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The importance of seed reserves for seedling performance: an integrated approach using morphological, physiological, and stable isotope techniques

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Abstract To investigate how seed reserves affect early seedling performance, we conducted a factorial greenhouse experiment using *Lithocarpus densiflora* (Tanoak). Seedlings were grown from large (5.8 ± 0.7 g) and small (3.2 ± 0.4 g) seeds and, following shoot emergence, seeds were either removed or left attached. Seedlings were harvested for quantification of biomass and $\delta^{13}\text{C}$ at seven time periods following seed removal (2, 4, 8, 16, 32, 64, 128 days) and seedling photosynthesis was measured three separate time periods (2–4, 49–82, 95–128 days after seed removal). Biomass increased for all seedlings, but the increase was significantly larger for seedlings with attached seeds than with removed seeds. Seed removal just after shoot emergence significantly decreased seedling biomass, but seed removal 64 days after shoot emergence had no effect on seedling biomass. Seedling photosynthesis per unit leaf area varied by time and seed presence, but not by seed size. At the first period, seedlings with attached seeds had significantly higher photosynthetic rates than seedlings with removed seeds, at the second period there was no effect of seed removal, and at the third time period seedlings with attached seeds had significantly lower photosynthetic rates than seedlings with removed seeds. Despite temporal variation in photosynthesis per unit leaf area, seedlings with attached seeds always had significantly greater leaf area than seedlings with removed seeds, resulting in significantly higher total plant photosynthesis at all three time periods. The $\delta^{13}\text{C}$ values of both the leaves and roots were more similar to that of the seed for seedlings with attached seeds than for seedlings with removed seeds, however, seed removal and seed size

strongly affected root $\delta^{13}\text{C}$. This study demonstrates that seed reserves have important effects on the early growth, physiology, and $\delta^{13}\text{C}$ of *L. densiflora* seedlings.

Keywords *Lithocarpus densiflora* · Seed reserves · Seedling growth and physiology · $\delta^{13}\text{C}$

Introduction

Numerous studies have documented that initial seedling growth is positively correlated with seed size (e.g. Howe and Richter 1982; Stanton 1984; Vaughton and Ramsey 1998; Sousa et al. 2003). Seedlings grown from large seeds have also been shown to have higher rates of establishment than those from grown small seeds (Black 1958; Grime and Jeffery 1965; Armstrong and Westoby 1993; Burke and Grime 1996). Despite the widespread correlation between seed size and seedling growth, other studies in which varying amounts of a seed's reserves have been experimentally or naturally removed report little negative effect on seedling growth and establishment (Jarvis 1963; Dalling et al. 1997; Mack 1998; Dalling and Harms 1999).

Unrelated to potential effects on initial seedling growth, larger seed size may be important in buffering loss of cotyledonary mass to seed predators (Rosenthal and Kotanen 1994) as well as attracting seed dispersers (Howe and Smallwood 1982). In addition to pre-germination seed predation, some large-seeded species such as oaks are also subject to post-germination seed removal (Bossema 1979). It is unclear how post-germination seed removal affects subsequent seedling growth. A number of studies found that post-germination seed removal had no effect on seedling growth (Jarvis 1963; Sonesson 1994; Andersson and Frost 1996); However, Bonsil (1998) documented that the growth of both *Quercus rugosa* and *Q. laurina* seedlings significantly decreased after post-germination seed removal.

Seedling physiology could also be influenced by seed size or post-germination seed removal, but research on this

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topic is limited. Burris et al. (1973) found that the photosynthetic rates of *Glycine max* L. seedlings were not significantly different among seed sizes. Similarly, Wulff (1986) reported similar photosynthetic rates between large- and small-seeded *Desmodium paniculatum* L. (D. C.) seedlings grown at high irradiance levels, but higher photosynthetic rates of small-seeded than large-seeded seedlings at low irradiance levels. Other physiological parameters that could vary with seed size include nutrient uptake and water use efficiency (Lloret et al. 1999). To our knowledge though, there have been no ecological studies that address how post-germination seed removal affects seedling physiology.

Tracking the movement of resources between seeds and seedlings is essential for quantifying the effects of seed reserves on seedling growth. Natural abundance stable isotopes of carbon, nitrogen, and oxygen can be used as resource tracers if potential sources have repeatable and distinct isotopic values (Dawson et al. 2002). With respect to seeds and seedlings, the carbon isotopic ratio of reserves mobilized from the seed must differ from that of the carbon fixed by the leaf. This is likely to be true when the environmental conditions under which the seed reserves were synthesized differ from when the seedling is growing (Smedley et al. 1991) or if specific compounds are preferentially mobilized from the seed to the seedling. Additionally, because seedlings switch from seed reserves to photosynthesis during development, one would predict that the isotopic ratio of the seedling should become increasingly different from the seed as it develops.

