J Plant Res (2003) 116:207–216
Digital Object Identifier (DOI) 10.1007/s10265-003-0089-3

ORIGINAL ARTICLE

Daisuke Kabeya · Akiko Sakai · Kiyoshi Matsui
Satoki Sakai

Resprouting ability of *Quercus crispula* seedlings depends on the vegetation cover of their microhabitats

Received: September 24, 2002 / Accepted: February 18, 2003 / Published online: April 17, 2003

Abstract To examine the effects of vegetation cover on the resprouting abilities of *Quercus crispula* seedlings, in each of three consecutive years, we artificially clipped seedlings growing in microhabitats with differing degrees of vegetation cover. We also investigated the relationship between the level of total nonstructural carbohydrate (TNC) and resprouting ability. Seedlings with clipped shoots in gaps produced larger resprouting shoots than those in the understory. Moreover, both the percentage of resprouting seedlings and the survival ratio in seedlings with clipped shoots were negatively correlated with the degree of vegetation cover. Seedlings stored high levels of TNC, especially in their roots, and their TNC levels were negatively correlated with the degree of vegetation cover. There were also positive relationships between the TNC levels in their roots and the degree of resprouting. Hence, we conclude that release from vegetation cover enhanced the resprouting ability of *Q. crispula* seedlings by increasing their levels of stored carbohydrate. The key variables affected were the resprouting ratio (the proportion of seedlings capable of producing new shoots) and the size of the resprouted shoots.

Key words *Quercus crispula* · Resprouting · TNC · Tree seedling

Introduction

Quercus crispula Blume is a common species in the Japanese cool-temperate deciduous forests. It is an intermediately shade-tolerant species and its seedlings can live for some years on the closed forest floor. Like other *Quercus* species, *Q. crispula* can resprout vigorously following shoot destruction. The vigorous resprouting characteristics of *Quercus* species are thought to be of adaptive value following large-scale disturbances such as wildfire (e.g., Abrams 1996), and their seedlings also resprout after small-scale disturbances (Crow 1992; Herrera 1995). In the Japanese deciduous forests, where humid conditions and wildfires occur relatively infrequently, the shoots of *Q. crispula* seedlings are often destroyed by herbivory or shoot dieback (Ida and Nakagoshi 1996; Wada et al. 2000), and resprout following such events (Sato 2000; Kabeya 2001).

Resprouting requires resources (Bond and Midgley 2001) and, although some studies have questioned their significance (Taylor and Pharis 1982; Garcia et al. 2001), there is evidence that carbohydrate reserves are consumed and used during the resprouting process in various plant species following shoot destruction (Danckwerts and Gordon 1987, 1989; Kays and Canham 1991; McPherson and Williams 1998; von Fircks and Sennerby-Forsse 1998). Further, the well-developed taproots of *Quercus* juveniles may act as carbon sources during resprouting (Crow 1988; Matsubara and Hiroki 1989; Kruger and Reich 1993; Sakai and Sakai 1998).

The indications that resprouting involves the use of carbohydrate reserves raises interesting questions as to how many *Quercus crispula* seedlings have the necessary resources and, thus, the ability to resprout in natural environments. The forest floors, where these seedlings usually grow, are heterogeneous in terms of various environmental factors, especially the light regime, due to variations in the presence/absence of canopy trees and understory vegetation, especially *Sasa* spp., in Japanese deciduous forests (Shidei 1974; Wada 1993; Abe et al. 2001). Thus, since a seedling's carbohydrate reserves are likely to depend on the

---

D. Kabeya[1] (✉) · A. Sakai[2]
Department of Ecology and Evolutionary Biology, Graduate School of Science, Tohoku University, Aoba, Sendai 980-8578, Japan

K. Matsui[3]
Mount Hakkoda Botanical Laboratory, Graduate School of Science, Tohoku University, Sendai, Japan

S. Sakai
Graduate School of Life Science, Tohoku University, Sendai, Japan

Present addresses:
[1]Forestry and Forest Products Research Institute, Kiso Experiment Station, 5473-8 Kisofukushima, Kiso, Nagano 397-0001, Japan
Tel. +81-264-223203; Fax +81-264-222542
e-mail: kabeta@ffpri.affrc.go.jp
[2]Graduate School of Environment and Information Sciences, Yokohama National University, Yokohama, Japan
[3]Biological Laboratory, Nara University of Education, Nara, Japan

environmental conditions of its microhabitat, resprouting abilities might differ markedly among seedlings growing in different microhabitats. Differences in resprouting ability of the seedlings would be expected to affect their population dynamics, but few previous studies have examined whether such differences occur as the result of heterogeneities in microenvironmental conditions on the forest floor.

