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Arbuscular mycorrhizal fungi alter plant allometry and biomass–density relationships

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• *Background and Aims* Plant biomass–density relationships during self-thinning are determined mainly by allometry. Both allometry and biomass–density relationship have been shown to vary with abiotic conditions, but the effects of biotic interactions have not been investigated. Arbuscular mycorrhizal fungi (AMF) can promote plant growth and affect plant form. Here experiments were carried out to test whether AMF affect plant allometry and the self-thinning trajectory.
• *Methods* Two experiments were conducted on *Medicago sativa* L., a leguminous species known to be highly dependent on mycorrhiza. Two mycorrhizal levels were obtained by applying benomyl (low AMF) or not (high AMF). Experiment 1 investigated the effects of AMF on plant growth in the absence of competition. Experiment 2 was a factorial design with two mycorrhizal levels and two plant densities (6000 and 17 500 seeds m^{-2}). Shoot biomass, root biomass and canopy radius were measured 30, 60, 90 and 120 d after sowing. The allometric relationships among these aspects of size were estimated by standardized major axis regression on log-transformed data.
• *Key Results* Shoot biomass in the absence of competition was lower under low AMF treatment. In self-thinning populations, the slope of the log (mean shoot biomass) vs. log density relationship was significantly steeper for the high AMF treatment (slope = −1·480) than for the low AMF treatment (−1·133). The canopy radius–biomass allometric exponents were not significantly affected by AMF level, but the root–shoot allometric exponent was higher in the low AMF treatment. With a high level of AMF, the biomass–density exponent can be predicted from the above-ground allometric model of self-thinning, while this was not the case when AMF were reduced by fungicide.
• *Conclusions* AMF affected the importance of below-ground relative to above-ground interactions and changed root vs. shoot allocation. This changed allometric allocation of biomass and altered the self-thinning trajectory.

Key words: Arbuscular mycorrhizal fungi, biomass–density relationship, canopy radius–biomass allometry, root–shoot biomass allometry, *Medicago sativa*, self-thinning.

INTRODUCTION

As a crowded stand of plants develops, some individuals die, while others grow, a phenomenon called density-dependent mortality or 'self-thinning'. The quantitative relationship between the number of survivors and their mass can be modelled with the allometric equation: $M = kN^{\gamma}$, where M is mean survivor biomass, N is density, and γ and k are parameters (Yoda *et al.*, 1963), referred to as the allometric exponent and the allometric coefficient, respectively. This relationship is usually plotted and fit to data in its log–log form $\log M = K + \gamma \log N$, where $K = \log k$.

The exponent of the biomass–density relationship, γ, was for many years considered to be a universal constant, even though there is evidence that it varies among species (Weller, 1987*a*). This variation may help to explain why there is no agreement about the value of this exponent among those who claim it is a universal constant. A simple geometric model predicts that $\gamma = -3/2$ (Yoda *et al.*, 1963), while the proponents of metabolic theory predicted it to be −4/3 (Hutchings, 1983; Enquist *et al.*, 1998). Since plant growth is allometric, not isometric (Weller, 1987*b*; Weiner and Thomas, 1992), researchers have constructed allometric models to explain variation in the self-thinning exponent (Miyanishi *et al.*, 1979; Weller, 1987*b*). Miyanishi *et al.* (1979) suggested that the biomass–density exponent depends on the allometry between plant biomass and canopy dimensions such as height or radius. This can be expressed mathematically by setting the canopy radius proportional to M^{Φ}, where Φ can be variant to reflect allometric growth, and the thinning exponent $\gamma = -1/(2\Phi)$, and γ equals $-3/2$ only if shape is truly invariant (isometric growth, $\Phi = 1/3$).

The allometric theory of self-thinning developed from shoot biomass and canopy dimensions assumes that above-ground interactions drive self-thinning (Weller, 1987*b*). Whether below-ground interactions affect self-thinning through allometry is not well known because we do not have equivalent relationships between root biomass and root 'canopies' (soil volume occupied), because of the difficulties of such measurements (McPhee and Aarssen, 2001; Morris, 2003). The effects of crowding on the allometric relationship between shoot and root biomass are not known, and may provide useful

information, even for a theory of self-thinning based on above-ground allometry.