To address how variation in seed reserves influences seedling growth, physiology, and carbon isotope ratios, we manipulated seed size and presence of *Lithocarpus densiflora* (Hook. & Arn.) Rehder seedlings in a factorial greenhouse experiment. We predicted that seedlings from large seeds and with attached seeds would have a larger biomass than those from small seeds or removed seeds. Similarly, we predicted that seedling photosynthesis would be highest for seedlings with large and attached seeds because the seed carbon reserves augment seedling carbon income and therefore enhance carbon fixation rates despite the costs of mobilizing seed carbon (i.e. having higher respiration). Finally, we predicted that isotopic values of the leaves and roots should be more similar to the seed for seedlings with attached seeds than for those with removed seeds, especially during early seedling development when the growth of the seedling would be most dependent on seed reserves.

Materials and methods

Experimental design

In October 2002, acorns (hereafter referred to as seeds) of *L. densiflora* (hereafter referred to as *Lithocarpus*) were collected from multiple trees within a 1,000 m² area along Bolinas ridge on Mount Tamalpais (37°54'N, 122°37'W), Marin County, Calif., USA. Seed viability was determined

in the laboratory by immersing the seeds in water and removing all of those still floating after 5 min (Nyandira and McPherson 1992). Viable seeds were surface sterilized in a 10% bleach solution for 5 min, air dried, and stored at 4°C until planting. From the pool of collected seeds, 200 large and 200 small seeds were haphazardly selected. The large seeds had a weight of 5.80±0.69 g (mean ±1 SE) and the small seeds had a weight of 3.15±0.41 g. Seed weight (i.e. seed reserve and shell) and seed reserve weight were highly correlated ($r^2=0.972$, $P<0.001$, $n=20$).

Field soil was collected from the same area as the seeds and mixed 2:1:1 with Turface (Profile Products, Buffalo Grove, Ill., USA) and fine sand. The soil mixture was used to improve soil aeration and provide uniform soil moisture conditions. In December 2002, seeds were planted into D40 Deepot cells (Stuewe, Corvallis, Ore., USA) in the greenhouse and watered at least twice a week for the duration of the experiment to maintain high soil moisture. Seedlings were grown in non-supplemented light conditions at an average daily temperature of 20°C and relative humidity of 55%. Pots were moved at least once every 2 weeks to control for environmental variation across the greenhouse bays.

In all of the experiments described below, we manipulated seed reserves using two treatments. The seed size treatment compared seedlings with incremental differences in seed reserve mass, while the seed presence treatment compared seedlings with removed or attached seeds. For the seed size treatment, the average (±1 SE) difference in seed reserves between seedlings with large and small seeds was 1.4±0.04 g. Because of the factorial design of the experiments, the seed presence treatment included both large and small seeds. To determine the mass of the attached seeds, we took the average of the large and small seed masses (2.33±0.04 g). Since seedlings with removed seeds had an average seed mass of 0 g, the average difference in seed reserve mass of seedlings with attached and removed seeds was 2.33±0.04 g. As such, the seed presence treatment represented a greater difference in seed reserve mass than the seed size treatment. For all experiments, seed removal involved removal of the principle storage organ for the seedling, i.e. the cotyledon, which was encapsulated in the acorn below the soil surface.

To examine the effect of seed reserves on *Lithocarpus* seedling biomass, we conducted two independent experiments. The first experiment tested how early seed removal affected seedling growth, while the second experiment tested how early versus late seed removal affected seedling growth. For the first experiment, seed removal occurred when the seedlings' largest leaf reached 3 cm in length (approximately 1 week after shoot emergence). We used this length because we wanted to insure that seedlings with removed seeds would have the ability to fix their own carbon and we had previously determined that the chlorophyll content of 3 cm long leaves was approximately equivalent to that of fully expanded leaves (P. Kennedy, unpublished data). In the first experiment, there were five seedlings per treatment (treatments: large seed

removed, large seed attached, small seed removed, small seed attached) per time. The seedlings were harvested 2, 4, 8, 16, 32, 64, 128 days after the seed removal ($n=140$). For the second experiment, seed removal occurred at two time periods. Seeds were removed either when the seedlings' largest leaf was 3 cm or 64 days after that. There were eight seedlings per seed removal treatment (treatments: large seed removed, large seed attached, small seed removed, small seed attached) as well as an additional eight large-seeded seedlings and eight small-seeded seedlings for which the seeds were not removed ($n=48$). For the second experiment, all seedlings were harvested 128 days after the seedlings' largest leaf was 3 cm. Harvested seedlings were gently rinsed to remove soil, air dried at 80°C for 48 h, and then weighed for leaf, stem, and root biomass (g). The two experiments are hereafter referred to as "early removal staggered harvest" and "early vs. late removal single harvest".