In this study, we investigated the effect of vegetation cover on the resprouting abilities of *Quercus crispula* seedlings. Our study had two main aims: firstly, to quantify the differences (if any) in resprouting abilities after artificial shoot destruction (in shoot clipping experiments) in seedlings growing under different levels of vegetation cover (i.e., in a canopy gap without *Sasa* and under closed canopies with and without *Sasa*); and, secondly, to assess the relationship between the level of carbohydrate reserves in the seedlings' roots and their resprouting abilities. To survive shoot destruction, plants must not only produce new shoots, but the resprouted shoots must also be large enough and sufficiently productive to allow them to re-establish. Therefore, to evaluate the resprouting abilities of the seedlings, we monitored three key variables: the proportion of seedlings that can create new shoots following shoot destruction (the resprouting ratio); the size of the shoots produced by the resprouting seedlings; and the survival rate after shoot destruction (the re-establishment ratio).

Materials and methods

Plant material

Quercus crispula is a common deciduous broad-leaved tree species in the cool temperate forests of Japan. The species shows a flush-type shoot elongation pattern (Kikuzawa 1983), and the seedlings' shoots usually elongate once in spring on natural forest floors. However, amongst seedlings growing under favorable light conditions (e.g., in a gap created by a tree fall), there is often a further episode of shoot elongation at some stage during the growing season. Considerable numbers of seedlings lose their shoots through herbivory and shoot die back, especially during the autumn and the following early spring (Kabeya 2001). The species has hypogeous cotyledons, and the seedlings produce at least two lateral buds around the root collar (i.e., below ground). In addition, they have four or five scaly leaves with lateral buds on their epicotyl. Following shoot destruction, they can resprout from these dormant buds. Thus, aboveground damage usually causes very little meristematic limitation in this species, although Hasegawa (1984) reported that *Q. crispula* seedlings could not resprout when they were clipped below the root collar.

Table 1. Attributes of study sites

Site	Canopy	*Sasa*	Percentage of vegetation cover in 1997 ($n = 3$) as mean ± SE	Significant difference[a]
Site 1	Gap	Dead	90.5 ± 1.16	a
Site 2	Closed	Dead	93.9 ± 0.40	b
Site 3	Closed	Dead	95.4 ± 0.69	bc
Site 4	Closed	Alive	97.7 ± 0.44	c

[a] Different letters mark significant differences between sites at the $P < 0.05$ level according to the Tukey-Kramer test

Study site

The study site was located on the eastern side of Lake Towada, a caldera lake, in Aomori Prefecture, northern Japan (40°26′N, 140°56′E). The mean temperature and annual precipitation (calculated over 1983–1998) at the nearest AMeDAS station (40°26′N, 140°54′E) were 7.7°C and 1,480 mm, respectively. We established the study site in a secondary forest dominated by *Quercus crispula*, in which canopy trees were approximately 30 cm in diameter at breast height and 20 m in height. The forest understory is generally covered with *Sasa* (*S. kurilensis*) in this area (1.5–2.0 m in height), but in 1995, a mass flowering followed by death of *Sasa* occurred here (Makita et al. 1995). Subsequently, the light regime on the forest floor changed dramatically, except in areas with small surviving patches of living *Sasa*. In 1996, four sites with different levels of vegetation cover were laid out on the forest floor within 300 m of each other. Site 1 was under a canopy gap without *Sasa* cover, sites 2 and 3 were under a closed canopy without *Sasa* cover and site 4 was under a closed canopy with surviving *Sasa* cover (Table 1). The size of each site was about 20 m × 20 m except for site 1, which was located in a single gap (approximately, 10 m × 15 m), and the environment within each site was nearly homogenous.

