

# Dual colonization of *Eucalyptus urophylla* S.T. Blake by arbuscular and ectomycorrhizal fungi affects levels of insect herbivore attack

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## Abstract

- 1 Eucalypts are an important part of plantation forestry in Asia, but in south China productivity is very low. This is due to infertile soils and lack of indigenous symbiotic mycorrhizal fungi. The genus *Eucalyptus* is unusual because it forms both arbuscular (AM) and ectomycorrhizal (ECM) associations.
- 2 *Eucalyptus urophylla* saplings were grown with and without AM (*Glomus caledonium*) and ECM (*Laccaria laccata*) fungi in a factorial design. Two experiments were performed, one to simulate nursery conditions and the other to simulate the early stages of plantation establishment. Plant growth was measured over 18 weeks and levels of insect attack recorded.
- 3 The AM fungus reduced tree growth in the early stages, but the effect appeared to be transient. No effects of ECM were detected on tree growth, but the ectomycorrhiza reduced colonization by the arbuscular mycorrhiza. AM fungi appear to be rapid invaders of the root system, gradually being replaced by ECM.
- 4 Both fungal types affected levels of damage by insect herbivores. Of most importance was the fact that herbivory by the pest insects *Anomala cupripes* (Coleoptera) and *Strepsicrates* spp. (Lepidoptera) was decreased by ECM .
- 5 It is suggested that mycorrhizal effects on eucalypt insects may be determined by carbon allocation within the plant. Future studies of eucalypt mycorrhizas need to take into account the effects of the fungi on foliar-feeding insects and also the effects of insect herbivory on mycorrhizal establishment.

## Introduction

The genus *Eucalyptus* is notable from an economic and ecological point of view. Many species are grown as plantation forests in countries such as China, where fast growing eucalypts may provide a sustainable resource for the paper pulp industry (Zhou, 1995). Since the 1980's, more than 1,000,000 ha of eucalypt plantations have been established in southern China, with a current planting rate of about 100,000 ha per annum (Xu *et al.*, 2000). These trees may also provide afforestation on denuded and degraded land areas, aiding soil stabilization in these regions (Dell *et al.*, 2000).

In China, many eucalypt plantations occur on soils of low pH and exceptionally low nutrient availability (Brundrett, 2000; Chen *et al.*, 2000a). As a result, the productivity of these plantations is very low, being only about 25% of the average world value (Brown *et al.*, 1997). Eucalypts in China respond dramatically to fertilization, particularly P, N and trace elements such as boron (Dell & Malajczuk, 1994; Xu *et al.*, 2000). In addition to soil nutrients, it has been found that Chinese soils are deficient in mycorrhizal fungi (Dell *et al.*, 2000) and that inoculation with ectomycorrhizal species can enhance growth and sapling establishment (Zhong *et al.*, 2000).

The genus *Eucalyptus* is ecologically interesting because it is one of the relatively few genera that can form an association with both arbuscular (AM) and ectomycorrhizal (ECM) fungi (Lodge, 2000). These fungi can increase eucalypt growth through a process of improved nutrient acquisition, (especially P and N), although the effect varies dramatically with the species of *Eucalyptus* studied and the fungi inoculated (Adjoud *et al.*, 1996; Lu *et al.*, 1998). Furthermore, the importance of each association differs in the life of the plant. In general, AM fungi colonize seedlings and these are replaced after a few months by ectomycorrhizas through a process of competition (Bellei *et al.*, 1992; Dos Santos *et al.*, 2001). Chen *et al.* (2000b) have shown how these fungi interact to determine the growth of *E. urophylla* during the first four months of growth. AM species colonized first and had little effect on ECM colonization, but ECM fungi subsequently reduced the colonization levels of AM species. The succession of mycorrhizas did not appear to compromise growth effects and the greatest growth responses were seen in plants colonized by both types of mycorrhiza. Therefore, in theory, an ideal strategy for outplanting of this species would be to inoculate with both types of mycorrhiza (Brundrett, 2000).

Both AM and ECM fungi have been shown to have effects on foliar-feeding insects (Gehring & Whitham, 2002). These effects may be positive or negative, depending on the mode of feeding and degree of specialism of the insect studied. For both types of fungal association, generalist chewing insects respond in a negative fashion to mycorrhizal colonization of their hosts, while specialist chewing or sucking insects show increases in performance on mycorrhizal plants (Gange *et al.*, 2002b). These effects have been linked to mycorrhizal-induced changes in plant chemistry, either through changes in secondary metabolites (Gange & West, 1994) or alterations in plant nitrogen content (Gange & Nice, 1997; Rieske, 2001). Given that both AM and ECM fungi can increase plant size and nitrogen content of eucalypt foliage (Aggangan *et al.*, 1996a; Chen *et al.*, 2000b), we formulated the hypothesis that mycorrhizal plants would be more attractive to insects and thus suffer higher levels of attack (Schoonhoven *et al.*, 1998). It is important to know if this occurs, for if it does, any beneficial effects could be nullified, given the amount of damage that insects can inflict on eucalypts in China (Zhenghong, 2003).

A feature of the insect-mycorrhizal literature is that only one experiment has ever compared the effects of AM and ECM fungi simultaneously on herbivory by an insect. Gehring & Whitham (2002) reported that AM colonization of hybrid cottonwood trees (*Populus angustifolia* x *P. fremontii*) reduced populations of a specialist aphid, *Chaitophorus populicola*, while ECM colonization enhanced aphid numbers, relative to controls. Unfortunately, the experiment does not seem to have been fully factorial, and no data were given for aphid populations on trees colonized by both AM and ECM fungi. In this paper, we describe the first experiment to examine AM and ECM fungi and the interactions between them, on foliar-feeding insect attack of *E. urophylla*. An understanding of how these different fungi affect insect performance may go some way to unravelling the complex and little understood phenomenon of dual mycorrhizal plants (Lodge, 2000).

## **Materials and methods**

### **Preparation of study organisms**

Two experiments were conducted between January and July 2000. Experiment 1 consisted of a controlled garden study, designed to grow *E. urophylla* in typical nursery conditions (Dell, 2000). Experiment 2 was a field outplanting of *E. urophylla*, designed to mimic the early establishment phase of a plantation. Both experiments took place in southern China.

Seeds of *E. urophylla* were collected from a plantation in south China in 1995 (seed lot no. 14531). They were surface sterilized in 0.25 % NaOCl for 15 min before being washed in sterile water. Seeds were sown into trays containing an autoclaved mixture of sand, peat and vermiculite (2:1:1.5 v/v), and watered once with a balanced nutrient solution (Chen *et al.*, 2000b). Thereafter, trays were maintained at 12% soil moisture content with tap water at 25°C until germination occurred.

The AM fungus was *Glomus caledonium* (T.H. Nicolson & Gerd.) Trappe & Gerd., (isolate Gc90068), isolated from a *Eucalyptus* plantation in south China and propagated in pot culture on the roots of *Trifolium* plants. Plants were allowed to die, through cessation of watering, and 20 g of the dry soil, containing colonized roots, hyphal fragments and spores was used as the inoculum. The ECM fungus was *Laccaria laccata* (Scop. ex. Fr.) Berk. (isolate L1439) isolated from the same plantation and maintained in sterile liquid culture on Modified Melin Norkans medium (Brundrett *et al.*, 1996). The mycelial slurry was fragmented in a blender for 30 s and 20 cm<sup>3</sup> was used as the inoculum.