To examine the effect of seed reserves on *Lithocarpus* seedling photosynthesis, we measured photosynthetic ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) rates at three time periods during seedling development. The first sampling period was 2–4 days after seed removal, the second was 49–82 days after removal, and the third was 95–128 days after removal (the wider range of variation in the latter two time periods is due to measurements being taken on all plants on the same day). The same five seedlings per treatment (treatments: large seed removed, large seed attached, small seed removed, small seed attached, $n=20$ total) were measured at the second and third time periods; however, the first time period measurements were done on a second set of seedlings grown from the same pool of seeds. The seedlings from the first time period came from different acorns because they were planted after the initial photosynthesis experiment was in already progress. Due to difficulty in germinating a sufficient number of seedlings from small seeds at this later time point, only seedlings from large seeds were grown (treatments: large seed removed, large seed attached, $n=7$ per treatment). At each sampling period, measurements were taken on one leaf per seedling using a Li-6400 portable gas exchange system (Li-Cor, Lincoln, Neb., USA). All measurements were made holding photon flux density at either 1,000 or 1,200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, reference carbon dioxide concentration at 400 $\mu\text{l l}^{-1}$, leaf temperature between 26 and 30°C, and flow rate at 500 mmol s^{-1} . Two additional physiological measures were also calculated: total plant photosynthesis ($\mu\text{mol s}^{-1}$), which was calculated by multiplying photosynthesis per unit leaf area ($\mu\text{mol m}^{-2} \text{s}^{-1}$) by total leaf area (m^2) and water use efficiency (WUE), which was calculated by dividing photosynthesis unit leaf area by stomatal conductance.

To examine the effect of seed reserves on *Lithocarpus* seedling carbon isotope ratios, we analyzed seeds, leaves, and roots. We sampled three randomly selected seedlings per treatment (treatments: large seed removed, large seed attached, small seed removed, small seed attached) and time (2, 4, 8, 16, 32, 64, and 128 days after seed removal).

From each dried seedling, a sample of each seed was shaved off; a sample of each leaf was cut off, and three random 1 cm sections of the tap root and an equivalent quantity of fine roots were removed ($n=252$ total). The respective seed, leaf, and root samples were placed in separate screw-type security capsules with one stainless steel ball bearing and ground in a Wig-L-Bug Crescent Amalgamator (Dentsply International, Surrey, UK) for 2–5 min into a fine powder. Four milligrams of powder of each sample were placed in a tin capsule (COSTEK, Valencia, Calif., USA) and samples were analyzed for isotopic ratios via elemental analyzer/continuous flow mass spectrometry (ANCA/SL elemental analyzer coupled with a PDZ Europa Scientific 20/20 Mass Spectrometer). The isotope ratio is expressed in "delta" notation (‰), where the isotopic composition of a material relative to that of a standard on a per mil deviation basis is given by, $\delta^{13}\text{C}=(R_{\text{sample}}/R_{\text{standard}}-1)\times 1,000$, where δ is the isotope ratio and R is the molecular ratio of heavy to light isotope forms. The standard for carbon is V-PDB.

Carbon isotopic values for all comparisons are best used in a relative manner because plants were grown in the greenhouse and therefore well-watered and subjected to air within the greenhouse bay that was slightly depleted in ^{13}C due to respiration. Here there could be a slight offset ($\sim 0.4\text{‰}$) because our analysis of the $\delta^{13}\text{C}$ of greenhouse air was -8.3 to -8.5‰ (well mixed air is usually -8‰ ; see Dawson et al. 2002). Though the offset would be very small, we acknowledge that it could have had some influence on our final $\delta^{13}\text{C}$ values. For this reason, comparisons with $\delta^{13}\text{C}$ of field grown plants should be made cautiously.

Statistical analyses

The "early removal staggered harvest" experiment was analyzed using a three-way fixed-factor analysis of variance (ANOVA). The predictor variables were seed size (large and small), seed presence (removed or attached), and time (2, 4, 8, 16, 32, 64, 128 days after seed removal). Total seedling biomass was the response variable. The data were $\log(X+1)$ transformed to improve variance homogeneity, however, the variances remained slightly heterogeneous (Cochran's C -test, $0.01 < P < 0.05$). Although this assumption could not be met, large and balance designed ANOVAs are generally robust to slight departures from homogeneity (Underwood 1997). Significant two-way interactions were tested a posteriori using analysis of covariance (ANCOVA) to determine if the slopes were significantly different.

The "early vs. late removal single harvest experiment" was analyzed using a two-way fixed factor ANOVA. The predictor variables were seed size (large and small) and seed presence (early, late, and no removal). The response variable was total seedling biomass. Variances were determined to be homogenous (Cochran's C -test, $P > 0.05$). A Tukey HSD test was used for a posteriori comparisons of means.

The effect of seed reserves on *Lithocarpus* seedling photosynthesis was analyzed using a series of one- or two-way fixed factor ANOVAs. For the first sampling period (2–4 days after seed removal), the predictor variable was seed presence (removed or attached). For the second and third sampling periods (49–82 and 95–128 days after seed removal, respectively), the predictor variables were seed size (large and small) and seed presence. For both analyses, the five response variables were photosynthesis per unit leaf area, stomatal conductance, total leaf area, total plant photosynthesis, and WUE. In each ANOVA model, the variances were determined to be homogenous after the data were log or $\log(X+1)$ transformed (Cochran's *C*-test, $P>0.05$).