To quantify the degree of vegetation cover at these sites, hemispherical photographs were taken at a height of 20 cm at three randomly chosen points at each site on 6 September 1997. The chosen points were spaced more than 10 m apart at all sites except for site 1, where the spacing was 5 m. The photographs were then digitized using an image scanner, and the vegetation cover of each site was calculated using the GLA (version 2.0) software of Frazer et al. (1999). The vegetation cover at the first, second, third, and fourth sites was 90.5%, 93.9%, 95.4%, and 97.3%, respectively (Table 1). On 12 August 2001, we took photographs again, at points near those where we had taken the first set, to check whether the vegetation cover had changed. We found there were still significant differences in the vegetation cover between the sites ($F = 7.5$, $P = 0.01$, data not shown), although the differences between them had diminished. Thus, we consider the vegetation cover to have significantly differed between the sites throughout the study period of 1996–1998.

Clipping experiment

To examine the effect of vegetation cover on the resprouting ability of the seedlings, a clipping experiment was carried out in 1996, 1997, and 1998 for a single cohort of *Quercus crispula* seedlings that germinated in 1995 at each site. Thus the seedlings were 1, 2 and 3 years old in the respective years of the clipping experiment. Shoot dieback and herbivory of *Q. crispula* seedlings tend to occur in early stages of the growing season (D. Kabeya, unpublished data). Hence, we conducted clipping experiment just after the completion of the spring shoot flush in each year (on 7 July 1996, from 14 June 1997 to 16 June 1997 and on 8 June 1998). The shoots of seedlings in which leaf expansion had finished were removed at ground level, approximately 2 cm above the root collar. The numbers of clipped seedlings at each site were 10, 60, and 20 in 1996, 1997, and 1998, respectively. In each year previously unclipped seedlings were selected for clipping. All clipped seedlings were marked and observed monthly during the growing seasons to see whether they were resprouting. A few seedlings that were clipped in 1997 resprouted in the following season (1998), but they were treated as seedlings that did not resprout in this study. We then calculated the resprouting ratio, mortality ratio, and re-establishment ratios of the seedlings, as follows:

resprouting ratio = number resprouting/number clipped

mortality ratio = number that died after resprouting/ number that resprouted

re-establishment ratio = number surviving at the end of the growing season/number clipped

If clipped seedlings resprouted we counted their leaves and measured the lengths of their resprouting shoot and largest leaf lamina (maximum lamina length) monthly in each year. In 1997, however, the maximum lamina length was measured once at the end of the growing season. For the seedlings that resprouted two shoots simultaneously (none of them produced three or more shoots), the sum of the shoot length and the total number of leaves of the two shoots were used in the statistical analyses.

Carbohydrate analysis

To examine the effects of vegetation cover on carbohydrate reserves in the *Quercus crispula* seedlings and that between resprouting ability and carbohydrate reserves, we collected 10 unclipped *Q. crispula* seedlings (from the 1995 cohort) from each site in each of the three years. The samplings were carried out at the same time as the shoot clipping. Hereafter, we refer to these seedlings as the control seedlings to distinguish them from the clipped seedlings. In the morning, seedlings that had just completed their leaf expansion were collected, brought back to the laboratory, separated into leaves, stems and roots, and put in an oven within 24 h of collection. They were then dried at 70°C for 3 days and weighed. To determine the concentration of total nonstructural carbohydrates (TNC) in the roots and stems, starch was hydrolyzed to glucose enzymatically (Ono et al. 1996). Roots and stems were then ground using a Wiley mill, passed through a 65-mesh screen and dried in the oven for 24 h. Samples (2–3 mg) were homogenized with 80% ethanol. After evaporation to remove ethanol, they were suspended in 0.5 ml of 0.2 M KOH and placed in boiling water for 30 min. After cooling, 2.00 ml of 1.0 M acetic acid was added. To digest starch to glucose, 0.5 ml of *Rhizopus* amyloglucosidase (Sigma, St. Louis, Mo., USA.) solution (35 U/ml in 50 mM Na acetate buffer, pH 4.5) was added, and the tubes were incubated at 55°C for 30 min. After digestion, the tubes were placed in boiling water for 1 min and then centrifuged. The supernatant was transferred to another tube, and its total sugar content was determined by the phenol–sulfuric acid method (Dubois et al. 1956). Absorbance was read at 490 nm in a spectrophotometer (UV-16A, Shimadzu, Japan). Glucose solution was used as a calibration standard. Root and stem TNC pools, i.e., the total amounts of TNC in each part, were calculated by multiplying the concentration of TNC by the dry mass of the respective parts.

