SUPPRESSION OF *VENTURIA INAEQUALIS* (APPLE SCAB) ASCOSPORE PRODUCTION USING AUTUMN-APPLIED FUNGICIDES

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ABSTRACT

In autumn 2006 apple leaves with visible scab (black spot) symptoms were collected at the time of leaf fall and dipped in fungicides at concentrations equivalent to field application rates. The leaves were overwintered on gravel and ascospore production measured on three dates during the following spring using suction spore traps. Of ten fungicides tested, myclobutanil, trifloxystrobin, captan, dodine, tolyfluanid and cyprodinil either reduced or delayed ascospore production by *Venturia inaequalis* during the following spring, compared with a water-dipped control. Metiram, dithianon, urea and copper hydroxide had a minimal effect on ascospore production. Leaves from a separate orchard trial produced significantly fewer ascospores after myclobutanil treatment than after captan treatment. Captan and tolyfluanid are recommended for autumn application to control scab because they significantly reduced or delayed ascospore production and have a low risk of selecting resistant strains of *V. inaequalis*. Although also effective, myclobutanil, trifloxystrobin, dodine and cyprodinil should not be used for autumn application because of the risk of fungicide resistance.
Keywords: Integrated Fruit Production, apple black spot, overwintering, spore trapping, ascospore maturation.

INTRODUCTION

Fungicides applied during spring and summer are used to control scab (black spot) in apple orchards, but can leave trace residues of fungicides on harvested fruit. A major objective for the New Zealand apple industry is to produce fruit with no detectable chemical residues. Santitation measures applied during autumn or winter, such as urea, leaf removal, leaf shredding, heat treatment or ground covering can also reduce scab the following season by reducing spring ascospore production (MacHardy 1996; Holb et al. 2006). However, sanitation measures are often expensive to apply and can have negative environmental impacts. Autumn application of modern site-specific synthetic fungicides is also effective (e.g. Biggs & Warner 1990), but their overuse in orchards since the 1970s has led to selection of resistance in *V. inaequalis* in some at-risk fungicide groups. For example, resistance to benzimidazoles (e.g. Ross & Newberry 1985) and demethylation inhibitors (e.g. Hildebrand et al. 1988) is well documented in some countries.

This study investigated the ability of fungicides applied to apple leaves during leaf fall in autumn to suppress *V. inaequalis* ascospore production in New Zealand apple orchards. The main fungicide groups registered for use on apples in New Zealand were investigated to provide a comprehensive comparison of fungicide efficacy, even though some of the fungicides should never be used in autumn because of the risk of resistance.

METHODS

Ascospore production from fungicide-dipped leaves

Scabbed leaves of 'Royal Gala' apple were collected from an unsprayed orchard in May 2006 at the time of leaf fall. Thirty leaves were placed in a single layer between two 40 × 25 cm sheets of galvanised "chicken wire" (mesh size = 13 mm) (Manktelow & Beresford 1995). Each of five replicate meshes was dipped once for 30 s in one of 10 fungicides, including urea, or in water as an untreated control (Table 1). The 5% urea solution gave the equivalent amount of urea to that used as two applications of 2.5% urea to apple trees during leaf fall. This amount has been shown to suppress ascospore production (Beresford & Wood 2000) while avoiding damage to apple buds (Wood & Beresford 2000).

The leaves were overwintered outside, away from fungicide sprays on a gravel bed to minimise leaf degradation. In spring, the leaves were saturated with water on three separate occasions (26 September, 30 October and 22 November 2006) and each mesh was covered with a suction spore trap (Horner & Horner 2002). Spore traps were operated for 1 h with an air flow rate of 10 litres/min. The trap orifice was 5 cm above the leaf mesh and was contained in a clear plastic box (31 × 22 cm and 20 cm high). Ascospores were deposited onto glass microscope slides and were counted on two transects across the spore imprint band on each slide, at 400× magnification.

