Effect of partial defoliation on *Cylindrocarpon destructans* infection of grapevines

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Abstract One-year-old grapevines of Sauvignon blanc grafted to rootstocks 101-14 or Schwarzmann were used to evaluate the effects of partial defoliation and inoculation with *C. destructans* on disease severity, incidence and dry weight accumulation. Assessment 8 months after treatments showed that disease incidence was not affected by the defoliation treatments. However, disease severity was significantly highest for the highly defoliated plants ($P=0.043$). Incidence and severity were similar for Schwarzmann (29.3 and 8.4%, respectively) and 101-14 (31.0 and 14.9%, respectively). Root dry weight was affected by the defoliation treatments ($P=0.000$), being significantly lower in the highly defoliated plants than the moderately defoliated and undefoliated plants (15.6, 18.5 and 19.9 g, respectively). The practice of leaf removal in young vines clearly needs to be managed when black foot is a recognised risk factor for newly planted vineyards.

Keywords black foot, shoot trimming.

INTRODUCTION

Black foot, caused by *Cylindrocarpon* species, is one of the most destructive grapevine pathogens since it causes chlorosis, decline and eventually death of young grapevines in many newly planted vineyards worldwide. Black foot is recognised by the dark brown discoloration in the belowground trunk portions of affected vines, which can extend across the whole trunk and up to 10 cm above ground (Halleen et al. 2003; Oliveira et al. 2004). Scheck et al. (1998) concluded that death seemed to be inevitable when vines less than 10 years of age were infected with *C. destructans*.

Investigations into the disease cycle of grapevine black foot have shown that it is soil-borne. However, disease risk for young grapevines in nurseries and vineyards, is increased by stresses, including those caused by environmental and

vineyard management factors, such as poor drainage, soil compaction and inadequate planting holes, which cause poor root development, as well as poor nutrition, heavy cropping of young vines, high temperatures during summer and effects of other pests and pathogens (Scheck et al. 1998; Stamp 2001; Oliveira et al. 2004). Stamp (2001) concluded that processes of nursery propagation and vineyard establishment placed young vines under almost continuous stresses due to the wounds made during grafting, rooting processes, trimming during growth in the nursery field, uprooting, extended cold storage and establishment in the vineyard.

A standard practice for managing grapevines in cool climate regions is the removal of foliage several times a season, by shoot trimming and

removal of leaves from around fruit clusters (Hunter et al. 1995). Schoeneweiss (1981) reported that incidence and severity of stem and crown cankers and dieback diseases were usually increased in woody plants that were stressed, and that susceptibility to root rots was particularly increased through high intensity defoliation. Hunter et al. (1995) showed that early and harsh defoliation in one season resulted in lowered root storage carbohydrate levels and the premature mobilization of carbohydrate stored in the roots, trunks and canes. These practices are generally believed to have no adverse effects on grapevines, as the photosynthetic activity in the older leaves is increased and the sugar and organic acid levels in the fruit remain largely unchanged (Hunter et al. 1995). However, Marcais & Breda (2006) reported that the loss of leaf photosynthetic area through defoliation stressed plants by decreasing the availability and concentrations of photosynthates, which compromised their resistance to other biotic and abiotic stresses. The objective of this research, therefore, was to determine the effects of partial defoliation of young grapevines on black foot disease in young grapevines.

MATERIALS AND METHODS

Plant preparation

Plants of Sauvignon blanc scion wood grafted to two rootstock varieties, 101-14 or Schwarzmann, were purchased from a commercial nursery as 8-month-old, dormant, bare-rooted vines. They were planted in late winter (August 2006) into 4 litre pots containing a 50:50 mix of soil, which was sourced from the Lincoln University vineyard (Wakanui silt loam), and a potting mix [(80% horticultural bark (grade 2): 20% pumice (grade 3, 1-4 mm)]. The soil/potting mix contained 2 kg/m³ of a 3-4 month fertiliser (Osmocote Exact, Scotts Australia Pty Ltd; (16:5:9.2) (N:P:K)), 1 kg/m³ agricultural lime and 1 kg/m³ Hydraflo (Scotts Australia Pty Ltd). The vines were grown in a greenhouse (15-30°C) with high pressure sodium lamps (Son-T Agro 400, Philips) during 4 am to 12 pm and from 4 pm to 8 pm each day from March 2007 until harvest (July 2007). Vines were watered daily and flower buds were removed to prevent reduction of carbohydrate stores within the vines.

Defoliation treatments

Five months after the initial planting, vines were partially defoliated by removing leaves from all scion shoots above the fourth node. The three levels of defoliation were 0 (no leaves removed), 1 (every third leaf removed) and 2 (two of every three leaves removed). The treatments were repeated three times at 3-week intervals on newly developed sections of shoots.