The effect of seed reserves on *Lithocarpus* seedling $\delta^{13}\text{C}$ values was analyzed using a four-way fixed factor ANOVA. The predictor variables were seed size (large and small), seed presence (removed or attached), time (2, 4, 8, 16, 32, 64, 128 days after seed removal), and sample type (seed, leaf, root). The variances were determined to be homogenous (Cochran's *C*-test, $P>0.05$). All statistical tests were performed using SYSTAT 10.0 (SYSTAT Software, Richmond, Calif., USA) and considered significant at $P=0.05$.

Results

Seedling biomass

Seed reserves significantly affected *Lithocarpus* seedling biomass, but the effects varied through time (time \times seed presence: $F_{6,112}=4.415$, $P<0.001$; time \times seed size: $F_{6,112}=3.043$, $P=0.009$) (Fig. 1). Seedling biomass increased whether the seed was removed or not; However, the overall increase was significantly larger for seedlings with attached seeds than those with removed seeds

Table 1 Effects of time, seed size, and seed presence on the physiology of *L. densiflora* seedlings. The time periods are as follows: *I* 2–4 days after seed removal, *II* 49–82 days seed removal, *III* 95–128 days after seed removal. Total plant photosynthesis is calculated as the product of photosynthesis and leaf area and water

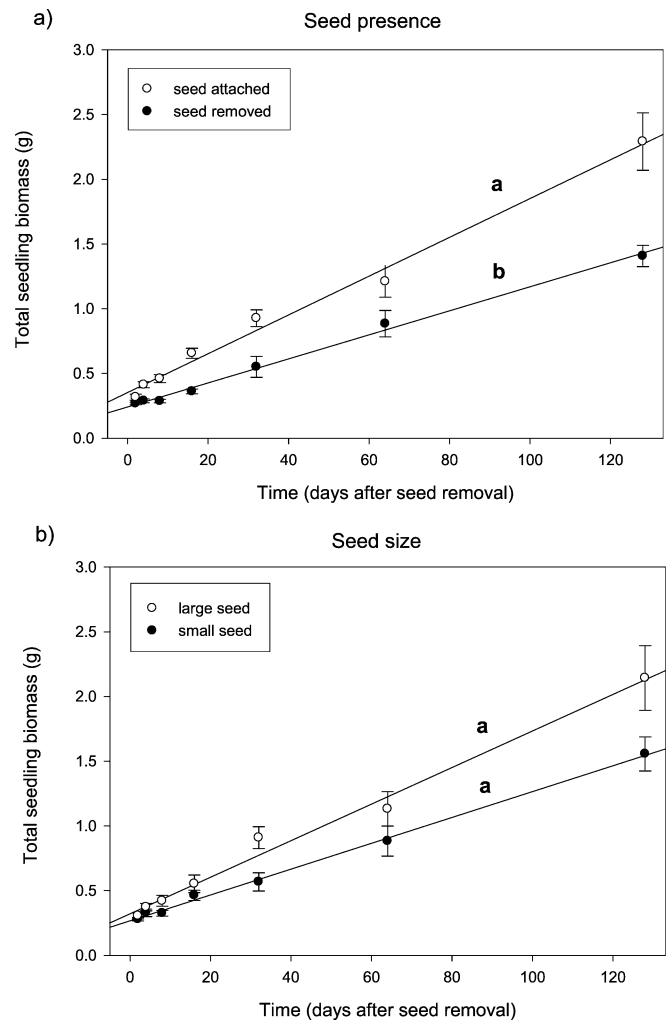
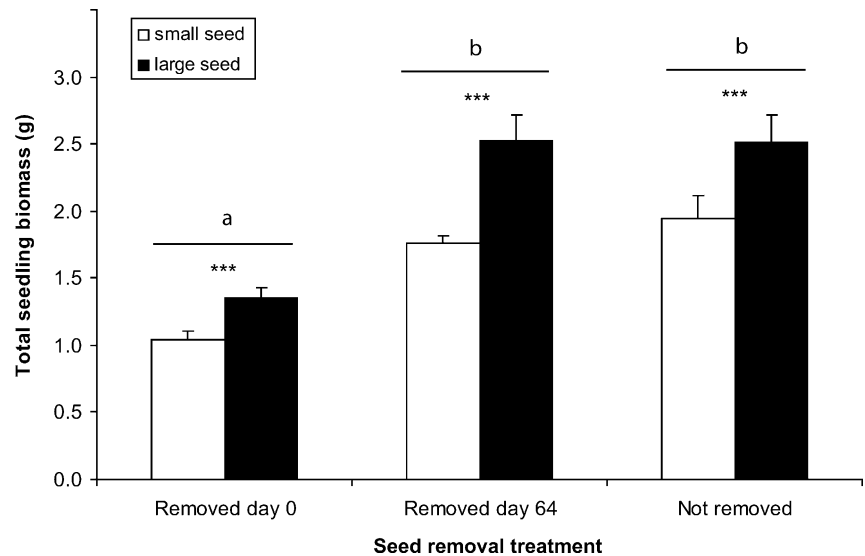


