

## TREATMENT OF OVERWINTERING APPLE LEAVES TO REDUCE PRIMARY INOCULUM OF APPLE BLACK SPOT

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### ABSTRACT

Field trials were conducted in Hawke's Bay and Lincoln into methods of treating over-wintering apple leaves to reduce ascospore production by the apple black spot pathogen, *Venturia inaequalis*. The leaf treatments comprised three levels of nutrient amendments (including a water control) and five levels of saprophytic fungal isolates (including a no-fungus control), in a factorial design. Leaves were left to over-winter on the orchard floor, and in spring the *V. inaequalis* ascospores released were trapped on glass slides and counted. Ascospore numbers were reduced ( $P < 0.05$ ) by the leaf amendment urea, which alone caused 73% reduction, but not by the Bio-Start™ product. The effect of fungal isolates was not significant ( $P = 0.12$ ), although when combined with the water treatment, the isolates, *Chaetomium*, *Phoma* and *Epicoccum* spp. and *Trametes versicolor* reduced numbers of ascospores by 33, 27, 15 and 28%, respectively, compared to the no-fungus control. When combined with urea, the *Chaetomium* isolate reduced ascospore numbers by 92 and 82% compared to the nil fungus/water control treatments in Hawke's Bay and Lincoln, respectively, indicating that this treatment has potential for reducing primary inoculum of apple black spot.

**Keywords:** *Venturia inaequalis*, ascospores, leaf litter, urea, *Chaetomium*.

### INTRODUCTION

Apple black spot, caused by *Venturia inaequalis* (Cooke) Wint., continues to cause major economic losses in apple crops worldwide. Control strategies able to prevent pseudothecial development, and subsequent ascospore release from over-wintered, infected apple leaves, have the potential to reduce development of the disease in spring and to reduce the need for fungicide applications. Methods that attempt to target leaf litter include removing dead leaves, burning or scorching them on the orchard floor, and cultivating them into the soil or shredding them to enhance decomposition (MacHardy 1996). Application of nitrogenous compounds has also been found to reduce primary inoculum of apple black spot. Many authors have reported the beneficial effects of autumn applications of urea (Burchill 1968; Carisse et al. 2000; Beresford et al. 2000). Ross & Hamlin (1965) reported that nitrate and ammonium salts, and amino acids were equally effective to urea, completely preventing development of pseudothecia. MacHardy (1996) concluded that nitrogenous compounds caused increases in decomposition of litter because the treated leaves accumulated more micro-organisms and were favoured by earthworms, which fragmented and removed them from the soil surface.

The use of micro-organisms to decompose litter or to act as antagonists to the pathogen during its period of saprophytic growth within the litter, has also been investigated. In laboratory trials, many fungi which originated from apple leaf litter

and soil were found to inhibit vegetative growth of *V. inaequalis* (Ouimet et al. 1997), as well as to soften leaf tissues, and to suppress pseudothecium formation (Phillion et al. 1997). However, in field trials only a few of the isolates were effective at reducing the numbers of ascospores. In Canada, Heye & Andrews (1983) reported that *Athelia bombacina* and *Chaetomium globosum* decreased numbers of ascospores by 100 and 90% respectively, and Carisse et al. (2000) reported that *A. bombacina*, *Microsphaeropsis*, *Ophiostoma*, *Trichoderma* spp. and urea reduced ascospore numbers by 97, 84, 52, 75 and 97%, respectively.

In New Zealand, a recent study by Tshomo et al. (2003) has identified local fungal isolates that were able to enhance decomposition of apple leaf discs under laboratory conditions. The field trial reported here investigated the effects of applying the most promising treatments to apple leaf litter, on numbers of ascospores released in spring.

## MATERIALS AND METHODS

A field trial set up in two apple-growing areas, Hawke's Bay and Lincoln, investigated the effects of four isolates of *Chaetomium*, *Epicoccum*, *Phoma* and *Trametes versicolor* (L.) Pilat and the leaf amendments, urea and the mixed Bio-Start™ products, Mycorrcin™ and Digester™. These treatments have been fully described by Tshomo et al. (2003). For both sites, senescing leaves, heavily-infected with *V. inaequalis*, were picked just before leaf fall in April 2001, from an unsprayed block of mature Red Delicious apple trees at Lincoln University, and were stored in paper bags at 4°C until needed.

