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The role of seed reserves in arbuscular mycorrhizal formation and growth of *Leucaena leucocephala* (Lam.) de Wit. and *Zea mays* L.

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Abstract We show here that seed reserves in *Leucaena leucocephala* (Lam.) de Wit. and *Zea mays* L. (maize) are important for mycorrhizal formation and seedling growth. Seed reserve removal reduced mycorrhizal formation markedly in *Leucaena* but not in maize, except at 15 and 45 days after seed reserve removal. Partial or total removal of seed reserves decreased plant growth and tissue nutrient concentrations in both hosts. Nodule number in *Leucaena*, which was related positively to plant biomass and mycorrhizal infection levels, was reduced when one or both cotyledons were severed. *Leucaena* seedlings without or with partial seed reserves had higher nutrient use efficiencies throughout seedling growth. But such an effect was observed only initially in maize. Seed reserve removal increased the specific absorption rates of nutrients in both hosts. Phosphorus absorption rate was significantly and positively related to root infection levels in both *Leuceana* and maize. Though the growth rates of plants without seed reserves were low initially, these plants had higher growth rates during later stages. We conclude that seed reserves are not only important for seedling growth, but also for mycorrhizal formation and nodulation.

Key words Arbuscular mycorrhiza · Cotyledon · *Leucaena* · Maize · Nodulation · Seed reserves

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Introduction

The embryo is wholly dependent on the transfer of storage materials from the cotyledons and endosperm during seedling development. In hypogeal species, the function of the cotyledons is completed after the transfer of the available stored nutrients. In addition, the cotyledons of many epigeal species expand, develop chlorophyll after emergence and become efficient photosynthetic organs (Marshall and Kozlowski 1976). Plant taxa with small or poorly provisioned seeds are thought to be highly mycorrhizal dependent during seedling growth and establishment, when the demand for nutrients is high relative to nutrient uptake (Koide 1991; Allsopp and Stock 1992). Alternatively, seedlings from large seeds have a high rate of root extension and are able to establish themselves independently of soil nutrients and mycorrhizae during the early stages of seedling growth (Allsopp and Stock 1995).

Although earlier studies examined the relationship of seed weight to seedling growth (Marshall et al. 1986; Wulff 1986) and mycorrhizal infection levels (Peat and Fitter 1993; Allsopp and Stock 1995), information about the regulatory role of seed reserves on arbuscular mycorrhizal (AM) formation and function is scanty. Cuenca et al. (1990) suggested that large cotyledons and their persistence after germination might prevent plants from becoming entirely dependent on roots for their nutrients and, hence, cause those plants to respond to a lesser degree to AM. In many nursery experiments, especially with cocoa, the cotyledons were severed to enhance the mycorrhizal response (Azizah Chulan and Ragu 1986; Azizah Chulan and Kamal 1988). This assumed positive response between cotyledon removal and mycorrhizal response, however, does not always seem to hold. Janos (1980) found no mycorrhizal response in decotyledonised *Pentaclethra macroloba* (Willd.) Ktze., and *Virola koschnyi* Warb., which are strongly mycorrhizal dependent, and he speculated that seed reserves are important for mycorrhizal formation and seedling growth. Unfortunately, the effect of cotyledon removal on mycorrhizal colonisation was not presented in that study (Janos 1980). Calculated carbon costs suggest that 4–17% of the photosynthates translocated to the roots is used by the mycorrhizal fungi. This carbon cost can often be seen as a growth depression in young seedlings if inadequately supplied with nutrients (Peng et al. 1993). Recently Ba et al. (1994) showed that ectomycorrhizal formation in *Afzelia africana* Sm. seedlings was affected by complete or partial cotyledon excision. Since seed reserves are related to seedling growth under competitive and stressful conditions (Marshall et al. 1986), we present here a test of the hypothesis that detachment of seed reserves could help plants in early mycorrhizal response and benefits. Further, this study compares the effect of seed reserves on mycorrhizal infection and nutrient uptake in strongly mycorrhizal-dependent *Leucaena* and weakly mycorrhizal-dependent maize.

