

The Potential for the Use of VA Mycorrhizae in Nursery Crop Production

Victor J. Galea and Richard C. D. Poli

Plant Production Department, University of Queensland Gatton College, Lawes, Queensland, 4343

This paper reviews experimental work on the benefits of VA-mycorrhizae in nursery crop production.

INTRODUCTION

About a century ago, several biologists noticed that some plant roots that were extensively invaded by fungi, were not diseased. The name mycorrhiza (fungus root) was thus coined in 1885 (Kendrick, 1992). Mycorrhizas are the association between plant roots and the hyphae of various naturally occurring, soil inhabiting fungi which operate together in a symbiotic partnership. The nature of this partnership is such that the plant provides the fungal component with nutrients (sugars, amino acids, vitamins etc.), while the fungal component assists the plant with the uptake of water and nutrients such as phosphorus (P) and zinc (Zn) (Brown, 1992) which are often limiting for plant growth. It is estimated that over 90% of all higher plants species are normally mycorrhizal (Kendrick, 1992) making this the "normal situation." Although naturally occurring in soil, mycorrhizae do not spontaneously form in the soilless media used for container grown plants. Their incorporation into such systems could potentially improve growth and survival rates by restoring a missing component which is the norm in natural communities.

There are two major categories of mycorrhizae; ectomycorrhizae and endomycorrhizae. Ectomycorrhizae are fungi which can be grown in artificial culture (like mushroom spawn) and occur largely on woody plants in families such as Pinaceae, Fagaceae, and Betulaceae (pines, oaks and birches; Linderman, 1981) and *Eucalyptus* (Kendrick, 1992).

The group of mycorrhizae most relevant to nursery and horticultural crops, however, are the endomycorrhizae. These fungi produce loose networks of hyphal strands which are often associated with the plant's feeder roots making their presence undetectable without the use of a microscope. Often known as Vesicular Arbuscular Mycorrhizae (VA mycorrhizae or VAM) these fungi produce a range of microscopic structures within and outside of the plant root (Fig. 1). The arbuscule is a branched feeding organ which penetrates the root's epidermal and cortical cells and allows the two-way exchange of nutrients between the fungus and the plant. The vesicle (which does not occur in all instances) contains oil droplets and is thought to be a storage organ. VA mycorrhizal fungi are obligate symbionts, they can only exist in the presence of living plants, and as such, they cannot be grown in artificial culture. In general, VA mycorrhizal fungi are capable of colonising a wide range of plants (St. John and Evans, 1990), thus a single isolate may be used to colonise a wide range of plant species.

