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Vesicular-Arbuscular and Ectomycorrhizas on the Annual Composite, *Podotheca angustifolia*

P.A. MCGEE

Department of Agricultural Biochemistry, Waite Agricultural Research Institute, University of Adelaide Glen Osmond, S.A. 5064, Australia

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Abstract

In a soil in which there were similar numbers of propagules of vesicular-arbuscular and ectomycorrhizal fungi, high soil temperature influenced which fungi initiated mycorrhizas. High soil temperatures were associated with a reduction in the spread of an ectomycorrhizal fungus along the root system of *Podotheca angustifolia*. Water content of the soil did not appear to influence either the type of mycorrhizal infection initiated or the rate of spread of fungi along the roots.

On seedlings of *P. angustifolia* inoculated with vesicular-arbuscular and ectomycorrhizal fungi, there was a competitive relationship between the fungi. *Acaulospora laevis* did not impart a significant growth increase to infected over uninfected plants. Spread of *A. laevis* was reduced in the presence of *Peziza whitei* and *P. whitei* increased the growth of infected plants, both alone and in the presence of *A. laevis*.

Keywords: VA mycorrhizas, ectomycorrhizas, *Podotheca angustifolia*, competition

1. Introduction

Many plant species have been observed with both vesicular-arbuscular mycorrhizae (VAM) and ectomycorrhizas (Newman and Reddell, 1987; Harley and Smith, 1983). In some woody perennial species it appears that roots of seedlings are initially infected by VAM fungi and that over time, ectomycorrhizas predominate (Lapeyrie and Chilvers, 1985; Read et al., 1977). Furthermore, some species known to form both vesicular-arbuscular (VA) and ectomycorrhizas in pot culture, may be found in the field with either or

both associations (McGee, 1986). These observations suggest that competition (*sensu* Chilvers et al., 1987) between the two types of association is affected by environmental conditions.

Many aspects of the environment, including soil temperature and soil water content, affect the formation and spread of mycorrhizas (Slankis, 1974; Reid, 1979) and many fungi are adapted to specific environments. Thus, *a priori*, each association could be expected to react differently to environmental variables.

The annual composite, *Podotheca angustifolia* (Labill.) Less. has both VAM and ectomycorrhizas throughout its growing season at a study site in semi-arid, South Australia (McGee, 1986). However, the proportion of root infected with each type of mycorrhiza varied. At the study site variation in soil temperature and rainfall (McGee, 1987) may have accounted for the different proportions of each type of mycorrhiza. *P. angustifolia*, therefore, seemed an ideal host to examine the formation of mycorrhizas in field soil and in pot culture in the presence of mixed inoculum. The effect of soil temperature and soil water content on the initiation of VAM and ectomycorrhizas on the same root system was examined.

2. Materials and Methods

Sandy soil (Uc 1.21; Northcote, 1979) was collected from the study site during summer when it was dry, thoroughly mixed, sieved through a 5 mm mesh and stored dry. The soil had low nutrient concentrations (McGee, 1987) and when dry, less than 0.5% water content. Referred to as natural soil, the mixed and sieved soil was used to examine the effect of soil moisture and temperature on the initiation of infection by mycorrhizal fungi. Where soil free of mycorrhizal fungi was required, soil was collected from below 10 cm deep, sieved, autoclaved and then stored dry for at least six months before use.

Seed of *Podotheca angustifolia* was collected from one plant in the field and stored dry. Single seeds were germinated on the surface of moistened, autoclaved sand and seedlings transplanted when the roots were approximately 2 cm long. Pot cultures of the VAM fungus *Acaulospora laevis* Gerd. and Trappe were established from spores on *Trifolium subterraneum* L. in autoclaved soil. Pot cultures of the ectomycorrhizal fungus *Peziza whitei* (Gilkey) Trappe were established by placing a plug of cultured fungus against the root of a seedling of *Melaleuca uncinata* R. Br. in autoclaved soil.