### **Nursery experiment**

Experiment 1 was set up in early January 2000. One hundred 25 cm diameter (volume 10 l) plastic pots were each lined with a plastic bag and filled with 2,500 g of the autoclaved sand:peat:vermiculite mix (above). There were four treatments, consisting of Control (no inoculum added), AM inoculated, ECM inoculated and inoculation with both fungi. For AM inoculation, 20 g of the soil mixture was placed in a layer 3 cm below the final surface of the potting mix. ECM inoculation consisted of 20 cm<sup>3</sup> of mycelial slurry applied close to the roots of the planted seedling. Dual inoculated plants received both 20 g and 20 cm<sup>3</sup> of inoculum and there were 25 replicates of each treatment.

Two 14 d old *E. urophylla* seedlings were planted into each pot and after a further two weeks, the weaker seedling was removed. Plants were given supplementary fertilizer every two weeks, as described by Dell & Malajczuk (1995). They were watered daily with tap water to maintain 12% soil moisture content (Dell, 2000). The pots were placed in an outside arena and arranged in a randomised block design with one replicate of each treatment per block.

Recordings were taken after 6 and 12 weeks and at the final harvest, after 18 weeks. On each sampling occasion, sapling height and total leaf number were measured. The length (L) and width (W) of a random sample of 25 leaves were measured *in situ* on each plant. These measurements were used to estimate the area (y) of each leaf from the equation  $y =$

$0.897L*W + 6.34$  ( $r^2 = 0.934$ ,  $P < 0.001$ ), calculated on a sample of 250 leaves, removed from other non-experimental saplings. Insect attack took three distinct forms, edge chewing by larvae of two species of unidentified Lepidoptera (Geometridae), holes near the centre of the leaf caused by adults of *Anomala cupripes* (Coleoptera: Scarabaeidae) and feeding within a leaf roll by larvae of *Strepsicrates* spp. (Lepidoptera: Tortricidae). The percentage of leaves on each tree that had suffered each form of damage was calculated on each date. Therefore, the data are not cumulative, but represent the extent of attack at different time intervals. Observations were made between the sampling periods and no other forms of insect attack (chewing or sucking) were observed.

After 18 weeks, saplings were carefully removed from pots and their roots washed free of soil. A sub sample was examined for mycorrhizal colonization, while the remainder of the plant was air dried to constant weight and total biomass measured. Roots were cleared by soaking in a solution of 2.5% KOH overnight. ECM colonization was examined under light microscopy and the percentage of mycorrhizal root tips recorded. Ectomycorrhizal tips were very obvious, being a silvery white in colour. For AM recording, roots were examined at  $\times 200$  using a Zeiss Axiophott epifluorescence microscope, fitted with a UV lamp and filters, giving a transmission of 455-490 nm blue. Under these conditions, the arbuscules fluoresce (Ames *et al.*, 1982), with measurements being more reliable than conventional stains (Gange *et al.*, 1999). Arbuscular colonization was recorded using the cross-hair eye piece method of McGonigle *et al.*, (1990), with a minimum of 200 intersections observed per slide.

Dry leaf material was ground to a powder and P content measured by the molybdenum blue method, following an acid digestion (Allen, 1989). Total N content of foliage was measured by semi-micro Kjeldahl digestion, followed by the indophenol-blue reaction (Allen, 1989).

### **Field experiment**

The experiment took place at Zhenhai Forest Farm, near Kaiping, Guangdong Province, P.R. China. An area of land was cleared of vegetation by burning, but not ploughed. The soil was a lateritic red soil, with a P level of  $3.1 \pm 0.56$  mg kg<sup>-1</sup> (bicarbonate extractable) and pH of 3.93. Saplings were grown and inoculated with the four mycorrhizal combinations in an identical fashion to those in the nursery experiment (above), and maintained for 10 weeks post inoculation. They were transferred to the field site in early April 2000 and there were 25 replicates of each treatment.

The experimental site measured 45 m x 45 m (2,025 m<sup>2</sup>) and within this, saplings were planted out in a randomised block design, with one replicate of each treatment per block. Saplings were planted 3 m apart and were given 50 g of urea, 150 g of superphosphate, 30 g KCl, 3 g of boric acid and 2 g of ZnSO<sub>4</sub> as recommended by Xu *et al.* (2000). This was done to aid tree establishment, as nutrient deficiency is a common reason for tree failure in soils of low pH in southern China (Dell *et al.*, 1995). No additional fertilizer or water was given during the experiment.

Saplings were then grown for 18 weeks and recordings taken after 6 and 12 weeks and at the final harvest. On each sampling occasion, tree height and leaf number were counted, a random sample of leaves measured for leaf area analysis and the percentage of leaves that had suffered the three forms of insect attack counted. At the final harvest, saplings were carefully removed from the soil, ensuring that as much of the root system as possible was removed and dried to constant weight. Sub samples of roots were used for mycorrhizal recording (above), while total P and N contents were measured in the dry foliage after total biomass had been recorded.

### **Statistical analysis**

All analyses were conducted using plants as replicates. For leaf area, where many measurements were taken per plant, we calculated the mean for each plant prior to analysis. All data sets were tested for normality and homogeneity of variances. Percentage data (insect attack and mycorrhizal colonization) were subjected to the angular transformation prior to analysis (Zar, 1996). Count data (leaf number per tree) was subjected to the square root transformation, while tree height was logarithmically transformed. The main effect of each fungal treatment on tree growth over time was examined with a Repeated Measures Analysis of Variance. Single parameters (biomass, colonization and P and N content) were examined with Two Factor ANOVA. Insect data sets contained many zero values on the first sampling date (week 6) and these data were omitted from the analysis. Furthermore, as this left only two dates, with clear interactions over time, we analysed insect attack separately on each date. We appreciate that this may increase the likelihood of committing a Type I error and so the *P* value was adjusted downwards using the Bonferroni correction (Simes, 1986).

### **Results**

### **Mycorrhizal colonization**

Small amounts of AM and ECM colonization were found in treatments not inoculated with these fungi (Fig. 1), presumably due to wind borne spores in the nursery experiment or colonization by indigenous fungi in the field site. However, background levels of both fungi were very low in the field site, as shown by colonization in control trees (Fig. 1c,d). Overall, colonization levels by both types of fungi tended to be higher in pot grown nursery plants than they were in the field (Fig. 1).

Inoculation with ECM had a significant negative effect on AM colonization in the nursery ( $F_{1,96} = 4.67, P < 0.05$ ) (Fig. 1a) and field ( $F_{1,96} = 7.63, P < 0.01$ ) (Fig. 1c). In both cases, AM colonization in the dual inoculation treatment was lower than that of the single AM inoculation, leading to a significant interaction term between the fungi (nursery:  $F_{1,96} = 5.91, P < 0.05$ ; field:  $F_{1,96} = 7.09, P < 0.01$ ). However, inoculation with AM fungi had no effect on colonization levels by ECM (Fig. 1, b,d).

### **Growth of nursery trees**

No effect of either fungus was seen on tree height (Fig. 2a). However, in nursery plants, AM inoculation decreased total leaf number ( $F_{1,96} = 6.24, P < 0.05$ ) (Fig. 2b). The effect was most apparent in the early parts of the experiment, and by the final sampling date had disappeared, leading to a weak interaction term between treatment and time in the analysis ( $F_{2,192} = 2.69, P = 0.06$ ). ECM had no effect on total leaf number in nursery grown saplings.

A similar pattern was seen with leaf area (Fig. 2c), where saplings inoculated with the AM fungus produced much smaller leaves, although only in the early part of the study ( $F_{1,96} = 4.71, P < 0.05$ ). By week 18 of growth this effect was no longer apparent, leading to a significant interaction term with time ( $F_{2,192} = 7.55, P < 0.001$ ). ECM inoculation had no effects on leaf area throughout the experiment.

