SOURCES OF VARIATION IN A FIELD EVALUATION OF THE INCIDENCE AND SEVERITY OF OLIVE LEAF SPOT

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ABSTRACT

Incidence (% infected leaves) and severity (number of lesions/leaf) of olive leaf spot disease, caused by *Spilocaea oleagina*, were assessed every 2 weeks on 20 trees in a Canterbury olive grove for 12 weeks during summer 2003/04. All the trees were infected by olive leaf spot disease (OLS) and although disease incidence and severity varied between trees ($P<0.001$), it did not vary between branches over time ($P=0.088$). There was a strong correlation ($R^2=0.869$) between disease incidence and severity. It was estimated that at least five trees and 50 leaves/tree were required to correctly estimate the mean values of the parameters measured. Throughout the duration of the experiment, no new leaf lesions formed and although old lesions increased in size ($P<0.001$), spore numbers decreased from 5×10^4 to 1×10^2 conidia/cm^2 of lesion and viability of conidia declined from 55 to 10%.

INTRODUCTION

Olive leaf spot (OLS), also called peacock spot disease, is caused by the fungus, *Spilocaea oleagina*, Castagne (Hughes) (syn. *Cycloconium oleagina*). It is widespread in all olive growing regions of the world, and has been known in the Mediterranean areas for over a century (Bernès 1923). OLS usually occurs on the upper surface of the olive leaf. As the spots expand and coalesce to cover a large proportion of leaf area, leaves often senesce and are shed from the tree prematurely. Leaf spots are usually more abundant on the lower parts of olive trees, and many shoots in these parts become completely defoliated. Recurrent infections often cause poor growth and dieback of defoliated twigs (Miller 1949; López- Doncel et al. 2000).

The influence of leaf cardinal point location on the distribution of OLS disease has been well documented. In the region of Setif, Algeria, OLS infection was reported to be more severe on north facing leaves as a result of their prolonged surface moisture retention and lower temperature (Guechi & Girre 1994). In New Zealand, OLS disease was found to be more abundant on south facing leaves, in trees with large dense canopies and in some cultivars, although all cultivars were affected (MacDonald et al. 2000).

The assessment of disease levels is usually by incidence or severity. "Disease incidence is the proportion (0 to 1) or percentage (0 to 100) of diseased entities within a sampling unit. Severity is the quantity of disease affecting entities within a sampling unit" (Seem 1984). For many plant diseases, only disease severity estimates are considered to give an accurate indication of their effects on the plants or of the efficacy of control treatments. Many different methods of estimating disease severity have been developed by various researchers although visual estimates of severity have been used almost exclusively. Estimates of severity are frequently based on lesion area but may also be based on lesion number. Seem (1984) noted that mean lesion counts per entity can be used to provide a true measure of severity, even though they cannot be expressed as a proportion or percent. However, visual estimates of disease severity can vary substantially between assessors whereas assessment of disease incidence is faster and more objective (Nutter 1997).

The purpose of this study was to develop a robust sampling and disease evaluation technique that tracked OLS development in further field trials. Since a study by MacDonald et al. (2000) found a high correlation ($P<0.001$) between numbers of OLS-infected leaves per tree, numbers of lesions per leaf and diseased leaf areas, and OLS lesions are known to expand very slowly, assessment of disease severity as the number of lesions per leaf was considered to be valid for this study. The relationship between disease incidence (% leaves infected) and severity was determined, and the rates of lesion development and spore production were also monitored.

MATERIALS AND METHODS

Field assessment was conducted during the summer of 2003/04 on 20 olive trees (6 years old, cv. Barnea) in the centre two rows of an olive grove in Canterbury, New Zealand. On each tree, seven branches were randomly selected on the south side at eye level, and labelled. At each fortnightly assessment, all the fully-expanded matured leaves on one branch for each tree were removed and taken to the laboratory for evaluation of disease incidence, severity and latent infection. Incidence was assessed by determining the percentage of infected leaves and severity by counting the number of lesions on each leaf (Teviotdale & Sibbett 1995). Lesion size was measured using a digital calliper (Mitutoyo Digimatic Caliper, Japan). To determine latent infection, 10 leaves randomly selected from each branch sample were dipped in 5% NaOH for 30 min (Shabi 1994) and examined for characteristic black spots.

Conidium numbers and their viability were determined on a randomly selected sub-sample (10 leaves with visible lesions) by washing conidia from the leaf lesions cut from each leaf vigorously for 1 min in 1 ml of distilled water. Conidia were counted (haemocytometer) in four sub-samples from each suspension, and their number/cm^2 leaf lesion calculated. To determine conidium viability, the 10 spore suspensions were pooled, the concentration adjusted to 5×10^4 conidia/ml and 100 µl aliquots plated (three replicates) onto olive leaf extract agar (Saad & Masri 1978). After incubation at 20°C for 48 h, 100 conidia/plate were examined using a light microscope at ×200 magnification, and the percentage germination recorded. A conidium was considered germinated if the length of the germ tube exceeded half the length of the conidium.

Analysis of variance (ANOVA) and model fitting were conducted using GenStat 7.2.

RESULTS AND DISCUSSION

All the trees were infected by olive leaf spot disease, but disease levels varied between trees ($P<0.001$), ranging from 1 to 52% for number of infected leaves and 0.01 to 1.7 for numbers of lesions/leaf. The variation in the levels of OLS between the trees was not due to a row effect ($P=0.236$) and may have been due to canopy size and density. It is known that the denser the olive tree canopy the greater the humidity and moisture retention capacity within and between the trees. This makes the trees more favourable for OLS infection (Teviotdale & Sibbett 1995; MacDonald et al. 2000). Therefore in future trials, olive trees of similar age/size and canopy density should be selected in order to reduce the between-tree variability of OLS disease.

