Growth of *Quercus robur* seedlings after experimental grazing and cotyledon removal

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SUMMARY

Grazing of leaves and stem or removal of the cotyledons, partly or completely, are two possible dangers for seedlings of *Quercus robur* that could influence their growth and survival. Acorns rely to a great extent on caching by animals for their dispersal. It has been suggested that the large nutrient reserve in the acorn is used to recover from grazing, or to grow longer to reach the light from a cache in the soil. We designed two experiments in which we studied (i) the effects on the seedlings of artificial grazing and removal of cotyledons and (ii) the effects of grazing after reduction of the cotyledons with acorns sown at different depth. Manipulated seedlings in both experiments produced a new leaf-set. The necessary resources are taken from the development of the shoot and the root system, except after severe defoliation when some resources were taken from the cotyledons. Defoliation and grazing caused the shoot and root system to become smaller and probably more vulnerable to, e.g. grazing, trampling or drought. Caching depth only affected emergence day. These results suggest that the cotyledons are not of vital importance for regrowth of the seedlings, but they may be of primary importance for dispersal of acorns.

Key-words: acorn, caching depth, grazing, resource allocation, seed predation.

INTRODUCTION

Factors regulating the survival of tree seedlings are important for the entire process of establishment and thus also for the composition and distribution of trees in a forest (e.g. Liu 1993). The pedunculate oak *Quercus robur* L. has an important impact on the biodiversity, particularly in boreo-nemoral forest landscape, since many cryptogams, invertebrates and vertebrates occur here in association with living or dead oaks.

Seedlings of *Q. robur* are often eaten or damaged by various animals, both invertebrates (Watt 1919; Ashby 1959; Tanton 1965; Shaw 1974) and vertebrates (Morgan 1991). We have observed that seedlings of *Q. robur* are sometimes completely defoliated, especially when growing near mature conspecific trees. Vertebrate grazing occurs during the whole growing season and is not restricted to the leaves. Rabbits and hares usually bite off the top of the shoot. Browsing by cattle, sheep or voles damages the seedling more severely as these animals cut off the stem close to the ground (Mellanby 1968; Shaw 1974; Ostfeld & Canham 1993).

It is likely that seedlings are more sensitive to defoliation than established trees, and resources necessary for regrowth of new leaves are probably taken from the stem, the root or the cotyledons (as *Quercus* have hypogeic germination). It is argued that the cotyledons act as the only nutrient source during the first year and thus have an important impact on seedling growth, especially on poor soils or if the seedling is grazed (Ovington & MacRae 1960; Brookes *et al.* 1980). It has also been argued that large seeds can establish from greater soil depth than small ones as they contain more stored nutrients (Vander Wall 1993). However, some papers have questioned the importance of cotyledons for early growth of oak seedlings (Jarvis 1963; Steele *et al.* 1993; Sonesson 1994).

Acorns are often cached by birds and rodents and buried in scatterhoards in the soil or litter layer (Jensen & Nielsen 1986; Stimm & Böswald 1994).

In Europe, the jay, *Garrulus glandarius*, locates oak seedlings and eats the cotyledons leaving the seedling otherwise undamaged (Bossema 1979), but other predators bite off the basal part of the acorn (cf. Turček 1956). Steele *et al.* (1993) showed that the acorns of *Q. phellos* germinate normally and develop normal seedlings even if half of the acorn is lost before germination, for instance, due to predation. However, Sonesson (1994) planted acorns of *Q. robur* in soils of different fertility and found that removal of the cotyledons in the seedling stage did not have any negative effect on the size of the sapling in its second year in any soil type.

This paper focuses on the hypotheses that the cotyledons are of vital importance during the first season, and that cotyledon resources could be used for regrowth after shoot damage or for penetrating the soil. Further, we suggest that seed manipulation by predators should make the seedling more susceptible to grazing. We experimentally assess the effects of grazing and cotyledon manipulation (removal, partly or completely) and burial of acorns, on growth and partitioning of biomass of *Q. robur* seedlings.

