

# Nutrient availability induces contrasting allocation and starch formation in resprouting and obligate seeding shrubs

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## Summary

1. Woody plant responses to crown removal in fire-prone vegetation are of two types: resprouting (resprouters) or killed (obligate seeders). Obligate seeders maximize their fitness by ensuring they are reproductively mature before the next fire; resprouters invest in structures that increase their chance of surviving the next fire.

2. We tested whether seven congeneric pairs of resprouter and obligate seeder shrubs differ in plant size, leaf traits, water relations, biomass allocation and root starch formation in response to resource availability. This was tested by examining the interactive effects of water and nutrient availability in a factorial experiment.

3. Plant size (dry mass and shoot length), leaf area and stomatal conductance did not differ consistently among resprouters and obligate seeders, but were affected by water and nutrient availability. Leaf-area ratio and specific leaf area were not affected consistently by nutrient or water availability, and did not differ among obligate seeders and resprouters.

4. Biomass allocation responded to resource availability and differed between resprouter and obligate seeder pairs. Nutrient addition reduced allocation to root mass, and resprouters consistently allocated more than obligate seeders to root mass. Leaf mass ratios were higher in obligate seeders, and were maintained at the expense of stem mass when nutrients limited growth.

5. Starch concentrations in the roots of resprouters were higher than in obligate seeders. Resprouters increased the concentration of starch in their roots when growth was limited by nutrients, but obligate seeders did not. Resprouters appear to develop a starch-storage capacity through both reserve formation and accumulation mediated by nutrient availability.

6. Allocation, rather than growth differences, provide some insight into why resprouters are often more common than obligate seeders, especially on fertile sites. Seedlings of resprouters may be advantaged at fertile sites because allocation to roots and starch reserve formation enable them to be better competitors after fire. We predict that the interplay of biomass allocation, reproduction, starch storage and disturbance frequency provide a framework for patterns of coexistence in shrubs in fire-prone ecosystems.

*Key-words:* fire ecology, plant function types, plant strategies, sprouting ability, starch accumulation, resource partitioning

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## Introduction

Patterns of resource allocation to growth, reproduction and maintenance are functional traits in plants

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that are under strong selection pressure. Comparative studies of resource allocation among conspecifics have long served as a powerful method for examining convergence in functional traits. A functional trait that is being increasingly recognized is the ability of woody plants to resprout after disturbance (Bellingham & Sparrow 2000; Pausas *et al.* 2004), giving rise to the concept of a persistence niche in plant communities (Bond & Midgley 2001). In particular, there is a strong

dichotomy of response to fire where taxa either resprout (resprouters) or are killed by fire (obligate seeders) in fire-prone vegetation (Vesk & Westoby 2004). The selective factors that result in this dichotomy are not well understood, but it seems that resprouting ability is a labile trait that is independent of phylogeny (Bond & Midgley 2003; Vesk & Westoby 2004; Clarke *et al.* 2005).

It has long been suggested that obligate seeders (plants killed by fire) maximize their fitness by ensuring they are reproductively mature before the next fire (Wells 1968). Conversely, resprouters maximize their fitness by allocating resources to structures that increase their chance of surviving the next fire. Reviews have generally concluded that obligate seeders devote more resources to growth and reproduction, whereas resprouters devote more resources to below-ground storage structures to facilitate resprouting following disturbance (Bellingham & Sparrow 2000; Bell 2001; Bond & Midgley 2003). Several studies have also found that resprouters devote proportionally larger amounts of root tissue to starch storage than do obligate seeders (Pate *et al.* 1990; Pate *et al.* 1991; Bowen & Pate 1993; Bell & Ojeda 1999; Verdagner & Ojeda 2002), although some do not report this (Cruz *et al.* 2003a, 2003b). Nevertheless, there have been no controlled manipulative experiments to determine how growth and allocation in resprouters and obligate seeders vary in response to the interactive effects of nutrient and water availability. In particular, the response of storage to manipulations of nutrients and water has not been tested, nor has the allometric effect of plant size been factored into analyses.

The ability of resprouting species to respond to disturbance appears to depend on storage of total non-structural carbohydrate (TNC) and a bud bank. The two processes resulting in the storage of TNC are accumulation and reserve formation. Chapin *et al.* (1990) defined accumulation as the increase in compounds because resource acquisition exceeds demand for growth and maintenance. Reserve formation, on the other hand, competes with growth, maintenance and reproduction allocation. An increase in the proportion of photosynthate allocated to storage as non-structural carbohydrates in stressed environments, compared with optimum environments, represents accumulation. In contrast, a lack of difference in allocation to storage between stressed and non-stressed plants would indicate that all stored TNC represents true reserve formation (Chapin *et al.* 1990; Wyka 2000). It is generally assumed that resprouting structures (e.g. lignotubers) result from reserve formation, but it is unclear whether it is reserve formation or a form of accumulation, or a combination of both.

The aim of this study was to test whether pairs of resprouters and obligate seeders differ in their size, morphology, allocation and root starch formation when resource availability changes. Thus the effect of the environment is partitioned from the effect of genotype

(resprouter *vs* obligate seeder). The phylogenetic consistency of resprouter *vs* obligate seeder contrasts was examined by using seven congeners and testing for a statistical interaction of genera, trait (resprouting *vs* obligate seeding) and manipulations of resource availability (nutrients and water).