Mesh bags, 22 cm x 30 cm, were made of insect screening (vinyl-coated fibreglass yarn, 0.275 mm thick, with 1.8 mm x 1.4 mm mesh, Permathene Ltd, Auckland), and were fastened together with heavy-duty 9 mm stainless staples. The 30 apple leaves per bag were selected indiscriminately from the storage paper bags, thereby using a mix of leaf sizes in each bag, and were laid flat with their edges overlapping slightly, to completely cover the bag area. The filled leaf bags were soaked for 5 min in the same concentrations of leaf amendment solutions as described by Tshomo et al. (2003) and then air-dried overnight at room temperature.

Inoculum of the fungal isolates was prepared by macerating seven-day-old cultures in water, using a stomacher blender, as described by Tshomo et al. (2003). Leaf bags were laid out in a single layer on moist newspaper on laboratory benches and then inoculated, one drop of mycelial suspension or water per leaf. Bags were immediately misted with tap water and covered with a double layer of moist newspaper. They were left for 5 days at room temperature (14-20°C) to allow the fungi to colonise the leaves, the surface of the newspaper being sprayed with water twice daily to keep them moist. The pre-colonised leaf bags were then placed on the ground in apple orchards, a commercial Hawke's Bay orchard (Crosses Road, Havelock North) and the same abandoned apple orchard from where the leaves had been collected at Lincoln University. For each treatment there were six replicates (180 bags in total). The leaf bags were laid out on 6 July 2001 in a randomised split-plot design, in the spaces between rows of 3-5 year old trees, on bare ground that had been treated with the residual herbicide, simazine (1.1 kg a.i./ha). The bags were pegged to the ground using 7.5 cm furniture staples, individual leaf bags being separated by 20 cm and replicate blocks by about 1 m, and were left to overwinter until late October 2001. No further pesticides were applied during the trial.

In spring, when ascospores were mature and there had been at least 3 days of dry, sunny weather, the bags of leaves were wetted to run-off, and immediately afterwards, four glass microscope slides were randomly placed onto each leaf bag. Slides were left for 2-3 hours, before being collected into vertical slide boxes. These spore-trapping procedures were used in the Hawke's Bay on 24 September and 5 October, and in Lincoln on 6 and 19 October. At the first spore-trapping event at Hawke's Bay, two extra slides were laid out on all leaf bags of all treatments and these were used for development of a spore-counting technique.

The ascospores were generally found in clumps, randomly dispersed over the slide, and so it was not possible to accurately count them *in situ*. The method developed for

mounting the ascospores involved 'sweeping' a 20 µl drop of water containing 0.1% Tween 20 several times across the slide with a cover slip, before scraping it to the middle and mounting the suspended spores under the same cover slip. This technique resulted in the spores being more uniformly dispersed, with few (3% of total ascospores) being left outside the cover slip.

A method of sub-sampling fields of view (FOV) was developed. In a small experiment, all FOV under the cover slip were counted for two slides from two blocks of two treatment combinations, and their totals plotted in a grid. Results showed that the ascospores were relatively evenly distributed, and so an accurate estimate of total numbers could be made when only 30 FOV, at 400 x magnification, were evenly selected across the cover slip. The FOV for counting the remaining slides were selected by beginning at one corner of each cover slip, and skipping two FOV after every one counted, in both horizontal and vertical directions.

The total number of spores from the 30 FOV was recorded for each slide and the number of ascospores per cm<sup>2</sup> calculated. These values were log transformed and the data from spore trapping at times 1 and 2 were analysed separately within the sites by analysis of variance (ANOVA) using GenStat, before being stacked and analysed with a nested ANOVA. Significance comparisons between treatments were done with Fisher's LSD test (P=0.05).

