South African Journal of Plant and Soil 2017, 34(5): 333–338

SOUTH AFRICAN JOURNAL OF PLANT AND SOIL
ISSN 0257-1862 EISSN 2167-034X
http://dx.doi.org/10.1080/02571862.2017.1317853

Use of adjuvants and fungicide application timing for the control of wattle rust (*Uromycladium acaciae*) in *Acacia mearnsii* plantations in South Africa

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Acacia mearnsii **(black wattle) plantations in South African cover approximately 110 000 ha, with *Uromycladium acaciae* (wattle rust) found from Limpopo to the Western Cape of South Africa. This disease of black wattle causes reductions in growth, and mortalities with severe infections. Although a limited number of fungicides have been screened for the control of wattle rust, these contain similar active ingredients and need to be applied repeatedly to be effective. Not only is this costly, but there is also the possible development of fungicide resistance. In October 2015 a trial was initiated in southern KwaZulu-Natal to determine the effectiveness of varied application schedules and adjuvants of fungicide for the management of wattle rust. The main aim was to reduce the application interval. The trial was laid out in a randomised complete block design with three replicates. The 2 × 4 factorial combination consisted of two adjuvants application schedules (42 or 56 d between application) and four adjuvants (none; poly-1-*p*-menthene; borax + orange oil; poly-1-*p*-menthene and borax + orange oil). Three additional treatments were included where one was a control (no fungicides applied), and the other two additional treatments had fungicides applied according to the recommended 28-day schedule (one application commencing in October and the other in November). Wattle rust had a significant impact upon groundline diameter and biomass index but not height. All of the adjuvants and application schedules were effective in managing wattle rust, but did not increase the period before re-application was required. The most effective fungicide application used will therefore be based upon cost and in a manner that will reduce the likelihood of acquired resistance developing in wattle rust populations.**

Keywords: borax, Forest Stewardship Council, resistance management

Introduction

Acacia mearnsii De Wild. (black wattle) plantations in South African cover approximately 110 000 ha, of which 78% belong to commercial farmers, 18% to three corporate companies and 4% to small-scale growers (Chan et al. 2015). The wood is used primarily for pulp milling, and to a lesser extent for charcoal and firewood, with the bark used as a source of vegetable tannin extract for the tanning of leather products as well as for adhesives (Chan et al. 2015; DAFF 2015). Together with various *Pinus* and *Eucalyptus* species, black wattle is significant for smaller timber growers as reliance on multiple species and products provides a means of flexibility and reduced susceptibility to market price fluctuations.

The land available for plantation forestry is limited in South Africa due to forestry being in competition with other land-use activities, with planting and water-use permits issued on a limited basis by the South African Government (Govender 2007). Optimal production of black wattle on the available land base is therefore necessary to satisfy the market demands and established industries that are dependent on black wattle (Dunlop et al. 2003). To achieve this, mitigating abiotic and biotic risks is one component that can aid in achieving optimum yields. Drought, hail, snow and frost are abiotic risks to black wattle, which can largely be avoided by not planting in areas where these risks occur (Sherry 1971; Davidson 1989). A number of pests and diseases also occur on wattle and cause reduced growth and mortalities. Wattle bagworm (*Kotochalia junodi* Hyalaerts), brown wattle mirid (*Lygidolon laevigatum* Reut.) and various lappet moths of the Lasiocampidae family are common pests of black wattle in South Africa (Dunlop and MacLennan 2002). Common diseases of black wattle include *Ceratocystis albofundus* Wingfield, De Beer & Morris and various *Phythoptora* and Botryosphaeriaceae species (Roux and Wingfield 1997). Research has been conducted for the management of these pest and diseases and has been successful in reducing losses in black wattle plantations.

The most recent disease of black wattle is a rust fungus identified as *Uromycladium acacia* (Cooke) P.Syd. & Syd. (wattle rust) (McTaggart et al. 2015). Since detection in 2012, wattle rust has spread throughout the entire wattle growing region of KwaZulu-Natal, as well as being recorded from Limpopo to the Western Cape (McTaggart et al. 2015). The disease affects trees of all age classes causing a reduction in growth and mortality if severe.