Materials and methods

Seed source

Seeds of *Leucaena* [*Leucaena leucocephala* (Lam.) de Wit.] were procured from the Institute of Forest Genetics and Tree Breeding, Coimbatore and those of maize (*Zea mays* L.) from Tamil Nadu Agricultural University, Coimbatore. The seeds of maize were soaked in water for 12 h period prior to sowing. *Leucaena* seeds were scarified with 98% H_2SO_4 for 30 min and washed in several changes of water prior to soaking in water.

Substrate

The soil used for this experiment was an Alfisol and had a pH of 7.2 (1 : 1, soil : water). The soil nutrients determined according to Jackson (1971) and Davis (1962) were 1.73 mg total nitrogen (N) kg^{-1}, 1.0 mg available phosphorus (P) kg^{-1} and 23.1 mg exchangeable potassium (K) kg^{-1}. The soil had an indigenous mycorrhizal fungal population of 93 propagules g^{-1} of soil assessed according to Porter (1979). The indigenous AM flora consisted of *Acaulospora scrobiculata* Trappe, *Glomus aggregatum* Schenck & Smith emend Koske, *G. geosporum* (Nicol. & Gerd.) Walker, *Scutellospora heterogama* (Nicol. & Gerd.) Walker & Sanders, and *Sclerocystis sinuosa* Gerd. & Bakshi.

Treatments

Two presoaked seeds were sown into 20-cm diameter pots filled with 3 kg of soil. After emergence, the seedlings were thinned to 1 plant per pot. After the unfolding of the first pair of leaves, when the export of the reserves is mostly directed to the roots (Ampofo et al. 1976; Marshall and Kozlowski 1976), the cotyledons were clipped wholly (two cotyledons) or partially (one cotyledon) at the stem surface. Similarly, the entire endosperm of maize was removed after the development of the first leaf. Seedlings with intact seed reserves served as controls. The treatments were arranged in a randomised complete block design with 10 replicates per treatment for *Leucaena* (60 pots) and 5 replicates per treatment for maize (25 pots). Plants were grown under natural light and were watered as needed to maintain the soil moisture at approximately maximal water holding capacity. No nutrients were added.

Harvests and measurements

Plants were harvested every 15 days up to a period of 75 days for maize, and up to 90 days for *Leucaena,* in both cases after seed reserve removal. At harvest, the soil from the roots were washed off carefully and the nodule number in *Leucaena* was counted visually. A weighed portion of each root sample was preserved in FAA solution for the assessment of mycorrhizal infection. Shoots (excluding seed reserves) and roots were separated and oven dried at 70 °C for 48 h for the determination of dry mass after recording their lengths. Total root lengths were determined by line intersect method (Tennant 1975).

The preserved root samples were cleared and stained for determining mycorrhizal infection by a modification of the method of Phillips and Hayman (1970). Fixed roots were cleared in 2.5% KOH, acidified with 5 N HCl, and stained with trypan blue (0.05% in lactophenol). The roots were left overnight in trypan blue lactophenol for staining, as against heating as described by Phillips and Hayman (1970). The AM infection was quantified according to the magnified intersection method (McGonigle et al. 1990).

The P concentrations in shoots and roots were determined by the molybdenum blue method (Jackson 1971) after wet ashing the dried samples in triple acid mixture (HNO_3, H_2SO_4, and 60% $HClO_4$). Tissue N was determined using a Technicon Auto Analyser II (Gedko International Ltd., UK) after digestion of samples with a mixture of selenium and concentrated H_2SO_4. Potassium was estimated by flame photometry (Davis 1962).

Nutrient-use efficiencies were calculated as the unit of biomass produced per unit of nutrient content (Koide 1991). The specific absorption rate (*SAR*), defined as the amount of nutrients absorbed per unit root mass (Gray and Schlesinger 1983), was calculated as follows :

$$SAR = \frac{Plant\ nutrient\ (\mu g)}{Root\ biomass\ (mg)} \mu g\ mg^{-1}$$

Relative growth rate (*RGR*) was calculated (Williams 1946) using the formula:

$$RGR = \frac{Log_e W_2 - Log_e W_1}{t_2 - t_1} mg\ mg^{-1}\ day^{-1}$$

where W_1 and W_2 are mean initial dry weight and final dry weight, respectively, and $t_2 - t_1$ is the time interval in days.