Inoculation

At 3 weeks after the third trimming treatment, the root systems of all the vines were wounded and inoculated with a mixed isolate conidial suspension (10⁶/ml) of *C. destructans* (four isolates) or sterile distilled water (SDW; control). The cultures were grown on potato dextrose agar (PDA; Difco) for 7-14 days at 20°C in the dark, then washed with SDW containing 0.01% Tween 80 (polyoxyethylene 20 sorbitan mono-oleate; BDH Chemicals Ltd), while gently scraping the surfaces with sterile glass slides to loosen conidia. Each resultant suspension was filtered through a sterile sieve of 150 µm mesh and the conidium concentration adjusted to 10⁶ conidia/ml using a haemocytometer. The viability of the conidia was confirmed at 97% by counting colony forming units (CFU) that developed when spread onto PDA and incubated for 2 days at 20°C in the dark. The inoculum was prepared by mixing equal volumes of conidial suspensions from all isolates.

The vine root systems were wounded with an asparagus knife driven vertically into the soil at four equidistant points approximately 8 cm from each trunk. Each vine was inoculated by pouring 50 ml of the mixed conidial suspension, or SDW, onto the soil surface close to the wounding sites, followed by 50 ml of tap water to help distribute conidia into the soil. The potted vines were replaced in their original positions, which were allocated by a completely randomised block design with 6 treatments and 10 replicates.

Assessment

After 6 months (July 2007), grapevine plants were uprooted, washed to remove all soil and air dried for 2 h at room temperature. The roots and shoots (without leaves and petioles) were then cut from the trunks, dried in a 50°C oven to constant weight

(14 days) and then weighed. The bottom 20 cm of each trunk was immersed in 70% ethanol for 30 s, 0.35% sodium hypochlorite for 5 min and 70% ethanol for 30 s to surface sterilise it (Halleen et al. 2003). After drying in a laminar flow cabinet, the lowest 1 cm of each trunk base was discarded and a 1–2 mm transverse piece was sliced from the basal end of the trunk (0 cm) and from 5 cm above the base. The 0 cm section was cut into four pieces, which were placed equidistantly around the edge of a plate containing PDA with chloramphenicol (250 mg/litre) and half of the 5 cm piece was placed in the centre of the same plate. Plates were sealed with clingfilm and incubated at 20°C with 12:12 h cycles of darkness:white light for 7 days.

Identification of *Cylindrocarpus* spp.

Fungal isolates growing from the trunk segments were presumptively identified as *C. destructans* by colony morphology. To confirm identities, at least 30% of these colonies were randomly selected for subculture and the accuracy of identification was confirmed by morphologies of their conidia, as described by Halleen et al. (2004).

Statistical analysis

Since symptom development was not well advanced by the time of harvest, presence of *C. destructans* in a vine was taken as evidence of disease incidence, and the proportion of infected wood pieces at 0 cm as disease severity. Data were analysed by analysis of variance, with terms appropriate to the design and two-way interactions amongst the factors of interest. Where significant main effects or two-way interactions

were identified, the significance of differences between treatment means was determined by Fisher's protected least significant difference tests (LSD) at $P \leq 0.05$. All analyses were conducted with SPSS® for Windows® V13.0 and the mean values shown are those estimated by the programmes.

RESULTS

Disease incidence and severity at 0 and 5 cm above trunk bases

The three defoliation treatments did not affect *C. destructans* disease incidence at 0 cm or 5 cm from the trunk bases (data not shown) ($P > 0.05$). The three defoliation treatments also did not significantly affect ($P = 0.138$) disease severity, although highly defoliated plants had notably greater disease severity than the undefoliated and moderately defoliated plants, which were similar (Table 1).

When the 0 cm severity data of undefoliated and moderately defoliated treatments were combined and the analysis repeated, there was a significant effect of the defoliation treatments ($P = 0.043$; Table 1), with significantly greater severity in highly defoliated plants than those with moderate to nil defoliation. There was no difference in *C. destructans* incidence at 5 cm for the highest defoliation (17.9%) than those of the combined undefoliated and moderately defoliated group (12.6%; $P = 0.551$) in the second analysis.

Rootstock variety had no significant effect on disease incidence or disease severity, being similar for Schwarzmänn (29.3 and 8.4%, respectively) and 101-14 (31.0 and 14.9%, respectively).

Table 1 Mean *C. destructans* disease severity (% infected wood pieces) of grapevine rootstocks at 0 cm from the stem base after three levels of defoliation. Data are combined averages for inoculated and non-inoculated plants. Results are presented for the same data in two separate analyses.

Analysis one		Analysis two	
Defoliation	Infected wood pieces (%)	Defoliation	Infected wood pieces (%)
0	7.15	0 + 1	7.60 a
1	8.15		
2	19.65	2	19.65 b
P-value	0.138		0.043

¹Values followed by different letters are significantly different at $P \leq 0.05$ (according to Fisher's Protected LSD).

Cylindrocarpon destructans inoculation did affect disease incidence ($P=0.037$), with 40.5% and 19.5% incidence in inoculated plants and non-inoculated plants, respectively, and disease severity ($P=0.031$), with 17.9% and 5.4%, respectively.

