Seedling Performance in Two Oak Species

The Effects of Seed Size, Cotyledon, Reserves, and Herbivory

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The role of seed size in seedling performance has received consider able attention (Foster, 1986; Mazer, 1989; Seiwa and Kikuzawa, 1991; Westoby, Jurado, and Leishman, 1992), and differences in seed size among species have been found to relate to the ecological conditions in which plants establish.

Some of the patterns detected in comparisons among species are also found within species. A positive relationship among seed size and seedling establishment and growth has been reported in a variety of species (Weis, 1982; Stanton, 1984; Weller, 1985), including oaks (McComb, 1934; Tecklin and McCreary, 1991), but there have been few attempts to explore the relationship between seed size and the performance of oak seedlings under a variety of ecological conditions.

Oaks are frequently dispersed by jays and small rodents, which are also their main seed predators. It has been argued that the rapid germination (nondormancy) and establishment of white oaks is a mechanism that allows escape from post-dispersal seed predation, since it permits the seedling to escape seed recovery by caching animals (Barnett, 1977; Fox, 1982). However, the probabilities of survival and continued growth of a seedling after detachment of the acorn (cotyledons) may be affected by the amount of reserves originally available to the seedling. Herbivory, frost, drought, and pathogens are other common challenges facing a seedling early in its life and its ability to cope with them is likely affected by the presence or absence of cotyledons, the size of the seed from which it originated, and the time elapsed since germination.

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Quercus rugosa (subgenus *Leucobalanus*, *Quercus*) is a 15-20 m tall tree, widely distributed in Mexico, that frequently coexists with *Q. laurina* (subgenus *Erythrobalanus*), a 15-30 m tall tree (González-Villareal, 1986; Bello and Labat, 1987). Both species can be found in pure oak stands or intermingled with pines at higher elevations.

In this study the effects of seed size and presence or absence of cotyledonary reserves on survival and growth of *Q. rugosa* and *Q. laurina* seedlings were evaluated, both in presence and absence of simulated herbivory. The objectives were to determine (1) whether differences in the ability of one-month-old seedlings to survive detachment of cotyledons are related to seed size, (2) whether reserves remaining in the cotyledons at time of detachment still contribute to survival and growth, (3) the interaction of herbivory and seed size on seedling survival and growth, and (4) the consequences of cotyledon removal on the response of the seedlings to simulated herbivory.

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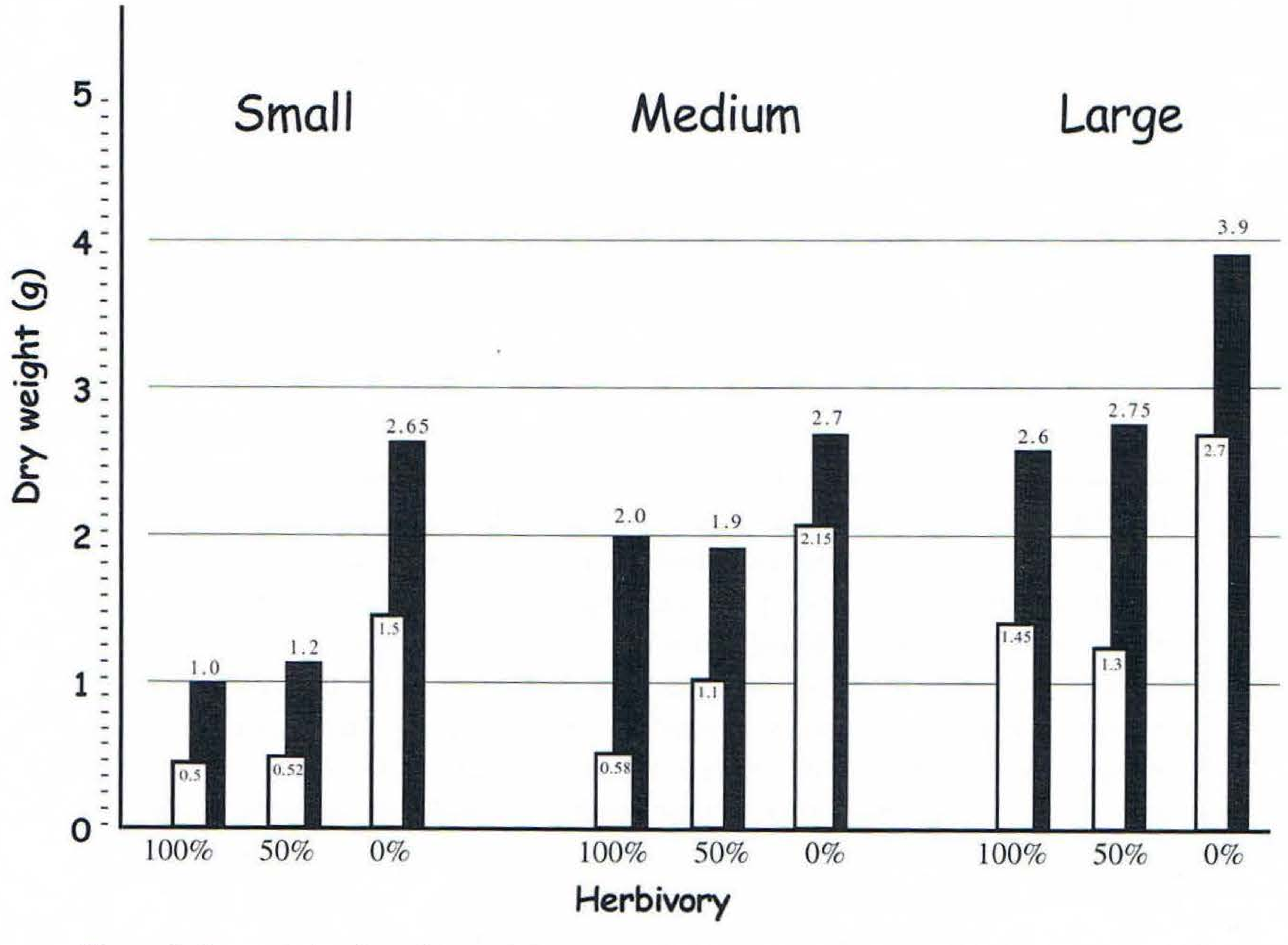

