AUTUMN-APPLIED UREA AND OTHER COMPOUNDS TO SUPPRESS *VENTURIA INAEQUALIS* ASCOSPORE PRODUCTION

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ABSTRACT

The effect of urea applied to apple leaves in autumn on production of ascospores of *Venturia inaequalis* (black spot) was quantified in four studies. Autumn urea at concentrations from 0-20% reduced ascospore production in spring in proportion to the log of the urea concentration. A single application of 5% urea sprayed onto fallen leaves gave an 88% decrease in ascospore production. Urea applied to apple trees before leaf fall significantly reduced black spot disease on leaves the following spring in cv. Fuji, but not in cv. Royal Gala. Ascospore production was reduced by the fungicide cupric hydroxide, but was not affected by a low concentration of fish fertilizer. Three methods of ascospore assessment were used to detect differences in ascospore production.
Keywords: *Venturia inaequalis*, apple scab, urea, fungicide control, ascospore discharge.

INTRODUCTION

The effectiveness of urea in inhibiting the ascospore stage of *Venturia inaequalis* has long been known (Burchill *et al.* 1965; Burchill 1968). Urea is fungicidal against *V. inaequalis*, it maintains a high nitrogen content in fallen apple leaves, which prevents formation of overwintering pseudothecia, and it promotes leaf decomposition by stimulating populations of micro-organisms, mainly bacteria (Crosse *et al.* 1968). Urea can either be sprayed onto leaves in the autumn before they fall from trees, or sprayed directly onto leaves lying on the ground in autumn or early spring. Autumn application appears to be more effective than spring application (MacHardy 1996), possibly because it provides opportunities for suppression of *V. inaequalis* at several life cycle stages.

The effectiveness of urea on New Zealand apple cultivars and under New Zealand growing conditions has not been confirmed. This paper reports four studies which quantified the suppression of ascospore production by urea, using three different methods of ascospore assessment, and investigated the effects of urea in reducing incidence of black spot disease in apple trees.

MATERIALS AND METHODS

Effect of urea rate and other compounds on ascospore production

Black spot-infected apple leaves of cv. Braeburn were collected in South Auckland in June 1990 just after they had fallen to the ground. Leaves were placed into 30×20 cm nylon mesh bags (mesh size ca 1 mm, 6 g fresh weight per bag) and bags were dipped for 30 s in the following treatment solutions: 1) Water control, 2) 2.5% urea, 3) 5% urea, 4) 10% urea, 5) 20% urea, 6) 5% fish fertilizer (Talgro, containing 5% N), 7) 0.1% cupric hydroxide (Kocide DF, containing 40% cupric hydroxide) or 8) 0.004% myclobutanil (Systhane, containing 40% myclobutanil). After dipping, the mesh bags were blotted dry and exposed on mown grass at meteorological sites at Broadfield, Canterbury and at Owairaka, Auckland from 19 June until 9 October 1990. There were 6 replicate bags per treatment per site.

A forced discharge method was used to assess ascospore production from leaves in the mesh bags. On 10 October 1990 the mesh bags were wetted for 5 min in water with 0.01% Tween 20, then blotted dry and placed in plastic bags with the tops tied on the laboratory bench, near a window but out of direct sunlight, for 4 h. Ascospores were washed out of each bag after 4 h with 100 ml of water containing 0.01% Tween 20. The washings were poured through a 45 µm sieve into a volumetric beaker. The volume of water retrieved was noted and later used to calculate numbers of ascospores per ml. Ascospores from each replicate bag were counted under the microscope at 400× magnification using a haemocytometer. Four samples were taken from each replicate and both sides of the slide were counted, giving eight counts per replicate. Leaves in the bags were air dried for 2 days, then weighed and ascospore numbers were expressed as numbers of ascospores per gram of leaf material. Ascospore counts were square root transformed before analysis.

Effects of urea and leaf fragment size on ascospore production and leaf decomposition

Black spot-infected apple leaves of cv. Royal Gala were collected from an organic orchard at the HortResearch Crosses Road Research Orchard, Hawkes Bay, in May 1999, shortly after they had fallen to the ground. Leaves were subject to two urea treatments (dipped for 30 s in 5% urea or in water as a control) and three leaf size treatments (intact, ca 4-8 cm^2 and ca 0.5-1.0 cm^2 leaf pieces) in a factorial design with three replicates of each treatment. Leaves were placed in 25 × 25 cm plots on bare ground within the rows of trees in a conventionally managed apple orchard where the background amount of black spot inoculum was low. Plots were covered with chicken wire mesh to prevent leaves being windblown. Leaves falling from surrounding trees were removed from plots once per week during autumn. Percentage of ground area covered by leaves was assessed on 5 October 1999 by placing a clear plastic sheet marked with a grid over plots, and scoring presence or absence of leaf litter beneath 100 grid points per plot.