In summary, our experiment tested whether: (1) plant size, leaf traits and water relations differ among obligate seeders and resprouters when resources are freely available; (2) biomass allocation and root starch differ among obligate seeders and resprouters in relation to resource availability of either water or nutrients; and (3) root starch formation occurs as a result of reserve formation and/or accumulation.

## Methods

### SPECIES SELECTION AND ESTABLISHMENT

Life-history contrasts of species were constructed using sclerophyllous shrub species with known post-fire responses from the New England Tableland Bioregion of eastern Australia (Clarke & Knox 2002). Seven species pairs were selected, based on seed availability, from four families all of which are long-lived (>10 years longevity) (Table 1). No estimates of phylogenetic distance among pairs were available because cladistic analyses of these genera have not been done. Two species pairs were used in the genus *Allocasuarina* to provide replication within a genus. One pair was from a more humid (H) region and the other from a sub-humid (SH) area. In general, the obligate seeder species have larger seed mass than the resprouters (Table 1).

Seeds were placed in a germination cabinet and germinated on pads moistened with deionized water after dormancy was broken for *Acacia*, *Hovea* and *Dodonaea*. Dormancy of *Acacia* and *Hovea* spp. was broken by scarification of the seed coat with a razor blade; dormancy of *Dodonaea* spp. was broken by imbibing seeds in hot smoked water. Following germination, seedlings were transferred to a glasshouse on the day following radicle emergence and planted into 90 mm diameter pots that had a depth of 500 mm (volume 3.2 l) filled with coarse, washed river sand. Seedlings were grown under uniform water conditions until 4 weeks of age, at which stage the water-limitation treatment was imposed as described below.

### EXPERIMENTAL DESIGN AND RESPONSE VARIABLES

A fully factorial randomized design was used to assess the effects of nutrient addition and water limitations on seven congeneric pairs of species in a glasshouse experiment. Two levels of nutrients were applied: 1 g l<sup>-1</sup> low-phosphorus Osmocote mixed into the sand growing medium; or no addition to a sand growing medium. Two soil moisture treatments were also applied: pots were watered to field capacity twice a week; or watered

**Table 1.** Study species listed by seed mass, family, fire response, type of resprouting and habitat (nomenclature according to New South Wales Herbarium)

Species (seed mass mg)	Seed mass (mg)	Family	Fire response	Habitat
<i>Allocasuarina littoralis</i>	2.6	Casuarinaceae	Resprouter (epicormic)	Dry sclerophyll forest (humid)
<i>Allocasuarina rigida</i> (eastern)	1.0	Casuarinaceae	Obligate seeder	Rocky outcrop (humid)
<i>Allocasuarina brachystachya</i>	1.2	Casuarinaceae	Resprouter (basal shoots)	Rocky outcrop and forest (sub-humid)
<i>Allocasuarina rigida</i> (western)	1.0	Casuarinaceae	Obligate seeder	Rocky outcrop (sub-humid)
<i>Acacia filicifolia</i>	12.4	Fabaceae section Botrycephalae	Resprouter (root suckers)	Dry sclerophyll forest
<i>Acacia latiseptala</i>	27.4	Fabaceae section Botrycephalae	Obligate seeder	Rocky outcrop
<i>Acacia implexa</i>	21.9	Fabaceae section Plurimerves	Resprouter (basal shoots)	Dry sclerophyll forest
<i>Acacia venulosa</i>	25.6	Fabaceae section Plurimerves	Obligate seeder	Rocky outcrop
<i>Dodonaea triquetra</i>	4.7	Sapindaceae	Resprouter (basal shoots)	Dry sclerophyll forest
<i>Dodonaea hirsuta</i>	5.2	Sapindaceae	Obligate seeder	Rocky outcrop and forests
<i>Hakea laevipes</i>	20.1	Proteaceae	Resprouter (basal shoots)	Dry sclerophyll forest
<i>Hakea macrorrhyncha</i>	58.5	Proteaceae	Obligate seeder	Rocky outcrop
<i>Hovea heterophylla</i>	14.8	Fabaceae	Resprouter (basal shoots)	Dry sclerophyll and grassy forest
<i>Hovea grantia</i>	42.5	Fabaceae	Obligate seeder	Rocky outcrop

to field capacity once the soil water potential approached  $-1.5$  MPa (which occurred approximately every month). The moisture content of the soil at  $-1.5$  MPa was determined using the pressure-plate technique (National Soil Survey Center 1996). A known weight of water was added to the pots, so that the weight of pots could be used to determine when the soil approached  $-1.5$  MPa. For each level of each treatment there were six replicates resulting in an experiment with 4 treatments  $\times$  14 species  $\times$  6 replicates.

