EVALUATION OF AN ASCOSPORE MONITORING METHOD FOR *VENTURIA INAEQUALIS* TO IMPROVE APPLE BLACK SPOT FUNGICIDE MANAGEMENT

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ABSTRACT

Release of *Venturia inaequalis* ascospores was monitored from August to December over 3 years in three apple cultivars in five New Zealand apple growing regions by passive collection of naturally discharged ascospores on microscope slides placed above leaf litter samples. Cumulative counts of ascospores were subject to logit transformation and linear regression to determine dates that various cumulative percentiles were reached in different regions and seasons. Apple cultivar and site within a region had little effect on the timing of release. Region had a large effect, with later release in more southern regions, and there were large variations in the timing of release between seasons within a region. Most variation occurred in the dates that cumulative percentiles were reached, rather than in the rate of ascospore maturation. There was a non-significant trend for slower maturation in more southern regions.

Keywords: apple, *Venturia inaequalis*, black spot, ascospore release, disease management

INTRODUCTION

New Zealand research on a disease management system (Manktelow and Tate 1991) for apple black spot (*Venturia inaequalis*) has so far focused on weather-based infection period monitoring and weather forecasting (Beresford and Spink 1992; Laurenson and Manktelow 1991). The potential to reduce fungicide use through use of infection period information has been demonstrated (Beresford and Manktelow 1994). However, weather-based assessments of disease risk address only one aspect of disease management, and inoculum information is required to further improve black spot disease management, which relies on prevention of spring ascospore infection with fungicides.

General ascospore maturation patterns (Brook 1976) have been useful for establishing seasonal fungicide schedules, but more specific information is required to assist decisions relating to individual fungicide applications. Models developed overseas to predict ascospore maturation have not provided useful predictions under New Zealand conditions (Manktelow 1990). The research reported in this paper investigates a passive collection method for ascospore monitoring, and evaluates its use in relation to different apple cultivars, regions and seasons.

MATERIALS AND METHODS

Ascospore release was monitored by weekly counts of ascospores naturally discharged onto glass microscope slides, using a modification of Parham's (1932) method. Apple leaves were collected from beneath trees (80-90% leaf fall) that were known to have had high levels of black spot in the previous season. Twenty leaves were placed between two layers of galvanised wire mesh (2 cm grid, 350 x 250 mm), and

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care was taken to ensure that the upper sides of leaves on the orchard floor were kept uppermost in the meshes. The meshes were placed on herbicide-sprayed patches of ground in the apple orchard where the leaves were collected, in areas protected from fungicide or urea sprays. Fine gauze shade cloth was placed under the meshes to reduce loss of litter to earthworms and slugs.

From the last week of August, 4-5 glass microscope slides (76x26 mm) were placed on the meshes and secured with wire clips. Slides were removed for examination at weekly intervals and were replaced with clean slides in exactly the same position over the litter. Slides were mounted with water and a 60x22 mm coverslip and the ascospores in a single lengthwise traverse of each slide were counted at 200x magnification. Counts continued until the last week of December.

In evaluating the method, leaf meshes containing leaves collected and overwintered at the HortResearch Hawke's Bay Research Centre, Lawn Road, were distributed to different monitoring sites in early August when the first mature ascospores were observed in pseudothecial squash mounts (Gadoury and MacHardy 1982a). Some leaf meshes were also collected, overwintered and monitored in Nelson (Appleby Research Orchard) and Otago (Clyde Research Orchard). A total of 220 slides were examined from various combinations of treatments which included three apple cultivars (Gala, Braeburn, Fuji), three seasons (1992, 1993, 1994) and five regions (Auckland, Hawke's Bay, Nelson, Canterbury, Central Otago).

RESULTS

Ascospores counted at each sampling date were accumulated for each slide and the cumulative numbers were expressed as a percentage of the final cumulative total for each slide. Slides with total spore counts of less than 100 and those where spores were observed for fewer than five sampling dates were excluded from subsequent analyses. The cumulative percentage spore counts followed a sigmoid pattern as reported by Gadoury and MacHardy (1982b). These were linearised by taking logits of the cumulative percentages (Schwabe *et al.* 1989). Simple linear regression was used to fit day numbers (1=1 January) to the logit cumulative counts. The regression coefficients and intercepts were used to calculate the day numbers at which cumulative release reached 5, 10, 50, 90 and 95% for each slide and the durations of release from 10-90% and 5-95%. The replicate values of the slides on each litter mesh were used in analyses of variance to compare ascospore release patterns. In total, 123 out of the 220 slides were accepted for analysis. The relatively large number of slides which had too few ascospores to analyse statistically limited the comparisons which could be made.

Cultivar effects

In 1992, Braeburn and Gala leaves were compared at two sites 8 km apart (Lawn Road, Twyford) on the Heretaunga Plain in Hawke's Bay (Fig. 1A). The only difference between the cultivars was that Braeburn started releasing ascospores later than Gala at Lawn Road, as indicated by significantly later dates for 5% and 10% cumulative percentiles. There were no significant differences in the slopes of the regression lines between the two cultivars, indicating that the rate of maturation did not vary. Neither were there variations in the durations of release between the 10-90% or 5-95% percentiles.