Statistical analysis

In *Leucaena*, the growth, nodulation and mycorrhizal parameters were subjected to analysis of variance and the means were separated using Duncans Multiple Range Test (DMRT). The growth and mycorrhizal variable means in maize were subjected to Students *t*-test. The relationship between plant growth, nutrient, mycorrhizal and nodulation variables were assessed using linear regression analysis. Percent values of mycorrhizal colonisation were arcsine square root transformed prior to statistical analysis.

Results

Plant growth and relative growth rates

Removal of seed reserves reduced plant height and total root length in *Leucaena* and maize (Table 1).

Table 1 Effect of seed reserve removal on plant growth in *Leucaena* and maize. (*+2C*, *+1C*, *−C* with two, single and no cotyledons, respectively; *+SR*, *−SR* with and without seed reserves, respectively; *R* root, *S* shoot). Within each column means followed by different letter(s) are significantly ($P<0.05$) different according to DMRT for *Leucaena* and *t* test for maize

Growth parameters	Host	Treatments	Days after seed reserve removal					
			15	30	45	60	75	90
Plant height (cm plant^{-1})	*Leucaena*	+2C	6.58a	9.95a	13.29a	18.17a	21.03a	26.19a
		+1C	5.37ab	8.54ab	10.92b	13.99b	17.84b	23.31b
		−C	4.77b	7.00b	8.87c	10.95c	13.81c	17.77c
	Maize	+SR	11.10a	14.52a	17.40a	20.66a	24.50a	–
		−SR	9.20b	13.20b	14.00b	19.10b	24.12a	–
Total root length (m plant^{-1})	*Leucaena*	+2C	17.15a	19.64a	22.80a	27.23a	31.55a	38.46a
		+1C	12.43b	13.64b	18.00b	21.66b	26.10b	36.17b
		−C	10.51c	14.29b	16.11b	20.63b	22.01c	35.66b
	Maize	+SR	39.80a	45.60a	52.12a	65.30a	66.24a	–
		−SR	29.40b	43.00a	45.82b	53.80b	58.68b	–
Shoot dry weight (g plant^{-1})	*Leucaena*	+2C	0.049a	0.290a	0.499a	0.615a	0.944a	1.252a
		+1C	0.039b	0.158b	0.370b	0.597a	0.898b	1.297b
		−C	0.030c	0.147b	0.230c	0.466b	0.778c	1.174c
	Maize	+SR	0.160a	0.357a	0.515a	0.708a	1.164a	–
		−SR	0.157a	0.327a	0.506a	0.668a	1.138a	–
Root dry weight (g plant^{-1})	*Leucaena*	+2C	0.025a	0.053a	0.086a	0.120a	0.253a	0.444a
		+1C	0.015b	0.038a	0.064b	0.087b	0.196b	0.415b
		−C	0.011c	0.029b	0.031 c	0.034c	0.163c	0.276c
	Maize	+SR	0.157a	0.547a	0.753a	1.025a	1.151a	–
		−SR	0.141a	0.462b	0.649b	1.006a	1.156a	–
R/S ratio	*Leucaena*	+2C	0.524a	0.199a	0.171a	0.202a	0.268a	0.355a
		+1C	0.349b	0.289b	0.174a	0.145b	0.219a	0.320a
		−C	0.369b	0.197a	0.131a	0.073b	0.212a	0.235b
	Maize	+SR	0.982a	1.533a	1.467a	1.460a	0.991a	–
		−SR	0.894a	1.423b	1.288b	1.504a	1.026a	–

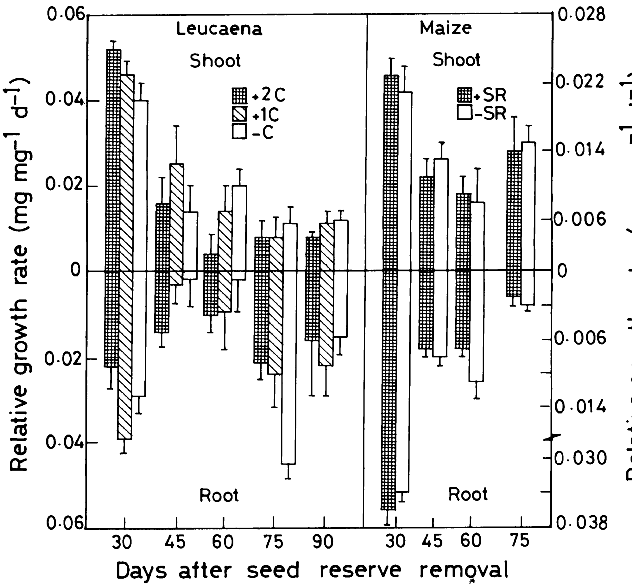

Fig. 1 Effect of seed reserve removal on relative shoot and root growth rates in *Leucaena* and maize (*+2 °C*, *+1 C*, *−C* with two, single and no cotyledons; *+SR*, *−SR* with and without seed reserves); *bars* ±SE