The slope of biomass–density relationships can be affected by abiotic environmental conditions such as soil fertility (Morris, 2002) and water availability (Deng *et al.*, 2006). In most studies on self-thinning, mortality occurs only after the plant stand reaches 100 % cover, and this 100 % cover is maintained during the process of self-thinning. However, this does not appear always to be the case in extreme environments (e.g. arid and infertile areas), where an open canopy is commonly observed even when plant biomass is at its carrying capacity (Fowler, 1986; Deng *et al.*, 2006). Under arid or infertile conditions, plants may allocate more biomass to below-ground structures to acquire limiting resources, and this leads to a lower absolute value of γ (Morris, 1991, 1996; Deng *et al.*, 2006). Thus, root–shoot biomass relationship can alter biomass–density relationships, especially under environmental stress (Morris, 2002).

Biotic interactions can affect plant performance (Smith and Read, 1997), but their effects on allometric patterns have not been well studied. Here we focus on arbuscular mycorrhizal fungi (AMF) and ask whether AMF can alter plant allometry and biomass–density relationships. Arbuscular mycorrhizal fungi form symbiotic associations with roots of most terrestrial plants. In exchange for energy in the form of reduced carbon from plant hosts, these fungi can facilitate plant uptake and transport of phosphorus and other relatively immobile soil nutrients, promote plant growth, enhance drought tolerance and reduce pathogenic infections. Arbuscular mycorrhizal fungi can also transport nutrient between plants through a common hyphal network, and mediate plant interactions (Smith and Read, 1997).

By increasing nutrient acquisition, AMF symbionts have been shown to promote plant performance and influence aspects of plant form such as biomass, height, canopy radius and allocation to above-ground vs. below-ground structures (Shumway and Koide, 1995; Smith and Read, 1997). Simply put, plant allometry will be changed if AMF promotes the growth of different parts (e.g. roots vs. shoots) at different rates. In a previous study with benomyl application to create lower mycorrhizal colonization, it was found that higher mycorrhizal colonization increased canopy extension for a given biomass in self-thinning populations of *Medicago sativa* L., and decreased the root:shoot ratio (Xu, 2010). Here, the results of experiments testing whether the variations in plant form were related to different radius–biomass allometry and root–shoot biomass relationship, and whether the effects of AMF on these allometric relationships alter the biomass–density trajectory during self-thinning, are reported.

MATERIALS AND METHODS

Soil and plant materials

The soil for the experiment was collected in an agricultural field at 30·12°N,120·66°E in Shangyu City, Zhejiang Province, south eastern China. The soil had a pH of 8·54 and contained 4·85 g kg^{-1} organic matter, 23·41 mg kg^{-1} extractable P, 0·84 g kg^{-1} total N and 231·1 mg kg^{-1} extractable K.

Medicago sativa L., a perennial legume, was used in this study. *Medicago sativa* requires phosphorus fertilization to grow well (Berg *et al.*, 2005; Suriyagoda *et al.*, 2010), and is highly dependent on AMF (Azcón *et al.*, 1991; Xu, 2010). Seeds of *M. sativa* L. were supplied by the Zhejiang Forestry Academy and were stored at 4 °C for 1 week before sowing.

Experiments

Experiment 1: effects of AMF on the growth of individual plants. This experiment was conducted with two AMF levels to assess the effect of AMF on the growth of individual plant of *M. sativa* in the absence of competition. The two AMF levels were 'low AMF', obtained by applying a fungicide that suppresses the AMF naturally present in soil, and 'high AMF' (no fungicide). There were four replicates for each treatment.

Mesocosm-sized containers (47·5 cm length × 34·5 cm width × 15·4 cm height) were filled with 20 kg of soil from the field. For the low AMF treatment, the fungicide benomyl (2 g dissolved in 2 L of tap water) was applied to the soil monthly to suppress AMF (Helgason *et al.*, 2007). In the remaining high AMF mesocosms, the same amount of tap water without fungicide was added. Ten seeds, which were surface sterilized with 1 % NaClO and distilled water, were sown in each mesocosm. Five days after germination, seedlings were thinned manually to one seedling per mesocosm. The mesocosms were arranged in a greenhouse under natural light and temperature conditions with an average air temperature of 18–30 °C during the course of the experiment from April to August. Plants were watered daily to keep soil moisture at 70–90 % of water-holding capacity. No additional nutrients were given during the experiment.