The time of assessment had no significant ($P=0.088$) effect on OLS disease levels. The mean number of infected leaves ranged from 8 to 16% and the number of lesions/leaf ranged from 0.11 to 0.22, indicating no differences between branches over time for the 2003/04 summer. Assessing the mature leaves on one branch on the south side of the tree was considered an adequate strategy for estimating incidence and severity and this should be used in future trials. This validates earlier research by MacDonald et al. (2000). From the variance figures generated by analysis of variance, it was estimated that at least 5 trees and 50 leaves/tree were required to accurately estimate the mean values of these parameters.

The relationship between disease incidence (% infected leaves) and disease severity (number of lesions/leaf) for OLS was best described by a second order polynomial regression (Fig. 1; $R^2=0.869$). Incidence data (% infected leaves) are faster to collect

with greater accuracy than severity data (number of lesions/leaf), particularly when leaves are wet, since the lesions are not clearly visible. Therefore the % infected leaves should be used for assessing the efficacy of any control measures tested in the future. If required, the number of lesions/leaf can be derived from Figure 1. However, under high disease pressure (>50%) the level of accuracy may be reduced.

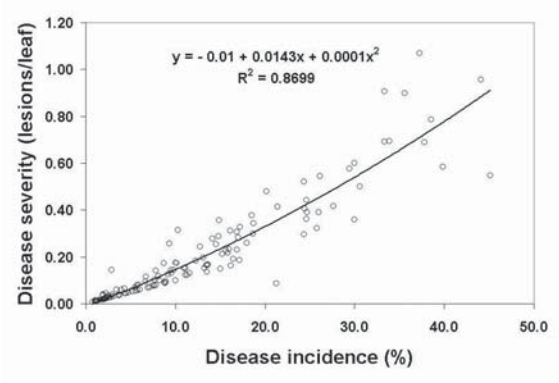

FIGURE 1: Relationship between OLS incidence (% infected leaves) and severity (number of lesions/leaf) across all trees and assessment times determined from a 12-week field assessment.

Lesion sizes did not differ significantly (P=0.095) between trees during the assessment period (data not shown), indicating that OLS lesion expansion was uniform between the trees. Although no new lesions on the leaves developed, latent infections were observed throughout the duration of the experiment. There was a significant (P<0.001) increase in the mean size of old lesions on the leaves for the first 8 weeks of disease monitoring (Fig. 2). Lesion sizes did not increase significantly from mid-December 2003 to 26 January 2004. This may have been due to the unusually hot and dry weather conditions recorded in Canterbury during the assessment period (Fig. 3). The results agree with previous overseas reports, which stated that during hot, dry summers the lesions stopped expanding and became dry, hardened, cracked or blistered (Miller 1949; Graniti 1993).

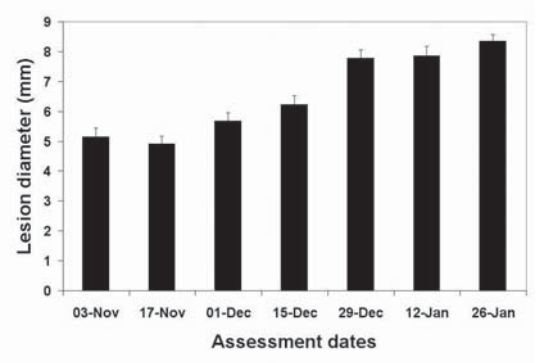

FIGURE 2: Mean size of *S. oleagina* leaf lesions during the summer of 2003/04. Bars represent the standard errors of the means.

The production of conidia by *S. oleagina* decreased over the period of assessment with the initial mean conidium numbers being 5×10^4 conidia/cm^2 of leaf lesion, whereas at the end of the experiment, the mean number of conidia was 1×10^2 conidia/cm^2. Conidium viability also followed a similar trend with spore germination declining from 55% to 10%. Conidium production and viability seemed to be related to environmental conditions, particularly rain and temperature, although lesion and conidium age could also be contributing factors. For example, in November 2003 the mean daily temperature ranged from 7 to 17°C and mean monthly rainfall was 36.7 mm. However, in January 2004 the mean temperature ranged from 12 to 25°C and mean rainfall was only 3.6 mm (Fig. 3). This result is consistent with Mediterranean research trials, which reported abundant conidium production during spring and autumn, but limited conidium production during the summer months (Laviola & Scarito 1993; Guechi & Girre 1994).

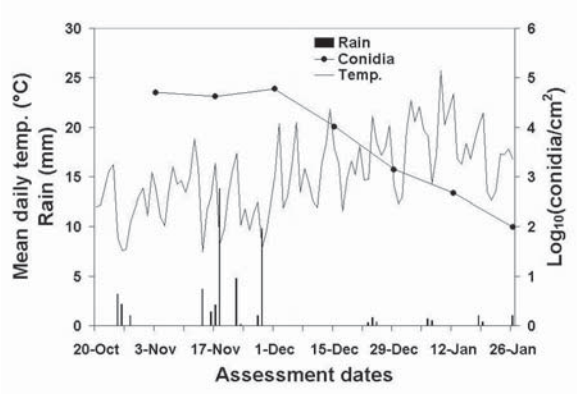

FIGURE 3: Conidium production by *S. oleagina* in relation to temperature and rain events during the months of October 2003 through to January 2004.

CONCLUSION

This study has shown that the main source of variation in OLS disease evaluation in the field in summer was between individual olive trees. There was a strong correlation between OLS incidence (% infected leaves) and severity (number of lesions/leaf). The % infected leaves measurement of disease incidence is less time-consuming than the number of lesions/leaf, and will be conducted on at least five trees and 50 leaves per tree in subsequent field trials evaluating fungicides for disease control.

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