Suitable fungicides were screened for managing wattle rust (Little and Payn 2016). As the products tested have a recommended 28-day re-application period, a number of fungicide applications would be required within each growing season. Besides being costly, fungicide resistance may also develop. The Fungicide Resistance Action Committee (FRAC 2016) recommend strategies to prevent the development of resistance and include avoiding repetitive use of a fungicide from the same fungicide group (or mode of action), mixing or alternating fungicides from separate fungicide groups (or modes of action), limiting the number and timing of treatment applications, avoiding eradicant use, maintaining recommended dose rates by the manufacturer, and integrating fungicide use with non-chemical methods (Brent and Holloman 2007).

The South Africa Forest Industry subscribe to the principles of environmental, social and economic responsibility set out by the Forestry Stewardship Council (FSC), with c. 85% of the plantations certified (FSA 2012). The FSC has guidelines regarding pesticide use in forestry (FSC 2005), with only those fulfilling these criteria being tested and considered for use. Of the fungicides that could be considered for the management of wattle rust, all contain azoxystrobin (methyl(αE)-2-[[6-(2-cyanophenoxy)-4-pyrimidinyl]oxy]-α-(methoxymethylene), a fungicide with a high risk for the development of resistance (FRAC 2016).

Methods used to avoid fungicide resistance are essential to ensure that those fungicides containing azoxystrobin remain effective for managing wattle rust. Pesticide efficacy and longevity may be improved through the use of adjuvants, which may (dependent on adjuvant) improve coverage, absorption, persistence on foliage, improve translocation and increase efficacy (e.g. Hart et al. 1992; Thompson et al. 1996; Young and Hart 1998; Maschhoff et al. 2000). The use of adjuvants to extend the period between re-applications per season is one particular resistance management strategy that may not only aid in maintaining long-term use of fungicides, but also has the potential to reduce costs.

To determine whether the use of adjuvants and reduced fungicide applications would be effective in managing wattle rust, a trial was established at Harding in southern KwaZulu-Natal in October 2015. Two adjuvants (poly-1-*p*-menthene, and borax (sodium tetraborate decahydrate) + orange oil) and three different application timings (28, 42 and 56 d between application) were tested with azoxystrobin (methyl(αE)-2-[[6-(2-cyanophenoxy)-4-pyrimidinyl]oxy]-α-(methoxymethylene) benzeneacetate) + difenoconazole (1-[[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole).

Materials and methods

A site on Sheepwalk farm in southern KwaZulu-Natal was located in the wattle-growing region of South Africa on an existing stand of black wattle (Table 1). The site was selected such that it fell within areas where wattle rust was known to occur. The site was also selected so that the trees were between 0.5 and 1.0 m in height at the time of trial initiation. This would allow for the continued manual spraying of the fungicides onto the foliage of the trees through the growing season when using a knapsack sprayer. In addition, the trees would be in their exponential growth phase, the period during which any negative/positive treatment impacts (if any) would most likely be expressed.

Table 1: Site characteristics for an *Acacia mearnsii* trial initiated in October 2015 in the southern KwaZulu-Natal for testing of adjuvants and application schedules for the control of wattle rust

Magisterial district	Harding
Plantation/trial name	Sheepwalk
Latitude	30°37′27.7″ S
Longitude	29°49′53″ E
Altitude (m asl)	957
Mean annual precipitation (mm)	898
Mean annual temperature (°C)	16.4
Aspect	Undulating, steep, south-facing slope
Selected soil physical and chemical properties	
Soil form	Inanda 1200
Soil depth (m)	1.2
Soil texture	Clay
Spacing (m)	2 × 3
Stems per hectare (sph)	1 666
Seed lot/orchard	PSO-10
Date planted	1 February 2015
Drought risk (%)	
>850 mm	45.1
<650 mm	13.7
Potential productivity	
Climate zone	WT2 (warm temperate)
Growing conditions for species planted	Optimum
Site index (age 5)	15