An ascospore suction trap was used to assess ascospore production from the plots. On 28 September and 4 October 1999, plots were sprinkled with water to saturate the leaves and each plot was covered with a suction spore trap, which sampled 10 litres of air per minute for 1 hour. Five areas of ground within the orchard rows that were soaked and covered with spore traps detected no ascospores, indicating that background ascospores did not influence the results. Ascospores were counted on nine transects across the spore imprint band on each slide, using 400× magnification. Ascospore counts were square root transformed before analysis.

Suppression of ascospores with 5% urea sprayed onto fallen leaves in autumn

Black spot-infected apple leaves were collected from beneath trees of cv. Braeburn at leaf fall in May 1997 and placed in a single layer between two layers of chicken wire mesh (40 × 25 cm). The four replicate meshes of each treatment were either sprayed with 5% urea on 23 May 1997 or were left untreated.

An exposed glass slide method (Manktelow and Beresford 1995) was used to assess ascospore production from the leaf meshes. Glass microscope slides (76 × 26 mm) were placed on the leaf meshes in the field (five slides per treatment) for 4 days from 11 September to 15 September 1997, after which they were removed and mounted in water with 60 × 22 mm coverslips. Ascospores which were released by natural rainfall were counted in three lengthwise transects of each slide at 200× magnification. Counts were transformed to square roots before analysis.

Field control of black spot using autumn-applied urea

A two year field trial at the Crop & Food Research Pukekohe Research Station in 8-year-old trees of the apple cultivars Royal Gala and Fuji investigated the effects of autumn-applied urea on black spot arising as a result of primary infection by ascospores. Each cultivar consisted of two rows of 50 trees and each treatment plot was four trees long by two rows wide (eight trees) for each cultivar.

Five urea treatments (0%, 2.5%, 5%, 10% and 20%) were applied as two replicate concentration gradients along the apple tree rows. The 0% urea treatment occurred at the outside ends of the rows and both 20% concentration plots were adjacent to each

other in the middle of the block. This gradient layout, as opposed to a randomized block layout, reduced the effects of inter-plot interference which could mask treatment differences if high and low disease levels occurred in adjacent plots. The block had an historically even incidence of black spot within each variety.

Urea was applied twice during leaf fall in 1997, over both varieties each time, on 29 May (25% leaf fall in Royal Gala) and on 16 June (75% leaf fall in Royal Gala). In 1998 urea was applied three times, on 18 May (25% leaf fall in Royal Gala), 1 June (50% leaf fall in Royal Gala) and 15 June 1998 (80% leaf fall in Royal Gala).

During both growing seasons all trees received a standard fungicide programme (7-10 day applications from green tip until early December and 14-21 day applications from December until harvest) of black spot and powdery mildew fungicides, including metiram, dodine and penconazole, applied at standard recommended rates (Foot 1998). Black spot was assessed, on all the leaves on 40 shoots per plot, as the percentage of leaves with one or more black spot lesions, on 11 December 1997 and on 23 December 1998.

RESULTS

Effect of urea rate and other compounds on ascospore production

Autumn urea at all concentrations significantly reduced ascospore numbers compared to the water control (Fig. 1). Numbers of ascospores/g leaf litter were inversely proportional to the log of the urea concentration and the following equations

