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Susceptibility of four grapevine rootstocks to *Cylindrocladiella parva*

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Abstract The susceptibility of four common grapevine rootstocks (101-14, Schwarzmann, 5C and Riparia Gloire) to *Cylindrocladiella parva* (black foot disease) infection was assessed in a pot experiment. The roots of 4-month-old callused rooted cuttings were wounded *in situ* and inoculated with 50 ml *C. parva* conidial suspension (10^6/ml) or sterile water (controls). After 6 months of growth, shoot dry weight was significantly higher for control plants (24.2 g) than for those inoculated with *C. parva* (16.5 g), but did not differ between rootstock varieties. Root dry weight was not significantly affected by *C. parva* inoculation, but root dry weight of 101-14 was significantly higher than other rootstocks. Incidence and severity of trunk infection were significantly affected by rootstock variety, being lowest in rootstock 101-14 plants than other rootstocks. None of the rootstocks tested was resistant to this pathogen.

Keywords black foot disease, disease severity, trunk infection.

INTRODUCTION

Black foot disease is a major problem worldwide, causing the decline and eventual death of young grapevines. A national sampling of symptomatic vines in New Zealand recovered *Cylindrocarpon/Ilyonectria* species (*C. destructans*, *C. liriodendri* and *C. macrodydimum*), and isolates identified as *Cylindrocladiella parva* from characteristic black foot symptoms (Bleach et al. 2007; Jones et al. 2012). *Cylindrocladiella parva* (syn *Cylindrocladium parvum;* Boesewinkel 1982) was recovered from the 101-14 rootstock of a symptomatic grapevine from a Central Otago vineyard and from the stem bases of 101-14 rootstock vines from an Auckland nursery (Jones et al. 2012). *Cylindrocladiella parva* is a common soil saprophyte and has previously been isolated from grapevines in New Zealand (Boesewinkel 1982; Gadgil et al. 2005), as well as roots and rootstocks of mature grapevines, cuttings and graft unions of grafted young grapevines in South Africa (van Coller et al. 2005) and mature grapevines in Spain (Agustí-Brisach et al. 2012). Although van Coller et al. (2005) reported that stem inoculations of green and 1-year-old grapevine shoots did not produce lesions that differed from the uninoculated control, Jones et al. (2012) demonstrated that root inoculations of grapevine rootstocks caused internal blackening of the stems, from which *C. parva* was isolated. Similarly Agustí-Brisach et al. (2012) reported inoculation of potting mix media resulted in reduced vigour, necrotic rot lesions and occasional mortality of grapevine seedlings.

This study assessed the susceptibility of four grapevine rootstocks commonly grown in New

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Zealand to *C. parva* applied by root inoculation with *C. parva* conidia.

MATERIALS AND METHODS

Fungal origin and inoculum production

Four single spore *Cylindrocladiella parva* isolates (LUPP1154, LUPP1155, LUPP1156 and LUPP1157) were used. All isolates were recovered from rootstocks of variety 101-14 in a vineyard nursery in Auckland and identity had been confirmed by sequencing rDNA, β tubulin and translation elongation factor genes (Jones et al. 2012). Spore suspensions were recovered from 3-week-old cultures on potato dextrose agar (PDA; Difco) and the conidium concentration adjusted to 10^6 conidia/ml based on haemocytometer counts, before combining them to give a mixed-isolate suspension. Conidial viability (92%) was determined by plating 100 µl of a 10^3/ml dilution of this mixed-isolate suspension on PDA and incubating at 20°C for 2 days.

Pathogenicity assay

Callused cuttings of four rootstock varieties, 101-14, Schwarzmann, 5C and Riparia Gloire, were obtained from a commercial nursery and rooted in trays of vermiculite on heat pads at 25°C. The rooted cuttings were then planted in 2.5 litre pots containing potting mix (80% horticultural bark (grade 2): 20% pumice (grade 3, 1-4 mm)). The potting mix contained 5 kg of an 8-9 month fertilizer, Osmocote Exact ((Scotts Australia Pty Ltd; (15:4.0:7.5) (N:P:K)), 1 kg agricultural lime and 1 kg Hydraflo (Scotts Australia Pty Ltd) per 1 m^3. Pots were arranged in a completely randomised block design with eight treatments and 10 replicates. The vines were grown on corrugated metal tables in a greenhouse (15-30°C) from January 2010 until harvest (November 2010), and placed under 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 May until July, after which they were allowed to naturally senesce until growth resumed in spring (Sept 2010). Vines were watered daily and all weeds removed by hand.

