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BIOLOGICAL CONTROL OF *CYLINDROCARPON SPP. ON GRAPEVINE ROOTS*

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INTRODUCTION

Cylindrocarpon spp. have been implicated both in 'black foot' disease in northern NSW (1) and in Young Vine Decline (YVD) disorder, a sporadic problem of grapevines of the Griffith region, Riverina, NSW, Australia. Soil contains significant populations of microorganisms with the ability to attack or suppress plant pathogenic fungi (2, 3). Fungistasis has been shown to be strongest in soils with high organic matter content and microbial activity, and soil bacteria have been implicated as the major cause of such fungistasis (3). Disease suppressive soils capable of controlling plant pathogens have been reported for a number of crops (2, 4, 5, 6) but this aspect of natural biocontrol has not yet been investigated in viticulture.

A vineyard field experiment (Wagga Wagga, NSW, Australia) showed that the culturable soil population able to inhibit *Cylindrocarpon* sp. *in vitro* was around seven times greater ($P < 0.001$) in soil with high organic C in comparison with soil containing low organic C (7). One isolate from the field experiment, *Streptomyces* sp. (MW555), decreased *in vitro* vegetative growth of grapevine pathogens *Cylindrocarpon liriodendri* and *Cylindrocarpon macrodidymum*. The actinomycete also demonstrated biocontrol activity against *C. liriodendri* in a long term *Vitis vinifera* cv. Chardonnay soil pot experiment. In order to study the interactions between the actinomycete and *C. macrodidymum*, a gnotobiotic pot experiment was conducted with Chardonnay rooted cuttings in sterile sand/peat.

MATERIALS AND METHODS

Two-month old Chardonnay rooted cuttings planted in coarse river sand/peat moss (1:1, v/v) were inoculated with *Streptomyces* sp. (MW555). The actinomycete was scraped from the surface of Yeast Extract Malt Extract agar, suspended in sterile deionised water (SDW) and adjusted to cell density 2.6×10^6 cfu/mL. A 5 mL aliquot of the suspension was pipetted onto the surface of the potting mix resulting in inoculum of approximately 2×10^4 cfu/g dry weight of potting mix, or 10^7 cfu per vine. Clear plastic cylinders with 0.2 μm filters were fixed over the pots to prevent contamination, and the pots were arranged in randomised complete blocks in a glasshouse with a temperature range of 12-20°C.

Three weeks after inoculation with MW555, a 1.5-cm auger was used to aseptically remove three cores of potting mixture from every pot and 0.3 g sterilised wheat germ was placed aseptically into the holes. Aliquots (1 mL) containing *C. macrodidymum* macroconidia and microconidia (10^5 spores/mL) in SDW were applied to the wheat germ and the holes were back-filled with the extracted potting mix. The fungal inoculum was prepared from a 21-day-old colony grown on Potato Dextrose Agar (Oxoid) (PDA) at 25°C in a 12 h light/dark cycle. There were four replicate vines for the following treatments: uninoculated (SDW only); inoculated with *C. macrodidymum* alone; inoculated with *C. macrodidymum* and *Streptomyces* sp. (MW555) together; and inoculated with MW555 alone.

The pots were destructively sampled 14 weeks after fungal inoculation. Root lengths were measured using a root scanner and the roots were scored for 'root health', where 0 = no disease symptoms; 1 = 1-25%; 2 = 26-50%; 3 = 51-75%; and 4 = 76-100% root rot. The roots were surface sterilised for 3 min with calcium hypochlorite (1% active chlorine) and rinsed three times with sterile deionised water. Twenty root pieces (3 mm long) from each pot were incubated on Dichloran Rose Bengal Chloramphenicol agar (DRBC) (Oxoid Australia, Adelaide). Fungi growing from the roots were transferred to PDA and incubated as above. Immediately after sampling, representative root samples were frozen in liquid N_2 and stored at -80°C for Cryo Scanning Electron Microscopy (cryoSEM). Root pieces were mounted on stubs with carbon paste and frozen in liquid N_2 slush, and then transferred to the cold stage of a CryoSEM (Cambridge Model S360; Cambridge Instrument Co., Cambridge, UK), etched for a few minutes at -85°C to show the cell outlines, cooled to -160°C, coated with gold and observed at 18 kV (8).