The reduction in total root length in response to seed reserve removal was more pronounced in maize (11%) than in *Leucaena*. A reduction of 17% and 32% in shoot dry weight was observed in *Leucaena* seedlings, respectively, after partial and complete cotyledon removal, despite higher growth rates of their seedlings (Fig. 1). A similar effect (22% and 52%) was observed in root dry weights after partial and total cotyledon removal. Although decotyledonised *Leucaena* seedlings exhibited higher root growth rates at 30 and 75 days, these seedlings had lower root growth rates at 45 and 60 days. Even though the removal of seed reserves slowed shoot growth rates between 15 and 70 days in maize, the shoot dry weight was not affected and in contrast root dry weight was reduced by 14–16% between 15 and 60 days despite of increased root growth rates.

Mycorrhizal colonisation

Cotyledon excision either partially or wholly reduced percentage mycorrhizal colonisation (%RLC), percentage root length with arbuscules (%RLA) and vesicles (%RLV) in *Leucaena* (Fig. 2). In contrast, removal of seed reserves did not significantly affect

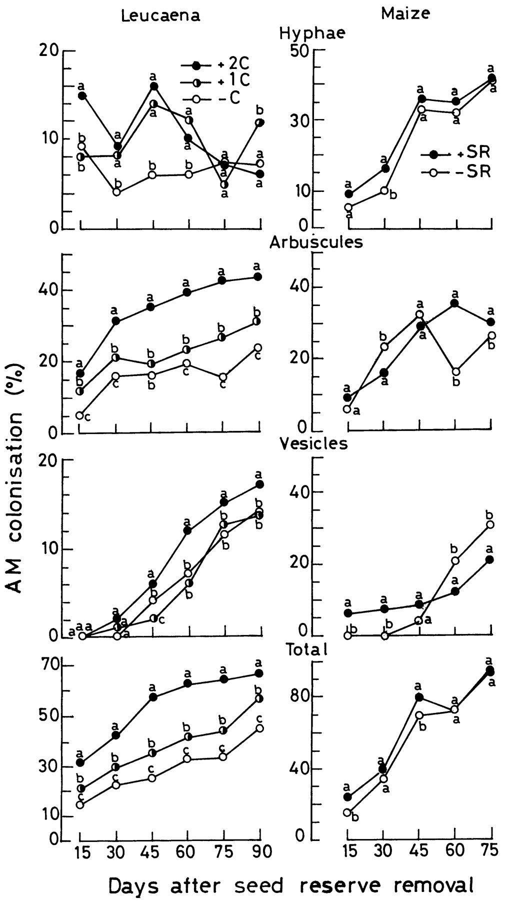

Fig. 2 Effect of cotyledon removal on AM colonisation in *Leucaena* and maize. Points followed by different letters are significantly ($P < 0.05$) different according to DMRT for *Leucaena* and *t* test for maize. Abbreviations as in Fig. 1