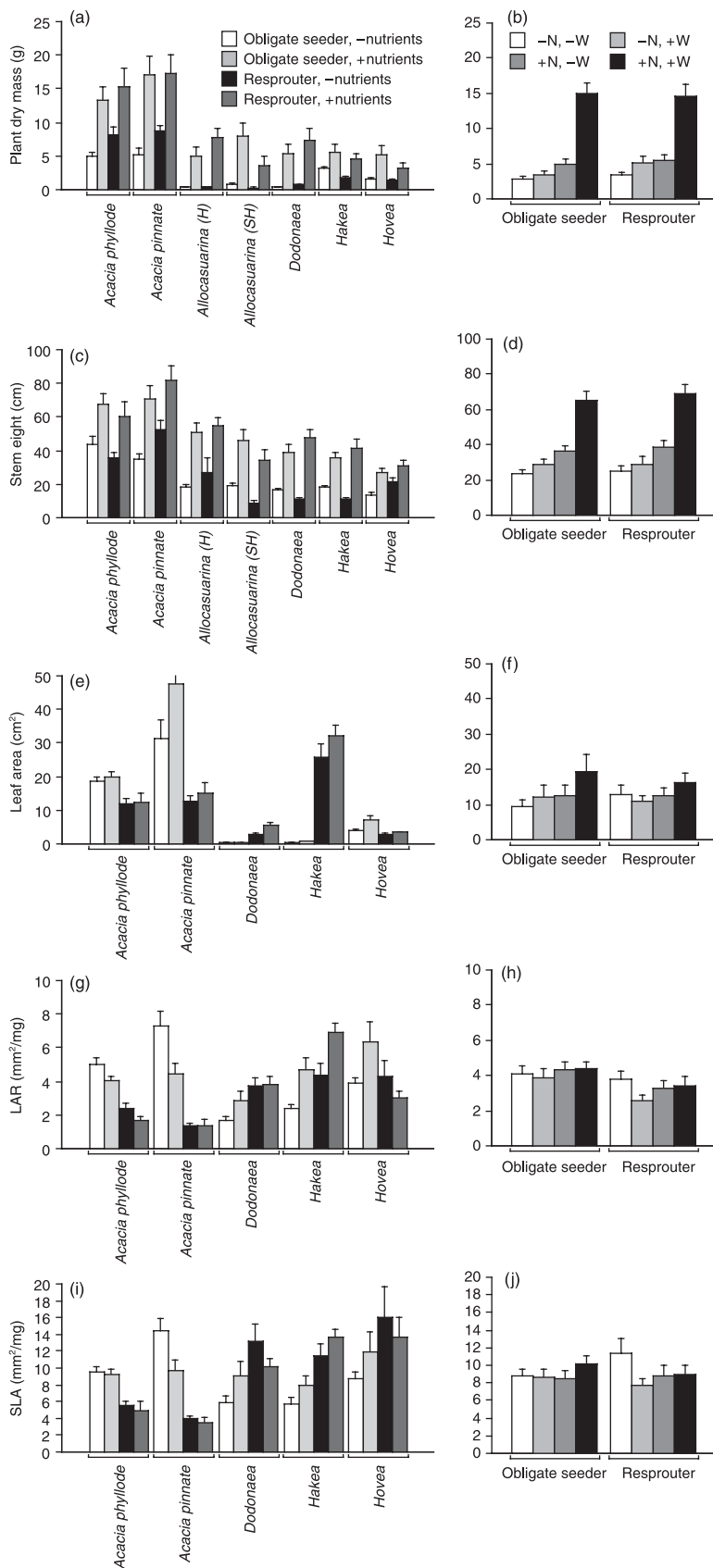
At approximately 4-week intervals, the height, leaf number and reproductive status of each plant were measured. Leaf numbers for *Allocasuarina* species were not measured because they have numerous cladodes. Stomatal conductance (Delta-T AP4 Porometer) was also measured during a water-stress phase at week 24 during mid-morning (09.00–11.00 h). At 30 weeks, plants were harvested, divided into roots, stems and leaves (cladodes and phyllodes), and dried for 24 h at 80 °C for dry weight determinations. Additional response variables for each plant were derived from primary measures: specific leaf area (SLA = leaf area/leaf dry mass); leaf area ratio (LAR = leaf area/total plant dry mass); stem mass ratio (SMR = stem dry mass/total plant dry mass); leaf mass ratio (LMR = leaf dry mass/total plant dry mass); root mass ratio (RMR = root dry mass/total plant dry mass). For *Allocasuarina* the term 'leaf' refers to the cladodes; for the phyllodinous *Acacia* 'leaf' refers to the phyllodes because both organs are functionally equivalent to leaves. Sub-samples of root material were ground for starch determination using the perchloric extraction method (Allen 1974). Both fine and coarse roots were combined and ground before subsamples were taken for starch analysis. The small amounts of both fine and coarse roots (<1 mg) in the water and nutrient-limited samples precluded analyses of TNC.

The day before harvest, two leaves of each species for each treatment were removed to determine the water potential of the leaves using thermocouple psychrometers (Morgan 1983). Plant samples and standards were sealed in chambers immediately after excision and immersed in a water bath at 25 °C for 4 h to equilibrate. Water potentials were determined after a cooling current of 2.5 mA was applied for 30 s, and the output read using a microvoltmeter.

#### STATISTICAL ANALYSES

Initially, we tested whether seedling stem height and seedling mass were related to seed mass by regression analyses to account for variation among genera in the analyses. No strong relationship was found at harvest (see Results). Thereafter, we used harvest mass as a covariate in all subsequent analyses.