Sampling was carried out 120 d after sowing. Shoots were separated from roots and were oven dried at 80 °C for 48 h and then weighed. Root colonization by AMF was tested by the grid-line intersection method modified from Giovannetti and Mosse (1980). Briefly, roots were cleaned in 10 % KOH (w/v) and stained in acid fuchsin, and then tested for the presence or absence of mycorrhizal infection (arbuscles, vesicles, coils or hyphae) under a compound microscope at ×200 magnification. The AMF colonization level was calculated as: AMF colonization (%) = no. of intersections colonized (hyphae, arbuscules, vesicles and hyphal coils)/total no. of intersections examined ×100 %.

A *t*-test was performed on shoot biomass and AMF colonization in SPSS V.10·0 with AMF level as the factor.

Experiment 2: effects of AMF on plant allometry and biomass–density relationship. This experiment was a 2 × 2 factorial design with two AMF levels as in expt 1, and two plant sowing densities: 6000 and 17,500 seeds m^{-2}. There were four replicates for each treatment.

The containers, quantity of soil and surface sterilization of seeds were the same as in expt 1. The seeds were mixed with sand and sown with a sieve to achieve a random spatial pattern. The two AMF treatments, the arrangement of the containers and the watering regimes were as in expt 1. The experiment ran from April to August.

Sampling took place 30, 60, 90 and 120 d after sowing. To avoid the first sampling affecting the later ones, each mesocosm

was divided into four 23·7 × 17·2 cm areas. At each sampling one of the four areas was randomly chosen and a square sample area was located in its centre (Shumway and Koide, 1995). There was at least 5 cm between sampling areas. The square area was increased over the course of the experiment as plants grew (25, 36, 100 and 100 cm^2 at 30, 60, 90 and 120 d after sowing, respectively) as described by Shumway and Koide (1995). All shoots within the area were cut at the soil surface and the plant number was counted. For root system sampling, a steel square of the same size as the sample for the shoot was inserted vertically into the soil and all roots within the square were collected. The collected roots were rinsed with tap water. Shoots and roots were oven dried at 65 °C for 48 h and weighed. Mean biomass of shoot and root was calculated as (total biomass)/(no. of individuals). A random sub-sample of 12 individuals was chosen from each mesocosm to measure the plant canopy radius (Morris, 1996), and the maximum diameter of the canopy for each individual plant was recorded. The radius was defined as half the diameter.

At the final sampling, AMF colonization of roots was quantified using the grid-line intersection method as in expt 1.

The allometric exponents or slopes and the intercepts were estimated by the standardized major axis (SMA; SMATR Version 2·0; Warton *et al.*, 2006) regression on log-transformed data. Comparisons of slopes of biomass–density relationship, canopy radius–shoot biomass relationship and root–shoot relationship between the two AMF levels were performed in SMATR. Pre-thinning vs. thinning data points were separated according to the criteria of Morris (1996), and only the data from self-thinning populations were included in the analyses (Morris, 2002). There were 24 'high AMF' data points and 20 'low AMF' data points in the analyses.

The slope of biomass–density relationship was predicted by the allometric model of self-thinning [$\gamma = -1/(2\Phi)$] (Miyanishi *et al.*, 1979), where γ is the biomass–density exponent and Φ is the canopy radius–shoot biomass exponent that was estimated from measurement in this experiment.

Two-way analysis of variance (ANOVA) was performed by SPSS (V.10·0) on AMF colonization, with AMF level and plant density as the two factors. Normality tests and a homogeneity test were performed before ANOVA. The least significant difference (l.s.d.) at the 5 % confidence level was used for comparisons.

RESULTS

Effects of benomyl on mycorrhizal colonization and plant growth in expt 1

Benomyl application significantly suppressed mycorrhizal colonization when *M. sativa* plants were grown individually ($t = 15·23$, $P < 0·001$; Fig. 1). Mycorrhizal colonization was reduced about 81 % by benomyl (Fig. 1A). Benomyl application also decreased shoot biomass about 27 % ($t = 3·16$, $P = 0·019$; Fig. 1B).

Effect of benomyl on mycorrhizal colonization in expt 2

Benomyl application decreased the AMF colonization ratio by about 85 % ($F = 463·22$, $P < 0·001$; Fig. 2). In the absence of fungicide, the AMF colonization rate was lower at high plant density ($F = 10·028$, $P < 0·01$; Fig. 2), but there was no effect of plant density with fungicide, so there was an interaction between benomyl application and plant density ($F = 11·07$, $P < 0·01$).