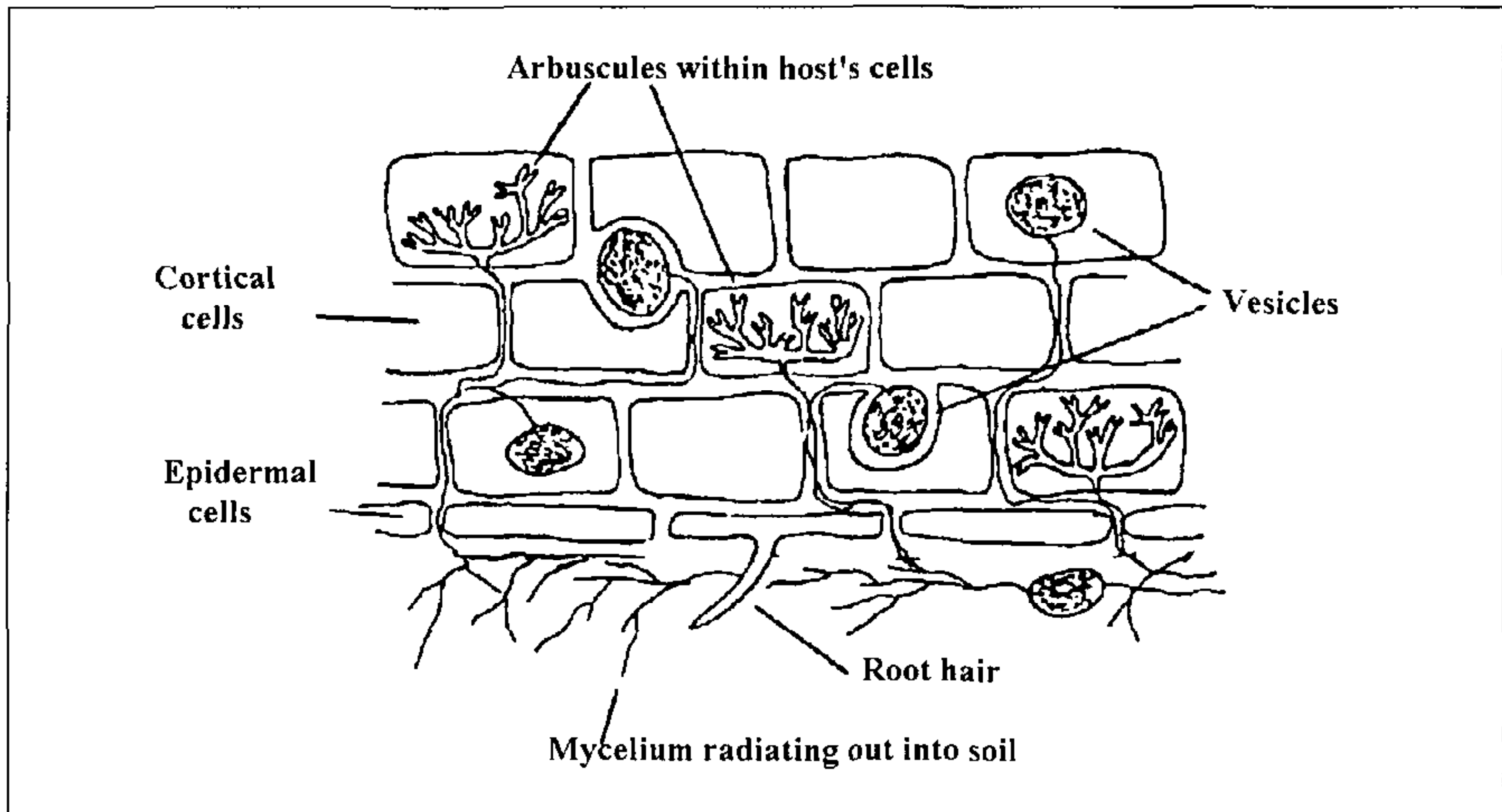


Figure 1. Diagram of a root section showing the features of VA mycorrhizas (Brown, 1992)

PLANT NUTRITION AND VA MYCORRHIZAE

Countless experimental trials have shown that plants colonised by mycorrhizal fungi are generally larger and have higher concentrations of phosphorous in their tissues (Krikun, 1991). The improvement in plant nutrition is due to the production of an extensive network of hyphae into the soil (or media) around the plant. Normally, the plant root removes phosphorous (P) rapidly from the region immediately surrounding the root. The hyphal network grows beyond this depletion zone and supplies phosphorous that is not normally available to the plant's roots alone (Brown, 1992; St. John and Evans, 1990). This effect can often be reduced by the addition of high levels of P as mycorrhizae are most active in P-deficient situations. Johnson (1982) showed that the addition of *Glomus fasciculatum* and *G. mosseae* to container-grown plants (woody ornamentals) could reduce the use of P fertilisers by 70% and reduce the levels of nitrogen (N), potassium (K), and micronutrient fertilisers by 30 to 40%. Maronek and Hendrix (1978) inoculated southern magnolia (*Magnolia grandiflora*) seedlings with *G. fasciculatus* in a pot experiment. Their results indicated that mycorrhizal plants grown at $\frac{1}{4}$ the normal fertiliser rate (1.1 kg m^{-3} Osmocote, 18-6-12) were twice as large as non-mycorrhizal plants grown at the full rate (4.5 kg m^{-3}). Ponton et al. (1990b) demonstrated that inoculation of Boston fern (*Nephrolepis exaltata*) with *G. vesiculiferum* resulted in growth levels similar to that achieved with a low rate of P addition. This lead the authors to suggest that mycorrhizal ferns were more efficient at P utilisation, which will have greater implications when fertiliser supplies become limiting at the end of the next century. Abbott and Robson (1985) advocate the comparison of mycorrhizal with non-mycorrhizal plants grown over a range of nutrient (P) levels. Comparisons can thus be made at a particular plant yield (horizontal comparison) rather than at a particular nutrient rate (vertical comparison). This allows the nutrient savings (due to mycorrhizal colonisation) to be determined when growing plants to a particular size (Abbott and Robson, 1985; Bolan et. al., 1983)(Fig. 2).