Figure 1. Dry weight of seedlings of Q. rugosa (dark) with cotyledons, (white) without cotyledons.

Table 1. Survival (proportion) of *Quercus rugosa* and *Quercus laurina* seedlings in the presence and absence of cotyledons (+/- cot.) at three levels of simulated herbivory. The numbers in parentheses indicate the expected values of the logistic model. Since herbivory did not have a significant effect in *Q. laurina*, the expected values are the same for all three herbivory levels.

	Seed size					
	Small		Medium		Large	
Herbivory	+ cot.	- cot.	+ cot.	- cot.	+ cot.	- cot.
			Quercus rugosa			
0 %	1.00	0.81	1.00	0.95	1.00	0.96
	(0.98)	(0.81)	(0.99)	(0.94)	(1.00)	(0.99)
50 %	0.91	0.52	1.00	0.73	1.00	1.00
	(0.92)	(0.51)	(0.98)	(0.80)	(1.000)	(0.96)
100 %	0.80	0.36	0.95	0.71	1.00	0.92
	(0.85)	(0.34)	(0.96)	(0.67)	(0.99)	(0.93)
			Quercus laurina			
0 %	0.75	0.22	0.69	0.23	0.83	0.35
50 %	0.85	0.21	0.67	0.07	0.83	0.06
100 %	0.50	0.11	0.56	0.00	0.95	0.06
	(.74)	(0.14)	(0.65)	(0.09)	(0.82)	(0.21)

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Materials and Methods

During January 1993, acorns were collected from below six different trees of both *Q. rugosa* and *Q. laurina* in the Ajusco Hills, south of Mexico City. Each viable acorn was marked with a number and its individual weight recorded. A subsample (N = 62 for *Q. rugosa*, N = 54 for *Q. laurina*) was oven dried for 48 hours at 80°C and used to evaluate the relationship between fresh and dry weight and the proportion of dry weight in the cotyledons. Fresh-weight data of all the seeds (1185 for *Q. rugosa*, 1075 for *Q. laurina*) were used to assign each seed to one of three size categories, small (<1.5 g), medium (1.5 - 2.5 g for *Q. rugosa*, 1.5 - 2.0 g for *Q. laurina*), and large (>2.5 g for *Q. rugosa*, >2.0 g for *Q. laurina*) within each species.

In May 1993, the seeds were soaked for 24 hours in a 1 g/L giberellic acid solution in order to synchronize germination, and placed horizontally on trays containing agrolita. Every other day the trays were watered and the seeds inspected to record the date of germination.