The reductions in leaf number and size caused by AM fungi were reflected in the final biomass of trees (Fig. 2d), with AM inoculation having a significant negative effect ( $F_{1,96} = 4.68, P < 0.05$ ). ECM inoculation had no effect on final biomass and there were no interactions between the fungi.

### **Growth of field grown trees**

Field grown trees were considerably taller and produced more leaves than did nursery grown specimens and final biomass was therefore higher too (Fig. 3). In the field, the pattern of fungal effects was almost identical to those found in nursery trees. However, unlike nursery



trees, AM inoculation significantly reduced tree height ( $F_{1,96} = 14.34, P < 0.001$ ) (Fig. 3a), while ECM did not affect this parameter.

AM inoculation reduced total leaf number ( $F_{1,96} = 11.96, P < 0.001$ ) (Fig. 3b) and leaf area ( $F_{1,96} = 10.54, P < 0.01$ ) (Fig. 3c). The pattern of leaf production was different between treatments inoculated with AM and those without, leading to significant interaction terms between AM treatment and date (leaf number:  $F_{2,192} = 11.14, P < 0.001$ ; leaf area:  $F_{2,192} = 9.32, P < 0.001$ ). By the end of the study, the effect of AM on leaf area had disappeared (Fig. 3c) and the leaf number of AM and non-AM treatments were also beginning to converge (Fig. 3b). AM fungi had a highly significant negative effect on biomass ( $F_{1,96} = 13.05, P < 0.001$ ) while ECM had no effect on final tree size (Fig. 3d).

### **Insect attack**

Damage levels varied through the experiment on nursery grown trees (Fig. 4). For edge chewing by the Geometrid larvae, there were highly significant effects of both AM ( $F_{1,96} = 26.04, P < 0.001$ ) and ECM ( $F_{1,96} = 57.42, P < 0.001$ ), as well as a significant interaction between them ( $F_{1,96} = 28.63, P < 0.001$ ) on week 12 (Fig. 4a). This was caused by the fact that damage levels were very high on trees inoculated with both fungal types, but neither fungus alone caused an increase in damage. These effects had disappeared by the end of the study, when larvae had pupated.

Centre chewing by *A. cupripes* adults was significantly reduced by ECM fungi at weeks 12 ( $F_{1,96} = 5.28, P < 0.04$ ) and 18 ( $F_{1,96} = 7.81, P < 0.01$ ) (Fig. 4b). Although AM fungi also appeared to reduce internal chewing, the effect was not significant, due to a relatively large amount of variation in the data. Leaf folding by *Strepsicrates* larvae was only common at the final date and at this time, ECM fungi significantly reduced the level of this form of attack ( $F_{1,96} = 5.42, P < 0.04$ ) (Fig. 4c).

Insect damage results from field-grown trees differed from those seen on nursery trees (Fig. 5). In the field, few significant effects were found at the levels set by the  $P$  value correction procedure (0.039). However, on week 12, ECM inoculation was found to significantly increase the levels of damage by the Geometrid larvae ( $F_{1,96} = 20.14, P < 0.001$ ) as was AM inoculation ( $F_{1,96} = 5.16, P < 0.04$ ) (Fig. 5a). This result was consistent with that found on nursery trees, although in the field, all fungal treatments increased damage, which was not so in nursery trees (Fig. 4a). A second consistent result was that ECM reduced the incidence of *Strepsicrates* damage at the end of the experiment ( $F_{1,96} = 4.78, P < 0.04$ ) (Fig. 5c).

### Nutrient contents of foliage

The effects of the fungi on P and N content of nursery and field trees were remarkably similar and results for field trees are presented in Fig. 6. All fungal combinations increased P content, with the AM inoculation ( $F_{1,92} = 14.52$ ,  $P < 0.001$ ) having a greater effect than that of ECM ( $F_{1,92} = 6.9$ ,  $P < 0.05$ ) (Fig. 6a). AM inoculation caused a considerable increase in foliar N content ( $F_{1,92} = 16.01$ ,  $P < 0.001$ ) as did ECM ( $F_{1,92} = 13.3$ ,  $P < 0.001$ ), but there was also a significant interaction between the fungi, as dual inoculation did not increase foliar N beyond that of either single fungal inoculation ( $F_{1,92} = 10.28$ ,  $P < 0.01$ ) (Fig. 6b).

### Discussion

Several important and hitherto unreported facts have emerged from this relatively simple study. The first is that AM inoculation of saplings had a detrimental effect on the growth of *E. urophylla*, although this effect appeared to be transient. Secondly, although ECM inoculation successfully initiated mycorrhizal colonization, no effects on plant growth were seen, but significant effects on insect herbivores were found. AM inoculation had little influence on insect herbivores, but when this did occur, the effect was the same as that caused by ECM. Finally, greater mycorrhizal effects were found on insects attacking nursery trees, but when field effects were found, these were consistent with the nursery results.

Species in the genus *Eucalyptus* are relatively unusual as they can form arbuscular and ectomycorrhizal associations at the same time. However, the benefit from forming the two different types of mycorrhiza seems to depend on many biotic and abiotic factors. Perhaps the most important biotic factor is the identity of the fungus that is used as inoculum. Adjoud *et al.* (1996) tested three AM fungi (*Glomus intraradices*, *G. mosseae* and *G. caledonium*) on 11 *Eucalyptus* species and found positive effects on growth in only 21% of the plant-fungus combinations. Moreover, in that study, *G. caledonium* failed to colonize *E. urophylla*, while in the current study, an isolate of this fungus did colonize the roots of saplings. Meanwhile, Chen *et al.* (2000b) found positive growth effects in *E. urophylla* with three AM fungi (*Glomus invermaium*, *Acaulospora laevis* and *Scutellospora calospora*). The effects were not equal between fungal species, with *A. laevis* producing the greatest response and *G. invermaium* the least. Such results clearly indicate that AM mycorrhizal species are more host specific than has previously been thought (Sanders, 2002). In the experiment reported here, *G. caledonium* reduced early sapling growth, although the effect appeared to be transient

and had virtually disappeared by week 18 of growth. Reduced growth of eucalypts by AM fungi is unusual but has been reported before (Lapeyrie *et al.*, 1992). Negative effects of AM on plants are reasonably common and usually result from particular host-fungus combinations, high colonization densities or certain environmental conditions, such as high soil P (Gange & Ayres, 1999). In the current experiment, the latter two explanations can be rejected easily, and one can only conclude that *G. caledonium* is not a particularly effective symbiont for *E. urophylla*. Indeed, a similar conclusion was reached by Ortas *et al.* (2002) with the same fungus inoculated on to orange (*Citrus sinensis*) trees. It is not known why some AM fungi can elicit negative growth effects in their hosts, but a likely explanation is that these AM species have a relatively high demand for carbon (Smith & Read, 1997).