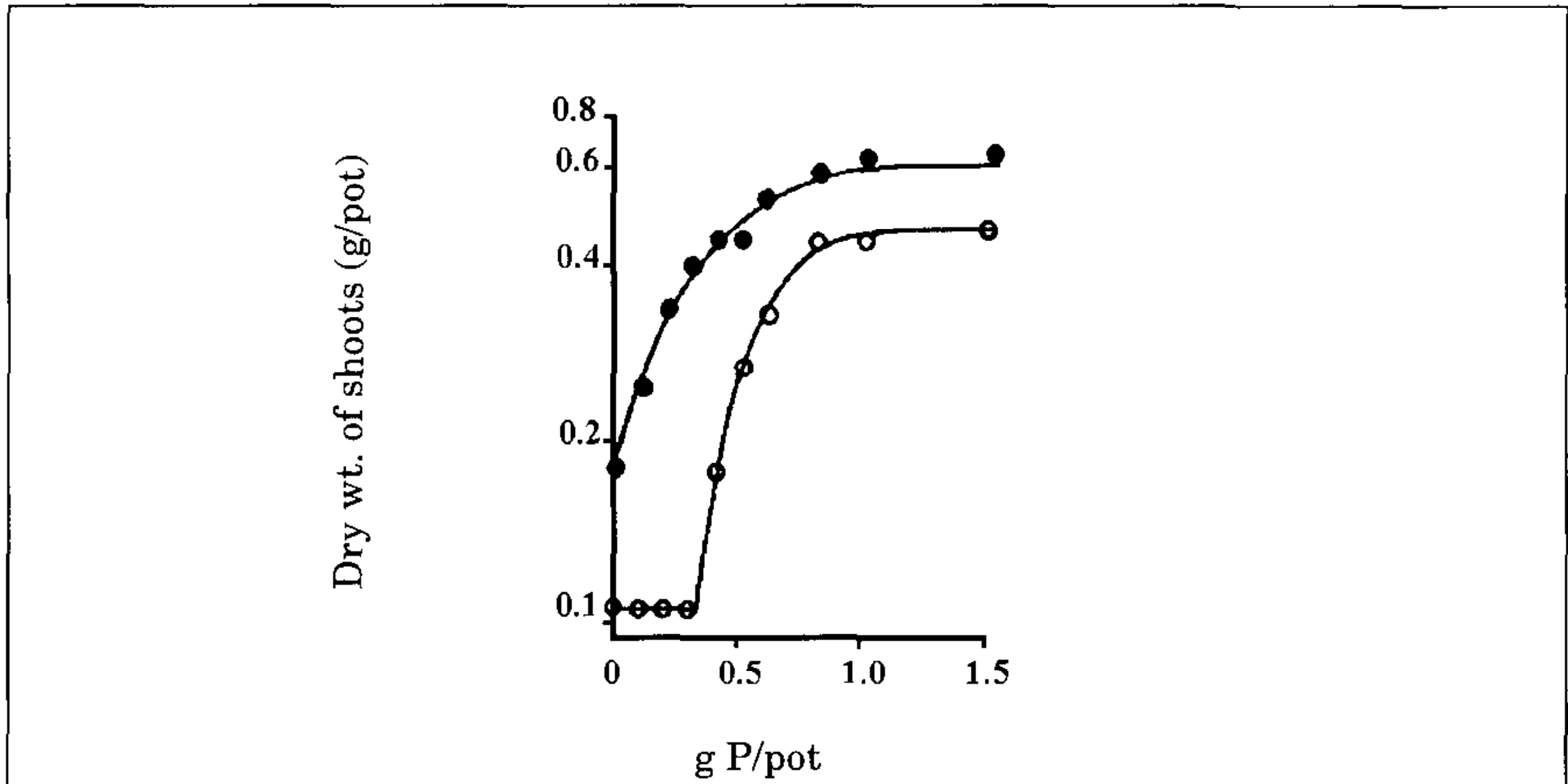


Figure 2. The effect of phosphate application and mycorrhizal inoculation on the dry weight of shoots of subterranean clover (Bolan *et. al.*, 1983). (● = inoculated plants, ○ = un-inoculated plants)

PROPAGATION SUCCESS AND VA MYCORRHIZAE

The benefits of VA mycorrhizae in propagation of arrow wood (*Viburnum dentatum*) was demonstrated by Verkade (1986), who found that root initiation and root weight were both increased in plants (cuttings) inoculated with *G. fasciculatum*. Improved rooting of cuttings in woody plants has been recorded elsewhere (Maronek *et al.*, 1981). There is considerable discussion in the literature about the role that mycorrhizae play in the hormonal balance of the plant. It has been shown that plants colonised by these fungi produce altered amounts of hormones such as gibberellin, abscisic acid, cytokinin and zeatin (Krikun, 1991). Although it is difficult to prove that the source of hormones is the fungal partner (Maronek *et al.*, 1981), the evidence suggests that mycorrhizal fungi influence the hormonal balance of the plants that they colonise.

