

# OPTIMISING THE MEDIUM FOR PRODUCING ARBUSCULAR MYCORRHIZAL SPORES AND THE EFFECT OF INOCULATION ON GRAPEVINE GROWTH

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## ABSTRACT

Arbuscular mycorrhizae form obligate symbioses with the majority of vascular plants. Propagation of these fungi relies on maintenance of pure pot cultures, typically sterile sand supplemented with a nutrient solution is used. However, river sand is inappropriate for New Zealand species as it is denser than soils and prone to water logging. Three different media (silica sand, pumice and potting mix) at two particle sizes (500-1000 µm and 1000-1400 µm) were mixed in nine combinations for the propagation of arbuscular mycorrhizae spores. The results showed that the silica sand/pumice medium (500-1000 µm, bulk density of 1) produced the greatest ( $P < 0.01$ ) number of spores (1.70/ml of substrate). The density of the media had the greatest effect on spore formation. Inoculations of grapevines with spores derived from these cultures significantly improved both root and shoot growth.

**Keywords:** arbuscular mycorrhizae, pot culture, media, media density, particle size, grapevine.

## INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are one of the most widely distributed and ecologically important fungal groups. These fungi co-evolved with vascular plants almost 400 million years ago and currently more than 80% of land plants form these symbiotic associations (Simon et al. 1993). Current research has shown that AMF have numerous benefits to plants, including increasing the absorption of mineral elements, enhancing pathogen defence and drought resistance (Jeffries et al. 2003). Grapevines are normally mycorrhizal and this partnership provides benefits to both the productivity and health of plants (Quarles 1999). However, due to difficulties in culturing and identifying mycorrhizae little is known about the interactions of mycorrhizal communities and agricultural practices.

Wine represents 10% of New Zealand's total horticultural exports and is the fifth largest horticultural export commodity (Anon. 2000). Winegrowers throughout New Zealand have adopted the Integrated Winegrape Production (NZIWP) scheme initiated in 1995. The scheme promotes environmentally sustainable vineyard practices, which enhance the "clean, green" image of New Zealand wines. These management practices protect the environment and reduce chemical, labour and machinery costs. Inoculation of vineyards with mycorrhizae would benefit NZIWP.

AMF must be cultured in the presence of a live host plant. In order to ensure culture purity, the growth medium must be sterile or AMF free. For this reason, the majority of research groups use sterilised sand augmented with a nutrient solution. At Lincoln University, researchers used sterile river sand for this purpose but the plants did not grow well as it was very dense and prone to water logging. The high density led to infestation of

algae and disease in the host plant. Typically, only 50% of the initiated cultures produced any spores and, of these, only 50% at usable rates of 0.5 to 1.0 spores/ml (H.J. Ridgway, unpubl. data). The study described below was undertaken to optimise the medium for spore production. The effect of the propagated AMF spores on grapevine growth was also demonstrated.

## MATERIALS AND METHODS

### Inoculum source

The inoculum was a commercial product called Vaminoc™ (MicroBio Ltd, Royston Herts, UK) and contained a mixture of three *Glomus* spp. The product was obtained from Agrimm Technologies (Christchurch, New Zealand) and consisted of fragments of colonised roots and spores in a clay support granule. This inoculum provided a uniform base for the media experiment, eliminating problems due to AMF batch variation.

### Potting media

Three different media (silica sand, pumice and potting mix) in nine combinations were tested. The silica sand and pumice (Egmont Seed Company Ltd, Christchurch) were sieved into two batches, one of 500-1000 µm and one of 1000-1400 µm. Both were checked for the absence of AMF spores by wet sieving using sucrose density centrifugation (Brundrett et al. 1996). The potting mix was supplied as a 4:1 ratio of bark to pumice and contained standard amounts of fertilisers, but did not contain phosphate. The potting mix was sterilised by autoclaving over 1 h using stepwise increases to ensure the centre reached 120°C for 30 min. The treatments were replicated five times in new 500 ml plastic pots as follows: (1) silica sand 500-1000 µm, (2) silica sand 1000-1400 µm, (3) pumice 500-1000 µm, (4) pumice 1000-1400 µm, (5) silica sand/pumice 500-1000 µm (70:30% v/v to reach a bulk density of 1), (6) silica sand/pumice 1000-1400 µm (50:50% v/v to reach a bulk density of 1), (7) silica sand/potting mix (50:50 v/v) 500-1000 µm, (8) silica sand/potting mix (50:50 v/v) 1000-1400 µm and (9) sterilised potting mix. Each pot was wetted with 50 ml of a 10% soil bacterial filtrate. The filtrate was made by mixing 100 g of soil in one litre of sterile water for 1 h and filtering to 1 µm.