In April 2010, 4 months after planting, the root systems of the ten replicates vines per treatment were wounded using 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 (10^6/ml), or sterile distilled water (SDW; control), over the soil close to the wounding sites, followed by 50 ml of tap water. The vines were grown for a further 6 months to allow symptoms to develop.

Assessments

The grapevine plants were uprooted, washed and air-dried for 2 h at room temperature. The roots and shoots were removed from each trunk, and placed in a brown paper bag, to be dried in a 50°C oven to constant weight (after 7 days).

The vine trunks were sterilised in batches of the same treatment, with the bottom 20 cm of each trunk being immersed in 70% ethanol for 30 s, in 0.35% sodium hypochlorite for 5 min and in 70% ethanol for 30 s. Each trunk was air-dried in a laminar air hood, and the root crown (1 cm) removed from the trunk base. A 1-2 mm slice of the basal end of each trunk (0 cm) was then cut into four uniform tissue pieces (~3 mm^2) and placed onto a PDA plate containing chloramphenicol (Sigma; 0.25 g/litre). Another sliver of trunk tissue taken 5 cm above the basal section was placed in the centre of the same PDA plate. Plates were incubated at 20°C with 12:12 h of dark:light, for 7 days, after which the tissue sections with characteristic *C. parva* colonies were counted. The *C. parva* isolates were identified by comparison with culture plates and conidia of the pathogens used for inoculation. Symptoms were not well developed so presence of *C. parva* in a vine was taken as evidence of disease incidence, and the proportion of infected wood pieces at 0 cm as disease severity.

Statistical analysis

Disease severity data were subject to a non-parametric Kruskal-Wallis test and disease incidence data (proportion of infected plants) to a Chi-square test. Most of the un-inoculated control plants had no infection and so were not included in the analysis of rootstock varieties. Root and shoot dry weight data were subject to analysis of variance (ANOVA) with treatment means compared using

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Fisher's protected least significant difference tests (LSD) at P<0.05. Chi-square analysis was carried out using Minitab 15 with all other analysis carried out using GenStat 12.2.

RESULTS

Root and shoot dry weights

Across all rootstock varieties, there was a significant difference (P<0.001) in shoot dry weight between control (24.2 g) and *C. parva* inoculation (16.5 g) (Table 1), but there was no rootstock variety effect (P=0.261) nor interaction (P=0.559). Root dry weight was significantly affected by rootstock variety, with 101-14 being highest. Across all rootstock varieties the root dry weights of inoculated vines (mean 28.3 g) were not significantly different (P=0.468) from the corresponding controls (mean 30.5 g), and there was no significant interaction between rootstock and inoculation (P=0.968).

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

Mean disease incidence at 0 cm and 5 cm was significantly higher in the *C. parva* treatment (77.5% and 55%, respectively) compared with the untreated control (2.5% and 0%, respectively). For the inoculated treatments, the significant variety effect in disease incidence at 0 cm for the rootstocks, was associated with incidence in 101-14 (40%) being lower than for all other rootstocks (80-100%; Figure 1). There was no significant effect of rootstocks (P=0.750) on disease incidence at 5 cm, being similar (40-60%) for all rootstocks.

Cylindrocladiella parva mean disease severity was significantly higher in the *C. parva* treatment (51.2%) than the un-inoculated controls (0.6%; Figure 1). For inoculated vines, there was a significant variety effect on disease severity, with means being lowest for 101-14 (25%) and highest for Riparia Gloire (75%).

DISCUSSION

The study showed that all tested rootstock varieties were susceptible to *C. parva* infection, with Riparia Gloire being the most susceptible and 101-14 the least, based on the disease incidence and severity data. All *C. parva* isolates were originally recovered from 101-14 rootstocks, which probably reflects the relative abundance of 101-14, being the most widely grown variety at the time (2005) of the initial sampling and isolation (Bonfiglioli 2005), rather than its relative susceptibility to *C. parva*. The previous pathogenicity study by Agustí-Brisach et al. (2012) provided no details of the rootstock variety used and so offers no opportunity for comparison.