Fig. 1a, b Effects of seed presence (a) and seed size (b) on the early growth of *L. densiflora* seedlings. Circles represent mean total seedling biomass ± 1 SE. The trend lines summarize the results of a

use efficiency is calculated as photosynthesis divided by conductance. Values are compared using one- or two-way ANOVAs at each time period (see text for details). Different letters indicate significant differences at $P<0.05$

| Time period | Seed size | Seed presence | Photosynthesis ($\mu\text{mol m}^{-2}\text{s}^{-1}$) | | Conductance ($\text{mmol m}^{-2}\text{s}^{-1}$) | | Leaf area (m^2) | | Total plant photosynthesis ($\mu\text{mol m}^{-2}\text{s}^{-1}$) | | Water use efficiency ($\mu\text{mol}/\text{mmol}$) | |
|-------------|-----------|---------------|--|--------|---|--------|----------------------------|--------|--|--------|--|---------|
| | | | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| I | Large | Removed | 1.67 | 0.26 a | 0.09 | 0.01 a | 0.13 | 0.01 a | 0.22 | 0.04 a | 25.75 | 5.83 a |
| I | Large | Attached | 4.80 | 0.71 b | 0.10 | 0.02 a | 0.19 | 0.02 b | 0.92 | 0.20 b | 56.52 | 16.57 a |
| II | Small | Removed | 9.18 | 0.48 a | 0.18 | 0.01 a | 0.36 | 0.05 a | 3.23 | 0.38 a | 60.15 | 4.40 a |
| II | Small | Attached | 9.25 | 0.81 a | 0.14 | 0.02 a | 0.57 | 0.06 b | 5.14 | 0.51 b | 66.00 | 6.45 a |
| II | Large | Removed | 9.96 | 0.42 a | 0.16 | 0.02 a | 0.29 | 0.01 a | 2.88 | 0.21 a | 57.02 | 2.58 a |
| II | Large | Attached | 9.37 | 0.79 a | 0.15 | 0.02 a | 0.61 | 0.06 b | 5.87 | 1.12 b | 68.97 | 7.71 a |
| III | Small | Removed | 8.46 | 0.61 a | 0.20 | 0.01 a | 0.37 | 0.03 a | 3.44 | 0.42 a | 50.88 | 3.06 a |
| III | Small | Attached | 7.80 | 0.10 b | 0.14 | 0.01 b | 0.57 | 0.11 b | 4.45 | 0.77 b | 57.72 | 2.11 a |
| III | Large | Removed | 10.55 | 0.39 a | 0.17 | 0.02 a | 0.32 | 0.04 a | 2.95 | 0.14 a | 51.86 | 2.49 a |
| III | Large | Attached | 7.84 | 0.39 b | 0.14 | 0.01 b | 0.61 | 0.05 b | 4.65 | 0.30 b | 54.52 | 1.41 a |

Fig. 2 Effect of the timing of seed removal on seedling growth of *L. densiflora* seedlings. Bars represent mean total seedling biomass ± 1 SE. Different letters above each seed treatment indicate significant differences as determined by a posteriori HSD Tukey tests ($P < 0.05$). Seed size also significantly affected seedling biomass within each seed removal treatment as indicated by the asterisks ($P < 0.001$). Day 0 is defined as the day in which the seedlings' largest leaf reached 3 cm (see text for details)



(Fig. 1a). Similarly, seedling biomass increased for seedlings with small and large seeds, but the overall increase between seed sizes was not significantly different (Fig. 1b). Interactions between seed size and seed treatment ($F_{1,112}=1.999$, $P=0.160$) and time, seed size, and seed treatment ($F_{6,112}=0.535$, $P=0.781$) were not significant.

The timing of seed removal also significantly influenced seedling biomass ($F_{2,41}=33.114$, $P < 0.001$) (Fig. 2). If the seed was removed when the length of the largest leaf was 3 cm, seedling biomass was significantly smaller than seedlings for which the seed was not removed. In contrast, if the seed was removed 64 days after the largest leaf was 3 cm long, seedling biomass was not significantly different from seedlings for which the seed was never removed. The biomass response was consistent across seedlings grown from small and large seeds (Fig. 2).

Seedling photosynthesis

The photosynthetic rates of seedlings at the first sampling period (2–4 days after seed removal) differed significantly by seed presence ($F_{1,12}=9.357$, $P=0.010$). Seedlings with attached seeds had a mean photosynthetic rate more than two times higher than those with removed seeds (Table 1). Because the leaf areas of the seedlings with attached seeds were significantly larger ($F_{1,12}=5.239$, $P < 0.041$), total plant photosynthesis of seedlings with attached seeds was also significantly higher than those with removed seeds ($F_{1,12}=24.305$, $P < 0.001$). Stomatal conductance and WUE did not differ significantly between seedlings with attached and removed seeds (stomatal conductance: $F_{1,12}=2.904$, $P=0.173$; WUE: $F_{1,12}=3.069$, $P=0.105$).