The first hypothesis to be tested was that plant dry mass varies with resprouting ability, and this was consistent among genera for treatments of water and nutrient availability. This was tested using a four-factor



**Fig. 1.** Mean values (+ SE) for (a,b) plant dry mass; (c,d) stem height; (e,f) leaf area; (g,h) leaf area ratio; (i,j) specific leaf area for species pairs of obligate seeders and resprouters. *Allocasuarina* was excluded from leaf measurements because it has cladodes. -N, no nutrients added; +N, nutrients added; -W, watering reduced; +W, water freely available (see Methods).

ANOVA with sprouting ability, genera, water and nutrients as factors. The second hypothesis to be tested was that plant growth (shoot length, leaf number, leaf area, SLA, LAR); allocation (LMR, RMR, SMR); water relations (conductance, water potential); and root starch (concentration, total starch) varied with resprouting ability and resource availability, independently of plant mass. This was tested using a four-factor analysis of covariance (ANCOVA) with sprouting ability, genera, water and nutrient limitation as factors, and total plant mass at the end of the experiment as the covariate. The covariate and dependant variables were transformed to their natural logarithm, except for ratio data where the arcsin square root of the variable was used. Where the covariate interactions were not significant ( $P > 0.1$ ) in the original model, each interaction term was removed and the model refitted sequentially. Where there was a significant interaction (only covariate and sprouting ability), this indicated heterogeneity of slopes, and the effect of sprouting ability on the response variable was treated cautiously. All data were checked for homogeneity of variance by plotting the residuals vs predicted values as recommended by Quinn & Keough (2002).

## Results

### VARIATION AMONG GENERA AND SEED SIZE

Seedling shoot height after 1 month of growth was closely correlated with seed mass ( $r^2 = 0.92$ ) across all treatments, and accounted for most of the variation among genera. In addition, the seed mass of obligate seeders was generally higher than that of resprouter species (Table 1;  $F_{1,74} = 11.45$ ,  $P < 0.01$ ), and this was also reflected in 1 month seedling heights. As time passed, however, harvest seedling mass was weakly related to seed mass ( $r^2 = 0.29$ ) and hence the variation in seedling mass at harvest among genera was not related to seed size. At the final harvest, *Acacia* species attained the greatest seedling mass and height compared with the other species across all treatments (Fig. 1a). Under non-limiting conditions, *Allocasuarina* seedlings, the smallest-seeded genus, attained seedling masses greater than *Hakea*, which had the highest seed mass (Fig. 1a).

### VARIATION AMONG RESPROUTERS VS OBLIGATE SEEDERS IN SIZE, FLOWERING, MORPHOLOGY AND WATER RELATIONS

There was no consistent difference in plant dry mass and stem height at harvest between obligate seeding species and those that resprout, as detected by a significant second-order interaction of sprouting, genera and nutrients (Table 2; Fig. 1a,c). The addition of nutrients and water increased total mass and stem height in all species (Fig. 1b,d). All nitrogen-fixing *Acacia* species had relatively high plant mass when nutrients were not

**Table 2.** Results of four-factor ANOVA for effects of resprouting ability, genera, nutrients and water availability for plant mass, and of four-factor ANCOVA for effects of resprouting ability, genera, nutrients and water availability for 13 response variables with plant mass as a covariate