The trial design consisted of a 2 × 4 factorial with three additional treatments, replicated three times and laid out as a randomised complete blocks design (Cochran and Cox 1968). The factorial combination consisted of application schedules (42 and 56 d between application) and four adjuvant combinations (none; poly-1-*p*-menthene; borax + orange oil; and poly-1-*p*-menthene & borax + orange oil) (Table 2). The three additional treatments consisted of one control with no fungicides applied. The other two additional treatments had fungicides applied according to the recommended 28-day schedule (one commencing in October and the other in November). Each treatment plot consisted of a single line of 22 trees, with the inner 20 trees being measured (two buffer trees at each end). In addition, there were single lines of non-treated trees on either side of the treated rows so as to act as a buffer between adjacent plots.

The fungicides were sprayed onto the foliage to just prior to run-off using a 16 L knapsack sprayer, fitted with an air-induction, twin-flat-fan TeeJet Turbo TwinJet® AITTJ60 nozzle. This nozzle was selected due to its ability for good canopy penetration and cover. The nozzle is also recommended for the broadcast application of fungicides where good drift control and coarse droplets are required (TeeJet Technologies 2015). Pressure was regulated to 1.5 KPa, resulting in a spraying volume of 669 L ha^{-1}. Rate of fungicide applied was 1 L ha^{-1} assuming a spray volume of 1 000 L water ha^{-1}. The dates when the fungicides were applied, together with the climatic conditions on the day of spraying were recorded (Table 3). Standard silvicultural practices were implemented in the trial, with weeding carried out as required to remove competition from unwanted

Table 2: Treatments, including adjuvant-related information, tested for the control of wattle rust in an *Acacia mearnsii* trial initiated in October 2015 in southern KwaZulu-Natal

Treatment	Fungicide trade name[1]	Fungicide active ingredient (g L^{-1})	Adjuvant trade name[1]	Adjuvant active ingredient (g L^{-1})	Rate of adjuvant ha^{-1} assuming spray volume 1 000 L ha^{-1}	Application period (d)	Spray starting date
1	Amistar Top®	Azoxystrobin (200 g L^{-1}) Difenoconazole (125 g L^{-1})	–	–	–	42	30 Oct 2015
2						56	30 Oct 2015
3	Amistar Top®	Azoxystrobin (200 g L^{-1}) Difenoconazole (125 g L^{-1})	Nu-Film P®	Poly-1-*p*-menthene (875 g L^{-1})	1 250 mL	42	30 Oct 2015
4						56	30 Oct 2015
5	Amistar Top®	Azoxystrobin (200 g L^{-1}) Difenoconazole (125 g L^{-1})	Orosorb®	Borax (10 g L^{-1}) Orange oil (50 g L^{-1})	208 mL	42	30 Oct 2015
6						56	30 Oct 2015
7	Amistar Top®	Azoxystrobin (200 g L^{-1}) Difenoconazole (125 g L^{-1})	Nu-Film P®	Poly-1-*p*-menthene (875 g L^{-1})	1 250 mL	42	30 Oct 2015
8			Orosorb®	Borax (10 g L^{-1}) Orange oil (50 g L^{-1})	208 mL	56	30 Oct 2015
Additional treatments (controls)							
9	Control	–	–	–	–	–	–
10	Control	–	–	–	–	–	–
11	Amistar Top®	Azoxystrobin (200 g L^{-1}) Difenoconazole (125 g L^{-1})	–	–	–	28	2 Oct 2015
12						28	30 Oct 2015

Table 3: Sequence of events in terms of assessments dates and the application of treatments in an *Acacia mearnsii* trial initiated in October 2015 in southern KwaZulu-Natal