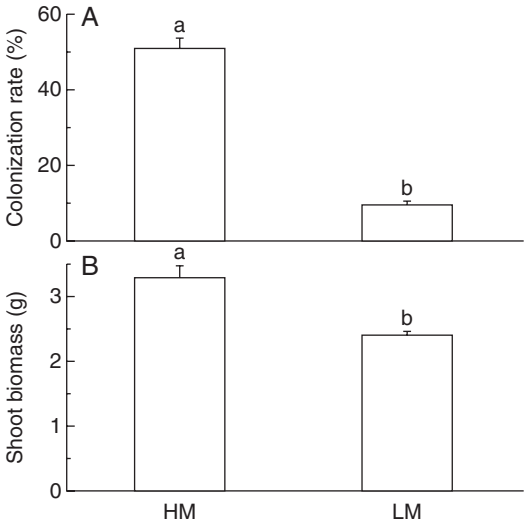

FIG. 1. Arbuscular mycorrhizal fungal colonization and shoot biomass of *Medicago sativa* at two AMF levels.

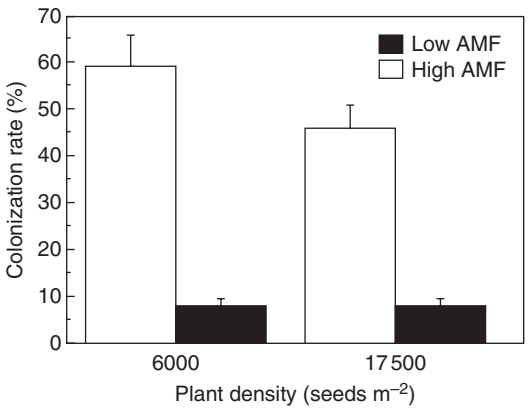

FIG. 2. Arbuscular mycorrhizal fungal colonization of *Medicago sativa* under different treatments. 'Low AMF' (treated with fungicide) and 'high AMF' (untreated), as indicated.

Canopy radius–biomass allometry in expt 2

The position of the fit log (canopy radius)–log (shoot biomass) line was higher under high AMF (Fig. 3, Table 1), while the slopes (Φ) of log (canopy radius)–log (shoot biomass) were not significantly different between the two AMF level treatments (test statistic = 0·361, $P = 0·543$). For a given shoot biomass, high AMF populations had larger canopies than low AMF populations (Fig. 3).

Root–shoot biomass allometry in expt 2

Plant biomass allocation to shoot and root was altered by reduced AMF (test statistic = 11·782, $P = 0·001$; Fig. 4,

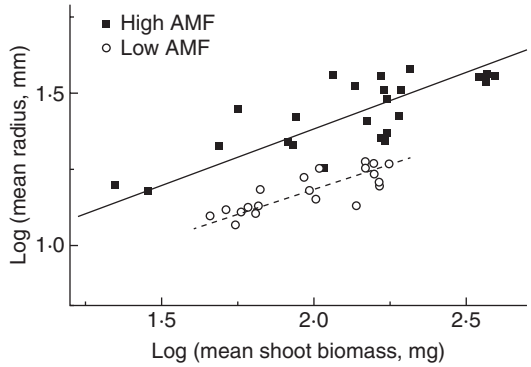

FIG. 3. Log (mean canopy radius)–log (mean shoot mass) relationships for *Medicago sativa* grown under 'high AMF' (no fungicide) and 'low AMF' (fungicide) treatments, as indicated. Data shown here are for populations undergoing self-thinning. Parameters fit by the standardized major axis are given in Table 2.

TABLE 1. *Regression parameter estimates (standardized major axis regression) of log (canopy radius) on log (shoot biomass) in populations of* Medicago sativa *L. undergoing self-thinning under 'high AMF' (no fungicide) and 'low AMF' (fungicide) treatments.*

AMF treatment	*n*	Intercept	Slope	95 % CI of the slope	r^2
'High AMF'	24	0·639	0·373	(0·282–0·493)	0·589
'Low AMF'	20	0·520	0·3315	(0·2494–0·4405)	0·661

TABLE 2. *Regression parameter estimates (standardized major axis regression) of log (root biomass) on log (shoot biomass) in populations of* Medicago sativa *L. undergoing self-thinning under 'high AMF' (no fungicide) and 'low AMF' (fungicide) treatments.*