### Inoculation

A 2.5 g quantity of Vaminoc™ was mixed into the top 4 cm of medium in each pot. Each pot was planted with a single, sterile seedling of white clover (cv. Huia) and inoculated with 1 ml of *Rhizobia* sp. culture to ensure nodulation and nitrogen fixation. The rhizobia suspension was prepared by dislodging a 3-day-old culture from a Petri plate of potato dextrose agar using 10 ml of sterile water and a drop of Tween 20. The solution was made up to a final volume of 50 ml with water. Pots were arranged in a randomised block in the glasshouse. After 2 weeks each pot was treated with 20 ml of 2× Hoagland solution minus phosphate (Hoagland & Arnon 1938).

### Harvest

After 5 months the aerial parts of the plants were removed and the pots dried for 2 weeks. Once the pots were dried, the roots were removed and the media collected. The roots were weighed and the average number of spores/ml of medium was determined (Brundrett et al. 1996). Results were analysed by ANOVA using GenStat Seventh Edition (Lawes Agricultural Trust, Rothamsted Experimental Station, UK, 2004).

### Grapevine growth trial

To assess the effect of mycorrhizal inoculation on grapevine growth a glasshouse experiment was established. Sixty own rooted cuttings of Chardonnay (clone 5) were placed in 2 litre plastic pots in a medium consisting of 50% silica sand (500-1000 µm), 30% (v/v) pumice (500-1000 µm) and 20% (v/v) potting mix (as described above). To each pot, 100 g was added of a dried pot culture containing 200 spores of *Glomus mosseae* (isolated from a vineyard, Christchurch, New Zealand) or 200 spores of Vaminoc™. Sterile pot culture medium without spores (100 g) was added to the control pots. The inoculum or sterile pot culture medium (controls) was mixed into the top 8 cm of medium prior to planting with a grapevine. Each treatment contained 20 replicate pots arranged

randomly in the glasshouse. After 4 months the trial was harvested and shoot/root fresh weight determined. Sample roots were stained to confirm that they had been colonised (data not shown). The results were analysed by ANOVA using GenStat Seventh Edition (Lawes Agricultural Trust, Rothamsted Experimental Station, UK, 2004).

## RESULTS

The results showed that, although all pots produced spores, there were substantial differences in spore formation and root growth between media (Table 1). The least spores were obtained from silica sand 1000-1400  $\mu\text{m}$  (treatment 2) and sterilised potting mix (treatment 9), but these were not significantly different ( $P>0.05$ ) from treatments 1, 3, 4, 7 and 8. The most spores ( $P<0.01$ ) were obtained with silica sand/pumice 500-1000  $\mu\text{m}$  (treatment 5), which was followed by silica sand/pumice 1000-1400  $\mu\text{m}$  (treatment 6). In both of these treatments the medium was at a bulk density of 1, which is similar to that of soil.

In contrast, the best root growth was observed in sterilised potting mix (treatment 9), followed by both other treatments containing the potting mix (treatments 7 and 8). These three had significantly greater root mass compared to the remaining six treatments, which were not significantly different from each other (Table 1).

**TABLE 1: Spore density (mean no. spores/ml) and root weight (g) of white clover growing in nine potting media treatments.**

Trt #	Treatment	Spore density	Root weight
1	Silica sand 500-1000 $\mu\text{m}$	0.32 a	7.1 a
2	Silica sand 1000-1400 $\mu\text{m}$	0.08 a	7.0 a
3	Pumice 500-1000 $\mu\text{m}$	0.14 a	3.7 a
4	Pumice 1000-1400 $\mu\text{m}$	0.23 a	5.6 a
5	Silica sand/pumice (bulk density of 1) 500-1000 $\mu\text{m}$	1.70 c	5.8 a
6	Silica sand/pumice (bulk density of 1) 1000-1400 $\mu\text{m}$	1.14 b	5.7 a
7	Silica sand/potting mix (50:50 v/v) 500-1000 $\mu\text{m}$	0.19 a	16.1 b
8	Silica sand/potting mix (50:50 v/v) 1000-1400 $\mu\text{m}$	0.54 a	13.1 b
9	Sterilised potting mix	0.08 a	22.9 c
	LSD ( $P<0.01$ )	0.55	3.9

Spores produced using treatment 5 were used in a grapevine growth trial to determine their effectiveness at improving plant growth. As part of this experiment two spore sources, the Vaminoc<sup>TM</sup> and a New Zealand *Glomus mosseae* strain were compared. The results showed that both AMF treatments significantly ( $P<0.05$ ) improved the growth of shoots and roots of own-rooted cuttings (Table 2). The two AMF treatments were not significantly different from each other in their ability to stimulate shoot and root growth.