Trial	Sheepwalk							
Assessment date	02/10/2015		08/01/2016				29/03/2016	
Tree age when assessed (d)	243		334				415	
Application date	1st 2 Oct 2015	2nd 30 Oct 2015	3rd 16 Nov 2015	4th 29 Nov 2015	5th 24 Dec 2015	6th 18 Jan 2016	7th 10 Feb 2016	8th 19 Feb 2016
Conditions at time of application								
Time of spraying (h)	14:42–16:57	10:28–11:41	15:40–17:10	08:40–10:55	10:48–13:50	12:04–15:11	08:57–10:43	09:07–09:51
Temperature shade (°C)	22.6	30.0	20.1	24.5	33.5	24.9	24.7	28.8
Relative humidity (%)	74.0	60.3	78.4	71.1	60.7	71.1	70.9	72.1
Wind speed (m s^{-1})	9.7	3.0	9.4	4.7	7.6	4.5	1.9	3.1

vegetation. As four plots were not weeded on one occasion, a covariate was used for the analysis of these plots.

Assessments

Tree height and groundline diameter were measured on three occasions, at trail initiation, three months after trial initiation and one month after the final fungicide application (Table 3). Biomass index was calculated as groundline diameter^2 × height and provides a good index of overall tree performance in young trees (Eccles et al. 1997). Tree condition was quantified through the visual estimation of a combination of all disease symptoms. A modified Braun Blanquet method (Kent and Coker 1996) for the estimation of the area of each tree affected by wattle rust was used, whereby increasing values are assigned, based on increasing cover (Table 4). This method of overall assessment of tree condition was selected as cover estimates are not biased by tree size. As the visual expression of any fungal pathogen varies according to the type, stage and severity of infection, a number of visible symptoms were scored so as to quantify disease expression. These were adapted from symptoms as described by Dick (2009) and included the scoring of trees for teliospore masses (brown pustules) on the leaves, deformed pinnules or pinnae and stem lesions (teliospore masses on the main stem, or branches) according to the volume of tree affected, where 0, 1, 2 and 3 were 0, 1–25%, 26–50% and >50% affected, respectively. As the trees showed signs of the presence of wattle rust disease from the 2015 growing season, the third assessment of tree condition and disease expression was calculated relative to the first assessment.

Table 4: *Acacia mearnsii* 'tree condition' was quantified through the visual estimation of a combination of defoliation, discolouration and disease symptoms. A modified Braun Blanquet method (Kent and Coker 1996) for estimation of the area of each tree affected was used, whereby increasing values were assigned, based on increasing cover affected

Tree condition score	Percentage area affected	Median value used for analyses
1	Rare: 1–2 leaflets (<5%)	1
2	Few: 3–4 leaflets (<5%)	2
3	Many: 5–10 leaflets (<5%)	3
4	Abundant: >10 leaflets (<5%)	4
5	5–12 %	8.75
6	12.5–25 %	18.75
7	25–50 %	37.5
8	50–75 %	62.5
9	75–100 %	87.5

Analysis

Plot means from the final assessment date were analysed as a 2 × 4 factorial with three additional treatments using Statistica for Windows (Dell 2015). Prior to the analysis, the data were checked to ensure that the assumptions for a valid analysis of variance were not violated (Table 5). Before any comparison between treatments, an *F*-test was carried out to determine the overall significance of the differences between all treatment means within the experiment. Only if the *F*-value was significant were treatment differences further investigated using least significant differences (LSDs). The first measurement was used in addition to the presence of weeds as covariates for subsequent measures. These were significant as covariates and therefore were included in the final analysis. The variate of biomass index received square root transformation prior to analysis so as to stabilise the variance. Changes in tree condition and disease expression were calculated relative to initial assessments (and expressed as a percentage) and are displayed using descriptive graphic techniques (Figures 1c and 2).