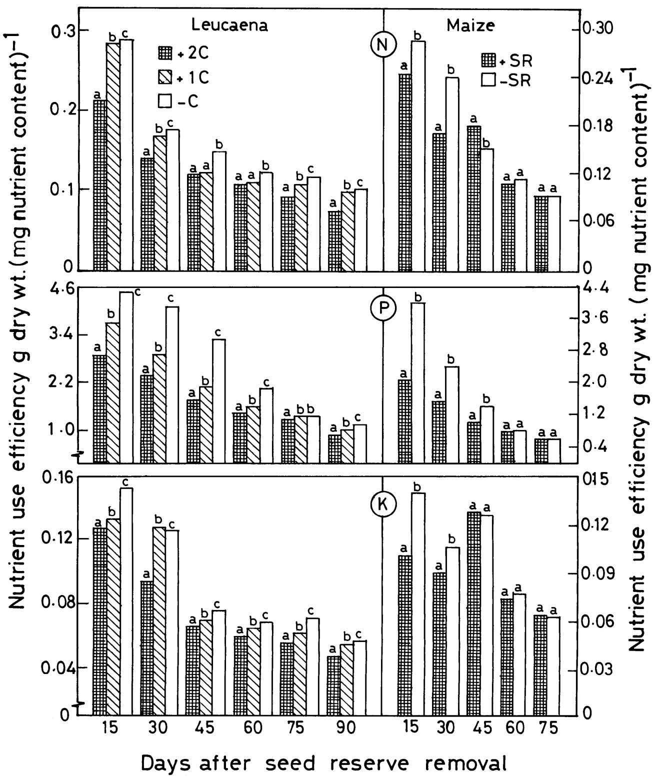

Fig. 3 Effect of seed-reserve removal on nutrient use efficiencies (dry mass per unit of nutrient) in *Leucaena* and maize. Bars with different letters are significantly ($P < 0.05$) different according to DMRT for *Leucaena* and *t* test for maize. Abbreviations as in Fig. 1

%RLC or percentage root length with hyphae (%RLH) in maize except during early stages. Although, seed reserve removal increased %RLA in maize between 15 and 45 days, it decreased by 52% at 60 days and was 22% lower than the control at 75 days. The %RLA varied in a linear manner in response to changes in %RLC in *Leucaena* ($r = 0.963$; $P < 0.001$) and maize ($r = 0.758$; $P < 0.05$). The %RLH was drastically affected in *Leucaena* after complete but not after partial cotyledon excision, which resulted in a twofold increase in %RLH at 90 days compared with other treatments. Complete removal of seed reserves delayed vesicle formation by 15 and 30 days in *Leucaena* and maize, respectively.

The %RLC was linearly related to plant biomass in *Leucaena* ($r = 0.763$; $P < 0.001$) but not in maize ($r = 0.235$; $P > 0.05$). The %RLC ($r = 0.726$ and $r = 0.946$, respectively; $P < 0.001$), %RLA ($r = 0.622$; $P < 0.001$ and $r = 0.703$; $P < 0.05$) and %RLV ($r = 0.870$ and $r = 0.845$; $P < 0.001$) were linearly related to root mass in *Leucaena* and maize.

Nutrient content and nutrient use efficiency

Decotyledonisation either partially or completely reduced nutrient contents of shoots and roots in *Leucaena* (Table 2), but increased nutrient use efficiency (Fig. 3). However, in maize, the increased nutrient use efficiency was limited to early stages of plant growth although the removal of seed reserves reduced nutrient contents of shoot and roots.

Nutrient SAR were higher in *Leucaena* seedlings with and without cotyledons at 30 and 60 days, respectively (Fig. 4). However, seed reserve removal in maize decreased nutrient SAR upto 60 days, except for K at 30 day. SAR was linearly related to %RLC in maize (Table 3), whereas for *Leucaena* such a relation existed only for SAR of P with %RLA and %RLC. Generally, SAR and nutrient use efficiencies were inversely related in both hosts.

Nodulation

Complete cotyledon excision delayed nodulation by 15 days in *Leucaena* and cotyledon removal either wholly or partially reduced nodule inverse (Fig. 5). Nodule

Table 2 Effect of seed reserve removal on shoot and root nutrient content of *Leucaena* and maize. For abbreviations, see Table 1