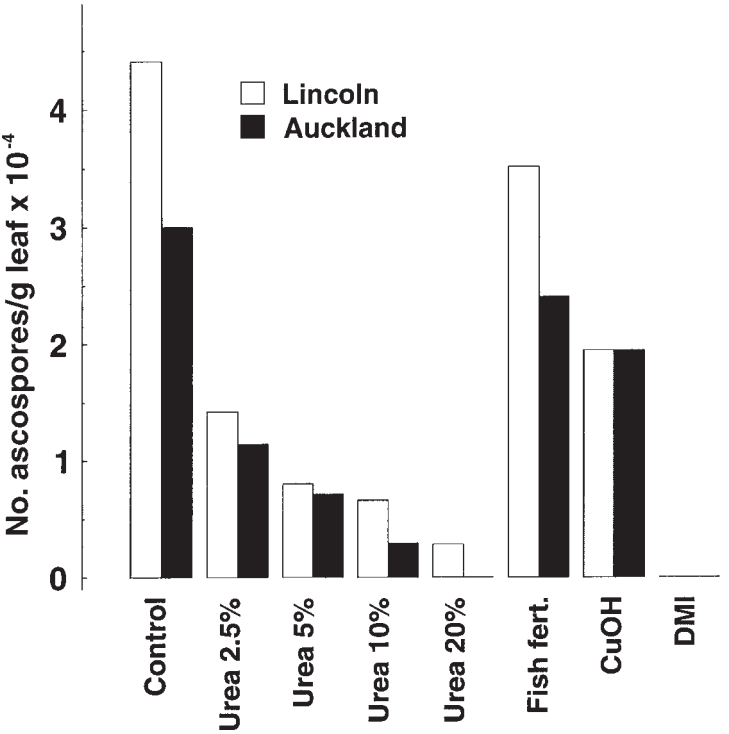

FIGURE 1: Numbers of ascospores/g of leaf litter (square root transformed) from leaves treated with different rates of urea, and with other compounds, then overwintered at Lincoln and Auckland. All treatments, except fish fertilizer, were significantly different from the control ($P<0.05$).

explained 98% and 99% of the variation in ascospore numbers for Lincoln and Auckland respectively:

Lincoln $\sqrt{\text{No. ascospores/g}} \times 10^{-4} = 2.41 - 1.846 \times \log_{10}(\%\text{urea}+0.1)$

Auckland $\sqrt{\text{No. ascospores/g}} \times 10^{-4} = 1.67 - 1.323 \times \log_{10}(\%\text{urea}+0.1)$.

Fish fertilizer did not significantly reduce ascospore numbers compared to the control, but cupric hydroxide did. The demethylation inhibitor (DMI) fungicide, myclobutanil, gave complete suppression of ascospore production.

There were significantly ($P<0.001$) fewer ascospores from leaves overwintered at Auckland than at Lincoln, but the treatment rankings at both sites were identical. The lower ascospore numbers from Auckland were associated with a faster rate of leaf litter breakdown than at Lincoln (data not presented).

Effects of urea and leaf fragment size on ascospore production and leaf decomposition

Ascospore numbers on suction trap slides were significantly lower from plots with 5% urea treated leaves than from plots with untreated leaves for both trapping dates (Table 1). Urea did not significantly affect the percentage of ground covered by leaves in spring. Ground area covered by leaves in spring was significantly lower in the small-fragment treatment, but leaf fragment size did not have a significant effect on numbers of ascospores per plot.

TABLE 1: Main effects in the factorial urea x leaf size experiment, showing mean numbers of ascospores (back-transformed) per transect and percentage ground area covered by apple leaves. The urea x leaf size interaction was not significant ($P>0.05$) for ascospore counts in Sept or Oct, nor for ground area covered by leaves.

	Number of ascospores counted		% ground area covered by leaves
	28 September	4 October	4 October
A. Main effect for urea			
with urea	1.7	1.9	56
without urea	16.7	35.1	53
P-value	P<0.001	P<0.05	P>0.05
B. Main effect for leaf size			
Intact leaves	8.2	15.1	58
medium-sized fragments	11.0	26.5	64
small-sized fragments	3.6	3.7	42
P-value	P>0.05	P>0.05	P<0.01

Suppression of ascospores with 5% urea sprayed onto fallen leaves in autumn

Spray application of urea to leaves on the ground resulted in the 5% urea treatment having significantly ($P<0.05$) fewer ascospores (back-transformed mean of 14 ascospores per transect) than the untreated control (back-transformed mean of 119 ascospores per transect). This represented an 88% reduction compared to the untreated control.

Field control of black spot using autumn-applied urea

In 1997 none of the urea concentrations had a significant effect on black spot incidence for either cultivar, with a mean incidence of 3.1% over both cultivars. In 1998 there was a significant trend ($P<0.01$) for black spot on Fuji trees to decrease with increasing urea concentration, black spot incidence being inversely proportional to the log of the urea concentration (Fig. 2). The trend was not significant in Royal Gala ($P>0.05$).