Two weeks after germination, seedlings were carefully transplanted to black plastic bags filled with homogenized soil from the study site. When seedlings were one-month old, each member of the three seed size classes was randomly assigned to one of six experimental combinations for a total of 18 treatments: three seed sizes (small, medium, and large), three levels of simulated herbivory (O, 50, or 100% of shoot length removed) and two levels of cotyledons (presence-absence). Cotyledon excision and simulated herbivory were applied simultaneously, at which time all seedlings had completed their first burst of shoot elongation and had expanded leaves.

Twenty-five seedlings of *Q. rugosa* were assigned to each of the 18 treatment combinations. Due to poor germination, only 17 *Q. laurina* seedlings were available for each treat-

Table 2. *Quercus rugosa*: F statistics and probabilities for ANOVAs.

Variable	Seed size		Herbivory		Cotyledons		Size x Herb.		Size x Cot.		Herb x Cot.		S x H x C	
	F	p	F	p	F	p	F	p	F	p	F	p	F	p
df treatment [a]	df 2		df 2		df 1		df 4		df 2		df 2		df 4	
Shoot height	22.81	< 0.00001	274.75	< 0.00001	98.32	< 0.00001	1.01	NS	3.45	0.033	6.46	0.002	2.50	0.043
Leaf area	28.76	< 0.00001	63.86	< 0.00001	97.56	< 0.00001	2.31	NS	0.27	NS	3.72	0.03	1.86	NS
Diameter	69.36	< 0.00001	111.45	< 0.00001	139.78	< 0.00001	5.03	0.006	0.96	NS	0.35	NS	3.32	0.01
No. leaves	3.89	0.02	33.47	< 0.00001	7.46	0.007	6.81	0.00003	2.44	NS	6.95	0.001	2.07	NS
Mean area per leaf	31.86	< 0.00001	[illegible]7.72	< 0.00001	96.09	< 0.00001	0.72	NS	0.31	NS	3.44	0.03	1.89	NS
Biomass														
Shoot	86.83	< 0.00001	186.45	< 0.00001	251.25	< 0.00001	2.68	0.03	0.89	NS	5.50	0.005	4.38	0.002
Leaves	48.97	< 0.00001	70.57	< 0.00001	141.89	< 0.00001	4.39	0.002	0.26	NS	4.06	0.02	3.24	0.01
Root	90.49	< 0.00001	86.60	< 0.00001	145.76	< 0.0001	4.37	0.002	0.48	NS	6.01	NS	3.12	0.02
Total	101.41	< 0.001	123.25	< 0.00001	219.18	< 0.00001	6.12	0.001	0.89	NS	10.34	0.00005	4.68	0.043

[a] df error: 235 for size variables, 233 for biomass variables

ment. During application of treatments, shoot height and number of leaves were recorded and those seedlings that kept their cotyledons were superficially unearthed and reburied in the same way as those from which cotyledons were removed.

Seedlings were placed on wire-mesh tables in a nursery covered with green plastic mesh in a completely randomized design, with a border row of extra seedlings around each table to minimize edge effects. Rainfall was supplemented by watering to maintain soil moisture. Beginning 3-4 weeks after application of treatments, seedling height and number of leaves were recorded twice a month and the length of each leaf was measured once a month. Total leaf area was estimated by means of a previously developed regressions of leaf area versus leaf length. Seedlings were harvested 5 months after treatments, and shoot length, basal diameter, and leaf area were measured. Seedlings were oven dried for 48 hours at 80°C and each seedling part (root, shoot, and leaves) was individually weighed.

Survival data was analyzed by means of logit analysis for binomial data (i.e., proportions). Variables measuring final size and biomass of each seedling part were analyzed by means of ANOVA. When necessary, data were transformed in order to fulfill the requirements of homoscedasticity for the ANOVAs. Relative height growth rate (RGR) was also analyzed. This was the height growth of each seedling relative to its initial height at the time the treatments were applied.

Results and Discussion

Seed weight

The two species differed significantly in their mean seed weights. The range of variation of seed sizes was quite different in the two species (mean = 1.99 g, SD = 1.14 for *Q. rugosa* and mean = 1.75 g, SD = 0.48 for *Q. laurina*). There was a strong correlation between acorn fresh and dry weights for both species (r^2 = 0.998 for *Q. rugosa*, r^2 = 0.997 for *Q. laurina*), as well as between total fresh weight and cotyledon dry weight (r^2 = 0.995 for *Q. rugosa*, r^2 = 0.992 for *Q. laurina*), suggesting that seed fresh weight is a good indicator of the amount of reserves available for seedling growth.