At the second sampling period (49–82 days after removal), there was no significant difference in seedling photosynthetic rates by seed presence ($F_{1,15}=0.525$, $P=0.480$) or seed size ($F_{1,15}=0.302$, $P=0.591$). However, because the leaf area of the seedlings with attached seeds was significantly larger than those with removed seeds

($F_{1,15}=33.389$, $P < 0.001$), the total plant photosynthesis of seedlings with attached seeds was significantly higher ($F_{1,15}=26.543$, $P < 0.001$). Neither stomatal conductance nor WUE were significantly different by seed presence or seed size (stomatal conductance: seed presence: $F_{1,15}=1.342$, $P=0.265$; seed size: $F_{1,15}=0.147$, $P=0.707$; WUE: seed presence: $F_{1,15}=2.708$, $P=0.121$; seed size: $F_{1,15} < 0.001$, $P=0.988$) and there were no significant two-way interactions.

In contrast to the first sample period, the photosynthetic rates of seedlings with attached seeds were significantly lower than those with removed seeds at the third sample period (95–128 days after removal; $F_{1,13}=22.544$, $P < 0.001$) (Table 1). Although seedlings with attached seeds had lower photosynthetic rates, their leaf area was significantly larger than those with removed seeds ($F_{1,13}=22.544$, $P < 0.001$), resulting in significantly higher total plant photosynthesis ($F_{1,13}=8.989$, $P=0.010$). Stomatal conductance also differed significantly by seed presence ($F_{1,13}=5.091$, $P=0.041$), with conductance being lower in seedlings with attached seeds than those for which the seed was removed. However, there was no significant difference in stomatal conductance by seed size ($F_{1,13}=3.245$, $P=0.093$). WUE did not differ significantly by either seed presence or seed size (seed presence: $F_{1,13}=4.406$, $P=0.056$, seed size: $F_{1,13}=0.240$, $P=0.632$) and there were no significant two-way interactions.

Seedling $\delta^{13}\text{C}$

The $\delta^{13}\text{C}$ of the seeds, leaves, and roots varied consistently, with seeds being more enriched in ^{13}C (-24.19 ± 0.09 , mean \pm SE) than either the roots (-26.54 ± 0.12) or the leaves (-27.79 ± 0.17). $\delta^{13}\text{C}$ signatures were significantly affected by sample type ($F_{2,227}=141.019$, $P < 0.001$), time ($F_{1,227}=89.192$, $P < 0.001$), seed size ($F_{1,227}=18.898$, $P < 0.001$) and seed presence ($F_{1,227}=15.972$, $P < 0.001$), however, there were a number of significant higher-order interactions. The seed size by

time interaction ($F_{1,227}=10.009$, $P=0.002$) showed high variability through time with only day 32 and 64 samples clearly differing in $\delta^{13}\text{C}$. By day 128, seedlings from large and small seeds had similar $\delta^{13}\text{C}$ making biological relevance of this interaction unclear. The seed size by sample type interaction ($F_{1,227}=3.175$, $P=0.044$) revealed no differences in $\delta^{13}\text{C}$ values for seed and leaf samples, but the root $\delta^{13}\text{C}$ values of seedlings with small seeds were significantly lower than those of seedlings with large seeds (Fig. 3). There was also a three-way interaction among sample type, time, and seed presence ($F_{1,227}=4.192$, $P=0.016$) (Fig. 4). Seedlings with attached seeds had root $\delta^{13}\text{C}$ values more similar to that of the seed than to that of the leaf through time, while seedlings with removed seeds had root $\delta^{13}\text{C}$ values more similar to that of the leaf than that of the seed through time. Additionally, the differences between leaf and seed as well as root and seed $\delta^{13}\text{C}$ were greater for seedlings with removed seeds. All other higher-order interactions were not significant.

Discussion

We found that variation in seed reserves significantly affected the biomass of *Lithocarpus* seedlings. Consistent with our predictions, seedlings from large seeds had significantly greater biomass than those with small seeds and seedlings with attached seeds had significantly greater biomass than those with removed seeds. These similar results are not surprising because the availability of a given resource in the seed is largely a function of the total seed mass (Kitajima and Fenner 2000). The positive correlation between seed size and seedling biomass documented here concurs with the results of numerous studies within the family Fagaceae (Aizen and Woodcock 1996; Kormanik et al. 1998; Bonsil 1998; Merouani et al. 2001). Furthermore, the seed reserve and seedling biomass relationship we documented is similar to Bonsil (1998), who found that cotyledon excision significantly reduced subsequent seedling growth of both *Q. rugosa* and *Q. laurina*.