Statistical analysis

We tested the effects of vegetation cover (Site), experiment year (Year), and possible interaction effects on plant dry mass, TNC concentrations and pools, and the size of resprouting shoots. Then the data were subjected to analysis of variance (Proc GLM or Proc ANOVA implemented in SAS software, SAS 1989), with Tukey-Kramer HSD post hoc tests. To test the difference in TNC concentrations between roots and stems, Wilcoxon's signed-rank test (Sokal and Rohlf 1995) was used. The TNC concentrations were analyzed after arcsine transformation. Kruskal-Wallis tests and Mann Whitney's U tests (Proc NPAR1WAY, SAS 1989) were used to assess the effect of Site on the time that resprouting started. A log-linear model (Proc CATMOD, SAS 1989) and Fisher's exact test (Proc FREQ, SAS 1989) were applied to test the effect of Site, Year, and their interactions on the resprouting ratio, the mortality ratio, and the re-establishment ratio. A sequential Bonferroni method (Sokal and Rohlf 1995) was applied for multiple comparisons. Correlation analysis and regression analysis (Proc CORR or Proc NLIN, SAS 1989) were conducted to analyze the relationship between the size of initial reserves and the resprouting ability of clipped seedlings. For all experiment years, the mean root TNC concentrations and pools of the control seedlings were considered equivalent to the initial TNC concentrations and pools of the clipped seedlings.

Results

Seedling growth and dry mass distribution

Site and experiment year (Year) were found to affect root, stem, leaf, and total dry mass of *Quercus crispula* seedlings, and to interact (Table 2). In the site in the canopy gap without *Sasa* (site 1), leaf, stem, root, and total dry mass of the seedlings generally increased in successive years (Fig. 1). In contrast, in the other three, closed canopy sites (sites 2, 3, and 4) dry mass did not significantly differ among years. The ratio of shoot to root was consistently within the range 1.4–1.9 and was not significantly different either between sites or between years (Fig. 1).

Table 2. *F* statistics for ANOVA of dry mass, levels of total nonstructural carbohydrates (*TNC*) in control seedlings and the size of resprouting shoots of clipped seedlings. TNC pools indicate the amount of TNC in the respective organs. Measured parameters of resprouting shoots are shoot length, number of leaves and maximum lamina length. Factors exerting the main effects identified by this test are site and experimental year

	F value (probability)					
	Site		Year		Site × Year	
Dry mass of control seedlings						
roots	54.3	(<0.001)	7.25	(0.001)	3.51	(0.003)
stems	22.1	(<0.001)	10.7	(<0.001)	4.29	(<0.001)
leaves	47.4	(<0.001)	3.53	(0.033)	4.50	(<0.001)
total	42.3	(<0.001)	7.81	(<0.001)	4.82	(<0.001)
shoot/root ratio	1.24	(0.299)	0.03	(0.972)	1.59	(0.158)
TNC concentrations						
roots	65.1	(<0.001)	26.8	(<0.001)	9.17	(<0.001)
stems	25.7	(<0.001)	17.8	(<0.001)	3.80	(0.001)
TNC pools						
roots	97.0	(<0.001)	6.03	(0.003)	4.04	(0.001)
stems	35.8	(<0.001)	7.20	(0.001)	3.71	(0.002)
Resprouting shoot parameters						
shoot length	18.4	(<0.001)	2.85	(0.603)	1.03	(0.410)
numbers of leaves	26.0	(<0.001)	1.47	(0.440)	0.44	(0.959)
maximum lamina length	28.5	(<0.001)	3.14	(0.046)	0.55	(0.770)

Table 3. Log-linear models of the influence of the site where the seedlings grew, the experimental year and their interaction on the percentage of clipped seedlings that resprouted (the resprouting ratio), the percentage of resprouting seedlings that died (the mortality ratio), and the percentage of clipped seedlings that resprouted and survived until the end of the growing season (the re-establishment ratio)

