COLONISATION OF APPLE ROOTS BY ARBUSCULAR MYCORRHIZAE IN SPECIFIC APPLE REPLANT DISEASE AFFECTED SOIL

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ABSTRACT

In New Zealand, specific apple replant disease (SARD) causes retarded tree growth and poor establishment in replanted apple orchards. In two pot experiments, arbuscular mycorrhizae (AM) colonisation of apple roots in different SARD treated soils was assessed. In the first experiment, AM colonisation was significantly lower in SARD soil compared with non-SARD soil. In this experiment, 45.6% of roots were AM colonised at planting and AM colonisation was increased in both soil types following chloropicrin fumigation or fungicide application. The second experiment used only SARD soil, and at planting, only 0.3% of roots were colonised with AM. AM colonisation was significantly greater in two commercial *Trichoderma* treatments (pellet and powder formulations) than untreated control, uninoculated blank pellets and chemical nutrient treatments. AM colonisation in fumigated soil was very low and remained similar to the initial root stock material.

Keywords: biological control, *Trichoderma* spp., chloropicrin fumigation.

INTRODUCTION

Specific apple replant disease (SARD) is a problem in replanted apple orchards in New Zealand (Fullerton et al. 1999) and many other parts of the world, causing poor tree establishment and delayed productivity. Different biological, physical and chemical factors have been implicated as the cause of SARD in different apple growing areas of the world, with the causal factor in New Zealand unknown.

Colonisation of apple roots by arbuscular mycorrhizae (AM) has been reported to be associated with healthy trees (Trappe et al. 1973), efficient nutrient uptake (Benson & Covey 1976; Covey et al.1981) and enhanced plant growth (Mosse 1957; Plenchette et al.1981). Earlier observations of apple roots have shown significantly lower mycorrhizal infection in SARD soils (Caruso et al. 1989; Catska & Taube-Baab 1994) and alleviation of disease symptoms by inoculation with AM fungi (Catska 1994). In the present study, colonisation of feeder roots by AM was compared between SARD and non-SARD soils with different treatments in two pot experiments.

MATERIALS AND METHODS

Apple rootstock material and AM colonisation assessment

Plant material used in both trials was ungrafted M26 apple rootstocks, held in the cool-store for three months after lifting from the stool beds. AM colonisation of rootstock material used in the two experiments was assessed prior to planting by staining (Smith & Dickson 1997) and using the grid-line intersect method (Giovannetti & Mosse 1980). Briefly, roots were cleared by autoclaving in 10% KOH solution followed by rinsing in water and then in 10% HCl solution. Roots were once again rinsed in water and immersed

in lactoglycerol blue (lactic acid, glycerol and water in the ratio of 30:30:40 with 63 mg of trypan blue/litre) for 30 min. Roots were dabbed between layers of filter paper to remove excess stain and preserved in lactoglycerol. Stained roots were transferred to a Petri dish on which a grid has been glued. Roots were spread to avoid overlap and observed under a stereo microscope. A minimum of 150-200 randomly selected feeder roots was assessed and no distinction was made between healthy and diseased roots. Original rootstock had very few fine roots and all the available roots were assessed. Percent AM colonisation was calculated as the ratio of root intersects and number of intersects in which the root contains mycorrhizal structures.

Pot experiment 1 (Nov 2003–May 2004) at Havelock North

Soil was collected (top 20–25 cm) from beneath apple trees in a Hawke's Bay orchard with a history of SARD. Non-SARD soil was collected from a nearby site with no recent history of apple cultivation. Soil samples collected 40-45 days prior to planting were sieved through a 15-20 mm mesh screen to establish a fine and uniform tilth. For each soil, there were three treatments: (1) untreated control, (2) fumigated and (3) fungicide drench. The fumigation treatment was applied to soil in sealed containers (using hand-injected chloropicrin at 15 ml/40 litre crate of soil) 3 weeks prior to potting. After one week, the sealed containers were opened to allow venting of the residual fumigant gas. For the next 2 weeks, fumigated soil was aerated regularly by slowly tipping the soil from one container to another. The fungicide drench consisted of a fungicide mixture targeting a wide range of fungi and was applied through a mist sprayer 5 days prior to potting by spreading soil to a thin layer (1-2 cm) on a bench. Fungicides applied (in mg active ingredient/kg soil) were metalaxyl (0.5), benomyl (2.0), difenconazole (0.2) and fludioxinil (0.3). Fungicide treatment was re-applied as a soil drench in early April 2004. Rootstocks were planted in November 2003 in the different soil treatments in 4.5 litre plastic pots and grown in the open on gravel. Trees were regularly watered (2-3 times per week) to avoid water stress. After 6 months growth and soon after the trees had become dormant, trees were lifted and roots from five replicates were carefully harvested to recover as many fine roots as possible. AM colonisation was assessed as described above.