Root and shoot dry weights

The root dry weights were significantly affected by the defoliation treatments ($P=0.000$), with highly defoliated plants having a significantly lower ($P\leq 0.05$) mean root dry weight (15.6 g) than the moderately defoliated and undefoliated plants (18.5 and 19.9 g, respectively), which were similar. The root dry weights were also affected by rootstock variety ($P=0.003$), with mean root dry weights for 101-14 and Schwarzmann being 19.2 g and 16.8 g, respectively. The interaction between rootstock variety and defoliation level was not significant ($P=0.062$). A significant interaction between inoculation and defoliation ($P=0.031$) was evident, with reduced mean root weights in some of the inoculated defoliation treatments (Figure 1).

Shoot dry weight was not influenced by defoliation treatments, inoculation with *C. destructans* or any related interactions. However, rootstock variety affected shoot dry weights ($P=0.000$), with means of 4.3 and 2.4 g for 101-14 and Schwarzmann, respectively.

DISCUSSION

Partial defoliation of young grapevines increased black foot severity in the trunk bases and was associated with a trend for decreasing root dry weights. Both effects are probably due to decreased carbohydrate concentrations in roots. Schoeneweiss (1981) also demonstrated a relationship between defoliation and disease development in cottonwood seedlings, which developed stem cankers after inoculation with *Cytospora* species and only when manually defoliated. Old et al. (1990) also showed that drought and defoliation in *Eucalyptus* species increased their susceptibility to stem cankers caused by *Endothia gyrosa* and *Botryosphaeria ribis*. Fewer and smaller cankers and lesions developed after inoculation of undefoliated trees compared with defoliated trees. The authors

Figure 1 Root dry weight (g) of *Cylindrocarpon destructans*-inoculated and non-inoculated ('no Cyl' and 'Cyl inoc') grapevine plants that received different defoliation treatments three times for each plant (0=no leaf removal, 1=one-third of new leaves removed, 2=two-thirds of new leaves removed). Analyses were conducted separately for the plants of rootstock varieties 101-14 and Schwarzmann. Different letters above bars indicate significant differences between values at $P\leq 0.05$ according to Fisher's Protected LSD.

concluded that the low carbohydrate levels in the stems of the stressed trees inhibited their normal compartmentalisation responses and callus formation, leading to greater symptom development in the stressed *Eucalyptus* trees.

Overall, disease incidence at 5 cm above the trunk base was not significantly different between defoliation treatments or rootstock variety, and incidence was less at 5 cm than at 0 cm, which was most likely due to the slow progression of the disease within the 8 months allowed for the experiment. In contrast, Halleen et al. (2004) reported vine death due to *Cylindrocarpon* black foot at 4.5 months after the grapevines were inoculated with the same concentration of a *C. destructans* isolate. However, those experiments were not as representative of the natural situation since plant roots were dipped in the conidium inoculum and the pots did not contain soil so lacked competition by soil microorganisms.

The reduced root dry weights found in the current experiment when high and moderate

levels of defoliation were applied concurs with research reported by Kliewer & Fuller (1973). In that study, when 0, 25, 50, 75 and 100% defoliation was used on potted grapevines, the root dry weights of 50 and 75% defoliated vines were significantly lower than those of the 25 or 0% defoliated vines with a clear trend in reducing weights associated with greater defoliation. The use of larger plants and the longer timeframe for that study (2 years) may have accounted for the greater significance of the results than in the current experiment.

The significant interaction between inoculation and defoliation level in this research, which was shown by the inoculated vines having reduced root dry weights in moderate and high defoliation treatments (compared to non-inoculated vines), may be explained by greater levels of *C. destructans* infection in vines that were partially defoliated. These infected vines probably lost their necrotic diseased roots, and had poor root development from necrotic crowns. Halleen et al. (2004) also reported that inoculation with *Cylindrocarpon* species, including *C. destructans*, significantly reduced both root and shoot mass in potted grapevines. In the present study, the differences in root and shoot dry weights were probably due to the greater vigour of 101-14 than Schwarzmann (Jackson & Schuster 2001), and may have accounted for the greater effects of defoliation seen in the Schwarzmann than 101-14 vines.

The lack of significant differences between treatments in this experiment was probably associated with few replicates and the use of soil, which was belatedly found to be infested with *C. destructans*, even though there had been no reported incidence of black foot in the grapevines growing in the source soil. The control plants were infected, albeit to lower levels than the inoculated plants, which resulted in great variability in the data and so confounded outcomes of the analysis. Use of potting mix, as used by Rego et al. (2001), or greater numbers of replicate plants, as used by Halleen et al. (2004), may have improved the significance of effects.

In summary, this research has confirmed previous vineyard observations that stress factors

such as defoliation can contribute to black foot disease severity in young vines. Therefore, management methods to prevent stress within new vineyards and propagation nurseries need to be investigated. They could reduce the economic losses, which have forced many grape growers to replant their young vines in the past (Sheck et al. 1998).

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