In contrast to AM studies, a number of experiments have demonstrated positive growth effects of ECM inoculation on eucalypts (Jones *et al.*, 1998; Lu *et al.*, 1998), including *E. urophylla* (Chen *et al.*, 2000b; Xu *et al.*, 2001). Only one of these studies inoculated AM and ECM fungi together (Chen *et al.*, 2000b), in which it was found that AM colonization levels peaked at 8 weeks after planting, with a subsequent decline, mirrored by an increase in ECM colonization. A significant negative effect of ECM on AM was found in that study, a feature also recorded in the current experiment. It would appear that AM fungi are rapid colonizers of eucalypt root systems, but that ECM fungi slowly outcompete these early colonizers in a process of fungal succession (Dos Santos *et al.*, 2001). One difference between our investigation and other studies is that previous ECM effects on growth were found over time scales shorter than in the current experiment. Thus, Jones *et al.* (1998) found effects after 89 d, Lu *et al.* (1998) after 110 d and Chen *et al.* (2000b) after 112 d. However, after 126 d in our experiments, no effect of ECM could be found. Our levels of colonization by *L. laccata* were considerably lower than those obtained by Chen *et al.* (2000b) with the closely-related *Laccaria laterita*, so it may be that a certain level of colonization is required to produce changes in host growth.

A number of factors can reduce the effectiveness of ECM fungi on eucalypts and these include low soil pH (Aggangan *et al.*, 1996a), competition with indigenous fungi (Aggangan *et al.*, 1996b) and lack of mycorrhiza helper bacteria (Dunstan *et al.*, 1998). The pH of the field site in our experiment was considerably lower than the optimum of 5.2 for growth enhancement of *E. urophylla* by *L. laccata* (Aggangan *et al.*, 1996a) and this may be another reason for the apparent ineffectiveness of ECM in our study. Competition with indigenous fungi is thought to be of prime importance in determining the success of field inoculated ECM on eucalypts (Brundrett, 2000), but it may have been less of a factor in the current

experiment. As with other Chinese soils (Chen *et al.*, 2000a), natural levels of both AM and ECM fungi appeared to be very low in our field site, given the levels of colonization obtained in control plants. Bacterial levels were not measured in our field soil.

One other factor that has never been taken into account and that could affect both AM and ECM colonization levels is insect herbivore attack. Studies have shown that foliage removal by insect herbivores can reduce AM colonization levels of herbaceous plants (Gange *et al.*, 2002a), while Gehring & Whitham (2002) summarise similar effects of insects on ECM colonization levels in trees. Eucalypt plantations in southern China are often subject to high levels of insect herbivory (Zhenghong, 2003) and in Australia, plantations are also subject to heavy pest attack (Baker *et al.*, 2003). We suggest that future studies investigating the role of insect herbivores in mycorrhizal establishment on outplanted eucalypts would be very rewarding.

The reverse interaction between mycorrhizas and insects, i.e. the effect of colonization on insect attack has been studied more widely (Gehring & Whitham, 2002). One of our original hypotheses, that mycorrhizal colonization would elevate plant N content and lead to increases in insect herbivore attack, appeared to be supported by results for edge chewing by Geometrid larvae. However, other forms of insect attack were unaffected by AM fungal colonization. This was perhaps surprising, given that significant effects of AM inoculation were found on plant stature. Many insects show positive correlations between attack rates and plant size (Schoonhoven *et al.*, 1998), but these were not apparent in our experiments. Instead, the most consistent finding was for ECM inoculation to decrease herbivore attack, with this effect being found for attack by *A. cupripes* in the nursery experiment and *Strepsicrates* attack in both experiments. These latter results may be of great importance, as both of these insects cause large amounts of damage to eucalypts in south China (Zhenghong, 2003). Protection against herbivore attack is thus a hitherto unseen benefit from inoculating eucalypts with ECM fungi.

One explanation for effects of mycorrhizas on foliar-feeding insects involves variation in host plant N content. When arbuscular mycorrhizas increase plant N, the effect on the insect is a positive one (Goverde *et al.*, 2000), when they decrease N, the effect is negative (Gange & Nice, 1997). However, the situation is more complicated with ectomycorrhizas, as Rieske (2001) found that in conditions of high nutrient availability, ECM functioned parasitically, leading to decreases in insect herbivore performance. In our study, ECM increased foliar N content, but this did not lead to increased attack rates by *A. cupripes* or *Strepsicrates* spp. Some insects do respond negatively to elevated N in their diet (Schoonhoven *et al.*, 1998) but

the inconsistency of effects between species suggests that this is unlikely here. Jones & Last (1991) suggested a variety of possible effects of ECM on insects, depending on the relative availability of soil nutrients and light. Under conditions of low soil nutrients and high light (the conditions in our experiments) they suggested that ECM would increase anti-herbivore defences, through carbon allocation to defences in the host plant. This hypothesis may provide an explanation for the negative effects of ECM found on *A. cupripes* adults and *Strepsicrates* larvae. However, the responses of insects to host plant secondary chemistry differ greatly depending on the degree of specialism of the insect (Schoonhoven *et al.*, 1998). If the edge-chewing Geometrid larvae were specialists on *Eucalyptus* then they might be expected to respond positively to mycorrhizal-induced changes in leaf chemistry. Overall, our data suggest little support for the mycorrhizal elevation of plant N hypothesis and seem to provide more support for the carbon allocation hypothesis of Jones & Last (1991). However, a detailed analysis of mycorrhizas on the carbon chemistry of eucalypts is required to really address this problem.

Whichever mechanism is correct, these results show that future studies of mycorrhizal effects on eucalypt growth should include a consideration of the insect herbivores present. These fungi clearly have the potential to influence insect herbivore attack rates, and experiments need to be performed in which fungal species and soil conditions (pH and nutrients) are varied, to determine which, if any, mycorrhizal combinations could be used to reduce potential pest insect levels. Furthermore, the effect of insects on mycorrhizal establishment also needs to be addressed, as this may be a hitherto unconsidered factor in eucalypt production (Brundrett, 2000).

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### **References**

- Adjoud, D., Plenchette, C., Halli-Hargas, R. & Lapeyrie, F. (1996) Response of 11 eucalyptus species to inoculation with three arbuscular mycorrhizal fungi. *Mycorrhiza*, **6**, 129-135.

- Aggangan, N.S., Dell, B. & Malajczuk, N. (1996a) Effects of soil pH on the ectomycorrhizal response of *Eucalyptus urophylla* seedlings. *New Phytologist*, **134**, 539-546.
- Aggangan, N.S., Dell, B., Malajczuk, N. & De La Cruz, R.E. (1996b) Soil fumigation and phosphorus supply affect the formation of *Pisolithus-Eucalyptus urophylla* ectomycorrhizas in two acid Philippine soils. *Plant and Soil*, **180**, 259-266.
- Allen, S.E. (1989) *Chemical Analysis of Ecological Materials*. Blackwell Scientific Publications, Oxford.
- Ames, R.N., Ingham, E.R. & Reid, C.P.P. (1982) Ultraviolet-induced autofluorescence of arbuscular mycorrhizal root infections: an alternative to clearing and staining methods for assessing infections. *Canadian Journal of Microbiology*, **28**, 351-355.
- Baker, S.C., Elek, J.A., Bashford, R., Paterson, S.C., Madden, J. & Battaglia, M. (2003) Inundative release of coccinellid beetles into eucalypt plantations for biological control of chrysomelid leaf beetles. *Agricultural and Forest Entomology*, **5**, 97-106.
- Bellei, M.D., Garbaye, J. & Gil, M. (1992) Mycorrhizal succession in young *Eucalyptus viminalis* plantations in Santa-Catarina (southern Brazil). *Forest Ecology and Management*, **54**, 205-213.
- Brown, A.G., Nambiar, E.K.S. & Cossalter, C. (1997) Plantations for the tropics - their role extent and nature. *Management of Soil, Nutrients and Water in Tropical Plantation Forests* (ed. by E.K.S. Nambiar and A.G. Brown), pp. 1-19. ACIAR, Canberra.
- Brundrett, M.C. (2000) What is the value of ectomycorrhizal inoculation for plantation-grown Eucalypts? *Mycorrhizal Fungi Biodiversity and Applications of Inoculation Technology* (ed. by M.Q. Gong, D. Xu, C. Zhong, Y.L. Chen, B. Dell and M.C. Brundrett), pp. 151-160. China Forestry Publishing House, Beijing.
- Brundrett, M.C., Bougher, N., Dell, B., Grove, T. & Malajczuk, N. (1996) *Working with Mycorrhizas in Forestry and Agriculture*. ACIAR, Canberra.
- Chen, Y.L., Brundrett, M.C. & Dell, B. (2000b) Effects of ectomycorrhizas and vesicular-arbuscular mycorrhizas, alone or in competition, on root colonization and growth of *Eucalyptus globulus* and *E. urophylla*. *New Phytologist*, **146**, 545-556.
- Chen, Y.L., Gong, M.Q., Wang, F., Chen, Y., Zhang, M., Dell, B. & Malajczuk, N. (2000a) Diversity of putative ectomycorrhizal fungi and arbuscular mycorrhizal fungi in *Eucalyptus* plantations in southern China. *Mycorrhizal Fungi Biodiversity and Applications of Inoculation Technology* (ed. by M.Q. Gong, D. Xu, C. Zhong, Y.L. Chen, B. Dell and M.C. Brundrett), pp. 21-28. China Forestry Publishing House, Beijing.