Factor	Total mass			Shoot length		Leaf number†			Leaf area†			LAR†		SLA†		LMR			RMR			SMR			Water pot.		Conductance‡		Starch conc.		TRS§	
	df	F	P	F	P	df	F	P	F	P	F	P	F	P	df	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	
Sprouting	1	0.1	NS	2.5	NS	1	0.2	NS	189.6	***	19.5	***	2.6	NS	1	60.6	***	68.8	***	1.0	NS	13.5	***	0.4	NS	241.8	***	125.5	***			
Genera	6	101.3	***	27.9	***	4	9.4	***	84.3	***	8.4	***	20.4	***	6	18.3	***	3.9	***	27.88	***	3.2	*	–	*	9.6	***	30.3	***			
Nutrients	1	444.6	***	158.4	***	1	12.3	***	0.7	NS	12.8	**	3.4	NS	1	16.5	***	0.1	NS	19.2	***	0.2	NS	1.9	NS	2.6	NS	5.8	NS			
Water	1	95.6	***	54.4	***	1	2.5	***	0.1	NS	3.2	NS	2.5	NS	1	0.1	NS	3.3	NS	14.3	**	6.1	*	25.1	***	0.3	NS	2.8	*			
Sprouting × genera	6	7.7	***	6.0	***	4	117.7	***	261.0	***	37.4	***	10.2	***	6	9.3	***	53.7	***	11.2	***	2.0	NS	–	–	5.6	***	18.3	***			
Sprouting × nutrients	1	1.9	NS	3.7	NS	1	0.2	NS	0.5	NS	3.3	NS	0.9	NS	1	0.5	NS	0.1	NS	0.5	NS	1.6	NS	1.0	NS	19.8	***	7.2	*			
Sprouting × water	1	2.3	NS	0.1	NS	1	0.1	NS	0.6	NS	3.7	NS	0.1	NS	1	2.4	NS	1.2	NS	1.2	NS	0.3	NS	1.1	NS	0.3	NS	2.5	NS			
Genera × nutrients	6	10.6	***	7.4	***	4	4.6	***	2.2	NS	8.3	***	3.4	*	6	11.3	***	11.6	***	4.8	***	4.1	**	–	**	3.9	***	15.4	***			
Genera × water	6	1.5	NS	2.3	NS	4	0.1	NS	2.2	NS	0.4	NS	0.8	NS	6	1.2	NS	0.1	NS	1.7	NS	1.1	NS	–	–	1.4	NS	3.7	NS			
Nutrients × water	1	91.0	***	23.3	***	1	1.7	NS	6.3	*	16.2	***	7.7	**	1	0.5	NS	1.0	NS	0.1	NS	0.3	NS	0.3	NS	0.9	NS	4.9	NS			
Sprouting × genera × nutrients	6	3.0	**	3.7	**	4	5.1	**	0.6	NS	1.2	NS	0.8	NS	6	0.4	NS	1.7	NS	2.0	NS	1.4	NS	–	–	3.0	**	11.1	***			
Sprouting × genera × water	6	1.9	NS	1.7	NS	4	2.0	NS	2.0	NS	0.5	NS	0.6	NS	6	1.2	NS	1.4	NS	1.6	NS	0.3	NS	–	–	0.6	NS	1.8	NS			
Sprouting × nutrients × water	1	5.5	NS	0.7	NS	1	5.4	NS	0.1	NS	0.1	NS	2.4	NS	1	0.1	NS	0.1	NS	0.2	NS	1.5	NS	15	NS	0.3	NS	0.1	NS			
Genera × nutrients × water	6	1.7	NS	1.3	NS	4	0.4	NS	2.2	NS	2.5	NS	0.7	NS	6	0.6	NS	0.9	NS	2.6	NS	0.5	NS	–	–	0.8	NS	2.4	NS			
Sprouting × genera × nutrients × water	6	0.6	NS	1.3	NS	4	0.7	NS	0.9	NS	0.2	NS	0.7	NS	6	1.5	NS	1.4	NS	2.4	NS	1.7	NS	–	–	0.8	NS	1.6	NS			
Ln dry mass	1	–	–	1.1	NS	1	11.8	***	6.9	**	18.8	***	12.4	***	1	2.5	NS	5.7	*	1.5	NS	0.4	NS	0.1	NS	0.1	NS	18.9	***			
Ln dry mass × sprouting	–	–	–	–	–	–	10.6	**	–	–	–	–	7.5	***	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–			
Residual df	241	–	–	240	–	176	–	–	178	–	177	–	177	–	238	–	–	242	–	236	–	141	–	38	–	113	–	113	–			
Transform	Ln + 1	–	–	Ln + 1	–	Ln + 1	–	–	Ln + 1	–	Ln + 1	–	Ln + 1	–	√Arcsin	–	–	√Arcsin	–	√Arcsin	–	Ln + 1	–	Ln + 1	–	Ln + 1	–	Ln + 1	–			

\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

All ANCOVA models were refitted if covariate interactions were non significant. Ln plant dry mass was the covariate.

†Note that *Allocasuarina* was not included as a genus in these analyses.

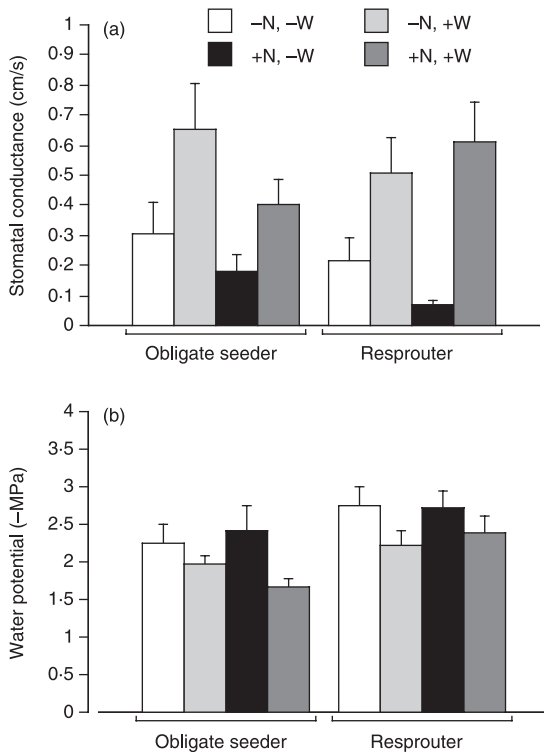
‡Genus not included due to unbalanced numbers of replicates.

§TRS = total root starch.

added, but other genera had much reduced plant mass (Fig. 1a). Two species flowered after 30 weeks of growth when water and nutrients were freely available, both of which were obligate seeders (*Allocasuarina*, *Dodonaea*), and a third obligate seeding species of *Acacia* had flower buds at harvest.

Leaf variables (leaf area, LAR, SLA) covaried with plant dry mass, but showed no consistent pattern of difference between obligate seeder and resprouter species with significant interactions of sprouting ability and genera (Table 2). Both *Acacia* obligate seeding species had higher leaf area, LAR and SLA than their resprouter pairs, but the converse was the case for the genera *Dodonaea* and *Hakea* (Fig. 1e,g,i). Leaf area was slightly enhanced by the addition of nutrients and water (Fig. 1f), but SLA and LAR were, in general, not enhanced (Fig. 1h,j). *Hakea* was the only genus that had increased SLA when nutrients were added for both congeners (Fig. 1i).

