylation inhibitors Bayfidan (triadimenol), Folicur (tebuconazole), Saprol (triforine), Systhane (myclobutanil), Tilt (propiconazole), and the quinone outside inhibitor Amistar (azoxystrobin). All of the multi-active ingredient fungicides were highly effective. The superiority of triadimenol confirms the results in South America against *P. psidii* on guava (Ruiz *et al.* 1991, Martins *et al.* 2011) and *Eucalyptus cloeziana* (Alfenas *et al.* 1993).

Oxycarboxin, triadimenol and triforine were reported as curative agents (i.e. eradicant) for *P. psidii* on guava (*Psidium guajava*) (Ruiz *et al*. 1991). Of these chemicals we observed an eradicant effect with triadimenol only. Of the other products tested for an eradicant effect Amistar Xtra (azoxystrobin + cyproconazole), Folicur (tebuconazole), Opus (epoxiconazole), Prosaro (prothioconazole + tebuconazole), Scorpio (tebuconazole + trifloxystrobin), and Tilt Xtra (propiconazole + cyproconazole) were the most effective fungicides.

Our field trials confirmed the efficacy of Amistar (azoxystrobin), Amistar Xtra (azoxystrobin + cyproconazole), Bayfidan (triadimenol) and Scorpio (tebuconazole + trifloxystrobin). The demethylation inhibitor Score (difenconazole) also effectively reduced the development of rust in the field trial.

Recently the label of Scorpio was extended to include myrtle rust. The same active ingredients in Scorpio, 200 g/L tebuconazole and 100 g/L trifloxystrobin, are also marketed as a consumer product called Zaleton. It would be desirable if a wider selection of active ingredients were registered and commercially available. The results of this investigation will be made freely available for fungicide manufacturers to use in support of extending label claims to include myrtle rust. The provision of this data will be especially important in the case of generic products such as triadimenol and triforine. Less incentive exists for manufacturers of generic products to invest in product label extensions because once changes are introduced they can then be 'imaged' by manufacturers of identical products without needing to supply data to support the new label claims.

Since myrtle rust spread to lemon myrtle growing areas, growers have had to rely on fungicides to protect their crops from disease. Chemical contamination has become a major issue and it will have to be managed to minimise disruptions to normal operations. It is to be hoped that chemicals will be available that are efficacious but are either not detectable or are below maximum residue limits (MRLs) at harvest. This trial has identified efficacious treatments for myrtle rust in lemon myrtle. The trial has also provided an indication of the residues that will occur when these chemicals are used. Residue levels could be compared with MRLs (should they exist) and allow appropriate treatments to be chosen. If MRLs do not exist for particular fungicides the trial data may be useful for getting MRLs established.

It is recognised that the accumulation of residues may be affected by many factors that were outside the scope of this study, such as the number of sprays applied during a season and the timing of spraying. Accordingly a broader field trial may be necessary to fully describe the extent of residues found in lemon myrtle following a fungicidal treatment programme.

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