- Dell, B. (2000) Management of ectomycorrhizal eucalypts in the nursery. *Mycorrhizal Fungi Biodiversity and Applications of Inoculation Technology* (ed. by M.Q. Gong, D. Xu, C. Zhong, Y.L. Chen, B. Dell and M.C. Brundrett), pp. 137-141. China Forestry Publishing House, Beijing.
- Dell, B., Aggangan, N.S., Lu, X., Malajczuk, N., Pampolina, N. & Xu, D. (2000) Role of ectomycorrhizal fungi in eucalypt plantations. *Mycorrhizal Fungi Biodiversity and Applications of Inoculation Technology* (ed. by M.Q. Gong, D. Xu, C. Zhong, Y.L. Chen, B. Dell and M.C. Brundrett), pp. 161-167. China Forestry Publishing House, Beijing.
- Dell, B. & Malajczuk, N. (1994) Boron deficiency in eucalypt plantations in China. *Canadian Journal of Forest Research*, **24**, 2409-2416.
- Dell, B. & Malajczuk, N. (1995) Fertiliser requirements for ectomycorrhizal eucalypts in forest nurseries and field plantings in southern China. *Mycorrhizas for Plantation Forestry in Asia* (ed. by M.C. Brundrett, B. Dell, N. Malajczuk and M.Q. Gong), pp. 96-100. ACIAR Proceedings no. 62, Canberra.
- Dell, B., Malajczuk, N. & Grove, T.S. (1995) *Nutrient Disorders in Plantation Eucalypts*. ACIAR, Canberra.
- Dos Santos, V.L., Muchovej, R.M., Borges, A.C., Neves, J.C.L. & Kasuya, M.C.M. (2001) Vesicular-arbuscular-/ecto-mycorrhiza succession in seedlings of *Eucalyptus* spp. *Brazilian Journal of Microbiology*, **32**, 81-86.
- Dunstan, W.A., Malajczuk, N. & Dell, B. (1998) Effects of bacteria on mycorrhizal development and growth of container grown *Eucalyptus diversicolor* F. Muell. seedlings. *Plant and Soil*, **201**, 241-249.
- Gange, A.C. & Ayres, R.L. (1999) On the relation between arbuscular mycorrhizal colonization and plant 'benefit'. *Oikos*, **87**, 615-621.
- Gange, A.C., Bower, E. & Brown, V.K. (2002a) Differential effects of insect herbivory on arbuscular mycorrhizal colonization. *Oecologia*, **131**, 103-112.
- Gange, A.C., Bower, E., Stagg, P.G., Aplin, D.M., Gillam, A.E. & Bracken, M. (1999) A comparison of visualization techniques for recording arbuscular mycorrhizal colonization. *New Phytologist*, **142**, 123-132.
- Gange, A.C., Stagg, P.G. & Ward, L.K. (2002b). Arbuscular mycorrhizal fungi affect phytophagous insect specialism. *Ecology Letters*, **5**, 11-15.
- Gange, A.C. & Nice, H.E. (1997) Performance of the thistle gall fly, *Urophora cardui*, in relation to host plant nitrogen and mycorrhizal colonization. *New Phytologist*, **137**, 335-343.

- Gange, A.C. & West, H.M. (1994) Interactions between arbuscular-mycorrhizal fungi and foliar-feeding insects in *Plantago lanceolata* L. *New Phytologist*, **128**, 79-87.
- Gehring, C.A. & Whitham, T.G. (2002) Mycorrhizae-herbivore interactions: population and community consequences. *Mycorrhizal Ecology* (ed. by M.G.A. van der Heijden and I.R. Sanders), pp. 295-320. Springer-Verlag, Berlin.
- Goverde, M., van der Heijden, M.G.A., Wiemken, A., Sanders, I.R. & Erhardt, A. (2000) Arbuscular mycorrhizal fungi influence life history traits of a lepidopteran herbivore. *Oecologia*, **125**, 362-369.
- Jones, C.G. & Last, F.T. (1991) Ectomycorrhizae and trees: implications for aboveground herbivory. *Microbial Mediation of Plant-Herbivore Interactions* (ed. by P. Barbosa, V.A. Krischik and C.G. Jones), pp. 65-103. John Wiley & Sons Ltd, Chichester.
- Jones, M.D., Durall, D.M. & Tinker, P.B. (1998) A comparison of arbuscular and ectomycorrhizal *Eucalyptus coccifera*: growth response, phosphorus uptake efficiency and external hyphal production. *New Phytologist*, **140**, 125-134.
- Lapeyrie, F., Garbaye, J., de Oliveira, V. & Bellei, M.D. (1992) Controlled mycorrhization of Eucalypts. *Mycorrhizas in Ecosystems* (ed. by D.J. Read, D.H. Lewis, A.H. Fitter and I.J. Alexander), pp. 293-299, CAB International, Wallingford.
- Lodge, D.J. (2000) Ecto- or arbuscular mycorrhizas - which are best? *New Phytologist*, **146**, 353-354.
- Lu, X., Malajczuk, N. & Dell, B. (1998) Mycorrhiza formation and growth of *Eucalyptus globulus* seedlings inoculated with spores of various ectomycorrhizal fungi. *Mycorrhiza*, **8**, 81-96.
- McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L. & Swan, J.A. (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist*, **115**, 495-501.
- Ortas, I., Ortakci, D. & Kaya, Z. (2002) Various mycorrhizal fungi propagated on different hosts have different effects on citrus growth and nutrient uptake. *Communications in Soil Science and Plant Analysis*, **33**, 259-272.
- Rieske, L.K. (2001) Influence of symbiotic fungal colonization on oak seedling growth and suitability for insect herbivory. *Environmental Entomology*, **30**, 849-854.
- Sanders, I.R. (2002) Specificity in the arbuscular mycorrhizal symbiosis. *Mycorrhizal Ecology* (ed. by M.G.A. van der Heijden and I.R. Sanders), pp. 415-437. Springer-Verlag, Berlin.