In 1994, ascospore release from Braeburn and Gala leaves overwintered in Hawke's Bay was compared in Hawke's Bay and Auckland at sites about 300 km apart. Release was significantly later in Hawke's Bay than Auckland (Fig. 1B) for both cultivars. In general, no significant differences were found in other cultivar comparisons, which included Fuji versus Gala in Hawke's Bay and Fuji versus Braeburn in Nelson in 1993, and a further set of Gala versus Braeburn in Hawke's Bay in 1994. The only exception occurred in the latter data set, where ascospore production from Braeburn leaves continued for about 1 week longer than from Gala ($P<0.05$). Both cultivars started discharging at the same time, but Braeburn had a slightly slower maturation rate (Braeburn, 0.153 %/day versus Gala, 0.184 %/day; $P=0.052$) leading to a later finish of ascospore release.

FIGURE 1: Relative timing for various cumulative percentiles of ascospore release for combinations of cultivars, regions and years. Points within each cumulative percentile followed by the same letter do not differ significantly ($P < 0.05$).

Regional effects

Gala leaves overwintered in Hawke's Bay in 1994 were discharged in Hawke's Bay and also distributed to Auckland, Canterbury and Central Otago for discharge (Fig. 1C). The timing of production varied significantly ($P < 0.001$) among regions with a trend for later release further south. Otago was the exception, where ascospore release started earlier than in Canterbury but finished about the same time. The mean maturation rate for the Otago regressions was lower than for the other sites, although not significantly (Auckland, 0.156, Hawke's Bay, 0.148, Canterbury, 0.142, Otago, 0.126 %/day; $P = 0.163$). The durations of release did not vary significantly for either 10-90% ($P = 0.112$) or 5-95% ($P = 0.112$), but they did show a consistent trend for longer duration further south. A further regional comparison was made between Hawke's Bay and Nelson in 1993 for Fuji leaves collected and overwintered at the respective sites. Release started at the same time, but in Nelson it continued later, with 90% and 95% dates being significantly later. Maturation rate in Nelson was lower, although not significantly ($P = 0.207$).

Seasonal effects

Ascospore release from Gala leaves collected, overwintered and monitored in Hawke's Bay was compared in 1992, 1993 and 1994 (Fig. 1D). Release occurred significantly earlier in 1992 than in the other two seasons ($P < 0.001$), but the rates of maturation were not significantly different ($P = 0.405$).

DISCUSSION

Only 56% of the slides counted yielded enough spores for reliable analysis. This was thought to be due to low disease levels in some trees from which leaf litter was collected, together with the random collection of litter. Selecting only diseased leaves could increase spore yields and increase the efficiency of the method. Counting more slide traverses could increase the number of spores detected but it was found that if the first count was low, then additional traverses of the same slide also tended to produce

low counts. It was therefore considered better to conduct single traverses of several different slides than count a larger area of each slide. This monitoring method provides information on relative numbers of ascospores available, rather than on the absolute amount of inoculum. Therefore its usefulness lies in identifying the beginning and end of the ascospore release season.

There were remarkably few differences between cultivars in the timing of ascospore release, considering, for example, that Gala leaves fall up to 1 month before Braeburn leaves. Since cultivar appears to have little bearing on time of ascospore release, monitoring data from one cultivar in a region would probably be representative of other cultivars. The low within-district variation in timing of ascospore release suggests that monitoring would be required at relatively few sites to obtain data relevant for a large area of similar climate.

Ascospore release was generally later in more southern regions and these results agree with Brook (1976) who found that ascospore production was delayed at cooler sites and in cooler seasons. Our data showed that the regional differences arose mainly from variations in the time of onset and finish, rather than in the rate (regression slope) of maturation. There was, however, a non-significant trend for a slower rate of maturation further south. The overwintering of leaves in Hawke's Bay followed by their distribution to other regions meant that differences which arose in timing of release were due to regional climatic differences from September onwards.

The large differences in timing of ascospore release between seasons emphasises the need for ascospore monitoring for managing fungicide programmes. In Hawke's Bay, for example, the earliest and latest dates that 5% release occurred were 8 September and 3 October, 25 days apart. For 95% release they were as early as 19 October and as late as 5 November, 17 days apart. In apple orchards with low levels of ascospore inoculum, 5% and 95% release dates probably define the period when fungicide protection is needed to prevent ascospore infection.

The ascospore monitoring method evaluated in this study is relatively simple, needing only a compound microscope and a short period of training to allow assessors to accurately identify *V. inaequalis* ascospores. This evaluation showed that sites within a climatic region are similar in the timing of release and that different apple cultivars behave similarly. The method is therefore suitable for monitoring the onset and end of ascospore release within regions and should provide useful additional information to allow apple growers to time fungicides for black spot control more efficiently.

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