Results and discussion

Although fungicide use has received attention within the South African forest industry, this has been focused on the management of fungal pathogens within nurseries or at establishment (planting), mainly for pines and eucalypts (Rolando 2006). Besides the screening of fungicides for the control of wattle rust in black wattle (Little and Payn 2016), little published literature could be found regarding research conducted on the use of fungicides during the post-establishment growth phase of plantation forestry species in South Africa. In contrast, fungicide use on trees grown for other uses (avocado, apple and citrus bearing trees) has received considerable attention in South Africa (e.g. Kotze 1981; Darvas and Kotze 1987; de Villiers et al. 1997). The use of adjuvants (e.g. Gent et al. 2003) and altered timing of fungicide application (e.g. Blandino et al. 2012) have both been used as management strategies for plant pathogenic fungi. However, little published literature could be found regarding research conducted on the use of these strategies for managing rust fungi in plantation forestry.

A site was selected in the black wattle commercial growing region of southern KwaZulu-Natal to test the effectiveness of different application schedules of fungicides, as well as the use of adjuvants for the management of wattle rust. Replicates were highly significant (*F*-prob < 0.01) and accounted for the highest portion of the variation for all tree growth variates (Table 5). As the replicates were blocked such to reduce variation associated with slope, this difference in tree growth is likely due to variations in soil depth along the slope profile.

By the end of the trial period (415 d), tree growth in terms of groundline diameter (sprayed treatments: $\bar{x}$ = 3.95 cm; control: $\bar{x}$ = 3.31 cm) and biomass index (sprayed treatments: $\bar{x}$ = 4 539; control: $\bar{x}$ = 3 021) were significantly impacted by rust (Table 5, Figure 1). Height was not

Table 5: Summary of analyses of variance showing means squares for selected tree variates at the final assessment date (415 d after planting) for an *Acacia mearnsii* trial initiated in October 2016 in southern KwaZulu-Natal for testing of adjuvants and application timing for the control of wattle rust. The first assessment (243 d after planting) and unweeded plots are included as covariates

Source of variation	df	Ht (m)	Gld (cm)	BI[1]
Replicates	2	0.423**	0.803*	558.011**
Control.Rest of treatments	3	0.084	0.84*	323.513**
Control.Adjuvant	3	0.0434	0.155	52.369
Control.Application timing	1	0.001	0.005	7.578
Control.Adjuvant.Application timing	3	0.043	0.022	5.669
Weeds covariate	(1)	0.0002	1.496**	454.478**
Measurement 1 covariate	(1)	1.051**	3.423**	2116.962**
Residual	29 (27)	0.063	0.184	57.785
Total	42			
Grand mean		2.46	3.88	64.96
Standard error of the difference (Control. Adjuvant.Application timing)		0.25	0.43	7.60
Coefficient of variation (units)		10.22	11.05	11.70
Levene's test for homogeneity of variance		1.87$	1.75ns	1.89$
Shapiro–Wilk test for normality		0.97ns	0.98ns	0.98ns