	χ^2 value (probability)					
	Site		Year		Site × Year	
Resprouting ratio	11.8	(0.003)	24.8	(<0.001)	9.1	(0.170)
Mortality ratio	1.9	(0.380)	15.7	(0.001)	6.6	(0.355)
Re-establishment ratio	9.0	(0.011)	42.0	(<0.001)	5.0	(0.542)

Clipping treatment

In all three years, most seedlings began to resprout within 2 months of clipping. However, the time that resprouting started differed significantly among the sites in 1996 ($P = 0.001$; Kruskal-Wallis test) and 1997 ($P = 0.004$), although the post hoc test indicated that the differences were only significant between one pair of sites in both years: resprouting starting earlier in site 4 than site 1 in 1996, and earlier in site 1 than site 3 in 1997. Following clipping, we observed that all resprouted shoots grew from dormant buds below the clipping point and no shoots grew from adventitious buds at the point of clipping or any other parts.

Site had significant effects on the resprouting ratio (Table 3). At site 1, more than 90% of the clipped seedlings resprouted during the experiments (Fig. 2A). When seedlings of all years were pooled, the resprouting ratio decreased with increases in vegetation cover (site 1 > site 2 ≥ site 3 > site 4, according to multiple comparison with Fisher's exact test at $P < 0.05$, Fig. 2A). Year also affected the resprouting ratio (Table 3), but only at site 4, where the resprouting ratio showed significant reductions in successive years (1996 > 1997 ≥ 1998 at $P < 0.05$).

Site was the only factor that exerted significant effects on the length and numbers of leaves produced by resprouting shoots (Table 2). Although both Year and Site significantly affected maximum lamina length (Table 2), according to the post hoc test, the mean maximum lamina length was not significantly different between experiment years. Among the sites, shoot length, numbers of leaves, and maximum lamina length were greater in the resprouting seedlings growing at site 1 than those at the other three sites (Table 4).

The mortality ratio of resprouting seedlings was only affected by Site (Table 3), and when seedlings of all years were pooled, the ratio was positively correlated with vegetation cover (site 1 ≤ site 2 < site 3 < site 4, Fig. 2B). In all study sites, almost all the seedlings that died after resprouting were killed by dieback caused by pathogenic fungal infections.

The re-establishment ratio was also affected by Site (Table 3). Like the resprouting ratio, the re-establishment