- Schoonhoven, L.M., Jermy, T. & van Loon, J.J.A. (1998) *Insect-Plant Biology - From Physiology to Evolution*. Chapman & Hall, London.
- Simes, R.J. (1986) An improved Bonferroni procedure for multiple tests of significance. *Biometrika*, **73**, 751-754.
- Smith, S.E. & Read, D.J. (1997) *Mycorrhizal Symbiosis*. Academic Press, San Diego.
- Xu, D., Bai, J. & Dell, B. (2000) Overcoming the constraints to productivity of plantation Eucalypts in southern China. *Mycorrhizal Fungi Biodiversity and Applications of Inoculation Technology* (ed. by M.Q. Gong, D. Xu, C. Zhong, Y.L. Chen, B. Dell and M.C. Brundrett), pp. 93-100. China Forestry Publishing House, Beijing.
- Xu, D., Dell, B., Malajczuk, N. & Gong, M.Q. (2001) Effects of P fertilisation and ectomycorrhizal fungal inoculation on early growth of eucalypt plantations in southern China. *Plant and Soil*, **233**, 47-57.
- Zar, J.H. (1996) *Biostatistical Analysis*. Prentice-Hall International, Upper Saddle River, New Jersey.
- Zhengong, P. (2003) Insect Pests of Eucalypts in China. *Eucalypts in Asia* (ed. by J.W. Turnbull), pp. 183-184, ACIAR, Canberra.
- Zhong, C., Gong, M.Q., Chen, Y., Wang, F., Malajczuk, N., Bougher, N., Grove, T. & Dell, B. (2000) Inoculation of mycorrhizal fungi for *Eucalyptus* plantations in southern China. *Mycorrhizal Fungi Biodiversity and Applications of Inoculation Technology* (ed. by M.Q. Gong, D. Xu, C. Zhong, Y.L. Chen, B. Dell and M.C. Brundrett), pp. 172-175. China Forestry Publishing House, Beijing.
- Zhou, G. (1995) Influence of tropical forest changes on environmental quality in Hainan province, P.R. China. *Ecological Engineering*, **4**, 223-229.

## Figure legends

**Figure 1** Colonization levels of *E. urophylla* by arbuscular mycorrhizal (AM) fungi and ectomycorrhizal (ECM) fungi in nursery and field grown trees. Key to treatments: Control: no mycorrhizal addition; AM: inoculation with the AM fungus *Glomus caledonium*; ECM: inoculation with the ECM fungus *Laccaria laccata*. Bars represent means  $\pm$  one standard error.

**Figure 2** Effects of dual mycorrhizal colonization on growth of nursery trees. (a) mean height, (b) Mean leaf number, (c) Mean leaf area and (d) mean final total dry biomass. Key to legend: Co: no mycorrhiza (control); AM: arbuscular mycorrhizal inoculation; ECM: ectomycorrhizal inoculation. Standard error bars omitted from time graphs for clarity.

**Figure 3** Effects of dual mycorrhizal colonization on growth trees in the field. (a) mean height, (b) Mean leaf number, (c) Mean leaf area and (d) mean final total dry biomass. Key to legend as in Figure 2.

**Figure 4** Effects of dual mycorrhizal colonization on insect herbivore attack on nursery grown trees. (a) chewing by Geometrid larvae, (b) chewing by *Anomala cupripes* adults, (c) leaf folding by *Strepsicrates* larvae. Key to legend as in Figure 2.

**Figure 5** Effects of dual mycorrhizal colonization on insect herbivore attack on trees grown in the field. (a) chewing by Geometrid larvae, (b) chewing by adults of *Anomala cupripes* (Coleoptera), (c) leaf folding by *Strepsicrates* spp. (Lepidoptera). Key to legend as in Figure 2.

**Figure 6** Effects of dual mycorrhizal colonization on P and N contents of foliage from field grown trees. Key to legend as in Figure 1. Bars represent means  $\pm$  one standard error.