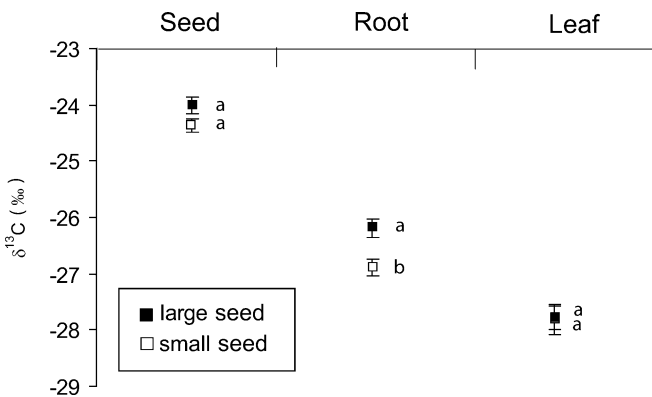


Fig. 3 Comparisons of $\delta^{13}\text{C}$ values of seeds, roots, and leaves of seedlings with large and small seeds. Squares represent mean $\delta^{13}\text{C} \pm 1$ SE across all time periods. Different letters indicate significant differences as determined by a posteriori HSD Tukey tests ($P<0.05$)

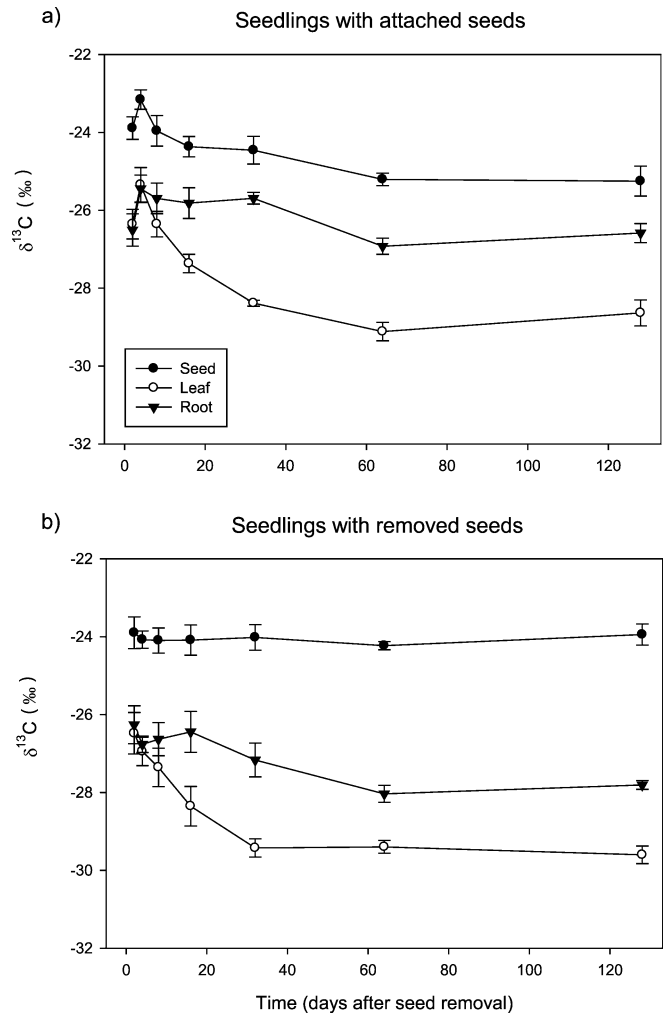


Fig. 4a, b $\delta^{13}\text{C}$ values of seeds, leaves, and roots for seedlings with attached (a) and removed seeds (b) through time. Circles represent mean $\delta^{13}\text{C} \pm 1$ SE at each time period (2, 4, 8, 16, 32, 64, and 128 days after seed removal)

The timing of seed removal also significantly affected seedling growth. Our results suggest that 2 months after the initial leaf flush, the positive effect of seed presence is no longer present. This result is consistent with Sonesson (1994), who found that after a period of 2 years, *Q. robur* seedling size was significantly affected by soil nutrient availability but not the presence of seed reserves. However, we also observed that seedlings with attached seeds still had greater growth than those with removed seeds after 4 months. We attribute this to the advantage that seed energy and nutrients provide towards the development of seedling leaf area. Because the rate of early plant growth is typically exponential, small initial differences in leaf area will cause increasingly larger differences in growth through time (Harper 1977; Weiner 1990). In a similar experiment to this study, Jarvis (1963) removed the cotyledons of *Q. petraea* after initial leaf flush and examined seedling weight after 1 year of growth. He showed that seedling size was positively correlated with initial seed size regardless of whether the cotyledons had

been removed. These results suggest that the effect of seeds on seedling growth occurs very early in development and that once seedlings have fully photosynthetically active leaves, they may quickly lose their dependence on cotyledonary reserves. It is important, however, to emphasize that seeds provide more than just carbohydrates to seedlings. Their role in seedling nutrient dynamics may reveal different patterns of reserve dependence. For example, Brookes et al. (1980) documented that *Q. robur* and *Q. petraea* seedlings were dependent on cotyledon potassium, magnesium, and phosphorus throughout their entire first year of growth.