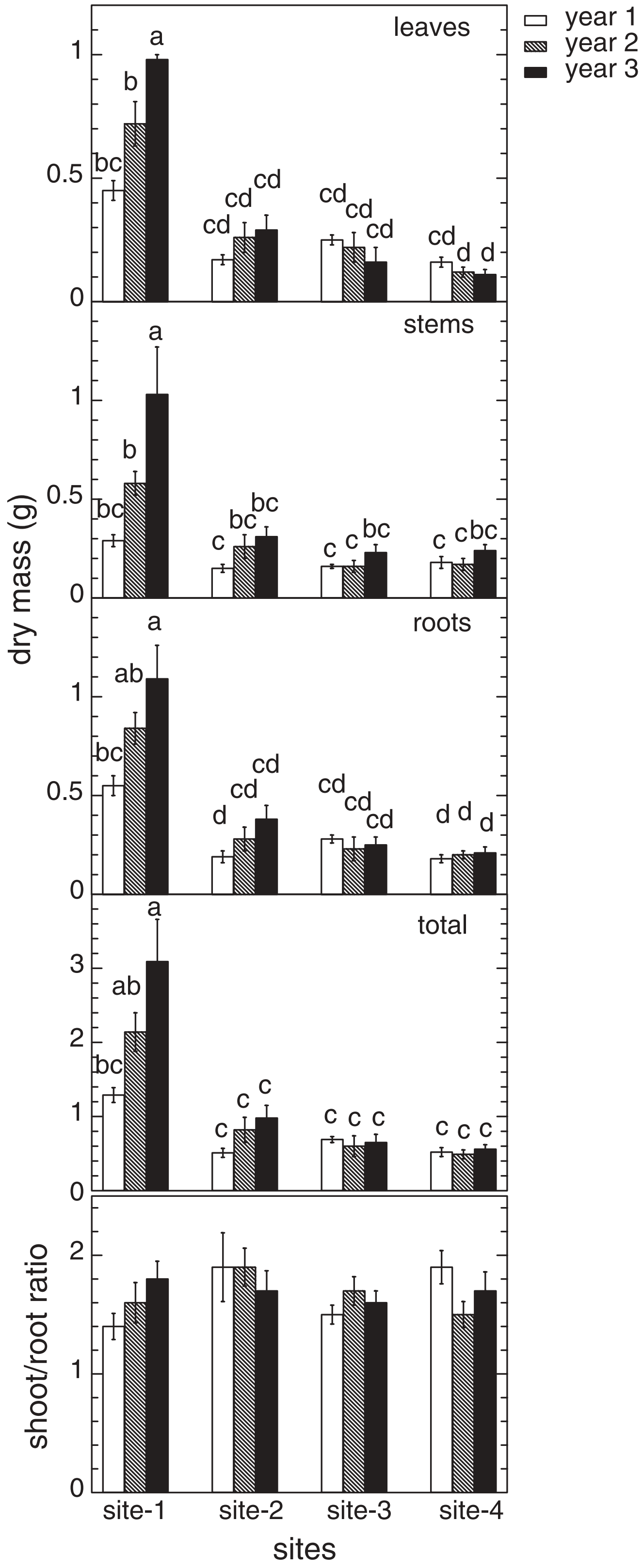

Fig. 1. Mean dry mass and shoot/root ratios of the control seedlings (*error bars* show standard errors, $n = 10$). The ANOVA results are shown in Table 2. Data points marked with different *letters* are significantly different at the $P < 0.05$ level according to the Tukey-Kramer test

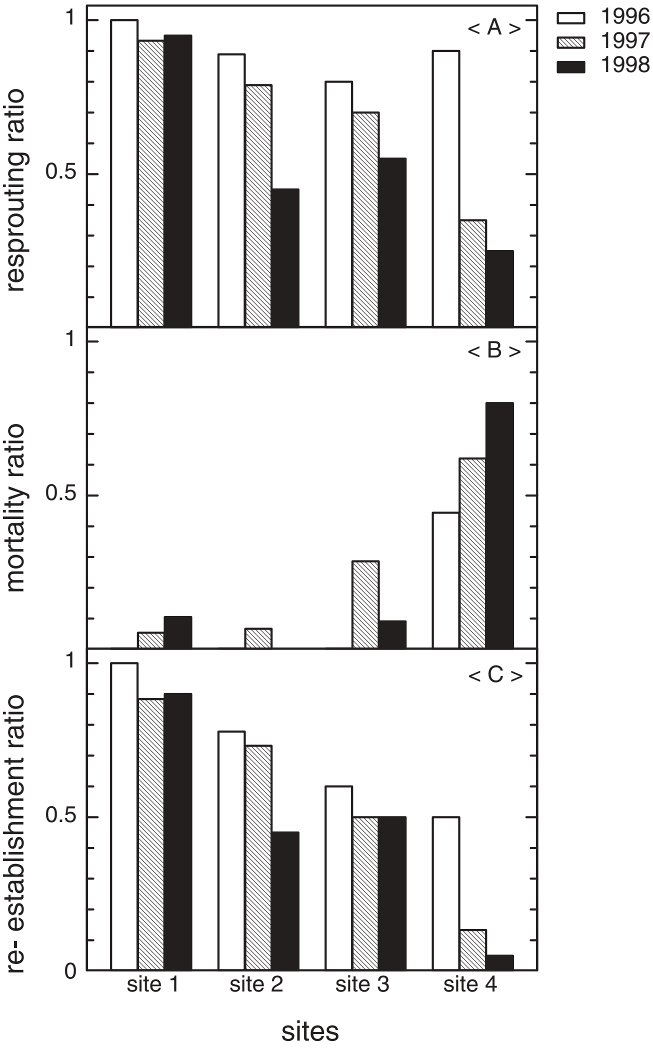

Fig. 2. **A** Percentage of resprouting seedlings amongst clipped seedlings (resprouting ratio). **B** Mortality rate of resprouted seedlings (mortality ratio). **C** Proportion of seedlings surviving at the end of the growing season (re-establishment ratio). Seedlings of the same cohort were clipped just after shoot development each year and their status was checked monthly during the growing season. The number of clipped seedlings at each site was 10 in 1996 (9 at site 2), 60 in 1997 (57 at site 2), and 20 in 1998

ratio decreased with increasing vegetation cover when data for all years were pooled (site 1 > site 2 ≥ site 3 > site 4, Fig. 2C). In addition, the re-establishment ratio was affected by Year (Table 3), according to multiple comparisons, although it was not significantly different between the experimental years at any individual site.