MATERIAL AND METHODS

Two greenhouse experiments (A and B) were performed. Artificial grazing and cotyledon removal were inflicted 3 weeks after emergence. Experiment A was performed in 1993 to test the effects of cotyledon removal during the first growing season in combination with different levels of browsing. Experiment B was performed in 1994 to test the effect of burial of acorns in combination with removal of the basal part of the acorn and defoliation. For experiment A we collected the acorns in October 1992. To minimize the genetic differences we used acorns from one single tree growing in a park in Uppsala. Visibly damaged acorns were discarded and the remaining acorns were stored at +5°C for about 6 months. For experiment B, acorns were imported from Poland because all the acorns collected in the autumn of 1993, including those from the tree used in the previous experiment, were infested with fungi. To minimize variation in the response to the planned treatments, only acorns around the mean weight were used.

Experiment A

A preliminary experiment carried out in 1991 indicated only negligible effects after 6 weeks, so an experiment was performed in which the effects of cotyledon removal were studied both after 6 weeks' growth and a moderate browsing treatment (only removal of leaves), and after a longer time (12 weeks' growth) and in combination with more severe levels of browsing: none, removal of top of stem and removal of whole stem.

Thus, one set was harvested 6 weeks after emergence and the remaining plants (including the more severe browsing treatments) were harvested 12 weeks after emergence, a period simulating a growing season.

The acorns had a mean fresh weight of 3·5 g, SD=0·79 and the average moisture content was 56·4%, SD=4·1 (n=31). Acorns were weighed, numbered and randomly assigned to a treatment in a factorial design and planted, just below the soil surface, one by one. Altogether 250 acorns were planted, 30 of which were kept in reserve as substitutes if any of the acorns originally assigned to treatment groups failed to germinate. Plants in the previous preliminary study appeared to develop large, deep root systems and thus, for this experiment a deeper type of container was used: a tube 35 cm long and 3 cm wide, each tube placed in a 2-litre plastic pot filled with a mixture of peat and sand poor in nutrients. This allowed the taproot to develop and later provided more space for the fine roots. The pots were placed in an 11 × 20 pattern and the pots were randomly rearranged every 2 weeks throughout the experiment. Each seedling was monitored 4–5 days per week and the soil kept moist with tap water. The seedlings usually finished the first elongation of the shoot, and had fully developed the first leaf set, 3 weeks after the shoot broke through the soil surface. This moment was thus chosen for the treatments, and each seedling was treated individually on the basis of its emergence day. The experiment started in February 1993. The length of the shoot was measured at treatment, and at 6 or 12 weeks (at harvest). The removed seedling parts at treatment (stem, leaves and cotyledons), and the seedling fractioned into acorn, root, stem and leaves, after harvesting, were oven-dried (80°C for 48 h) and weighed.

Experiment B

Longer planting tubes (70 cm) were used than in experiment A. The acorns had a mean fresh weight of 3·9 g, SD=1·33 (n=75) and the average moisture content was 41·7%, SD=4·3 (n=42). A total of 144 acorns were sown and randomly assigned to a treatment in a 2 × 2 × 2 factorial design. The acorns were sown 50 mm below soil surface or on the soil surface, with or without removal of the apical half of the acorn before planting. The experiment started in late April and 75 acorns produced a shoot and were available for the experiment. Each seedling was treated individually, based on its emergence day. The defoliation treatment (removal of leaves) was done 3 weeks after emergence. The experiment was harvested at 15 weeks after emergence.

Statistical analysis

The outcome of the experiment on partitioning of biomass was analysed with a general linear model procedure for unbalanced designs; SAS Proc GLM (SAS 1990) and ANOVA, SYSTAT (Wilkinson 1989). Mean acorn weight did not differ between treatment groups; however, there were variations in individual acorn fresh weights and thus two- or three-way analysis of covariance (ANCOVA) tests were used when applicable with acorn fresh weight as covariate (Steel & Torrie 1980; Wilkinson 1989).