Nutrients ($\mu g\ mg^{-1}$)	Host	Treatments	Days after seed reserve removal					
			15	30	45	60	75	90
Shoot N	*Leucaena*	+2C	5.12a	7.63a	8.91a	10.05a	12.10a	15.61a
		+1C	3.71b	6.71b	8.93a	9.91b	10.06b	12.03b
		−C	3.60b	6.10c	7.02b	8.52b	9.61b	11.02b
	Maize	+SR	4.82a	7.14a	9.32a	10.41a	13.73a	–
		−SR	4.61a	4.63b	8.03b	10.21a	13.91a	–
Root N	*Leucaena*	+2C	4.21a	4.34a	4.62a	5.40a	6.71a	8.30a
		+1C	3.30b	3.51b	3.81b	4.24b	4.50b	5.33b
		−C	2.81c	3.52b	3.63b	3.81b	4.11b	4.70c
	Maize	+SR	3.93a	4.03a	6.71a	7.08a	9.03a	–
		−SR	2.52b	3.81a	5.52b	6.91a	8.70a	–
Shoot P	*Leucaena*	+2C	0.41a	0.45a	0.60a	0.75a	0.81a	1.20a
		+1C	0.30b	0.39b	0.51b	0.68a	0.75ab	1.10b
		−C	0.25c	0.25c	0.31c	0.50b	0.68b	1.01c
	Maize	+SR	0.60a	0.90a	1.31a	1.80a	2.11a	–
		−SR	0.30b	0.60b	1.03b	1.72a	2.03b	–
Root P	*Leucaena*	+2C	0.23a	0.27a	0.38a	0.45a	0.73a	0.95a
		+1C	0.20a	0.22b	0.32b	0.40b	0.69a	0.83b
		−C	0.16b	0.20b	0.30b	0.38b	0.55b	0.73c
	Maize	+SR	0.50a	0.60a	0.80a	1.01a	1.30a	–
		−SR	0.20a	0.30b	0.50b	0.09a	1.26b	–
Shoot K	*Leucaena*	+2C	10.01a	12.25a	16.25a	18.03a	19.25a	24.03a
		+1C	9.26ab	8.67b	15.81a	16.25b	17.25b	20.01b
		−C	8.03b	8.03b	14.25b	15.01c	15.03c	18.81c
	Maize	+SR	18.03a	18.92a	21.61a	22.51a	25.21a	–
		−SR	13.11b	18.41a	21.46a	20.01a	20.15a	–
Root K	*Leucaena*	+2C	4.45a	5.25a	7.45a	9.01a	11.21a	18.81a
		+1C	3.75b	4.60a	6.25a	8.25a	10.21b	12.01b
		−C	3.03c	3.25b	4.25b	6.80b	8.25c	12.04b
	Maize	+ SR	1.51a	5.15a	5.15a	6.51a	7.53a	–
		−SR	0.80b	3.03b	5.20a	6.34a	7.34a	–

Table 3 Relationship between arbuscular mycorrhiza colonisation (*RC*), specific absorption rate (*SAR*) and nutrient use efficiencies (*NUE*, *PUE*, *KUE*) in maize (Y_1) and *Leucaena* (Y_2)

Variables	Equation	*r*
SAR – Nitrogen	$Y_1 = 16.623 + 0.833\ \mathrm{RC}$	0.300
	$Y_2 = -0.738 + 0.273\ \mathrm{RC}$	0.863***
	$Y_1 = 89.589 - 287.717\ \mathrm{NUE}$	−0.623**
	$Y_2 = 26.204 - 78.572\ \mathrm{NUE}$	0.889***
SAR – Phosphorus	$Y_1 = -0.336 + 0.079\ \mathrm{RC}$	0.530*
	$Y_2 = -0.832 + 0.050\ \mathrm{RC}$	0.921***
	$Y_1 = 5.957 - 1.146\ \mathrm{PUE}$	−0.737***
	$Y_2 = 2.879 - 0.764\ \mathrm{PUE}$	−0.803***
SAR – Potassium	$Y_1 = 29.958 + 1.382\ \mathrm{RC}$	0.275
	$Y_2 = 8.691 + 0.229\ \mathrm{RC}$	0.634*
	$Y_1 = 156.216 - 843.134\ \mathrm{KUE}$	−0.648***
	$Y_2 = 41.380 - 216.384\ \mathrm{KUE}$	−0.884***
NUE	$Y_1 = 0.347 - 0.005\ \mathrm{RC}$	−0.797***
	$Y_2 = 0.239 - 0.002\ \mathrm{RC}$	−0.457
PUE	$Y_1 = 6.159 - 0.101\ \mathrm{RC}$	−0.866***
	$Y_2 = 4.125 - 0.051\ \mathrm{RC}$	−0.895***
KUE	$Y_1 = 0.205 - 0.003\ \mathrm{RC}$	−0.794***
	$Y_2 = 0.142 - 0.0009\ \mathrm{RC}$	−0.600

*, **, *** Significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively

numbers were significantly ($P < 0.01$) and linearly related to root mass ($r = 0.560$), %RLC ($r = 0.744$) and P content of shoot ($r = 0.919$) and root ($r = 0.906$).