Transplant survival of micropropagated strawberry plants was significantly increased by inoculation with the VA mycorrhizal fungi *G. etunicatum*, *G. fasciculatum*, and *G. mosseae* (Chang, 1990). These fungi also increased the numbers of runners produced, flowering activity, and fruit yield over the non-inoculated control group. Chávez and Ferrera-Cerrato (1990) also working with micropropagated strawberries, evaluated three VA mycorrhizal fungi *G. macrocarpum*, *G. versiforme* and an unidentified *Glomus* species against four cultivars. In some cases, inoculated plants yielded better than the un-inoculated controls, while the reverse was true in other cases. Vestberg (1992) evaluated nine isolates of *Glomus* for their ability to increase production in micropropagated strawberries. Some isolates increased plant growth several fold over the un-inoculated plants with the differences persisting into the second year of the field planted trial. Micropropagated avocado plantlets showed improved survival rates when inoculated with isolates of *Glomus* spp. (Azcón-Aguilar *et al.*, 1992). Micropropagated pineapples (Guilemin *et al.*, 1992; Lovato *et al.*, 1992) and grapevines (Lovato *et al.*, 1992) have also been shown to have superior growth when compared to plants which are not inoculated with VA mycorrhizal fungi.

Survival of micropropagated Boston fern (*Nephrolepis exaltata*) rooted plantlets was increased when transferred into pots containing media inoculated with the VA mycorrhizae *G. intraradices* and *G. vesiculiferum* compared to un-inoculated controls (Ponton et al., 1990a). The potential benefits to plants generated by cuttings and micropropagation, of inoculation with VA mycorrhizae, are increased survival and plant vigour, which leads to a more uniform product and possibly a shortening of the production time required.

WATER UTILISATION AND VA MYCORRHIZAE

A much neglected area of mycorrhizal research has been the influence of this symbiosis on the water utilisation of plants. It has been clearly demonstrated in many species that a significant contribution to the plant's water supply is provided by the mycorrhizal fungus (Read, 1985) and that this is particularly important in arid-zone plants.

Through particularly elegant experiments, Hardie (1985) demonstrated that the removal of VA mycorrhizal hyphae (*G. mosseae*) from the roots of red clover affected their ability to maintain or increase transpiration levels. The importance of VA mycorrhizae (*G. intraradices* and *G. deserticola*) has also been demonstrated to increase drought tolerance in *Rosa* hybrid (Augé and Duan 1991; Henderson and Davies, 1990) and in capsicum (Davies et al., 1992). These results indicate the potential for using VA mycorrhizae to increase the drought hardiness of container-grown plants. In theory, such plants would be more tolerant of desiccation between watering periods. This would allow extended periods between irrigations and potential reductions in water use and waste water disposal problems in the commercial nursery.

PLANT DISEASE AND VA MYCORRHIZAE

There has been little work done on the interactions between VA mycorrhizae, nursery crops, and their pathogens. Evidence for the beneficial role of VA mycorrhizae in reducing disease levels comes mainly from other cropping systems. Iqbal and Nasim (1988) showed that cauliflower seedlings pre-inoculated with an unnamed VA mycorrhizal fungus had greater resistance to attack by *Rhizoctonia solani*. Champawat (1991) found that inoculation of cumin with *Gigaspora calospora*, *G. fasciculatum*, *G. mosseae*, or *Acaulospora laevis* reduced the severity of disease caused by *Fusarium oxysporum* f. sp. *cumini*. *Glomus fasciculatum* was also found to promote healthier roots in sweet orange challenged with the pathogen *Phytophthora parasitica* in low-P conditions (<15 mg P per g soil). Similar plants provided with higher levels of P (56 or 600 mg P per g soil) were not protected by the VA mycorrhizal fungus (Davis and Menge, 1980). Kendrick (1992) proposed three mechanisms by which plant disease severity is reduced by VA mycorrhizal fungi; (1) increased nutrition producing a plant more capable of resisting attack; (2) chitinolytic enzymes produced by mycorrhizal plants to digest senescent arbuscules in their tissues, may assist in destroying pathogenic fungal structures; (3) the presence of VA mycorrhizal fungi on the root system reduces the number of sites available for pathogen invasion. Although there are several reports of VA mycorrhizal fungi reducing the severity of pathogen attack, there are also some reports indicating that they can also increase the level of disease (Dehne, 1982).