The dates for spore trapping were chosen to span the ascospore maturation period for *V. inaequalis* in Hawke's Bay. An ascospore release prediction model (Beresford 1999, www.hortplus.com) was used to track potential ascospore release and the trapping dates represented 20%, 95% and 99% predicted ascospore release.

Ascospore production from orchard application of fungicides in autumn

Ascospore production was also measured from leaves of trees sprayed with fungicides during leaf fall in an apple field trial in Hawke's Bay (Beresford et al. 2008). Trees of 'Royal Gala' and 'Fuji' were sprayed on 17 and 24 May 2006 with either captan (96 g/100 litres) or myclobutanil (4.8 g/100 litres). There were two replicates of each fungicide treatment in each cultivar. Leaves with scab symptoms were collected from each cultivar at the time of leaf fall and placed in two replicate wire meshes, as described above. These were overwintered and spore trapping was used to measure ascospore production during spring, as described above.

Statistical analyses

Ascospore count data were analysed for treatment effects by analysis of variance, using the general linear model facility in Minitab version 15. Ascospore counts were transformed to log 10 to satisfy homogeneity of variance requirements.

RESULTS

Ascospore production from fungicide-dipped leaves

Six of the 10 fungicides tested significantly reduced numbers of ascospores compared with the untreated control at the time of the first trapping (Fig. 1). For the second trapping, myclobutanil significantly reduced ascospore counts, but captan, tolyfluanid and dithianon resulted in significantly higher counts compared with the control. For the third trapping, no fungicide treatment resulted in significantly different counts from the control. The relative increase in numbers of ascospores trapped in the second trapping compared with the first for some fungicides appeared to result from a delay in ascospore maturation. The total number of ascospores trapped over all dates showed significant reductions compared with the control only for myclobutanil ($P<0.001$) and trifloxystrobin ($P<0.01$). This further suggests that the differences between treatments for the first trapping were at least partly caused by a delay in ascospore maturation, rather than a reduction in overall ascospore production.

TABLE 1: Fungicides tested and described according to active ingredient, chemical group and application rate into which *Venturia inaequalis*-infected 'Royal Gala' apple leaves were dipped at the time of leaf fall in Hawke's Bay.

Fungicide	Active ingredient	% ai	Chemical group	Rate (ai/litre)
Untreated control	water	100	-	-
Captan FLO®	captan	48	cyclic imide	0.96 ml
Chorus®	cyprodinil	50	anilinopyrimidine	0.15 g
Delan® WG	dithianon	70	quinone	1.75 g
Dodine 400	dodine	40	guanidine derivative	0.32 ml
Euparen® Multi	tolyfluanid	50	sulphamide	1.25 g
Flint®	trifloxystrobin	50	Quinone outside inhibitor (strobilurin)	0.05 g
Kocide® 2000 DF	copper hydroxide	36	inorganic copper	0.27 g
Petrochem® Urea	urea	>99	primary amine	50.0 g
Polyram® DF	metiram	70	dithiocarbamate	1.05 g
Systhane 400WP	myclobutanil	40	demthylation inhibitor	0.048 g

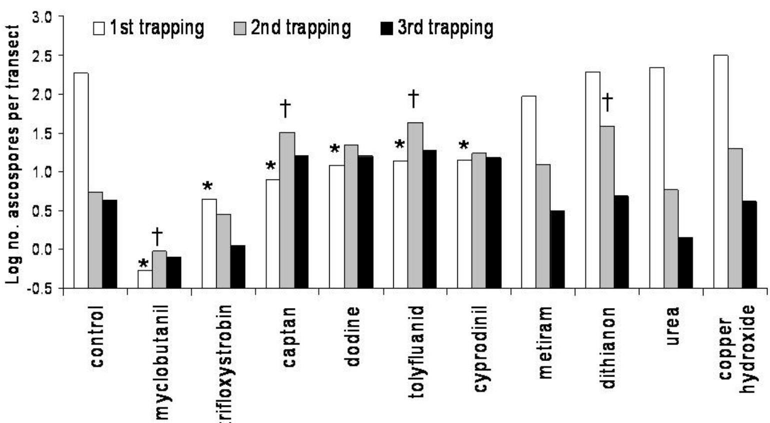

FIGURE 1: Log numbers of ascospores counted on spore trap slides for three trappings (26 September, 30 October and 22 November 2006) in the Hawke's Bay leaf dipping experiment. Treatments significantly different (P<0.05) from the control for the first trapping are indicated by* and for the second trapping by†.