Plants were grown in plastic lined pots in either a Wisconsin tank or a growth room or in plastic tubes with open ends, on a bed or autoclaved sand in a growth room. Wisconsin tanks were kept in a glasshouse with natural daylength and temperature and water temperatures were set at either 16, 20 or 30°C. In the growth room, plants grew during 12 hr days (20–22°C day, 16–18°C night) with a PAR of 240 μmol $m^{-2}sec^{-1}$. The temperatures were chosen to represent the variation found in the field at the study site at the start of the growing season in autumn (McGee, 1987). Plants in pots were watered twice or thrice weekly to weight with deionised water. Plants in tubes were watered to saturation daily with deionised water. Soil water content was determined prior to potting by mixing known weights of soil and deionised water. The soil water potential was determined by the filter paper method (Hamblin, 1981).

The fungi occurring in the field were previously identified. All the VAM fungi had hyphae wider than 1.5 μm. Ectomycorrhizal fungi with three different morphologies of infection were observed. All were Ascomycetes (broad, usually hyaline, thin walled hyphae with simple septa and an absence of clamp connections; Asco. I, II and III). Asco. I had hyaline hyphae that did not stain with Trypan Blue, though faint staining was occasionally left in the grooves between the hyphae of the mantle. Cells of the sheath were irregularly shaped with an angular appearance (see Fig. 6 of McGee, 1986). Asco. II had hyaline hyphae that retained some Trypan Blue, particularly away from the root. Cells of the sheath were sinuous and rounded (see Fig. 5 of McGee, 1986). Asco. III had brown hyphae and was probably a *Cenococcum* sp.

Number of fungal propagules

Numbers of propagules of VAM and ectomycorrhizal fungi present in the natural soil were estimated by a 'most probable number' (MPN) of propagules using a ten fold serial dilution with three dilutions (McGee, 1988). The trap plants, *P. angustifolia* and *Plantago drummondii* Decne. were chosen as they grow at similar rates, both occur at the study site and the latter only forms VAM (McGee, 1986). Trap plants were grown in open tubes on a bed of autoclaved sand. The test soil was placed as a band one cm below the soil surface, sandwiched between autoclaved soil. After 28 days, ten replicate plants were harvested, washed from the soil, cleared, stained in Trypan Blue (Phillips and Hayman, 1970) and examined for mycorrhizas.

Temperature and establishment of mycorrhizas

The effect of temperature on the germination of propagules and initiation of mycorrhizas was examined using single seedlings of *P. angustifolia* grown in pots of 1 kg of natural soil moistened to 10% by weight (approximately –0.001 kPa water potential, almost saturated). Five pots from each water bath (16, 20 and 30°C) were harvested at 10, 28 or 56 days and the roots cleared, stained and examined for mycorrhizas. The number of plants with VAM, each type of ectomycorrhiza, shoot dry weight and the percent root length with each type of mycorrhiza was determined.

Temperature and water content

Single seedlings were grown in pots of soil moistened to 5 (–0.008 kPa), 7.5 (–0.006) or 10% by weight with deionised water. Moisture was retained in the pots by placing a lid of a petri dish over each pot. There were six replicate pots of each treatment placed in each Wisconsin tank. Plants were harvested 28 days only, to reduce the chance of variation in plant size affecting rates of infection.

Inoculation with Acaulospora laevis and Peziza whitei

Singly and doubly inoculated seedlings of *P. angustifolia* were grown to examine the possibility of an interaction between VAM and ectomycorrhizal fungi. The quantity of inoculum to give an equivalent rate of initiation of mycorrhizas, was first determined. Single seedlings of *P. angustifolia* were placed over a pad of 0, 0.1, 0.25, 0.5 or 1 g of fresh mycorrhizal roots of either *Trifolium subterraneum* infected with *A. laevis* or *Melaleuca uncinata* infected with *P. whitei*, in pots of 500 g moistened, autoclaved soil. Four pots of each treatment were harvested after 3, 6 or 10 days and the roots examined for the presence of mycorrhizas.