The presence of seeds also significantly affected seedling photosynthesis and total leaf area. We believe the significantly higher photosynthesis of seedlings with attached seeds at the first sampling period may have been due to some resource within the seed that is important for photosynthesis or the more rapid development of leaves in young seedlings. Qualitatively, leaves on seedlings with attached seeds appeared to develop more rapidly, but we did not quantify this pattern. Larger leaf areas of the seedlings with attached seeds also resulted in significantly greater total plant photosynthesis. The same pattern was observed by Burris et al. (1973), who found that the photosynthetic rates of seedlings grown from different seed sizes were not significantly different, but that the leaf areas of the individuals from large seeds were greater, which resulted in higher total plant photosynthesis.

When both seed size and presence were manipulated (the second and third sampling periods), we found that only seed presence significantly affected seedling physiology. We believe this result is due to the nature of the two treatments rather than different effects of seed size and presence. The seed presence treatment had a greater effect on seed reserve mass than the seed size treatment, and thus caused a larger leaf area effect. Contrary to the previous two sampling periods, seedling photosynthesis per unit leaf area and stomatal conductance at the third sampling period was significantly lower for seedlings with attached seeds than with removed seeds. Reasons for these differences are not clear. Despite the lower photosynthesis per unit leaf area, total plant photosynthesis at the third sampling period was still significantly higher for seedlings with attached seeds.

The results of the isotope analyses further suggest that seed reserves have a significant impact on seedling growth. We found that the $\delta^{13}\text{C}$ values of the leaves and roots were more similar to that of the seed for seedlings with attached seeds than those with removed seeds. We believe that this lower difference is due to the direct movement of carbon from the seed to the seedling; however, $\delta^{13}\text{C}$ can also be influenced by changes in the water use efficiencies (WUE) of the seedlings (Farquhar et al. 1989). An increase in WUE via decreases in stomatal conductance and/or changes in photosynthetic biochemistry results in lower enzymatic discrimination against $^{13}\text{CO}_2$, thus higher WUE results in higher $\delta^{13}\text{C}$. However, we observed that seedlings with attached seeds had similar WUE to seedlings with removed seeds throughout the

duration of the experiment, which indicates that WUE was not the primary factor driving the differences in $\delta^{13}\text{C}$ between seedlings with attached and removed seeds.

The isotope results are also consistent with our prediction that the importance of the seed as a carbon source decreases through time. Two and 4 days after seed removal much of the carbon in the plant leaf is derived from the seed. The leaf $\delta^{13}\text{C}$ value rapidly diverges from that of seed as the seedling starts to photosynthesize at greater rates because photosynthate has a more negative $\delta^{13}\text{C}$ value than that of seed. In contrast, the roots continue to receive some carbon input from the seed up to day 64. In plants with attached seeds, the seed and root $\delta^{13}\text{C}$ values stabilize 64 days after seed removal, implying no further carbon exchange. There was also an overall difference in the root $\delta^{13}\text{C}$ between seed sizes, with seedlings with large seeds having a higher $\delta^{13}\text{C}$. We believe this difference was due to the larger amount of resources moved from large seeds to seedlings, resulting in $\delta^{13}\text{C}$ values more similar to those of the seeds than seedlings with small seeds.

In seedlings with attached seeds, the seed $\delta^{13}\text{C}$ values decreased with time, implying the movement of carbon compounds with high $\delta^{13}\text{C}$ values to the seedling. Of the major components of seeds, proteins and carbohydrates have significantly higher $\delta^{13}\text{C}$ values than that of the whole plant, while the $\delta^{13}\text{C}$ values of lipids are lower (Ghashghaie et al. 2001; Boutton and Yamasaki 1996). Given that acorns are composed of 10% protein, 57% carbohydrates, and 33% lipids (USDA 2003), our data suggest that the resources being moved from the seed are mostly carbohydrates and proteins. Higher carbohydrate than lipid movement from seeds to seedlings has also been observed in *Juglans regia* (Chenevard et al. 1994). Additionally, we observed that $\delta^{13}\text{C}$ values of both leaves and roots were 2‰ less enriched than that of the seed regardless whether the seed was attached or removed at day 2, indicating that initial leaf and root production comes from stored carbohydrates.

We have demonstrated that seed reserves have multiple effects that are important for the early performance of *Lithocarpus* seedlings. Future studies that more specifically identify the timing of when oak seedlings become autonomous from their seeds would deepen our understanding of the effects of post-germination seed removal. Additional work linking physiology and carbon transfer using techniques such as isotopic tracers would also further elucidate the role of seed reserves in seedling development. Finally, examining the specific composition of the carbon compounds transferred between the seed and seedling throughout seedling development would help clarify the physiological role of seeds in *Lithocarpus* seedling development.

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