POST-POINT-OF-SALE PERFORMANCE AND VA MYCORRHIZAE

Johnson (1982) referred to some earlier work where he observed improved establishment and survival of mycorrhizal plants over non-mycorrhizal plants in landscape soils. Western red cedar plants (*Thuja plicata*) grown in fumigated beds in a bare-root nursery were found to be stunted and deficient in VA mycorrhizae when compared to plants grown in non-fumigated beds. The mycorrhizal plants maintained their growth advantage up to 13 months after transplanting into the field (Berch et al., 1991). Stunted and chlorotic citrus seedlings growing in fumigated soils were found to be lacking VA mycorrhizae. Inoculation of these plants with *Endogone mosseae* resulted in normal growth being restored. After transplanting into a fumigated (methyl bromide) field site, the colonised plants were found to perform significantly better than the non-VA mycorrhizal plants (Kleinschmidt and Gerdemann, 1972). Davies (1982) has even suggested that the superior performance of VA mycorrhizal plants may even be translated into their commanding a premium price in the market place.

INOCULUM PRODUCTION

VA mycorrhizal inoculum can only be produced on the roots of live plants due to the obligate dependency of the fungal component on the plant. Generally such inoculum is produced by growing a host plant in media under glasshouse conditions. A small amount of VA mycorrhizal culture is introduced into the system and is multiplied accordingly. Fast growing tropical grasses such as Sudan grass and sorghum are ideal hosts (Morton et al., 1993). The container medium used should be relatively low in nutrients to encourage the spread of the fungus, rather than rapid plant growth (St. John and Evans, 1990). A major concern with this process is the contamination of the inoculum with unwanted pathogens such as *Fusarium*, *Rhizoctonia*, etc. High levels of hygiene must be maintained to reduce the possibility of this occurring as many fungicides, particularly the systemic compounds benomyl (Boatman et al., 1978; Smith, 1978) and thiophanate-methyl (Boatman et al., 1978), are known to be toxic to VA mycorrhizae.

Commercially produced inoculum is rapidly becoming available in several countries. NPI in Salt Lake City (Utah) produced a product called Nutri-Link (St. John and Evans, 1990) containing plant roots, hyphae, and spores of various VA mycorrhizal fungi. A similar product known as Dr. Kinko is produced in Japan. The Agricultural Genetics Company in the UK (Vam Inoc[®]) and Phytotec in Belgium also produce commercial inocula (Lovato et al., 1992). Typically the production cycle for such inocula is 2 to 4 months. The successful use of mycorrhizal fungi in nursery production will require identification and isolation of superior fungi, production of pathogen-free inoculum, development of inoculation techniques, and adjustments of cultural practices to ensure that the plant-fungal association is maintained (Maronek et al., 1981).

Experimental production of VA mycorrhizal inoculum has been achieved through the use of aeroponic systems (Jarstfer and Sylvia, 1992) and it is potentially possible through the use of other hydroponic systems. It is conceivable that through such systems, pathogen-free inoculum could be produced easily and processed in the form of colonised root material.

CONCLUSION

The benefits of VA mycorrhizae to plant growth have been clearly demonstrated by many workers. VA mycorrhizae have the potential to increase propagation success, produce healthier and more robust plants while reducing fertiliser inputs. Such plants are more resistant to disease attack, have greater drought tolerance, and potentially have better after-sales performance (and retail value). Initially, research must be conducted to examine the benefits of VA mycorrhizae to a range of commercially produced species, particularly as little work has been done in this area with Australian natives and many exotics. For example, a survey of 80 species of Australian Asteraceae (native daisies) found most of these to be capable (and some highly dependent upon) forming VA mycorrhizae (Warcup and McGee, 1983). A range of commercial inocula suited to the needs of the industry are being developed, and some of these are already commercially available. Inoculation with VA mycorrhizae will eventually become an integral part of nursery production systems.

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