Data were subjected to ANOVA and least significant differences were calculated at $P<0.05$ and $P<0.01$ using GenStat for Windows, 8th edition.

RESULTS

After fourteen weeks, all vines inoculated with *C. macrodidymum* alone had died and the infected roots were covered in black, sunken, necrotic lesions. In contrast, the vines inoculated with both *C. macrodidymum* and MW555, and the control vines (not inoculated, or inoculated with MW555 alone) appeared healthy above ground. *C. macrodidymum* was isolated from the roots of all vines inoculated with the fungus, thus satisfying Koch's postulates. *Streptomyces* sp. (MW555) was isolated from the roots of the vines inoculated with the actinomycete. The roots of the vines inoculated with *C. macrodidymum* alone were significantly shorter and more extensively rotted than those of the controls and the vines inoculated with both MW555 and *C. macrodidymum* (Table 1).

Table 1: Potted Chardonnay vines 14 weeks after inoculation[1].

	Total root length (cm)	Root rot [2]
Control	333 a	1.0 a
C. macrodidymum alone	28 b	4.0 b
C. macrodidymum plus *Streptomyces* sp. (MW555)	258 a	1.0 a
Streptomyces sp. (MW555) alone	305 a	0.8 a

[1] ANOVA performed for data from 4 replicate pots; [2] 0 = no disease symptoms; 1 = 1-25%; 2 = 26-50%; 3 = 51-75%; 4 = 76-100% root rot. Values within a column followed by the same letter are not significantly different based on l.s.d. ($P = 0.05$).

CryoSEM photos of roots inoculated with *C. macrodidymum* and MW555 together showed typical conidia of *C. macrodidymum* and hyphae of MW555 (Figure 1). Many *C. macrodidymum* conidia were penetrated by the hyphae of MW555, suggesting that *Streptomyces* sp. MW555 has antagonistic activity against *C. macrodidymum in vivo.*

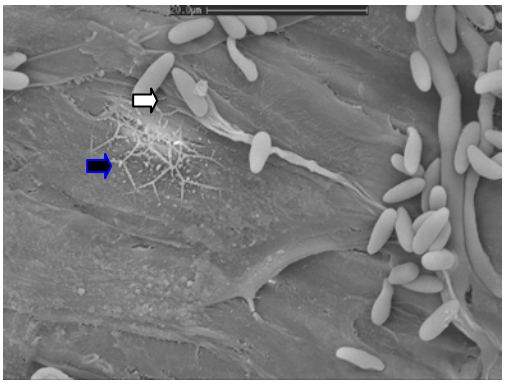

Figure 1: Chardonnay pot experiment. *Cylindrocarpon macrodidymum* conida plus *Streptomyces* sp. (MW555) hyphae in root cortical cells of healthy plant. *C. macrodidymum* conida (⇒) appear to have been penetrated by MW555 hyphae ( ). Scale bar = 20 μm.

DISCUSSION

The biocontrol agent *Streptomyces* sp. (MW555) increased the root length 9-fold and decreased the amount of root rot by 75%, indicating that it was highly antagonistic towards *Cylindrocarpon macrodidymum.* This study shows that MW555 may be a potentially useful root inoculum for grapevines at the nursery level. In addition, as we have shown that increasing vineyard soil organic matter increases the soil bacterial population (9), addition of organic amendments may be a strategy for increasing the naturally occurring 'suppressive' soil actinomycetes to improve vine health.

Further work will investigate the mechanisms for antagonistic activity and induction of plant defence genes by this potential biocontrol actinomycete.

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