Figure 1

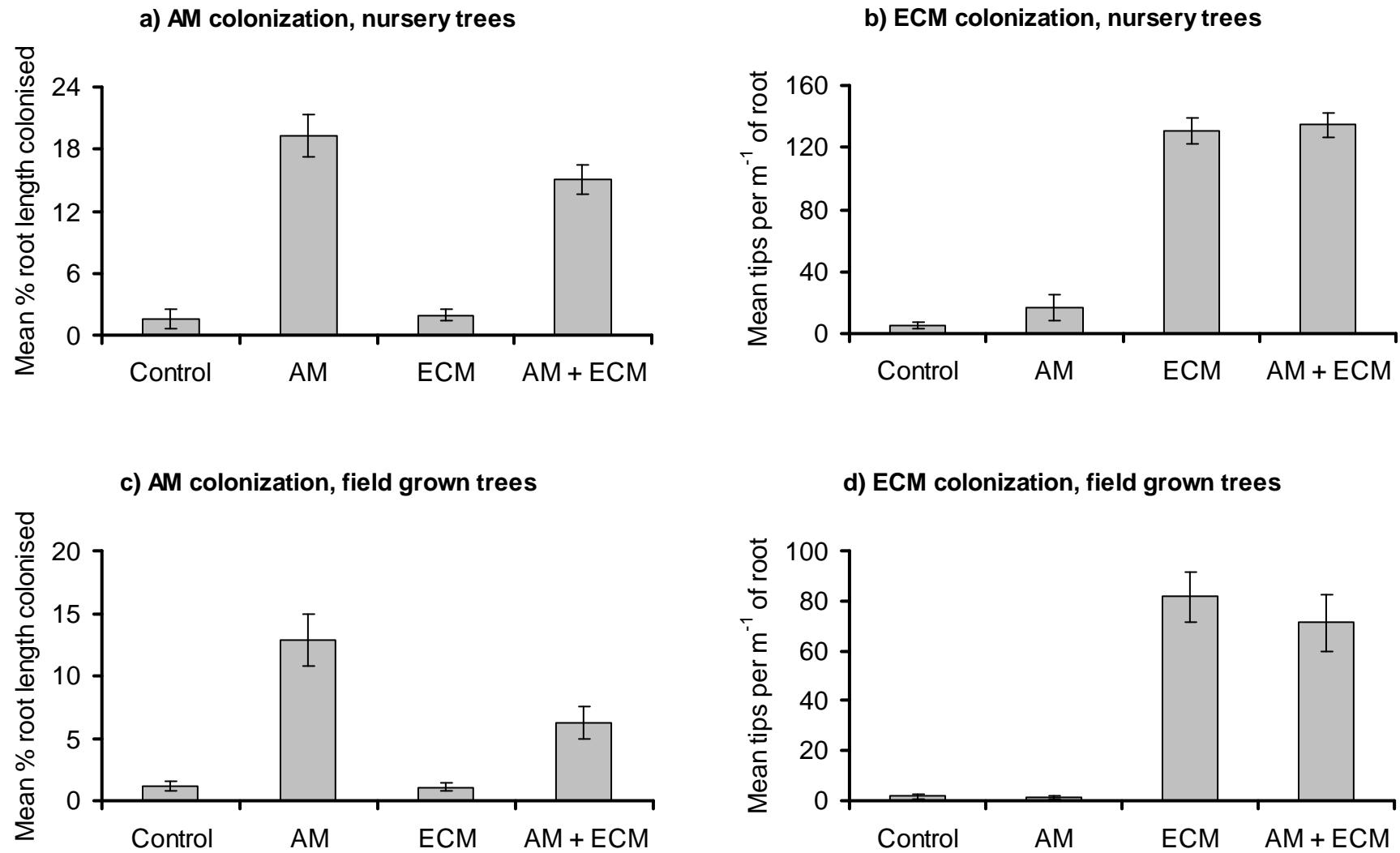


Figure 2

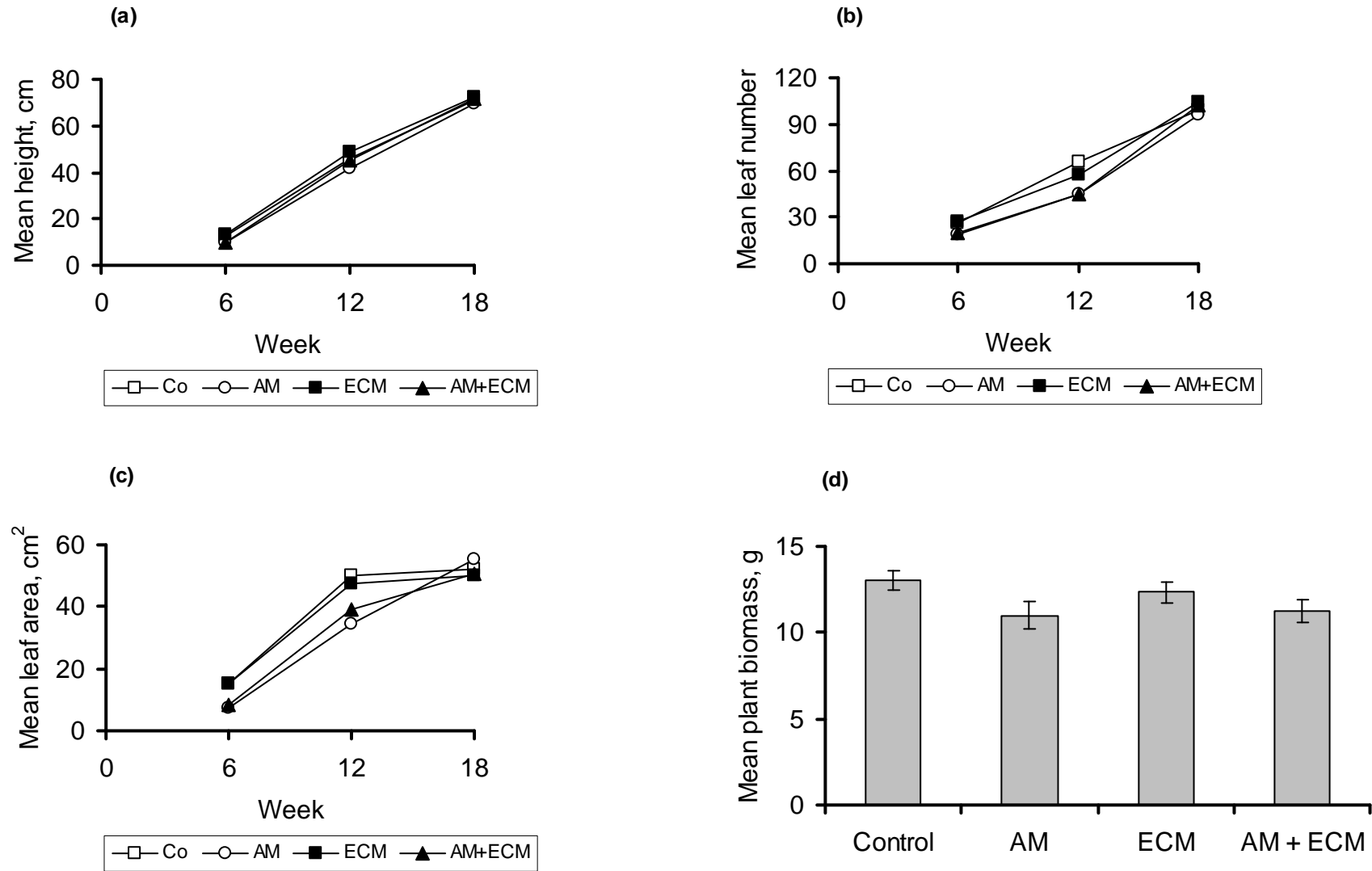


Figure 3

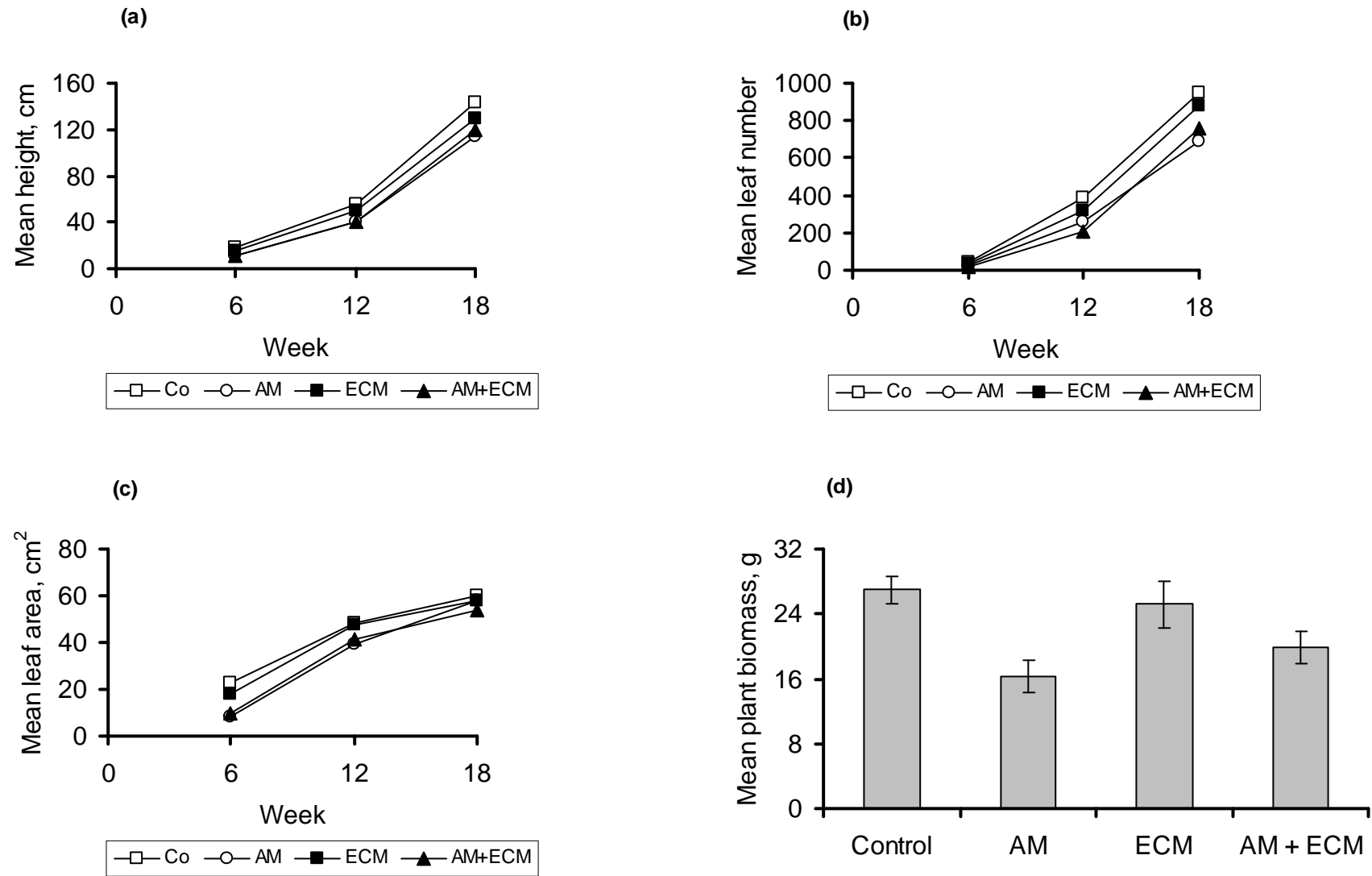


Figure 4