AMF treatment	*n*	Intercept	Slope	95 % CI of the slope	r^2
'High AMF'	24	0·5057	0·7790	(0·6635–0·9146)	0·867
'Low AMF'	20	−0·0203	1·1648	(0·9963–1·3618)	0·900

Table 2). In the high AMF treatment, plants allocated less biomass to roots, and the allometric relationship between root and shoot biomass lies below that of the low AMF treatment (Fig. 4). The slope of the log (root biomass)–log (shoot biomass) relationship was higher when fungicide reduced AMF ($P = 0·001$). In the high AMF treatment, plants allocated less biomass to roots than in the low AMF treatment (i.e. the fitted line lay below that of the low AMF treatment) and, as they grew, they allocated progressively less biomass to root (slope of log root vs. log shoot relationship significantly <1; Table 3). In the low AMF treatment, plants allocated more biomass to root growth, and this remained constant with growth (slope not significantly different from 1; Table 2).

Biomass–density relationship in expt 2

The biomass–density trajectory was significantly different between the two AMF level treatments (test statistic = 4·914, $P = 0·035$; Fig. 5, Table 3). The slope (γ) of the log

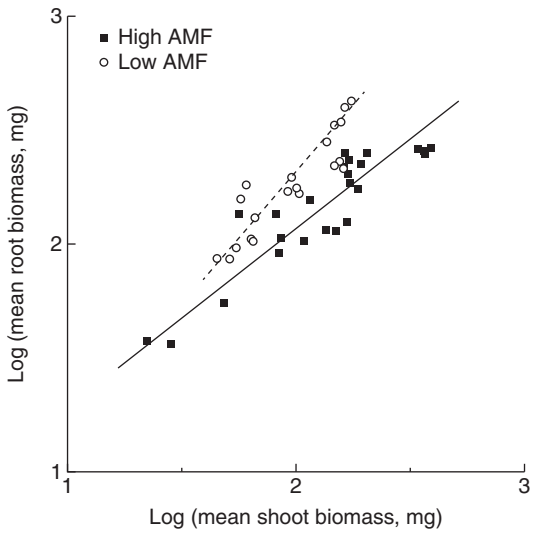

FIG. 4. Log (mean root biomass)–log (mean shoot mass) relationships for *Medicago sativa* grown under 'high AMF' (no fungicide) and 'low AMF' (fungicide) treatments, as indicated. Data shown here are for populations undergoing self-thinning. Parameter estimates are given in Table 3.

TABLE 3. *Regression parameter estimates (standardized major axis regression) of log (shoot biomass) on log density in populations of* Medicago sativa *L. undergoing self-thinning under 'high AMF' (no fungicide) and 'low AMF' (fungicide) treatments.*

AMF treatment	*n*	Intercept	Slope	95 % CI of the slope	r^2
'High AMF'	24	4·427	−1·480	(−1·746 to −1·255)	0·859
'Low AMF'	20	3·206	−1·133	(−1·349 to −0·951)	0·874

biomass–log density relationships was steeper in the high AMF treatment ($P = 0·035$; Table 3). The slope in the low AMF treatment was statistically different from $-3/2$, as this value was outside the 95 % confidence interval (CI) (Table 3).

In the high AMF treatment, the exponent of the shoot biomass vs. density (γ) relationship treatment estimated directly from the measurements on individual plants (Fig. 5, Table 3) was −1·480 (CI −1·746 to −1·255). The same exponent estimated indirectly from the allometric model of self-thinning [$\gamma = -1/(2\Phi)$] was −1·34, which is within the CI of the direct estimate. In the low AMF treatment, these two exponents were not close: the direct estimated γ was −1·133 (CI −1·349 to −0·951) (Fig. 5, Table. 3), while the indirect estimated γ was −1·51, which is outside the CIs of the direct estimate.