The relationship between *A. laevis* and *P. whitei* on the roots of *P. angustifolia* was assessed. A pad of 0.5 g of fresh mycorrhizal root inoculum of *A. laevis* and/or 0.25 g of fresh inoculum of *P. whitei* were placed under seedlings in 850 g of moistened, autoclaved soil in plastic lined pots. Pads of inoculum were used to ensure that spread of mycorrhizas was related to the growth of each fungus. There were four treatments; inoculation with *A. laevis* alone, inoculation with *P. whitei* alone, inoculation with both fungi or with a control of uninfected mixed roots. Ten replicates of each treatment were harvested after 6 weeks growth in the growth room. At harvest, shot

Table 1. The most probable number of propagules (MPN) per 100 g dry soil of vesicular–arbuscular and ectomycorrhizal fungi found using *Podotheca angustifolia* and vesicular-arbuscular mycorrhizal fungi using *Plantago drummondii* harvested after 28 days.

Host	Mycorrhizal fungus	MPN	95% Confidence interval
Pl. drummondii	VAM	13.3	5.7–30.9
Po. angustifolia	VAM	19.7	8.5–45.7
	Asco. I	17.0	7.3–39.4
	Asco. II	0.3	0.1–0.7
	Asco. III	0.2	0.1–0.6
	Total mycorrhizas	27.5	11.8–63.8

dry weight, root length, length of VAM, length of ectomycorrhiza (root intersect method; Giovanetti and Mosse, 1980) were estimated and the percent root length infected with VAM and ectomycorrhizas, calculated.

3. Results

Number of fungal propagules

There were similar numbers of propagules of VAM and ectomycorrhizal fungi trapped from the soil by *P. angustifolia* and similar numbers of propagules of VAM fungi using either host (Table 1).

Temperature and establishment of mycorrhizas

All plants at each harvest had VAM and except for one plant harvested at day 10, all had ectomycorrhizas formed by Asco. I. At 30°C, no ectomycorrhizas formed by Asco. II were observed and only small patches of ectomycorrhizas of Asco. I were present (Table 2). At 28 days the difference in shoot dry weight between plants grown at each soil temperature was not significant. By 56 days plants grown at 20°C had significantly higher shoot dry weight than those grown at 16 or 30°C. As major differences in plant growth may affect the infection process, the next experiment was harvested at 28 days.

Temperature and water content

One seedling at 30°in soil with 5% moisture content died prior to harvest (Table 3). Again, at 30°C, no Asco. II or III germinated and initiated infection and the spread of mycorrhizas of Asco. I was reduced. Soil water content appeared to have no effect on the germination and initiation of infection of fungal propagules at any temperature.

Table 2. Number of plants of *Podotheca angustifolia* observed with vesicular-arbuscular and ectomycorrhizas after 10, 28 or 56 days at 16, 20or 30°C and mean shoot dry weight and percent root length infected with vesicular-arbuscular and ectomycorrhizas at 28 or 56 days.

Temp °C	VAM	Ectomycorrhiza Asco. I	Asco. II	Total	Shoot dry weight (X±SEM) $g \times 10^{-2}$	Percent root length VAM	Ect.
					Day 10		
16	5/5	4	2	5			
20	5	4	5	5			
30	5	4	0	4			
					Day 28		
16	5	5	2	5	0.5±0.06	40	40
20	5	5	3	5	0.6±0.13	54	36
30	5	5	0	5	0.4±0.03	45	2
					Day 56		
16	5	5	4	5	2.4±0.2	80	30
20	5	5	4	5	4.7±0.6	46	70
30	5	5	0	5	4.2±0.2	68	12

Table 3. Number of plants of *Podotheca angustifolia* infected with vesicular-arbuscular and ectomycorrhizas, the mean percent infection and their shoot dry weight when grown in soil with a water content of 5, 7.5 or 10% in pots in water baths set at 16, 20 or 30°C for 28 days.

Temp °C	soil water content %	VAM	Ect.	% VAM	% Ect.	Shoot dry weight (mg) (X±SEM)
16	5	6/6	6/6	22	70	4.7±0.8
	7.5	6	6	23	68	5.4±1.0
	10	6	6	25	73	4.6±0.6
20	5	6	6	35	57	5.4±0.6
	7.5	6	6	27	63	5.4±0.8
	10	6	6	38	57	4.4±1.0
30	5	5/5*	5	52	6	3.5±0.2
	7.5	6	6	65	2	3.3±0.2
	10	6	6	65	5	3.9±0.4

*one plant died prior to harvest

Table 4. Dry weight of shoots, length of roots, length of VAM and ectomycorrhizas and percent root length infected with VAM and ectomycorrhizas of *Podotheca angustifolia* inoculated with *Acaulospora laevis*, *Peziza whitei*, both or neither after 6 weeks.