RESULTS

Experiment A

The acorns emerged 13–48 days after sowing. However, only 130 of 250 acorns emerged, leaving an average 10 plants per treatment. The low germination ability was caused by
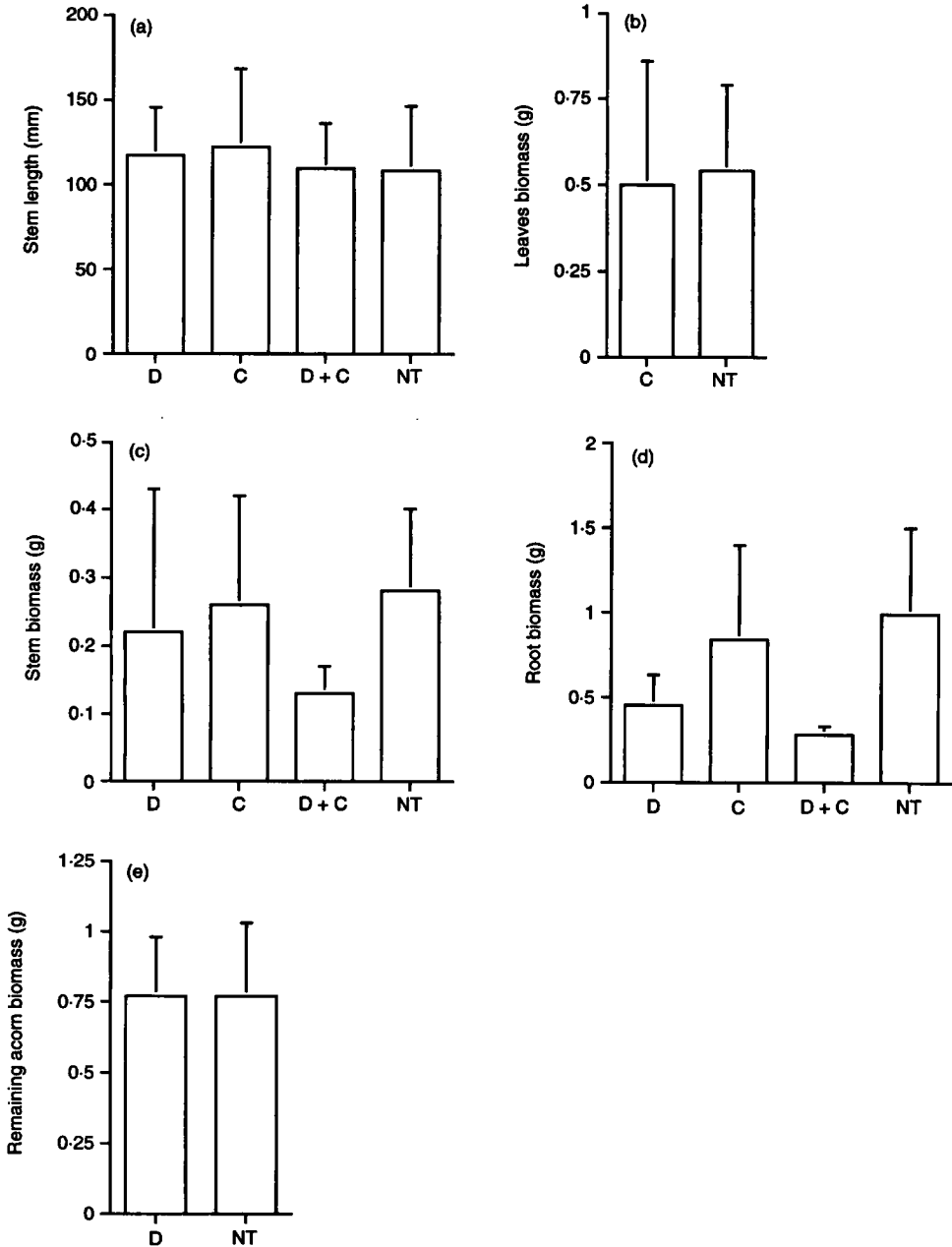
Fig. 1. Stem length and biomass of the *Q. robur* seedling fractions in experiment A, 6 weeks after shoot emergence. The treatments (defoliation or not, cotyledon removal or not) were applied 3 weeks after shoot emergence. (a) Stem length. (b) Leaf biomass. (c) Stem biomass. (d) Root biomass. (e) Remaining acorn biomass. D, defoliation; C, cotyledon removal; NT, no treatment.

mould. Three seedlings died during the experiment; all of them had clipped stem. The mean length of the seedlings was about 115 mm at the 6-week harvest (Fig. 1a) and about 140 mm at the 12-week harvest.