**TABLE 2: Root and shoot fresh weights (g) of grapevines growing in medium supplemented with no spores, Vaminoc<sup>TM</sup> spores or spores of a New Zealand *G. mosseae* strain.**

Treatment	Root	Shoot
Control	25.15 a	9.26 a
Vaminoc <sup>TM</sup>	36.19 b	12.98 b
<i>Glomus mosseae</i>	32.73 b	11.59 b
LSD ( $P<0.05$ )	4.30	2.25

## DISCUSSION

This study demonstrated that spore production of AMF on clover host plants varies significantly with the medium used. The best results were obtained with media at a bulk density of 1 and a particle size of 500-1000  $\mu\text{m}$ . The pots with the best spore production did not have the greatest root mass at harvest, suggesting that different criteria are pivotal for fungal spore versus root formation. Spores produced under these conditions were infective and capable of stimulating root and shoot growth in grapevine cuttings.

As a preliminary step to investigating the effect of AMF on plant growth and health, a large quantity of mature, healthy, pure spores must be obtained as an inoculum source. For this purpose pure pot cultures are commonly produced and have been initiated in a wide range of sterile media, including, sand, peat, perlite, vermiculite and glass beads (Gaur & Adholeya 2000). Many factors affect inoculum production including temperature (Liu et al 2004), light (Ferguson & Menge 1982), soil aeration (Saif 1981) and particle size (Gaur & Adholeya 2000). In this experiment, the effect of particle size and bulk density of the medium was assessed using mixes of silica sand, pumice and potting mix. The aim was to identify a reliable medium for production of bulk inoculum.

Soil aeration is a key factor driving infection, colonisation and metabolic activity of the AMF (Saif 1981). This was not optimal in the river sand media initially used for pot culture as it had a bulk density greater than that of soil (H.J. Ridgway, unpubl. data). The bulk density of sand was reduced by the addition of pumice to match that of soil. The results demonstrated that pots containing media of this density had significantly greater spore formation than pots denser (silica sand only) or lighter (pumice only). These results were consistent with those of Gaur & Adholeya (2000) who showed in their study that sand was the best substrate and perlite the worst for production of infective propagules. In contrast to the current study, they did not mix substrates and provided no information of the density of their substrates.

Two particle sizes were tested in this experiment to determine which was optimal for spore production. The results showed that, at a bulk density of 1, the 500-1000  $\mu\text{m}$  particles produced significantly more spores than the 1000-1400  $\mu\text{m}$  particle size. Greater spore formation in media of this particle size may be attributed to optimal aeration, drainage and oxygen supply (Saif 1981). The decrease in spore formation of the larger particle size may be explained by relatively less water retention and a propensity for dehydration, leading to fluctuations in soil moisture on fungal hyphae and consequently lower spore production. A similar trend was observed by Gaur & Adholeya (2000) who noted in their studies that a particle size of 500-780  $\mu\text{m}$  produced the best spore production. Interestingly, the trend associated with particle size was not evident in the pumice or silica sand only treatments, as the results from the two particle sizes did not differ significantly. This indicated that, for spore formation, bulk density may be a dominant factor to particle size.

Root mass was greatest in the media augmented with potting mix. It is likely that the greater available nutrients in these pots directly boosted root growth, whereas in those pots augmented with only Hoagland solution substantially fewer nutrients were available. This suggests that the nutrient status of the media is of secondary importance to density and particle size for AMF spore production.

Spores produced using the optimal media (treatment 5), together with a small amount of potting mix as a nutrient source, were used to inoculate rooted grapevine cuttings. The aim of this experiment was to demonstrate that the inocula produced was infective and suitable for use in trials designed to investigate the growth promotion effect of AMF. The results showed that the Vaminoc™ and endemic strain of *G. mosseae* were both capable of significantly improving the root/shoot growth. This result is consistent with international studies that have demonstrated AMF improve plant productivity (Jeffries et al. 2003).

In summary, the present results have identified a suitable medium for bulk production of pure, mature and infective AMF inoculum. The media is cheap, easily obtained and

sterilised and produces approximately 2 spores/ml of medium. Of particular interest was the observation that medium density had a greater effect on spore production than particle size and root mass.

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