Discussion

Removal of seed reserves either partially or completely slowed seedling growth more in *Leucaena* than in maize. The effect on *Leucaena* is in accordance with earlier reports emphasising the importance of cotyledon reserves for seedling growth in woody angiosperms (Ba et al. 1993; Allsopp and Stock 1995). After emergence, the epigeal cotyledons in *Leucaena* develop chlorophyll and persist for several weeks after the first foliage is produced. It is known that cotyledon photosynthesis plays an essential role in seedling development in woody angiosperms (Marshall and Kozolowski 1976). Deprival of cotyledon photosynthates wholly or partially may have affected *Leucaena* seedling development. Structural compounds which have no direct role either in photosynthesis or nutrient

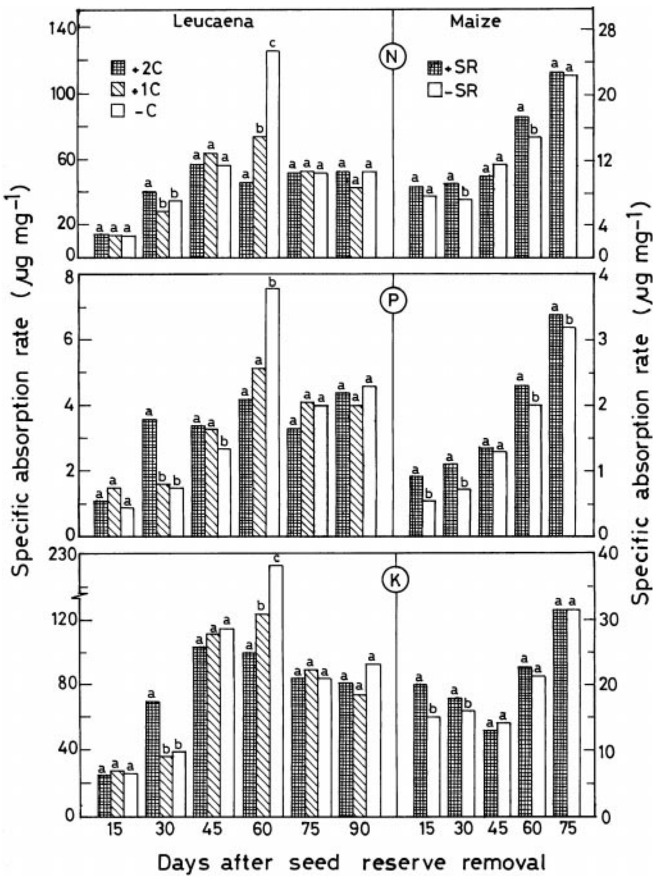

Fig. 4 Effect of seed reserve removal on specific absorption rates (seedling nutrient content / root dry weight) in *Leucaena* and maize. Bars with different letters are significantly ($P < 0.05$) different according to DMRT for *Leucaena* and *t* test for maize. Abbreviations as in Fig. 1

acquisition make up an increasing proportion of dry matter as plants increase in size, leading to the decline in shoot and root growth rates (Sanders 1993) in *Leucaena* and maize. Further, a marked increase in conversion efficiency of energy into biomass occurs under nutrient limitation more in roots than shoots, which can result in increased root growth rates (Kupier 1983), as observed in the present study.

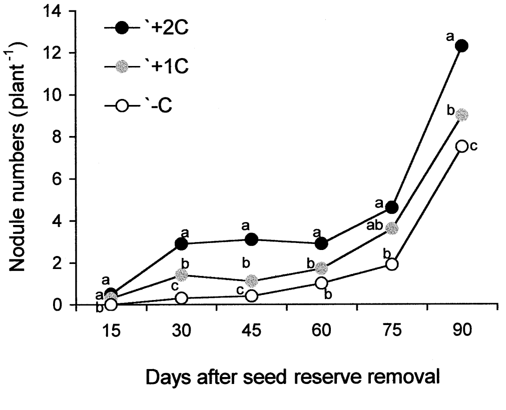

Fig. 5 Effect of cotyledon removal on nodulation in *Leucaena*. Points followed by different letters are significantly different according to DMRT ($P < 0.05$). Abbreviations as in Fig. 1