There were significant effects of water availability and sprouting ability on leaf water potential and stomatal conductance, but no effect of plant dry mass as a covariate (Table 2). In all species, water limitations reduced stomatal conductance, and leaf water potentials were slightly more negative (Fig. 2a,b). Resprouter species also developed more negative water potentials than obligate seeding species (Fig. 2b). While there



**Fig. 2.** Mean values ( $\pm$  SE) for (a) stomatal conductance; (b) leaf water potentials prior to harvesting for species pairs of obligate seeders and resprouters. -N, no nutrients added; +N, nutrients added; -W, watering limited; +W, water freely available (see Methods). Data pooled across five genera. Note water potentials are negative values: water-limited plants have more negative water potentials.

were significant interactions of nutrients and genera on water potential (Table 2), no consistent patterns were detected of nutrients affecting leaf water potentials or stomatal conductance.

#### EFFECTS OF RESOURCE LIMITATION ON ALLOCATION

The root mass ratio of resprouters was consistently higher for all genera, but varied in the magnitude of differences giving a significant first-order interaction of sprouting ability and genera (Table 2; Fig. 3a). No obvious difference in root architecture between obligate seeders and resprouters was observed when roots were washed. Nutrient addition reduced root mass ratios in both obligate seeders and resprouters for most genera, and this was prominent in *Allocasuarina*, *Dodonaea* and *Hakea* (Fig. 3b). In contrast, the leaf mass ratio was lower for most resprouters compared with obligate seeders (Fig. 3c), and nutrient addition had no consistent effect on relative leaf mass (Table 2; Fig. 3d). Stem mass ratio generally increased when nutrients were added, but there was no consistent difference among obligate seeders and resprouter species in stem allocation (Table 2; Fig. 3e,f).

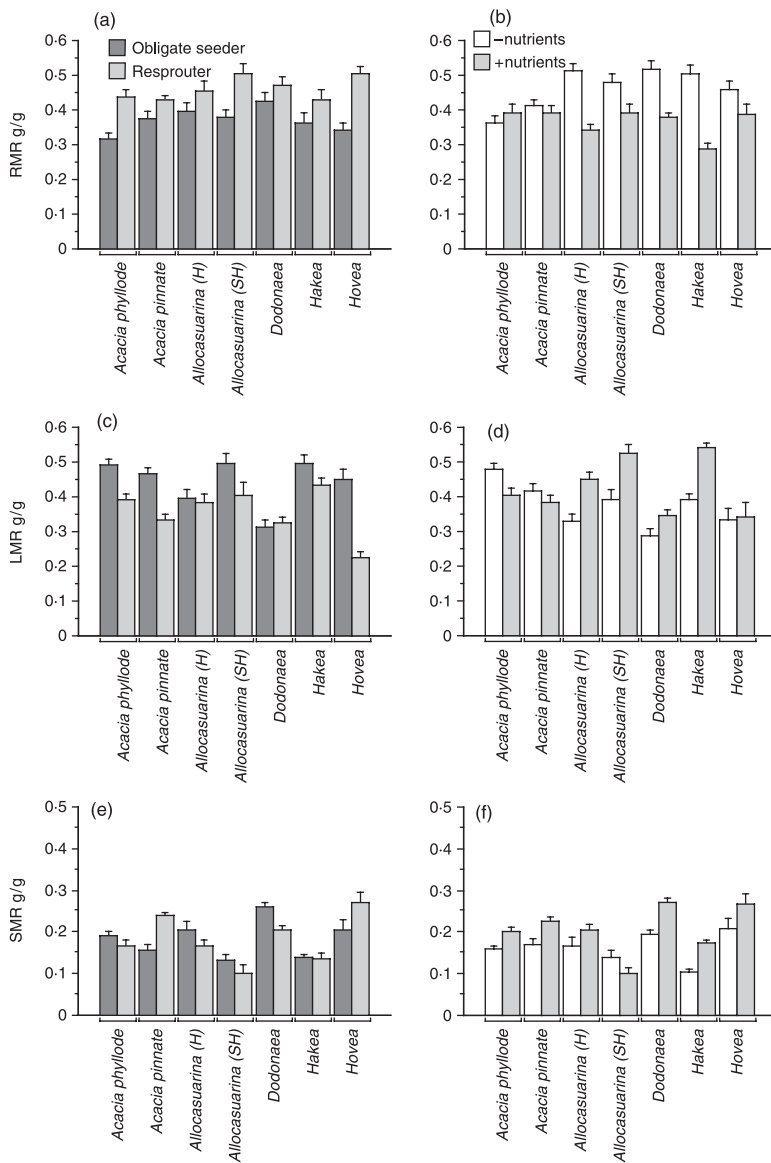
#### EFFECTS OF WATER AND NUTRIENT LIMITATION ON ROOT STARCH

Starch concentrations and total amounts of starch in roots were affected by nutrient limitation, and differed among resprouters and obligate seeders (Table 2). Considerable variation in starch concentration was also detected among and within genera (*Allocasuarina*) (Fig. 4a). There was a significant second-order statistical interaction between sprouting ability, genera and nutrient treatments. In other words, the effect of nutrient addition had different effects on resprouters and obligate seeders (Fig. 4a,b). The concentration of starch in the root tissues of resprouters was higher than in obligate seeders across all treatment levels of water and nutrients (Fig. 4b). The starch concentrations in obligate seeders were not affected by addition of nutrients or water (Fig. 4b). Resprouters, however, increased the concentration of starch in their tissue when nutrients were not added (Fig. 4b).