## RESULTS

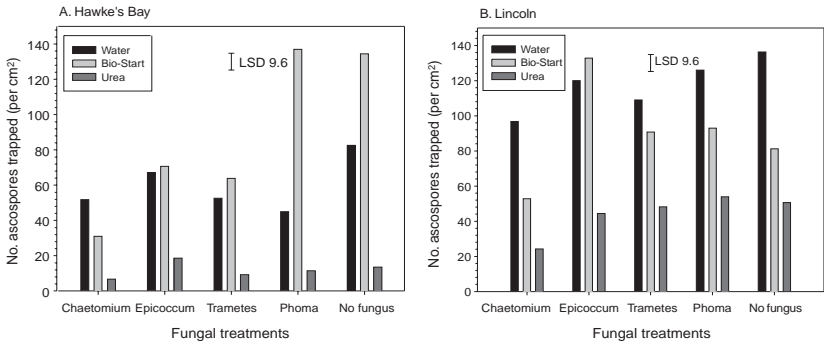
More ascospores were trapped at Lincoln than at Hawke's Bay, with 53 and 84 ascospores/cm<sup>2</sup> on slides from Hawke's Bay and Lincoln, respectively. Significance levels for the main and interaction effects are shown in Table 1. Of the leaf amendments, the Bio-Start™ treatment did not reduce numbers of ascospores trapped, whereas urea caused a reduction of 73% (P<0.05) compared to the water only treatment (Fig. 1). Although the fungal isolates did not cause an overall significant effect at either of the sites, they did cause reductions in numbers of ascospores compared to the nil fungus control. For *Chaetomium*, *Phoma* and *Epicoccum* spp. and *Trametes versicolor*, the respective reductions were 37, 46, 19 and 38%, in Hawke's Bay and 29, 8, 12 and 20% in Lincoln. The *Chaetomium*/urea treatment reduced ascospores trapped by 92 and 82% compared to the nil fungus/water control treatments in Hawke's Bay and Lincoln, respectively.

**TABLE 1: Probability values determined by ANOVA, showing the effects of leaf amendments and fungal isolates applied to apple leaf litter in late winter at two sites on numbers of ascospores trapped from litter the following spring.**

Site	Fungal isolates	Leaf amendments	Interaction (fungi x amendments)
Hawke's Bay	0.120	<0.001	0.247
Lincoln	0.054	<0.001	0.302

## DISCUSSION

The differences in numbers of ascospores trapped at the two experimental sites were probably due to the different climates at the two sites, since the periodicity of rainfall causes differences in ascospore maturation and release (MacHardy 1996). The significant effect of the nutrient amendment urea, which caused a 77% reduction overall in ascospore numbers in this trial, agreed with those from other field trials, in which urea reduced ascospore numbers by 65-95% in England (Burchill 1968) and 88% in New Zealand (Beresford et al. 2000). However, Bio-Start™ was ineffective despite the leaf decomposition effects described in recent European trials (G. Besamusca, pers. comm.).



**FIGURE 1: Numbers of *V. inaequalis* ascospores trapped/cm<sup>2</sup> on glass slides that had been placed onto apple leaf litter treated with a range of fungal isolates and the leaf amendments, urea, Bio-Start and water, in early winter in field trials in (A) Hawke's Bay and (B) Lincoln. Data are means of two trapping times in spring. Error bars shown are the Fisher's LSD ( $P < 0.05$ ) for comparing spore number between nutrient treatments.**

The effect of the fungal isolates in reducing numbers of ascospores was not significant overall. However, the *Chaetomium* isolate caused reductions of 87% in combination with urea, 33% with the water control, and 61% overall, results which were comparable with other reports. Miedtke & Kennel (1990) tested two isolates of *Chaetomium* and found that the reductions in ascospore numbers were about 30-40% and 65% respectively, while Heye & Andrews (1983) found that overall *C. globosum* caused a 90% reduction when tested with a range of nutrients. The other isolates tested in the current work were much less effective than *Chaetomium*, reducing ascospore numbers by 28, 27, and 15%, for *Trametes versicolor*, *Phoma* and *Epicoccum* spp., respectively. In laboratory trials, Pillion et al. (1997) found that reductions in ascospore numbers were 72, 21 and 99% respectively, for isolates of *Chaetomium*, *Epicoccum* and *Phoma*. However, their work was an *in vitro* trial, in which sterile leaf discs were inoculated with *V. inaequalis* conidia and incubated at room temperature for 3-4 weeks before inoculation with the isolates. The lack of competition and the more uniform and favourable environment was therefore likely to have improved the isolates' activities.

In this study, no attempt was made to recover the isolates from the field-incubated leaves at the end of the ascospore dispersal period, unlike the trial of Carisse et al. (2000). The failure of some isolates to be effective antagonists may have been due to their inability to survive in the external environment. In future trials, it is recommended that a wider range of saprophytic fungal isolates be selected from apple leaf litter, and that these isolates be applied in combinations and with nitrogenous compounds. The length of time for saprophytic pre-colonisation of leaves and the time for setting out in the field should also be studied. Once the leaves have been laid out in the orchard floor environment, survival of the isolates during winter should be determined by frequent sampling. From these isolates, further selections could identify those able to degrade apple leaf tissue quickly, and/or to antagonise *V. inaequalis* effectively, and so provide an additional tool in integrated disease management programmes.

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