$ *F*-prob < 0.10, * *F*-prob < 0.05, ** *F*-prob < 0.01, ns = non-significant

[1] Biomass index was square root transformed

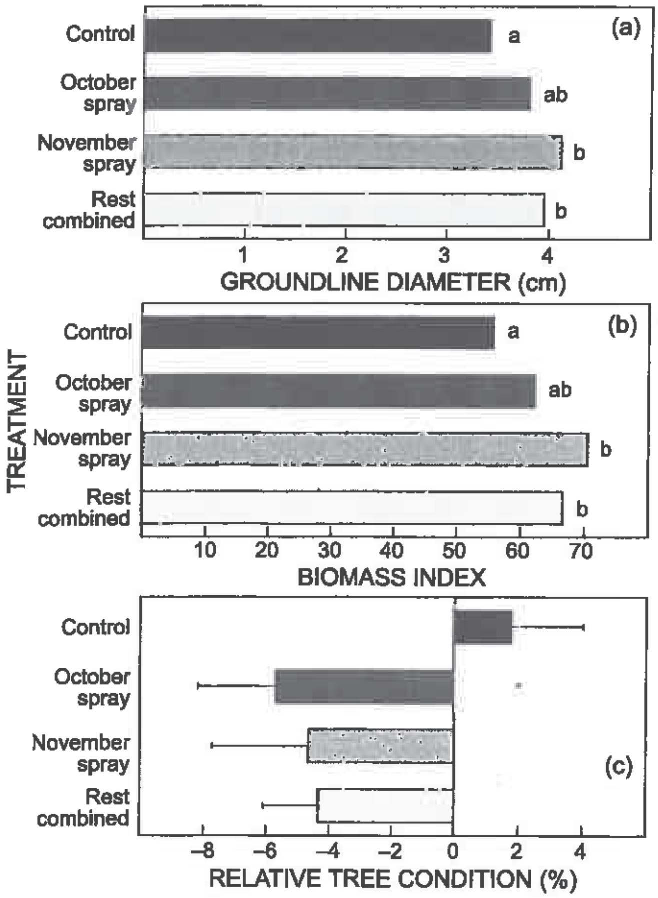

Figure 1: Groundline diameter (a), biomass index (square root transformed) (b) and relative tree condition (c) for an *Acacia mearnsii* trial initiated in October 2015 in southern KwaZulu-Natal for testing fungicide application schedules and adjuvants for the management of wattle rust. Letters beside bars in (a) and (b) indicate significance at $p < 0.05$. Standard error bars are included in (c)

as affected by rust infection (F-prob < 0.227) in either the sprayed treatments ($\overline{x}$ = 2.47 m) or control ($\overline{x}$ = 2.32 m). No significant difference was detected between the application schedules (biomass index: F-prob < 0.781) nor between the adjuvants tested (biomass index: F-prob < 0.594). However, spraying, which commenced in October on a 28-day schedule, had a lower growth response than when sprayed from November, albeit not significant, for both groundline diameter (F-prob = 0.860) and biomass index (F-prob = 0.781) in comparison with the other treatments (Figure 1). Lowered growth in the 28-day October treatment (7% for groundline diameter; 7.6% for biomass index) may have arisen from phytotoxicity, caused by too many applications of fungicide for the level of wattle rust observed. However, any phytotoxicity recorded was difficult to differentiate from the disease expression symptoms recorded (Figure 2). Phytotoxicity, as a result of fungicide use, was also suspected to have occurred in one (Commondale) of three trials implemented by Little and Payn (2016) where spraying commenced before wattle rust was observed in the trial. However, in both this trial, and those reported on by Little and Payn (2016), the application of fungicides had greater benefits than the effect of phytotoxicity. All of the fungicide application schedules and adjuvant mixtures were effective in controlling wattle rust. Although the fungicides were applied to trees already infected by rust, they were effective as a corrective treatment, as supported by Little and Payn (2016).

Tree condition, analysed at the third measurement relative to the first, was also reduced due to the application of fungicides in non-control plots (sprayed treatments: $\overline{x}$ = −4.9%; control: $\overline{x}$ = 1.8%) (Figure 1). Tree condition in the unsprayed control (1.8%) was notably lower in relation to a series of three trials (35.6%) conducted on the use of fungicides for the management of wattle rust (Little and Payn 2016). This is likely as a result of the difference in climatic conditions, particularly rainfall, experienced between the two sets of trials. Rust fungi epidemiology is known to be associated with climatic conditions, such as moisture, temperature and wind (Agrios 2005). Relative disease expression was reduced among all treatments, including controls (Figure 2). Reduced rainfall is likely to also have caused the lowered disease expression.

Application of fungicides needs to be timed according to climatic conditions associated with rust growth, as was indicated by the lowered growth response due to the 28-day application schedule commencing in October. This needs to occur in conjunction with the number applications best for managing the development of acquired resistance and that will not result in phytotoxicity.

Conclusion

Tree groundline diameter and biomass index were negatively impacted by wattle rust, irrespective of previous infection. Height was not significantly impacted by wattle rust, likely due to the low incidence of wattle rust observed. All of the adjuvants and fungicide application schedules tested were effective for the management of wattle rust. Phytotoxicity may arise from excessive applications when wattle rust infection is low. Adjuvants and application schedules will need to be selected according to the most cost effective strategy as well as in a manner to avoid resistance to fungicides developing. Monitoring the effectiveness of fungicide applications in field is essential for monitoring acquired resistance.