## Discussion

#### TRAITS' RESPONSE TO RESOURCE AVAILABILITY

The response to resource availability was pronounced for plant size, water relations and root allocation among congeners. This suggests the ability to adjust growth in response to resource availability was similar for obligate seeders and resprouters. Leaf traits (LAR, SLA) did not, however, respond consistently to resource availability, nor did obligate seeders have consistently



**Fig. 3.** Mean values (+ SE) for (a,b) root mass ratio; (c,d) leaf mass ratio; (e,f) stem mass ratio for species pairs of obligate seeders and resprouters (a,c,e), and for effects of nutrient addition where pairs are pooled (b,d,f).

higher LAR or SLA that would suggest higher growth rates. This is supported by similar measures in stomatal conductance among congeners when water and nutrients were freely available. Thus phylogeny and site factors are more likely than intrinsic differences between resprouters and obligate seeders to affect growth rates of these woody plants (cf. Bond & Midgley 2003). In *Hakea*, for example, there was scleromorphic plasticity in response to availability of nutrients, with increased sclerophylly (low SLA) when no nutrients were added; a pattern similar to the response of *Hakea* to sustained water stress (Groom & Lamont 1997). However, contrary to predictions, SLA was significantly lower for the obligate seeder compared with the resprouter species pair.

Differences among congener pairs were pronounced in biomass allocation and starch storage patterns,

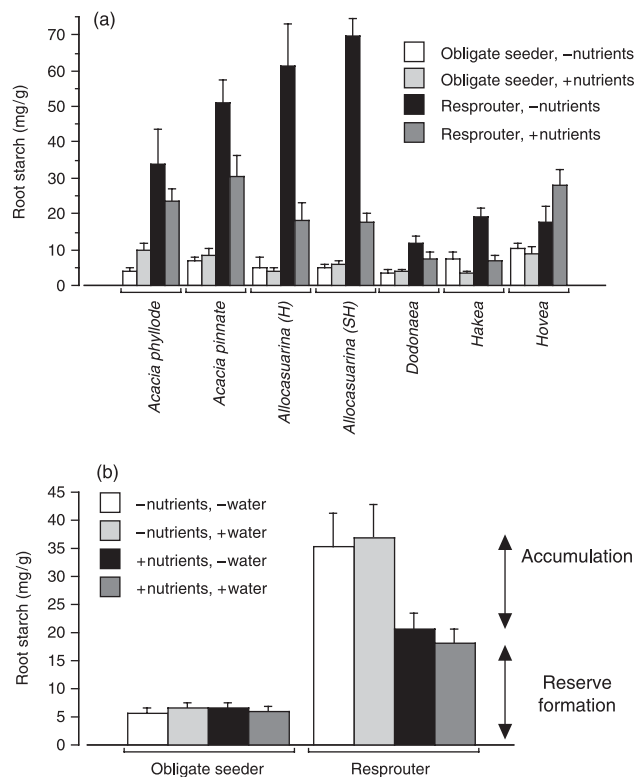
irrespective of resource availability, suggesting that these traits were under strong genetic control in relation to sprouting ability. In summary, resprouters had higher root mass allocation and starch root concentrations than obligate seeders. The significance of these results in relation to disturbance theory and community composition in fire-prone landscapes is elaborated below.

#### TRADE-OFFS IN GROWTH AND ALLOCATIONS

Both empirical data and theory predict that plants killed by fire will have higher relative growth rates and biomass compared with species that have the ability to resprout after disturbance (Bond & Midgley 2003). Previous studies, restricted to Mediterranean-type climates, found that seedlings of obligate seeders have greater biomass (Pate *et al.* 1990; Pate *et al.* 1991; Hansen *et al.* 1991; cf. Bell & Pate 1996), suggesting that obligate seeders have a higher relative growth rate. It has also been suggested that a cost in resprouting is reduced plant height (Midgley 1996; Kruger *et al.* 1997). We found no consistent differences in plant size, leaf traits and stomatal conductance among congeners, suggesting similar relative growth rates. What, then, are the trade-off mechanisms that allow (1) resprouters to increase their chance of surviving the next fire; and (2) obligate seeders to be reproductively mature before the next fire?

We found that resprouters allocate more biomass to roots than shoots, whereas obligate seeders maintain higher leaf mass allocation, irrespective of resource availability and allometric effects. This is consistent with numerous studies where resprouters have been reported to have greater root-to-shoot ratios than obligate seeders (Hansen *et al.* 1991; Bowen & Pate 1993). Both resprouters and obligate seeders increased their allocation to roots at the expense of stem mass when nutrients limited growth. Reduced stem mass in resprouters may also limit the ability of shrubs in nutrient-deficient soils to resprout from epicormic stem buds. This is supported by the observation that 88% of shrubs in the New England Tableland Bioregion, including all used in this study, resprout from basal buds (Clarke & Knox 2002; Clarke *et al.* 2005).