Our results clearly indicate that seed reserves can influence AM colonisation, suggesting a role in mycorrhizal formation. AM colonisation levels are a resultant of root growth and spread of infection, as suggested by the linear relation between mycorrhizal colonisation and root mass. Thus, factors which affect root growth can also influence mycorrhizal colonisation levels. The principal sink demand for seed reserves or cotyledon photosynthates is switched from leaf to roots with increasing age of the seedlings (Marshall and Kozlowski 1976) and any reduction or elimination of their translocation can significantly affect root growth and in turn mycorrhizal colonisation. Furthermore, when the translocation of seed reserves is reduced or eliminated, it may drastically modify the carbohydrate level in roots (Ba et al. 1994). This physiological modification could influence mycorrhizal formation and especially arbuscule development, which is dependent on carbohydrate concentrations within roots (see Schwab et al. 1991; Blee and Anderson 1998). Initially, the ratios of root length with arbuscules to total colonisation was higher in seedlings deprived wholly or partially of seed reserves. This indicates that a plant under stress is less able to affect arbuscules and thus they may function for longer (Smith et al. 1994).

Removal of seed reserves could have affected nodulation in *Leucaena* in three ways : firstly through its effect on root growth, as suggested by a linear relation. Secondly, the reduced P levels in response to low mycorrhizal colonisation might have affected nodulation, since nodulation and nitrogen fixation by the bacterial symbiont requires an optimal level of P in host tissues (Hayman 1986). Thirdly, the release of flavonoids or other chemical signals, which play an important role in rhizobia – legume interactions could have been affected, as their release is dependent on the host nutrient status (Phillips and Tasi 1992).

It is generally accepted that the most important effect of mycorrhizal colonisation on the host plant is the improvement of nutrient acquisition (George et al. 1995). Our results have shown that the nutrient content of maize by the end of the study was much less affected by the removal of seed reserves than that of *Leucaena*, which directly contrasts with the view that removal of seed reserves enhances the beneficial effect of AM (Cuenca et al. 1990). Maize with its efficient root system is less mycorrhizal dependent for its nutrient uptake than *Leucaena*, which lacks root hairs (Munns and Mosse 1980) and is strongly mycorrhizal dependent. A reduction in the mycorrhizal and nodule activity resulting from reduced photosynthate availability would explain the results observed.

Nutrient shortage could be overcome to a certain extent if plants allocated proportionally more biomass to roots than shoots, thereby increasing the size of the nutrient acquisition system or source and decreasing the relative size of the nutrient-utilising system or sink. This is evident in the present study, where removal of seed reserves either totally or partially reduced the root/shoot ratios in maize and *Leucaena*. Our results indicate that plants without seed reserves use nutrients more efficiently, which is likely to be part of an adaptive strategy of the plant species to the stress induced. The inverse relation observed between nutrient-use efficiency and mycorrhizal colonisation agrees with the results of Baon et al. (1993), but contradicts studies (Menge et al. 1978; Graham and Syvertsen 1985) in which plants with greater nutrient-use efficiencies were more mycorrhizal than their conspecifics with low nutrient-use efficiencies. Root absorption capacity or SAR often increase as nutrient availability declines or as shoot growth rates and nutrient demand increases (Chapin 1980; Koide 1993) and the present study confirms this trend. In addition, we deduce that an efficient nutrient usage may reduce SAR or nutrient uptake as indicated by an inverse relationship between these two variables.

In conclusion, this study demonstrates that changes in mycorrhizal colonisation and nutrient availabilities with seed reserve removal elicit a greater phenotypic response in mycorrhizal-dependent *Leucaena* than in less-dependent maize. The most significant effects were reduced mycorrhizal infection, nodulation, and nutrient accumulation, in addition to reduced biomass. However, greater efficiency of nutrient usage under initial nutrient limitation led to increased biomass production per unit of nutrient uptake, reflecting a physiological adjustment to seed reserve limitation, which might also have long-term implications. Hence, we reject the general hypothesis that severing the seed reserve enhances mycorrhizal response and conclude that in some plants seed-reserves are important for mycorrhizal formation and nodulation as well as for seedling growth.

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