DISCUSSION

Effects of fungicide

Benomyl application can suppress AMF colonization (Pimienta-Barrios *et al.*, 2003; Xu, 2010). Some experiments reported that pathogenic fungi (Callaway *et al.*, 2004) and other soil organisms such as root-feeding nematodes

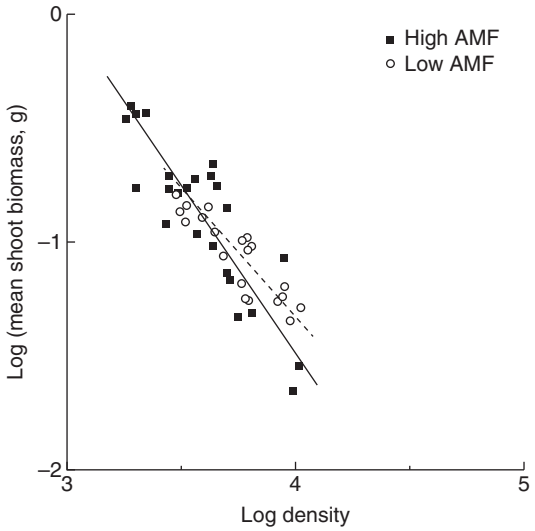

FIG. 5. Log (shoot biomass)–log (density) relationships for *Medicago sativa* populations grown under 'high AMF' (no fungicide) and 'low AMF' (fungicide) treatments, as indicated. Data are for populations undergoing self-thinning. Parameters of self-thinning lines fit by the standardized major axis are given in Table 1.

(van der Putten *et al.*, 1990) can also be affected by benomyl, while other experiments showed that benomyl application has little or no effect on the non-mycorrhizal plant and bacterial community (Hetrick *et al.*, 1986; Daleo *et al.*, 2008). If benomyl affects pathogenic more than mycorrhizal fungi, plant growth should be promoted, not suppressed (Harnett and Wilson, 1999). High AMF colonization may or may not result in a high symbiotic function because the interaction can fall along a continuum of mutualism to parasitism (Reynolds *et al.*, 2006). In our expt 1, when *M. sativa* plants were grown individually, benomyl application decreased mycorrhizal colonization and led to a lower biomass of individual plants, implying that the primary effects of benomyl are due to the suppressed AMF colonization.

AMF, allometry and the biomass–density relationship

Our results showed that the position of the canopy radius–shoot biomass line was lower (Fig. 3, Table 1), but the root–shoot position was higher in the low AMF treatment (Fig. 4, Table 2). These results suggested that the reduced AMF due to benomyl can change plant allometry. Plants develop allometric patterns in response to environmental constraints through resource allocation to maximize the uptake of limiting resources (Weiner, 2004). In the present study, the root–shoot allometric exponent was higher in the low AMF treatment (Fig. 4, Table 2). This is consistent with the patterns of self-thinning at low fertility (Morris, 2002) or low water availability (Deng *et al.*, 2006). Under low nutrient or water conditions, root competition may become more important than shoot competition. The effects of high mycorrhiza are similar to the effects of high soil nutrient levels, because mycorrhiza can extend their hyphae into the soil, giving access to more nutrients. In the low AMF treatment, plant nutrient acquisition may decrease. Thus, to expand nutrient acquisition, plants allocate more biomass to the root: as plants grow larger, they will have more root growth for a given amount of shoot growth.

It was found that the self-thinning trajectory was less steep in the low AMF treatment. Biomass–density relationships during the course of self-thinning are usually explained by competition for limited resources (Yoda *et al.*, 1963; Watkinson, 1980), which can be partitioned into shoot competition (for light) and root competition (for water and/or soil nutrients; Morris, 2002). According to the allometric model of self-thinning proposed by Miyanishi *et al.* (1979), the biomass–density exponent (γ) is related to the canopy radius–shoot biomass exponent (Φ) as $\gamma = -1/(2\Phi)$ when self-thinning is driven by shoot competition. It has been argued that root interactions may be driving self-thinning when there is no relationship between the canopy radius–biomass exponent and the biomass–density exponent (Morris, 2002). In the present study, the exponent of the shoot biomass vs. density (γ) relationship estimated directly from the measurements on individual plants was close to the exponent (γ) estimated indirectly from the allometric model of self-thinning [$\gamma = -1/(2\Phi)$] in the high AMF treatment, but these two estimates were not close in the low AMF treatment. These results imply that self-thinning is not driven solely by shoot competition under low AMF conditions, but that root competition also plays a role.