Quercus rugosa establishes in a broader range of microsites than *Q. laurina*. The larger seed-size variation of *Quercus rugosa* may partially account for its ability to establish in a mosaic of microsites with different physical and biotic conditions, and thus broaden its regeneration niche (sensu Grubb, 1977).

Seedling survival

Seed size had a clear effect on survival of *Q. rugosa*, with seedlings from large seeds having the highest survival and seedlings from small seeds the lowest (Table 1). Within each size class, survival was reduced by removal of the cotyledons and by increasing levels of shoot removal. The effects of cotyledon removal, seed size, and herbivory were highly significant, and the interactions between these variables were not.

Cotyledon loss caused greater mortality in *Q. laurina* and seed size did not compensate for this loss (Table 1). The effects of seed size and cotyledon removal were significant, with the predicted drop in survival due to cotyledon loss 60% in both large and small-seeded seedlings. Herbivory and the interactions among variables were not significant. Results for *Q. laurina* must, however, be treated with caution because some seeds and seedlings showed evidence of pathogen attack (probably a fungus), reducing survival even in seedlings from the control group.

The large drop in survival as a consequence of cotyledon removal in both species shows that cotyledons still contribute to seedling survival one month after germination, while the higher survival after cotyledon removal in *Q.*

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rugosa than in *Q. laurina* may indicate a more extended dependence on these reserves in the latter.

The results for *Q. rugosa* have implications for the hypothesis of non-dormancy as a means of escaping post dispersal seed predation, as the probabilities of surviving cotyledon detachment are significantly higher in large-seeded seedlings. Although predators would prefer to excise the cotyledon as early as possible, before its food reserves are consumed by the seedling, early excision of the acorn greatly reduces its chances of surviving, especially for small-seeded seedlings. Previous trials showed that seedlings are unable to survive if cotyledons are removed 7-15 days after germination.

Seedling growth

Quercus rugosa. The effect of seed size on height was evident throughout the growing season regardless of the level of herbivory. At the time the treatments were applied, there were already significant differences in height and number of leaves between seedlings coming from different seed sizes. Leaf development was greater in seedlings from medium and large seeds (median = 5 leaves) than from small seeds (median = 3 leaves).

There was a highly significant effect of the three factors studied on final shoot height, leaf area, basal diameter, number of leaves, and mean area per leaf (Table 2). The herbivory-

Table 3. ANOVAs on relative growth rates.

Effect	F	df	p
Q. rugosa			
Size	12.28	2	<0.001
Herbivory	792.57	1	<0.001
Cotyledons	59.65	1	<0.001
Size x Herb.	8.65	2	<0.001
Size x Cot.	4.34	2	0.014
Herb x Cot.	15.79	1	<0.001
S x H x C.	1.76	2	0.173
Error		215	
Q. laurina			
Size	1.03	2	0.362
Herbivory	59.95	1	<0.001
Size x Herb.	0.29	2	0.747
Error		62	

cotyledon interaction was significant for almost all variables, because the effect of increasing levels of herbivory was amplified by the absence of cotyledons.

The conspicuous effect of herbivory on shoot height persisted until the end of the experiment. It also reduced leaf area in all seed sizes, although little difference was observed between 50 percent and 100 percent herbivory. In large-seeded seedlings that kept their cotyledons, there was a noticeable recovery in leaf area towards the end of the growing season. The effects of herbivory were always mitigated by the presence of cotyledons, with 50-150 percent more final leaf area relative to seedlings without cotyledons.

The three variables studied had a significant effect on total, root, shoot, and leaf dry weight (Table 2). In all cases, the most conspicuous effect was due to cotyledon retention, followed by herbivory and seed size. The seed size-herbivory interaction was significant in all cases, due to the greater impact herbivory had on dry weight of small-seeded seedlings. Mean total dry weight responded in a manner similar to that of seedling parts. In general, the two levels of herbivory had a similar effect, but were clearly different from the control group (Fig. 1). There was also a trend for the effect of cotyledon retention to be more conspicuous when herbivory occurred.

Seed size, herbivory, and cotyledons, as well as all second-order interactions, had a significant effect on the relative growth rate (RGR) of *Q. rugosa* (Table 3). This analysis excluded seedlings without herbivory, due to differences in variability in the response of seedlings with and without herbivory. In general, seedlings with 100 percent herbivory had higher RGRs than those with 50 percent, and RGRs increased with seed size. The lack of cotyledons caused a reduction in RGRs in all cases, although this reduction was smaller in seedlings from large seeds. Differences between seedlings with and without cotyledons were more pronounced in the 100 percent than in the 50 percent shoot loss.