Pot experiment 2 (Nov 2004–June 2005) at Lincoln University, Canterbury

Soil collected as previously described, from the same Hawke's Bay SARD affected orchard as for experiment 1 was subjected to one of the following six treatments: (1) untreated control, (2) chloropicrin fumigation, (3) Gro-Chem DRH *Trichoderma* pellets (50 g/planting hole giving 7.2×10^5 cfu/g soil), (4) Gro-Chem uninoculated blank pellets (50 g/planting hole), (5) Gro-Chem DRH-F *Trichoderma* powder (50 µg/planting hole giving 7.9×10^5 cfu/g soil) and (6) NPK nutrients (a mixture of 2.7 g of ammonium nitrate, 2.8 g of super-phosphate and 0.88 g of potassium nitrate/planting hole). Gro-Chem products are made under license to HortResearch by Gro-Chem NZ Ltd, Porirua, New Zealand. For the *Trichoderma* powder formulation treatment, 500 µg of DRH-F was mixed in 10 g of sieved-soil and divided into 10 bags. Each 1 g bag was thoroughly re-mixed in 49 g of soil, which was again mixed with 1.5 litres of soil and used to fill-up around the rootstocks. All treatments were applied once at the time of planting in 6 litre plastic pots. The trial was conducted on gravel in a shadehouse (November 2004–July 2005) at Lincoln University, Canterbury, and regularly watered (2-3 times per week) to avoid water stress. After 7 months growth and soon after the trees had become dormant, roots from five replicates were harvested and AM colonisation was assessed as described above.

Experimental design and data analysis

Both trials were arranged as a randomised complete block design. Data were analysed using ANOVA (Genstat 7.0).

RESULTS

Pot experiment 1 (Nov 2003–May 2004) at Havelock North

Treatment effects are described in Table 1. AM colonisation of the initial rootstock material was 45.6%. In non-SARD soil, the roots were seen to maintain a similar level of colonisation to that observed for the initial rootstock material. However, in SARD soil, AM colonisation was reduced (16.0%) compared with the rootstock material and was significantly lower (P<0.05) compared with non-SARD soil.

Chloropicrin fumigation and fungicide application treatments were observed to increase AM colonisation in both SARD and non-SARD soils (Table 1). Marked differences were observed in the number of vesicles formed in feeder roots between fumigated SARD, non-SARD and SARD soils. Observation of roots indicated that feeder roots from fumigated SARD soil and all non-SARD soil treatments were generally white and healthy, while those from untreated and fungicide drench treated SARD soil, were predominantly brown or black with high levels of decay and sloughing-off of the cortex.

TABLE 1: The colonisation (%) of apple rootstock feeder roots by arbuscular mycorrhizae 6 months after planting in different soils treated with chloropicrin or a fungicide drench. All the values (except the original rootstock) are means of five replicates.

Soil type	Treatment	Colonisation (%)[1]
Original rootstock[2]		45.6
SARD	Untreated control	16.0 a
	Chloropicrin fumigated	62.6 c
	Fungicide mixture	56.2 c
Non-SARD	Untreated control	42.6 b
	Chloropicrin fumigated	89.4 e
	Fungicide mixture	78.6 d
LSD (P<0.05)		9.71

[1]Values followed by the same letter are not significantly different at P=0.05.
[2]Composite sample from 10 trees. Data not included in the statistical analysis because all other samples were from individual trees.

Pot experiment 2 (Nov 2004–June 2005) at Lincoln University, Canterbury

Treatment effects are described in Table 2. AM colonisation of the initial rootstock material was 0.3%. After 7 months of root growth, AM colonisation was highest in two commercial *Trichoderma* treatments (pellet and powder formulations) and significantly greater than untreated control and chemical nutrient treatments. In contrast, AM colonisation in fumigated soil was very low and similar to the initial rootstock material. Colonisation of roots by AM was high in the blank pellets treatment but this was not significantly different from the control and chemical nutrient treatments.