The slopes of the canopy radius vs. shoot biomass relationships for the two AMF were not significantly different in the present study. The allometric theory of self-thinning predicts that the self-thinning lines should also be parallel, with the plants with a lower canopy radius for a given shoot biomass having a self-thinning line with higher shoot biomass for a given thinning density (higher self-thinning line). This is what was initially observed, as self-thinning commenced at $n = 10^4$ plants m^{-2} in this study (Fig. 5). However, as thinning proceeded, there was more mortality per unit increment of biomass for plants in the low AMF treatment, giving a flatter slope. Thus while the packing of canopies observed for the high AMF plant populations matched the self-thinning line, in general agreement with the allometric self-thinning model, this was not the case for the low AMF plants, for which more plants died per increment of biomass than in the high AMF group. In the experiment of Morris (2003), when the nutrient supply was reduced, canopy packing models did not appear to be consistent with the thinning patterns observed. The hypothesis that root interactions can contribute to, or even drive self-thinning is an obvious alternative explanation. It may well be that the packing arguments developed for plant canopies apply to below-ground root 'canopies' as well; it is technically difficult if not impossible to estimate parameters for root extension in self-thinning stands, to test such models (Morris, 2003).

Arbuscular mycorrhizal fungi increased branching numbers of plants but did not alter the biomass–density relationship in *Abutilon theophrasti* (Shumway and Koide, 1995). Higher fertility leads to a lower survival rate and larger biomass in self-thinning populations of *Ocimum basilicum* (Morris, 2002). In our study, AMF altered not only the slope of the mass–density relationship, but also the speed at which self-thinning occurs. In the high AMF

treatment, there was more thinning, resulting in a lower final density and higher final mean biomass compared with the low AMF treatment (Fig. 5). The higher end point of the mass–density lines in the high AMF treatment was probably due to AMF-induced enhancement of shoot competition. 'High AMF' increased shoot branches, biomass and canopy radius (Supplementary Data Tables S1 and S2, available online). These enhancements of individual plant growth led to a greater overlap of individual canopies, increasing the intensity of competition among individual plants within the population and thus promoting self-thinning (Weiner *et al.*, 2001; Weiner and Damgaard, 2006).

Concluding remarks

Reduced AMF due to benomyl can increase allocation of biomass to roots for a given biomass, resulting in changes in allometric relationships between the canopy radius and shoot biomass, and between the root and shoot biomass. These altered allometric relationships led to a less steep log biomass–log density relationship when there was much less AMF. The exponent of the shoot biomass vs. density (γ) estimated indirectly from the allometric model of self-thinning was outside the CI of the direct estimate under low AMF, suggesting that self-thinning was influenced by roots. It is concluded that AMF levels can change plant allometry and alter the self-thinning trajectory. Roots become important for the self-thinning trajectory when AMF are suppressed.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following tables. Table S1: Mean shoot biomass, root biomass, canopy radius, branches per plant and leaf number per plant for *Medicago sativa* at 120 d after sowing. Table S2: Two-way ANOVA of the effect of plant density and AMF level on shoot biomass, root biomass, canopy radius, branches per plant and leaf number per plant for *M. sativa* at 120 d after sowing.

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LITERATURE CITED

Azcón R, Rubio R, Barea JM. 1991. Selective interactions between different species of mycorrhizal fungi and Rhizobium meliloti strains, and their efects on growth, N2-fixation (^{15}N) and nutrition of *Medicago sativa* L. *New Phytologist* **117**: 399–404.

Berg WK, Cunningham SM, Brouder SM, *et al.* 2005. Influence of phosphorus and potassium on alfalfa yield and yield components. *Crop Science* **45**: 297–304.

Callaway RM, Thelen GC, Barth S, Ransey P, Gannon JE. 2004. Soil fungi alter interactions between the invader *Centaurea maculosa* and North American natives. *Ecology* **85**: 1062–1071.

Daleo P, Alberti J, Canepuccia A, *et al.* 2008. Mycorrhizal fungi determine salt-marsh plant zonation depending on nutrient supply. *Journal of Ecology* **96**: 431–437.

Deng JM, Wang GX, Morris EC, *et al.* 2006. Plant mass–density relationship along a moisture gradient in north-west China. *Journal of Ecology* **94**: 953–958.

Enquist BJ, Brown JH, West GB. 1998. Allometric scaling of plant energetics and population density. *Nature* **395**: 163–165.

Fowler N. 1986. The role of competition in plant communities in arid and semiarid regions. *Annual Reviews of Ecology, Evolution, and Systematics* **17**: 89–110.

Giovannetti M, Mosse B. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytologist* **84**: 489–500.

Hartnett DC, Wilson GWT. 1999. Mycorrhizae influence plant community structure and diversity in tall grass prairie. *Ecology* **80**: 1187–1195.