The maintenance of leaf mass at the expense of stem mass is stronger in obligate seeders than in resprouters, especially when nutrients limit growth. This effect may account for more rapid reproductive maturity in obligate seeders, as we found flowering was initiated in three species (*Acacia*, *Casuarina*, *Dodonaea*) under the non-limiting (water and nutrient) treatment. However, the trade-off in allocation to early reproductive maturation and seed production requires longer-term experiments that take into account plant allometry, as resprouters are often multistemmed and have larger canopy volumes (Bond & Midgley 2003). In summary, both obligate seeders and resprouter seedlings appear to have similar growth rates, but rapid reproductive



**Fig. 4.** Mean values (+ SE) for (a) root starch concentrations for species pairs of obligate seeders and resprouters showing the effect of nutrient addition; (b) root starch concentrations for all treatment combinations pooled across seven species of obligate seeders and resprouters. Arrows show the interpretation of reserve formation vs starch accumulation where nutrients limited plant growth.

maturation in obligate seeders is likely to be a result of allocation to reproduction, while resprouters are slow to reach maturity due to root allocation.

#### STARCH RESERVE FORMATION AND ACCUMULATION

The trade-off in biomass allocation to roots is important in fire-prone environments if the storage product is to increase the chance of surviving the next fire (Bond & Midgley 2003). Surveys of restiads and shrubs in Mediterranean-type climates show that resprouters generally have more starch or other non-structural carbohydrates in their roots than obligate seeders (Pate *et al.* 1990, 1991; Bell & Pate 1996; Bell & Ojeda 1999; Verdagner & Ojeda 2002). We have extended this pattern to a non-Mediterranean biome across a diverse range of plant families, including replication within a lineage (*Allocasuarina*). Thus starch allocation is a highly labile trait influencing the demographic trade-offs in resprouting and obligate seeding.

The synergistic influence of water and nutrients on the ability of woody plants to store non-structural carbohydrates is little explored in fire-prone environments. The effect of starch depletion following defoliation is well documented (Miyaniishi & Kellerman 1986; Bowen & Pate 1993; Bell & Pate 1996), and the seasonal stress fluctuations (Cruz & Moreno 2001) and soil

nutrient status (Cruz *et al.* 2003a) have been explored in a narrow range of species. We restricted nutrient and water availability on seedlings in order to determine whether: (i) obligate seeders can accumulate starch; and (ii) resprouters have a true reserve formation, or if they can accumulate starch. Non-structural carbohydrate accumulation can be distinguished from reserve formation by manipulating plant growth rate experimentally and monitoring allocation to storage (Wyka 2000). An increase in the proportion of carbohydrates to storage in resource-limited plants indicates accumulation. By contrast, a lack of difference in allocation to storage between 'stressed' and 'non-stressed' plants suggests true reserve formation (Wyka 2000).

We found that the starch concentration in the roots of obligate seeders was not influenced by resource availability, and therefore that these plants do not have the capacity to accumulate starch and have only limited capacity for reserve formation. Conversely, resprouters accumulate starch when growth is limited by nutrients, and have a higher capacity than obligate seeders for reserve formation when nutrients are freely available. We did not detect any effect of water availability on starch accumulation because we used a fluctuating regime that could have resulted in starch being mobilized and used for growth when plants were rewatered. In summary, our results showed that obligate seeders do not accumulate starch, but resprouters have a starch-storage capacity driven by both the process of reserve formation and accumulation.

#### LANDSCAPE PATTERNS

Resprouters and obligate seeders are both present in fire-prone landscapes, yet some vegetation communities consistently have greater representation of one fire-response group (Kruger *et al.* 1997; Ojeda 2001; Clarke & Knox 2002; Clarke *et al.* 2005). An understanding of the trade-off in resource allocation involving resprouting compared with obligate seeders may allow the development of models to predict which environments promote the persistence niche (Bellingham & Sparrow 2000). Our results provide some insight into the reason why resprouter shrubs are more widespread than obligate seeders, and why resprouters are more common on fertile soils in Australia (Lamont & Markey 1995; Clarke & Knox 2002; Clarke *et al.* 2005; cf. Figure 1 of Bellingham & Sparrow 2000).

Seedlings of resprouters are predicted to be advantaged at fertile sites because allocation to roots and starch reserve formation may enable them to survive fires at shorter intervals than the time taken for obligate seeders to reproduce. Where longer fire intervals allow obligate seeders to reproduce and establish, their ability to recruit would be diminished because their growth would be dependent on seed mass rather than root storage. While resprouters have the ability to accumulate starch when nutrient availability is low, the total amount of starch in the roots is reduced. We

therefore predict that competitive ability between seedlings of obligate seeders and resprouting plants would be reduced at infertile sites. Hence this would account for the pattern of mixed coexistence of resprouters and obligate seeders on infertile soils where fire intervals allow regeneration of obligate seeders (Clarke *et al.* 2005). We suggest that the interplay of allocation, starch storage, reproduction and disturbance frequency provide a framework for patterns of coexistence in shrubs in fire-prone ecosystems.

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