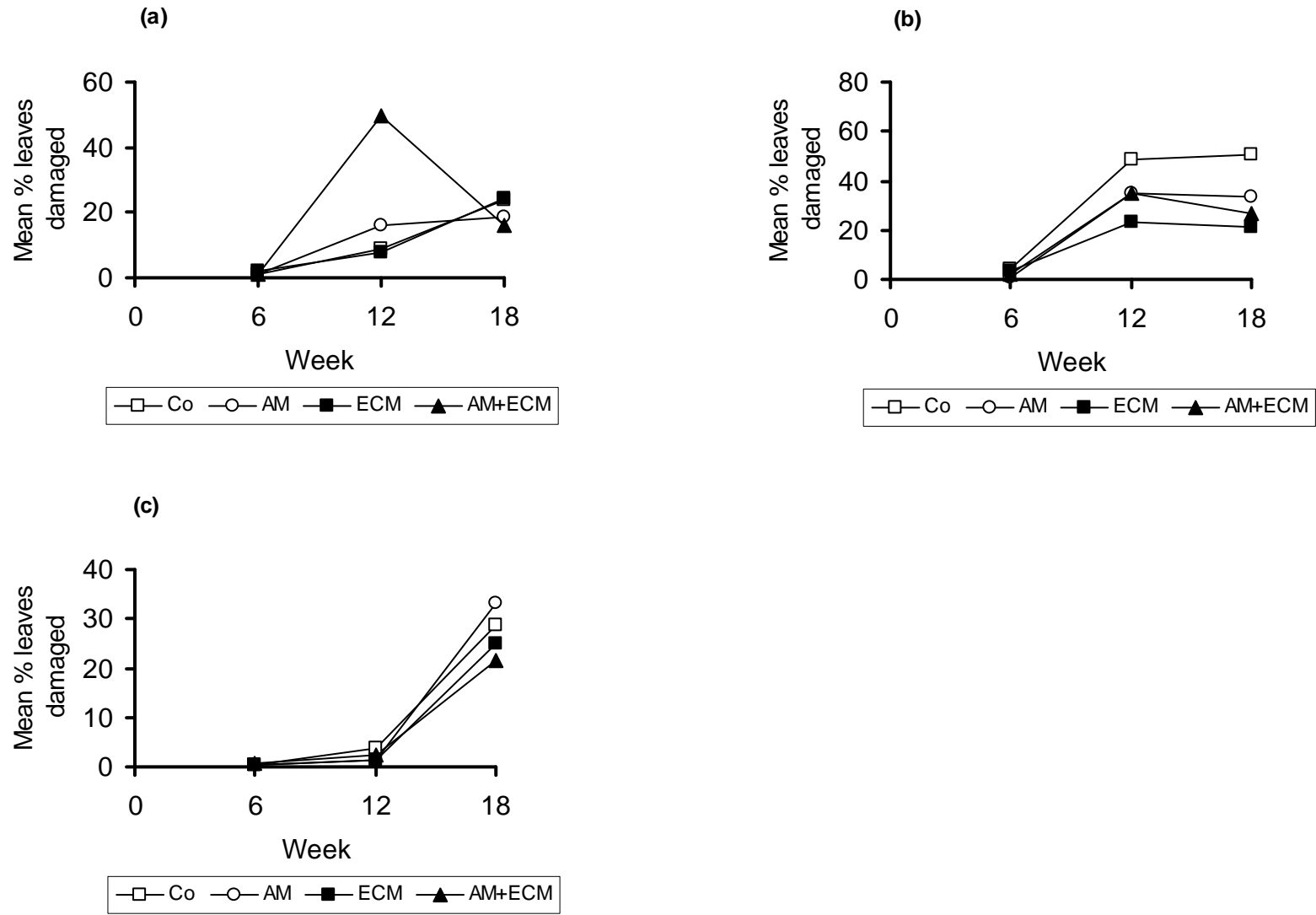


Figure 5

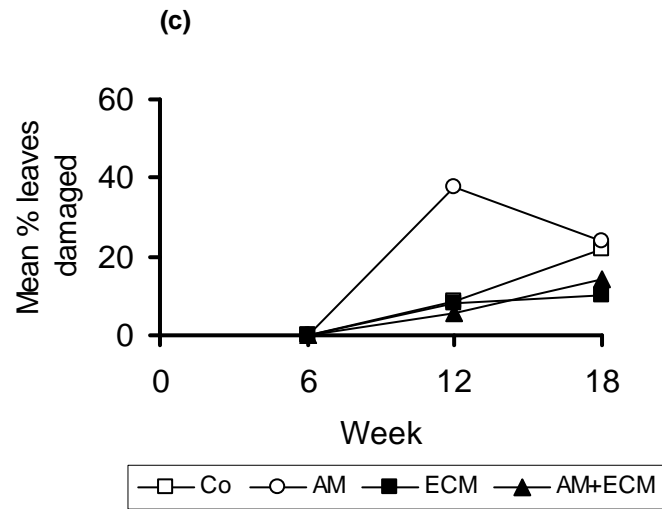
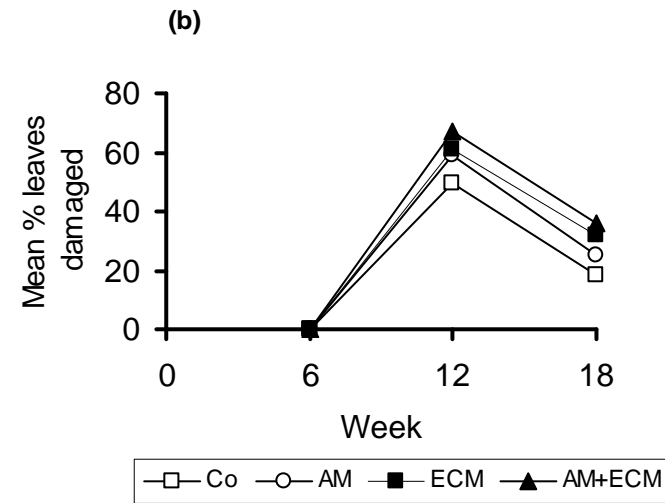
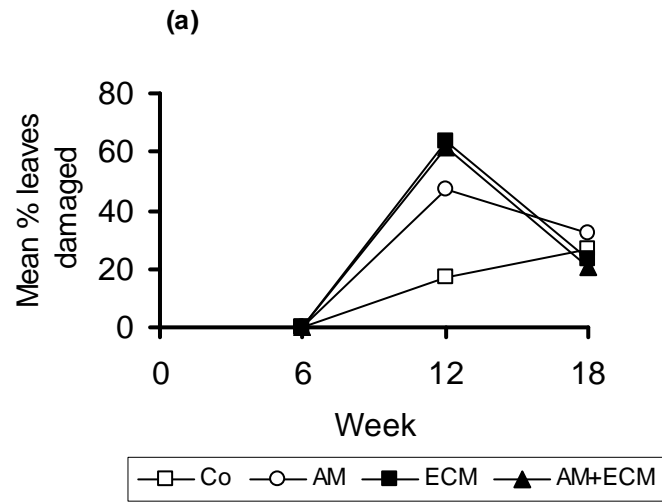


Figure 6