DISCUSSION

An, as yet, undefined factor of the SARD soil brought about a suppression of AM colonisation and this could be either due to poor root health (prior infection by root pathogens) or an artefact of root longevity. In SARD soil, a much more rapid turnover of roots was noticed, with a rapid decay and replacement cycle (I.J. Horner, unpubl. data). Thus, at any one point in time the average age of feeder roots in SARD soil will be much younger than fumigated or non-SARD soil and less likely to be colonised by AM fungi. Among fruit trees, apple roots have a relatively short life span with mycorrhizal colonisation reported to enhance root longevity through a number of different mechanisms, including defence against root pathogens (Eissenstat et al. 2000). Furthermore, development of endomycorrhizal relationships is essential for normal

TABLE 2: The colonisation (%) of apple rootstock feeder roots by arbuscular mycorrhizae 7 months after planting in SARD soil with different treatments. All the values are means of five replicates.

Treatment	Colonisation (%)[1]	
Original rootstock	0.3	a
Untreated control	40.4	b
Chloropicrin fumigation	0.4	a
Gro-Chem DRH *Trichoderma* pellets	60.4	c
Gro-Chem blank pellets	52.3	bc
Gro-Chem DRH-F *Trichoderma* powder	62.6	c
Nutrients (NPK)	42.3	b
LSD (P<0.05)	14.98	

[1]Values followed by the same letter are not significantly different at P=0.05.

growth and development of apple trees (Mosse 1957; Benson & Covey 1976; Covey et al. 1981). Caruso et al. (1988) also reported higher frequency of mycorrhizal infection in healthy trees than diseased trees from apple replant affected soils, while Catska & Taube-Baab (1994) confirmed this result in a comparative field study of virgin soil and soil prone to replant disorder.

In this study, chloropicrin fumigation provided contrasting results in the two experiments, where original rootstocks with well developed or poor AM colonisation were planted. Rootstocks were grown in stool-beds, and the differences in the two seasons may reflect the random variation in AM colonisation of newly formed stool-shoot roots in sawdust. Soil contamination of sawdust could have contributed to well colonised stool-shoot roots in the first experiment. In both cases, chloropicrin might have effectively sterilised the soil, removing deleterious organisms and resulting in greater root health, but also native AM fungi. In experiment 1, due to the high initial AM colonisation of the root stock material, the reduction in competition allowed AM in the rootstock to proliferate and colonise the new roots more effectively, resulting in a higher AM colonisation at harvest. However, in experiment 2, initial colonisation of the rootstock by AM was very low and as the chloropicrin treatment removed the native AM fungi from the soil, the relatively low inoculum levels resulted in low AM colonisation at harvest. If a similar situation of low AM colonisation of rootstock occurs in an orchard, recolonisation (through contamination) of fumigated soils could be faster by plant pathogens than by symbionts like AM and could lead to serious root diseases. Similar results were reported in a review by Menge (1982), where most field fumigants were seen to eliminate AM fungi from the soil resulting in a reduction in root recolonisation.

Another marked difference between the two experiments was the amount of AM colonisation in the untreated SARD soil controls, where a substantial difference (16% in the first and 40% in the second) was observed. This difference corresponded well with root dry matter measured at the end of the growing period (4.6 g in the first and 10.2 g in the second experiment; D.R.W. Kandula, unpubl. data) and these differences could either be attributed to the quality of the rootstock or to the growing environment between the two seasons.

The fungicide treatment (a combination of metalaxyl, benomyl, difenconazole and fludioxinil) in the first experiment, gave an increase in AM colonisation of roots in both SARD and non-SARD soils, again possibly due to the suppression of deleterious micro-organisms thus allowing AM colonisation. Application of metalaxyl and benomyl were reported to have no significant effect in heavy root colonisation by mycorrhizal fungi in clones of apple rootstock MM106 (Lubraco & Schubert 2001), but higher rates of benomyl decreased mycorrhizal formation.

Application of *Trichoderma* spp. enhanced AM colonisation in pot experiment 2 and could be exploited further to improve the root quality. AM fungi in the present

study were not identified but Kandula et al. (2005) reported *Glomus mosseae* to be the predominant species in both organic and conventional apple orchards in New Zealand. *In vitro* studies by Calvet et al. (1992) reported enhanced spore germination rate and development of *G. mosseae* in the presence of *Trichoderma* spp. and the present results with both *Trichoderma* spp. formulations suggest an interaction between these two groups of fungi. Catska (1994) reported suppression of phytotoxic micromycetes and resultant replant symptoms by AM fungi. Future trials could test mixtures of *Trichoderma* spp. and AM fungi to alleviate SARD problems to some extent in New Zealand.

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