Ascospore production from orchard application of fungicides in autumn

'Fuji' leaves produced significantly fewer ascospores on the first and second trapping dates than 'Royal Gala' and none at all on the third date (Fig. 2). Both cultivars appeared to show the same delay in ascospore maturation that was observed for captan and myclobutanil in the leaf dipping trial. For 'Royal Gala', there were significantly fewer ascospores trapped from the myclobutanil treatment than from the captan treatment at the first trapping date, but there were no differences at the second or third trapping dates. Absolute numbers of ascospores between the leaf dipping and field-sprayed fungicide experiments cannot be compared because the diseased leaves came from different sources and different fungicide application methods were used.

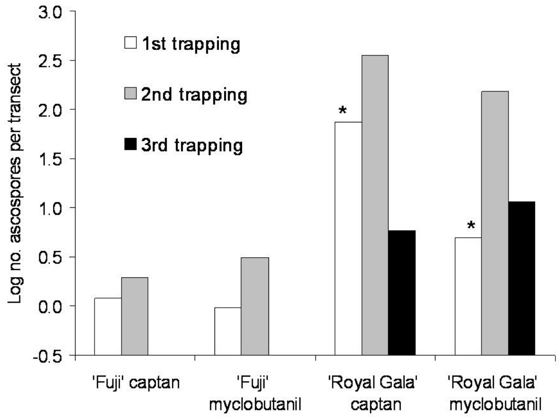

FIGURE 2: Log numbers of ascospores counted on spore trap slides for three trapping dates (26 September, 30 October and 22 November 2006) from apple leaves in a field trial treated with fungicides in autumn. Significantly (P=0.040) fewer ascospores were trapped from 'Fuji' than from 'Royal Gala'. Significant (P<0.05) differences between fungicide treatments within cultivars and trapping dates are indicated by*.

DISCUSSION

Four of the six fungicides that produced a significant effect on ascospore production, myclobutanil, trifloxystrobin, dodine and cyprodinil, are not suitable for autumn application in commercial orchards because of the risk of fungicide resistance (Martin et al. 2005). The other two, captan and tolyfluanid, are considered to have a low risk of selecting fungicide resistance and could therefore be suitable for leaf fall application in apple orchards. These fungicides appeared to delay ascospore maturation, rather than reduce the total number of ascospores produced. Beresford et al. (2008) showed that tolyfluanid applied to trees at leaf fall gave a significant reduction in leaf and fruit scab the following season. It is possible that delaying ascospore maturation could reduce scab risk if ascospore release occurs after the initial flush of susceptible apple leaf and fruit tissue between budburst and bloom has occurred. An additional advantage of using captan and tolyfluanid at leaf fall is that these fungicides have both been shown to reduce European canker, caused by *Neonectria galligena,* (van Zuidam & van Dijke 1996), which occurs in some New Zealand regions.

Three of the fungicides examined, metiram, dithianon and copper hydroxide, did not appear to suppress ascospore production. These are probably less suitable for autumn application for scab management, although copper hydroxide has previously been shown to give a slight but significant suppression of ascospore production in a leaf dipping experiment (Beresford & Wood 2000). Urea did not suppress ascospore production in this study and this was surprising, as its effectiveness for ascospore suppression has been well documented in other studies (e.g. Burchill 1968; Beresford & Wood 2000).

It is possible that the lack of efficacy was due to the mesh overwintering site being too abiotic to allow microbial breakdown of leaves. One mechanism by which urea suppresses overwintering of *V. inaequalis* is through the elevated nitrogen status of urea-treated leaves causing increased microbial activity, which results in increased leaf and pseudothecial decay (Burchill & Cook 1971).

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