DISCUSSION

These studies have shown that urea substantially reduces ascospore production of *Venturia inaequalis* in New Zealand apple cultivars under New Zealand climatic

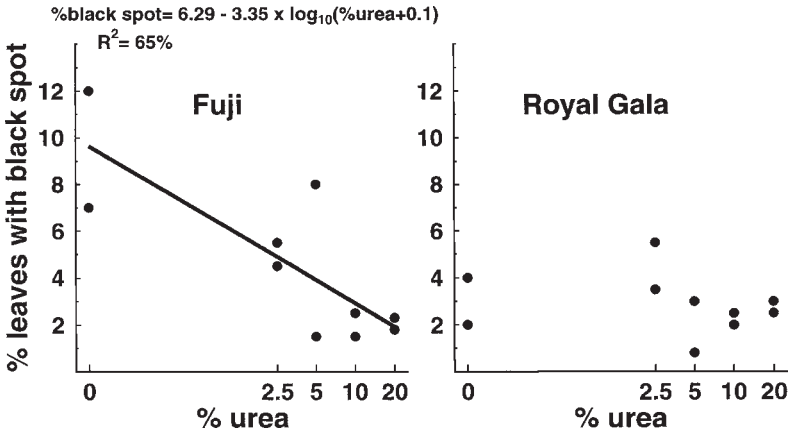

FIGURE 2: Black spot incidence on leaves in December 1998 at Pukekohe in response to different urea concentrations applied to trees in autumn 1997 and 1998. Black spot incidence in Fuji was inversely proportional to the log of the urea concentration, but in Royal Gala there was no significant trend.

conditions. Urea concentrations as low as 2.5% are effective, although the higher the concentration, the greater the ascospore suppression. Five percent autumn-applied urea gave reductions in ascospore production of 76%-88%, compared to untreated controls, at Lincoln, Auckland and Hawke's Bay. Reducing ascospore numbers to that degree should assist in black spot control. The Pukekohe trial showed a significant reduction in leaf black spot in response to urea in one of the two cultivars, in one of the two years. Although the reduction in disease was inconsistent in this small-plot trial, probably due to movement of leaves and dispersal of ascospores between plots by wind, use of urea in whole orchard blocks could be expected to give more consistent disease suppression.

When using urea in apples consideration must be given to the effects of additional nitrogen on tree nutrition and to possible damage to apple buds which can result from an excessive concentration of urea or from repeated urea sprays (Wood and Beresford 2000).

The fungicide cupric hydroxide, applied at the standard rate for winter use in dormant apple orchards (0.1%), reduced ascospore production, although not as much as urea. The tendency for copper to accumulate in orchard soils and its lesser ability to suppress ascospore production mean that autumn-applied copper is much less desirable than urea for black spot management. The DMI fungicide, myclobutanil, which gave complete ascospore suppression, was included for comparison with the other treatments. There is a high risk that *V. inaequalis* could develop resistance to DMI fungicides (Gaunt *et al.* 1996) and their use in autumn for black spot management is not advised.

Urea is unacceptable for use in "organic" orchards because of the way it is manufactured. Fish fertilizer was tested in this study to see if it could suppress ascospore production and provide an acceptable "organic" alternative to urea. The results were inconclusive, possibly because the amount nitrogen applied in the fish fertilizer was very low compared to that in the urea treatments, e.g. 5% urea (47% N) applied in 2,000 litres water/ha gives 47 kg N/ha, whereas 5% Talgro fish fertilizer (5% N, M. Daly, pers. comm.) gives only 5 kg N/ha. It is also possible that the form of nitrogen in fish fertilizer is not as effective in suppressing ascospore production as is urea. Investigation of higher fish fertilizer application rates and of other nitrogen-rich materials is required.

Size of leaf fragment did not significantly affect ascospore production and it appears that reduction of ascospores is more effectively achieved through the direct effect of urea on development of the fungus than through the indirect effect of leaf fragmentation. However, the loss of leaf area during winter was significantly greater in the treatment that had the smallest leaf fragments and it is likely that fragmenting leaves by mowing would enhance leaf decomposition and reduce overwintering of *V. inaequalis*. Mowing, as opposed to the leaf cutting method used in this study, would also mulch leaves with other organic matter, which might further enhance leaf decomposition. Although we were not able to demonstrate that urea enhanced the loss of leaf area in overwintered leaves, it is likely that urea combined with mowing would give greater reduction of ascospores than either method alone.

Three methods of ascospore assessment were successfully used in this study: 1) forced discharge by laboratory wetting of field-collected leaves, 2) field use of a suction spore trap with artificial wetting to release ascospores and 3) field-based passive trapping of ascospores released by natural rainfall. The latter, glass slide method has previously been used to determine the relative numbers of ascospores released in different parts of the growing season (Manktelow and Beresford 1995), but this study shows it can also be used to study the effects of leaf litter treatment on ascospore production.

In New Zealand, 1-2 applications of urea during leaf fall are recommended as a standard black spot management procedure in the Integrated Fruit Production manual for apples (Anon. 1998). The experiments conducted in this study support the usefulness of this practice and give insights into how urea use can be optimized to make it an effective component of black spot management programmes.

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