Using potted plants of grapevine rootstocks, Jaspers et al. (2007) showed that they varied in their susceptibility to a mixed inoculum of *Cylindrocarpon* spp., with 101-14 being highly susceptible, whereas Schwarzmann, 5C and Riparia Gloire had lower but more variable susceptibility. In field trials, incidence of rootstock infection differed between plots inoculated with conidia of the three *Cylindrocarpon* spp. and those with low levels of natural inoculum. In the inoculated plots, rootstock 5C was the least

Table 1 Shoot and root dry weight (g/pot) of four grapevine rootstock varieties after inoculation with *Cylindrocladiella parva*. Values are the mean of 10 replicates per treatment. Values within the rows or columns followed by the same letters are not significantly different.

	101-14		5C		Riparia Gloire		Schwart		Mean across rootstocks	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Control	26.5	38.0	26.9	27.4	23.3	29.1	20.0	27.4	24.2A	30.5
C. parvum	17.9	32.7	15.5	25.5	17.4	28.0	15.0	27.1	16.5B	28.3
Mean across treatments	22.2	35.4 a	21.2	26.4 b	20.4	28.5 b	17.5	27.3 b		

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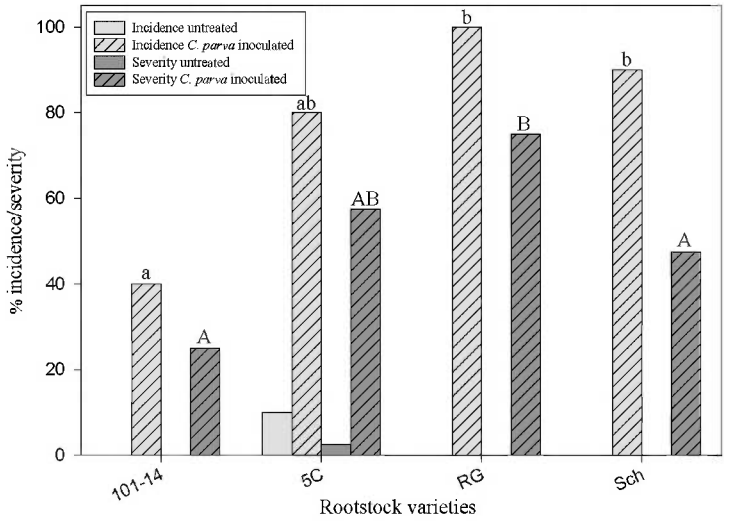

Figure 1 *Cylindrocladiella parva* disease incidence and severity (% infected wood pieces) at 1 cm from the stem base for four rootstock varieties. For *C. parva* inoculated rootstocks, disease incidence means followed by different letters (a-b) were significantly different according to Chi square test ($P<0.05$) and disease severity means followed by different letters (A-B) were significantly different according to Kruskal-Wallis test ($P=0.018$) followed by pairwise comparisons.

susceptible, and Schwarzmann, Riparia Gloire and 101-14 were more susceptible than 5C. In the non-inoculated plots, there were similar low levels of infection incidence (0-5%) across all varieties (Jaspers 2010). This therefore suggests that the relative susceptibility of rootstocks to fungal pathogens causing black foot infection depends on many factors including inoculum level and environmental conditions.

Although disease incidence at 0 cm above the stem base significantly differed between rootstock varieties, this was not seen at 5 cm above the trunk base, probably due to the slow spread of the pathogen during the 6-month-growth period. Similar results were observed for *Cylindrocarpon* spp. after 4 months of growth, when disease incidence at 5 cm above the trunk base was significantly lower than observed at 1 cm above the trunk base (Probst et al. 2012). In contrast, Agustí-Brisach et al. (2012) reported rapid development of disease in grapevine seedlings, with symptoms such as reduced vigour, occasional mortality and necrotic root lesions, and recovery of *C. parva* isolates from roots of inoculated plants 20 days after inoculation with *C. parva* colonised wheat grains. Since those authors recovered *C. parva* from roots, and not the rootstock stem as in the current study, this may account for some of the differences reported in the rates of symptom development.

Cylindrocladiella parva has also been reported to be a soil saprophyte and a pathogen of a broad range of plants, including *Eucalyptus* spp, black walnut (*Juglans nigra*), common oak (*Quercus robur*) and *Pinus* spp. It has also been isolated from roots of *Fragaria* sp., *Macademia integrifolia*, *Persea americana*, *Prunus* sp. and *Telopea speciosissima* (Crous 2002; Gadgil et al. 2005; Scattolin & Montecchio 2007). Due to the wide host range and the potential for the pathogen to survive on dead organic material in the soil this pathogen has the potential to be a major issue for the viticulture industry. However, detailed investigation on the epidemiology of this pathogen has not been carried out.

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