Helgason T, Merryweather JW, Young JPW, Fitter AH. 2007. Specificity and resilience in the arbuscular mycorrhizal fungi of a natural woodland community. *Journal of Ecology* **95**: 623–630.

Hetrick BAD, Kitt DG, Wilson GWT. 1986. The influence of phosphorus fertilization, drought, fungus species and non-sterile soil on mycorrhizal growth responses in tallgrass prairie plants. *Canadian Journal of Botany* **64**: 1199–1203.

Hutchings MJ. 1983. Ecology's law in search of a theory. *New Scientist* **98**: 765–767.

McPhee CS, Aarssen LW. 2001. The separation of above- and below-ground competition in plants. A review and critique of methodology. *Plant Ecology* **152**: 119–136.

Miyanishi K, Hoy AR, Cavers PB. 1979. A generalized law of self-thinning in plant populations. *Journal of Theoretical Biology* **78**: 439–442.

Morris EC. 1991. Self-thinning and competition intensity over a gradient of nutrient. *Journal of Ecology* **79**: 903–923.

Morris EC. 1996. Effect of localized placement of nutrients on root competition in self-thinning populations. *Annals of Botany* **78**: 353–364.

Morris EC. 2002. Self-thinning lines differ with fertility level. *Ecological Research* **17**: 17–28.

Morris EC. 2003. How does fertility of the substrate affect intraspecific competition? Evidence and synthesis from self-thinning. *Ecological Research* **18**: 287–305.

Pimienta-Barrios E, Del Castillo-Aranda MEG, Munoz-Urias A, Nobel PA. 2003. Effects of benomyl and drought on the mycorrhizal development and daily net CO_2 uptake of a wild platyopuntia in a rocky semi-arid environment. *Annals of Botany* **92**: 239–245.

van der Putten WH, Maas PWTh, van Gulik WAM, Grinkman H.1990. Characterization of soil organisms involved in the degeneration of *Ammophila arenaria*. *Soil Biology and Biochemistry* **22**: 845–852.

Reynolds HL, Vogelsang KM, Hartley AE, Bever JD, Schultz PA. 2006. Variable responses of old-field perennials to arbuscular mycorrhizal fungi and phosphorus source. *Oecologia* **147:** 348–358.

Shumway DL, Koide RT. 1995. Size and reproductive inequality in mycorrhizal and nonmycorrhizal populations of *Abutilon theophrasti*. *Journal of Ecology* **83**: 613–620.

Smith SE, Read DJ. 1997. *Mycorrhizal symbiosis*, 3rd edn. San Diego: Academic Press.

Suriyagoda LDB, Ryan MH, Renton M, Lambers H. 2010. Multiple adaptive responses of Australian native perennial legumes with pasture potential to grow in phosphorus- and moisture-limited environments. *Annals of Botany* **105**: 755–767.

Warton DI, Wright IJ, Falster DS, Westoby M. 2006. Bivariate line-fitting methods for allometry. *Biological Reviews* **81**: 259–291.

Watkinson A. 1980. Density-dependence in single-species populations of plants. *Journal of Theoretical Biology* **83**: 345–357.

Weiner J. 2004. Allocation, plasticity and allometry in plants. *Perspectives in Plant Ecology, Evolution and Systematics* **6**: 207–215.

Weiner J, Damgaard C. 2006. Size-asymmetric competition and size-asymmetric growth in a spatially explicit zone-of-influence model of plant competition. *Ecological Research* **21**: 707–712.

Weiner J, Thomas SC. 1992. Competition and allometry in 3 species of annual plants. *Ecology* **73**: 648–656.

Weiner J, Stoll P, Muller-Landau H, Jasentuliyana A. 2001. The effects of density, spatial pattern and competitive symmetry on size variation in simulated plant population. *American Naturalist* **158**: 438–450.

Weller DE. 1987*a*. A reevaluation of the −3/2 power rule of plant self-thinning. *Ecological Monographs* **57**: 23–43.

Weller DE. 1987*b*. Self-thinning exponent correlated with allometric measures of plant geometry. *Ecology* **68**: 813–821.

Xu LM. 2010. *Phenomena and mechanism of arbuscular mycorrhizal fungi mediating plant density effect under different water levels*. Masters Thesis, Zhejiang University, China.

Yoda K, Kira T, Ogawa H, Hozumi K. 1963. Self-thinning in overcrowded pure stands under cultivated and natural conditions. *Journal of Biology, Osaka City University* **14**: 107–129.