	Inoculum			
	Nil	*A. laevis*	*P. whitei*	Both
Number of plants	10	8	9	7
Shoot dry wt.				
Mean ($g \times 10^{-4}$)	144	158	490	496
SEM	18	26	38	35
Length of roots				
Mean (cm)	402	428	1736	1461
SEM	43	68	274	177
Length of VAM				
Mean (cm)	0	88	0	29
SEM		42		9
Length of ecto				
Mean (cm)	0	0	293	198
SEM			63	30
% VAM				
Mean	0	16.3	0	2.3
SEM		4.3		0.7
% Ect.				
Mean	0	0	14.9	13.1
SEM			2.3	1.0

Inoculation with A. laevis and P. whitei

The minimum time to initiate infection arose from several combinations, that with the least inoculum being 0.5 g of roots infected with *A. laevis* and 0.25 g of *P. whitei*.

Eight plants singly inoculated and seven plants doubly inoculated were found with VAM. Nine plants singly inoculated and ten plants doubly inoculated were found to be ectomycorrhizal. Four trends in infection and growth of *P. angustifolia* when inoculated with either or both *A. laevis* and *P. whitei* were apparent (Table 4). *A. laevis* by itself did not significantly increase shoot dry weight and root length. *P. whitei* significantly increased both shoot dry weight and root length of the host ($p>0.05$) both alone and in association with *A. laevis*. The percentage root length infected with *A. laevis* declined ($p>0.025$, Mann-Whitney U test) in the presence of *P. whitei*, though when measured as total root length infected, the decline is only significant at $p>0.1$. The percentage root length infected with *P. whitei* was

not significantly affected by the presence of *A. laevis* on those pants that became infected by *A. laevis*.

4. Discussion

Clearly, with five replicates it is hard to be certain, but these data support the hypothesis that mycorrhizal initiation and mycorrhizal fungi are influenced by soil temperature but not by soil water content at levels commonly found in the field at the start of the growing season in semi-arid South Australia. They also suggest that there is a competitive relationship between VA and ectomycorrhizas on single root systems and that environment influences the relationship.

The data suggest that propagules of VAM fungi germinate independently of propagules of ectomycorrhizal fungi. Though it needs to be tested, it is also likely that different ectomycorrhizal fungi also germinate independently. The relationship appears to be competitive by the time mycorrhizas are established. This is not surprising as the root supplies energy for growth and development of the fungi. The precise mechanism of competition is unclear. Chilvers et al. (1987) suggest that the ectomycorrhizal mantle blocks out the VAM fungi. Such a complete physical barrier does not occur in *Podotheca angustifolia*. Ectomycorrhizas of *P. angustifolia* rarely ensheath entirely the root circumference, short laterals are never observed and the sheath is rarely more than one cell thick. Root tips extend much more rapidly than the fungal mantle and there are always areas of the root surface, both at the root tip and along the root that might become infected by VAM fungi. While such an ectomycorrhiza is not commonly described outside Australia, they have been demonstrated elsewhere to promote positive growth response in their hosts (Kope and Warcup, 1986; Warcup and McGee, 1983). Thus the action of a physical barrier, if it occurs must be in addition to another mechanism of competition in hosts with partial sheaths.

It is unfortunate that a more aggressive VAM fungus was not isolated from the study site. Though five other spore forming VAM fungi were observed (McGee, 1989), all fungi failed to impart significantly faster growth than uninfected plants in the growth room. Whether this is because of the nature of the fungi at the study site or because of environmental conditions (such as low light in the growth room), (Son and Smith, 1988), is not known.

While ectomycorrhizas or VA mycorrhizas or both may be found on individual hosts in the field, these data only indicate some mechanisms controlling infection. While the environment is important, the genetic aspects have

not been examined and relations between genetics and environment must be considered. Also, much work is required to determine the precise mechanisms controlling the relationship between VA and ectomycorrhizas on one root system. It is likely that environmental factors will interact with the genetic components of the system. Elucidation of such mechanisms will do much to improve our understanding of the processes of infection of VA and ecotomycorrhizas.

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