Fungicides currently registered for the control of wattle rust contain azoxystrobin, difenoconazole and cyproconazole. As cyproconazole is no longer available for use by forest companies certified through FSC (as

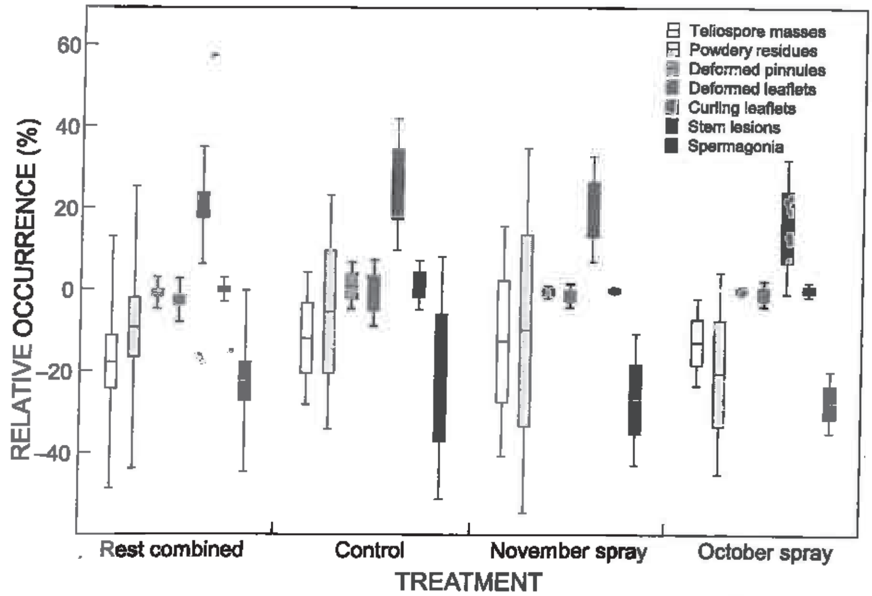

Figure 2: Relative occurrence for an *Acacia mearnsii* trial initiated in October 2015 in southern KwaZulu-Natal for testing fungicide application schedules and adjuvants for the management of wattle rust. Treatments means are shown as solid bars, the 95% confidence levels by the boxes, and the standard deviation by the bars

determined by FSC standards and criteria), azoxystrobin and difenoconazole are the only fungicides available for use. To avoid resistance developing for these two fungicides, alternative fungicides from different groups/modes of action need to be tested for their effectiveness in managing wattle rust. This will allow a selection of fungicides that can be used in combination and/or alternatively that will reduce the likelihood of resistance developing. Combining multiple methods of managing wattle rust, such as planting resistant/tolerant varieties of black wattle with the use of various fungicides, will also contribute to reducing the likelihood of resistance.

Recommendations

Testing a number of additional fungicides from different fungicide groups/modes of action to those tested for the management of wattle rust needs to be conducted. This will increase the number of fungicides that can be used in mixtures and/or alternatively to avoid acquired resistance developing in wattle rust populations.

Due to the influence of climatic variables on wattle rust occurrence, further trials to test the use of adjuvants and altered timing is recommended under different climatic conditions in order to understand the influence weather has on treatments.

Acknowledgements — The South African Wattle Growers for providing the financial support for this work, NCT member Kim Bouverie for supplying the land for carrying out the trial and for assistance provided during trial implementation, and in particular, Craig Norris (NCT) for initiating this piece of research. Dr Gerhard Verdoorn (Griffon Poison Information Centre) for his technical advice regarding the products tested in terms of certification criteria, Prof. Jolanda Roux (Forestry and Agricultural Biotechnology Institute, University of Pretoria) for assistance in linking symptoms assessed to fungus, and René Jacobs (Syngenta) for supplying the products and supporting technical information used. The above are also thanked for their comments regarding the initial draft of this document.

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Received 19 October 2016, revised 17 March 2017, accepted 5 April 2017
Associate Editor: Roy Neilson