Table 1. ANCOVA of length and biomass of leaves, stem and root, and biomass of the remains of the acorns for *Q. robur* seedlings in A at 6 weeks. Treatments were: defoliation (D) or not and cotyledon removal (C) or not. Afw=acorn fresh weight (covariate)

Measured variable	Source of variation	d.f.	F	P
Shoot length	Defoliation	1	0·02	NS
	Cotyledon removal	1	0·06	NS
	D × C	1	1·04	NS
	Afw	1	0·32	NS
	Error	34		
Leaf biomass	Defoliation	1	38·39	***
	Cotyledon removal	1	0·11	NS
	D × C		0·03	NS
	Afw	1	0·0004	NS
	Error	33		
Stem biomass	Defoliation	1	4·47	*
	Cotyledon removal	1	1·44	NS
	D × C	1	1·14	NS
	Afw	1	2·17	NS
	Error	34		
Root biomass	Defoliation	1	18·45	***
	Cotyledon removal	1	1·50	NS
	D × C	1	0·02	NS
	Afw	1	1·33	NS
	Error	34		
Remaining acorn biomass	Defoliation	1	2·72	NS
	Afw	1	62·65	***
	Error	18		

*$P<0·05$; ***$P<0·001$.
NS, not significant.

The 6-week harvest showed no main effects of cotyledon removal (Fig. 1, Table 1) but stem and root biomass was significantly lower in the defoliated seedlings (Fig. 1c, d and Table 1). There was no reduction in acorn weight after defoliation (Fig. 1e, Table 1). The 12-week harvest showed that the seedlings were able to survive and respond to severe grazing levels. Seedlings that had their top or whole stem removed managed to produce new, although smaller, stems and leaves. The only significant main effects of cotyledon removal was a decrease in stem length and biomass (Table 2). The remains of acorns did not decrease in weight between the 6- and 12-week harvest ($F=1·52$, $P=0·23$). In all grazing treatments, root biomass was slightly lower when acorns were removed (Fig. 2), although this effect was not statistically significant ($P=0·061$, Table 2). There were no cases of interactions between grazing and cotyledon removal.
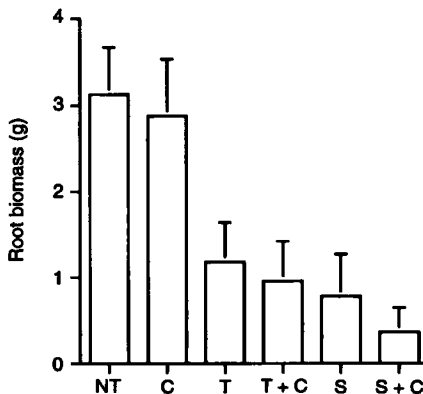
Experiment B
When acorns were halved there were significant main effects in all cases but for leaf biomass. Intact acorns emerged significantly later, grew taller and stem and root biomass was significantly higher than in seedlings from reduced acorns. Furthermore, the loss of acorn biomass were significantly greater in intact acorns. Reduced cotyledons

Table 2. Two-way ANCOVA of stem length, biomass of leaf, stem, and roots of *Q. robur* seedlings in experiment A at 12 weeks. Treatments were: grazing (G), seedling artificially grazed (levels: none, top or whole stem); cotyledon (C), cotyledon removed from the seedling or not. Afw, acorn fresh weight (covariate)

Measured variable	Source	d.f.	*F*	*P*
Shoot length	Grazing	2	16·61	***
	Cotyledon	1	11·42	**
	G × C	2	1·11	NS
	Afw	1	1·05	NS
	Error	47		
Leaf biomass	Grazing	2	40·30	***
	Cotyledon	1	0·37	NS
	G × C	2	0·39	NS
	Afw	1	9·00	**
	Error	46		
Stem biomass	Grazing	2	50·74	***
	Cotyledon	1	6·89	**
	G × C	2	0·04	NS
	Afw	1	0·01	NS
	Error	46		
Root biomass	Grazing	2	99·14	***
	Cotyledon	1	3·70	NS
	G × C	2	0·09	NS
	Afw	1	0·96	NS
	Error	44		
Remaining acorn biomass	Grazing	2	0·14	NS
	Afw	1	170·36	***
	Error	23		