The proportion of dry weight accounted for by the root (root-weight ratio) in seedlings of *Q. rugosa* that did not experience herbivory differed significantly with seed size (small = 0.47, medium = 0.48, large = 0.53). The presence or absence of cotyledons did not affect this relationship. This result reinforces the view that in oaks the seed reserves are quickly directed to the root (McComb, 1934; Grime and Jeffrey, 1965), where they remain available for seedling resprout (Matzuda and McBride, 1986; Crow, 1988; Walters, Kruger and Reich, 1993) after disturbance-caused death of aerial biomass. The fact that root biomass was reduced by herbivory may reflect the utilization of stored reserves in the production of a new shoot.

Quercus laurina

The low number of seedlings surviving cotyledon detachment did not allow a sound comparison between the growth parameters of seedlings with and without cotyledons, so comparisons were made only among those treatments with cotyledons. As with *Q. rugosa*, one month after germination there were already significant differences in height and leaf number among seedlings from small, medium, and large seeds.

Differences in height due to herbivory were maintained in seedlings of the three size classes throughout the growing season. Relative height growth rates were not significantly affected by seed size (Table 3). The growth of seedlings without cotyledons could only be observed in a few individuals but the results suggest that cotyledon loss had a great impact on both seedling height and leaf area, as was the case for *Q. rugosa*.

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Final leaf area and basal diameter of *Q. laurina* were both significantly affected by seed size and herbivory (Table 4). Total number of leaves was significantly affected by seed size, and seedlings from large seeds had an average of 3.5 more leaves than seedlings from small- or medium-sized seeds. Mean area per leaf was affected by herbivory, and seedlings without herbivory had larger leaves.

With the exception of shoot weight, seed size was more important than herbivory in the biomass attained at the end of the season, although both variables had significant effects on almost every seedling part (Table 4). The strong effect of herbivory on shoot weight, as on height, indicates that seedlings are unable to recover from loss of aerial tissue in one growing season.

As with *Q. rugosa*, *Q. laurina* seedlings from large seeds had a clear advantage in most cases, while there were not always differences between seedlings from small- and medium-sized seeds. Herbivory resulted in a clear decrease in total dry weight. Seed size did not have a significant effect on root-weight ratio in *Q. laurina*.

Conclusions

In both species there was a clear effect of seed size on seedling growth, both initially and at the end of the growing season. In *Quercus rugosa*, the consistent increase in RGRs with seed size both in seedlings with and without cotyledons shows the importance of having a large supply of reserves from the very start. The fact that seed-size did not affect RGR in *Q. laurina* suggests that differences among seed size classes in height at 6 months are determined principally during the first month.

In both species the removal of 50 percent of the shoot resulted in the loss of most leaves and frequently produced shoot dieback. This, combined with the higher subsequent RGR of plants in the 100 percent shoot loss treatment, resulted in seedlings from both levels of herbivory having similar final size and biomass.

The relationship between seed and seedling size in oaks has been described before (McComb, 1934; Triphati and Kahn, 1990; Tecklin and McCreary, 1991). This study shows that in *Q. rugosa* the capacity of seedlings to recover from herbivory is mediated by seed size. Nevertheless, differences in height and biomass

Table 4. *Quercus laurina*: F statistics and probabilities for ANOVAs.

Variable	Seed size F	Seed size p	Herbivory F	Herbivory p	Size x Herb. F	Size x Herb. p
df treatment [a]	df 2		df 2		df 4	
Leaf area	9.97	0.0001	4.14	0.02	1.89	NS
Diameter	7.25	0.001	4.43	0.015	1.56	NS
No. leaves	3.78	0.03	0.57	NS	0.89	NS
Mean area per leaf	2.10	NS	4.14	0.02	0.55	NS
Biomass						
Shoot	18.22	< 0.00001	42.27	< 0.00001	2.19	NS
Leaves	12.76	0.00002	4.02	0.02	1.00	NS
Root	19.98	< 0.00001	1.99	NS	1.22	NS
Total	26.09	< 0.0001	15.11	< 0.00001	1.94	NS

[a] df error: 77

between uncut seedlings and those that suffered artificial herbivory were maintained until the end of the study period. Welker and Menke (1990) also found that severely defoliated *Quercus douglasii* seedlings did not accumulate biomass to levels that approached non-defoliated seedlings.

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