**P<0·01; ***P<0·001.
NS, not significant.

Fig. 2. Root biomass of *Q. robur* seedlings in experiment A, 12 weeks after shoot emergence in relation to the damage inflicted on the seedlings. Treatments (cotyledons removed or not; top (apical meristem) removed or not; and whole stem removed or not) were applied 3 weeks after shoot emergence. NT, no treatment; C, cotyledon removed; T, top removed; S, whole stem removed.

Table 3. Mean values (according to Tukey's adjusted least square mean) and ANOVA of emergence day, shoot length, stem biomass, total biomass, root biomass, leaf biomass and biomass loss in the acorn of *Quercus robur* seedlings in experiment B. Treatments were: cotyledon reduction or not (C); acorn burial (B), acorn buried 50 mm below soil surface or placed on soil surface; defoliation (D), seedling defoliated or not

Source		Adj LS mean						
		Emergence (days)	Stem length (mm)	Stem biomass (g)	Total biomass (g)	Root biomass (g)	Leaf biomass (g)	Acorn biomass loss (g)
Cotyledon	Intact	27·77	121·20	0·41	3·88	2·16	0·41	0·96
		***	*	***	***	***	NS	***
	Reduced	20·42	101·98	0·30	2·44	1·39	0·37	0·57
Burial	Surface	19·76	94·48	0·29	3·32	1·95	0·41	0·68
		***	***	***	NS	NS	NS	*
	Buried	28·43	128·70	0·43	2·99	1·60	0·38	0·85
Defoliation	No	—	110·89	0·38	3·41	2·03	0·46	0·76
			NS	NS	*	**	*	NS
	Yes	—	112·29	0·33	2·90	1·52	0·32	0·77
C × B	Intact, surface	20·00						
	Intact, buried	35·54						
	Reduced, surface	19·53						
	Reduced, buried	21·00						
		***	NS	NS	NS	NS	NS	NS
C × D	Intact, not defoliated	—		0·40	3·81	2·22		
	Intact, defoliated	—		0·42	3·94	2·10		
	Reduced, not defoliated	—		0·37	3·02	1·83		
	Reduced, defoliated	—		0·24	1·86	0·94		
			NS	*	***	*	NS	NS
D × B	Surface, not defoliated	—		0·26				
	Surface, defoliated	—		0·31				
	Buried, not defoliated	—		0·51				
	Buried, defoliated	—		0·35				
			NS	**	NS	NS	NS	NS

*$P<0.05$; **$P<0.01$; ***$P<0.001$.
NS, not significant.

in combination with defoliation affected the root and stem biomass negatively (C × D interactions in Table 3).

Acorn burial affected emergence day negatively and seedlings with buried acorns grew in total taller, and the stems weighed significantly more, than seedlings with acorns on the soil surface. The significant interaction (cotyledon reduction × acorn depth) indicates that the delay due to burial was stronger in intact acorns than in reduced ones (Table 3). Buried acorns showed significantly higher stem biomass when the plant was not defoliated. Moreover, acorns lost more in weight when they were buried (Table 3).

Defoliation resulted in significantly reduced total biomass, root and leaf biomass and in interaction with defoliation halved cotyledons resulted in significantly reduced stem, root and total biomass. Acorn biomass loss was not affected by defoliation (Table 3).

DISCUSSION

The results of this study confirm our hypothesis that cotyledons are of importance during the first growing season but, in contradiction to our second hypothesis, the seedlings only use a minor part of the cotyledons for regrowth of leaves or even the whole shoot, after damages. It is also in agreement with the findings of Sonesson (1994) and Steele *et al.* (1993) that cotyledons are 'oversized' for the requirements of the germination process. Our results show that acorn removal or reduction do affect above-ground growth negatively after one growing season, but the time for acorn removal or reduction is important. Caching depth had no impact on total biomass, but burial had a negative effect on both root and stem biomass if, at the same time, cotyledons were reduced in combination with grazing. The loss of acorn biomass when acorns were buried, we explain as resources were taken from cotyledon for shoot growing up to the soil surface. Not surprisingly, emergence days for buried acorns were delayed, but this could be important for oak seedlings which are cached but not transported away from oak-tree canopies. As Shaw (1974) pointed out, most insect defoliation on oak seedlings occurs by caterpillars falling from the trees immediately overhead. We suggest that caching makes it possible for the seedlings to escape the most severe attacks of defoliation in time instead of space.

All seedlings in experiment A reached the same length regardless of treatment. This means that the shoots of the defoliated seedlings were more slender as their stem mass was reduced by *c.* 50%. In experiment B, on the other hand, acorns sown deeper in the soil became taller than the ones sown on the soil surface, although the above-ground part is shorter. That shoot elongation is given priority could be viewed as a mechanism to avoid competition for light with the lower field layer; seedlings of *Q. robur* are not shade-tolerant (Jones 1959). A shorter shoot, and a darker environment, would also increase the risk for attacks of oak mildew, *Microsphaera alphitoides* (Vaartaja 1962; Shaw 1974). A thinner shoot diameter due to defoliation was also found for *Q. rubra* seedlings by Wright *et al.* (1989) and resulted in increased winter mortality for the seedlings. Thus, the advantage of a longer, more slender shoot seems to be more important than the increased risk for damage caused by grazing or trampling on a shorter shoot. We will further test the relevance of our results in a field experiment in which the seedlings are browsed during winter; we will also test the use of acorn resources in an interaction situation with competition and grazing.

The smaller root system resulting from defoliation is important as the seedlings are likely to be more sensitive to drought and to repeated defoliation. Oak seedlings in

natural situations are known to survive repeated removal of the above-ground parts and could remain in a suppressed state for several years (Merz & Boyce 1956; Andersson 1991). A well-developed root system is therefore important as a source of carbohydrates for regrowth after grazing. This has also been shown in first-year seedlings of *Q. rubra* where defoliation, especially in combination with root damage, was fatal (Wright *et al.* 1989).

Our experiments illustrate two kinds of naturally occurring seed predation: the jay snapping off the cotyledon from an already developed seedling, as Bossema (1979) describes, is an example of cotyledon removal, as in experiment A. Our data show that removal of the cotyledons had no effect on the seedlings when only the leaves were removed. When the cotyledons were removed, the biomass of the leaves and the stem did not become smaller than those in the control group (Table 1). After a longer time and as a response to the more severe grazing, we found an influence of the cotyledons on root growth (Table 2). We interpret these results as indicating that resources were taken from the cotyledons for root development but not for development of the stem or leaves. The lack of interactions between grazing and cotyledon removal on above-ground parts in experiment A leads to the conclusion that cotyledons have not been used for regrowth of the above-ground parts. Brookes *et al.* (1980) showed that small amounts of mineral nutrients continued to be transferred to the seedlings from the cotyledons after the development of the first leaf set, even though the transfer of carbohydrates had finished. On the other hand, Sonesson (1994) showed that removal of the cotyledons had no effect, not even on root biomass, in the second year of seedlings growing in different soil types, from nutrient-rich to poor. Thus, it seems that the cotyledons play a minor role after the seedlings have developed their first leaf-set.

The other kind of seed predation, cotyledon reduction, as in experiment B, occurs naturally when a mouse bites off part of the acorn before seed germination. We have no reasonable explanation for the greater mass loss in intact cotyledons but our results show that an acorn with partly reduced cotyledons can establish successfully, although these seedlings are smaller and are more vulnerable to grazing. This emphasizes our conclusions that cotyledon resources are only used during the first season of seedling development and that superfluous resources are not used for resprouting after grazing. A possible evolutionary explanation is that the large size of the acorn has evolved as a means to attract dispersers. This supports the suggestion of Steele *et al.* (1993) that the cotyledons have a role similar to that of the elaiosomes in many ant-dispersed seeds (e.g. Hughes & Westoby 1992), redirecting predation from more vulnerable parts of the seed.

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