TNC concentration and TNC pools just after completion of leaf expansion

Concentrations of TNC in roots just after completion of leaf expansion were significantly affected by Site and Year, and these factors interacted (Table 2). Root TNC concentration tended to increase with reductions in vegetation cover (Fig. 3). Stem TNC concentration was affected by Site

Table 4. Resprouting shoot parameters of the seedlings (shoot length, number of leaves, and maximum lamina length). ANOVA results are described in Table 2. Data related to seedlings of different years were pooled for each site. Each value was obtained at the end of the growing season of each year, except for dead seedlings, for which resprouting shoot measurements taken a month before the observation of seedling death were used

Site	Sample size	Mean ± SE of shoot length (cm)	Significant difference[a]	Mean ± SE of number of leaves	Significant difference[a]	Mean ± SE of maximum lamina length (cm)	Significant difference[a]
Site 1	81 (79[b])	8.9 ± 0.36	a	3.7 ± 0.19	a	5.2 ± 0.13	a
Site 2	48 (45[b])	6.6 ± 0.30	b	2.6 ± 0.17	b	4.1 ± 0.15	b
Site 3	44 (31[b])	5.8 ± 0.22	b	2.3 ± 0.11	b	3.6 ± 0.19	bc
Site 4	16 (9[b])	6.5 ± 0.24	b	2.2 ± 0.16	b	3.0 ± 0.24	c

[a] Means with different letters are significantly different at the $P < 0.05$ level (Tukey-Kramer test)
[b] Sample size for maximum lamina length

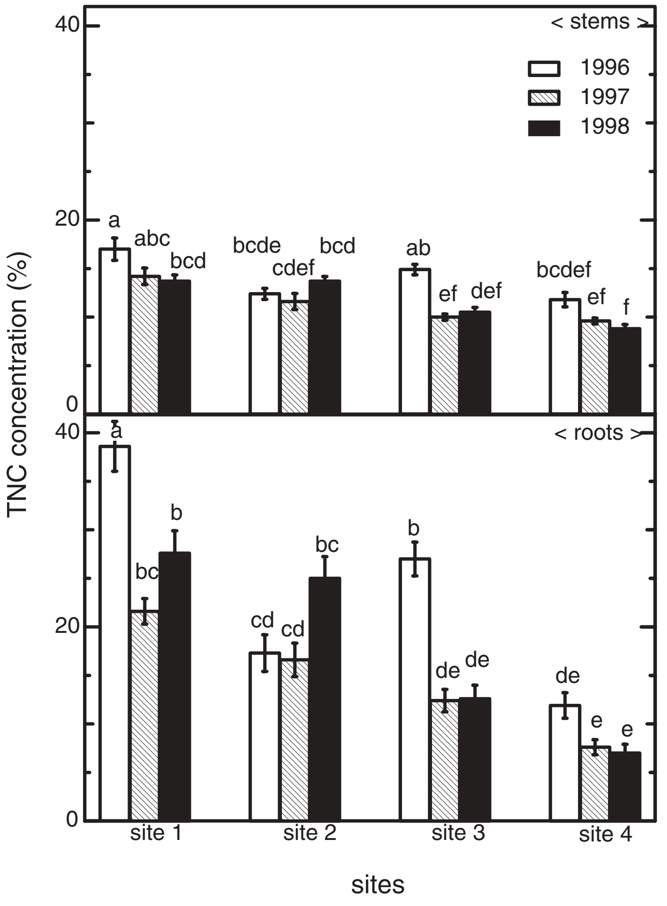

Fig. 3. Mean total nonstructural carbohydrate (TNC) concentrations in the stems and roots of the seedlings (*error bars* show standard errors, $n = 10$). The results of ANOVA are shown in Table 2. Columns with different *letters* are significantly different at the $P < 0.05$ level according to the Tukey-Kramer test

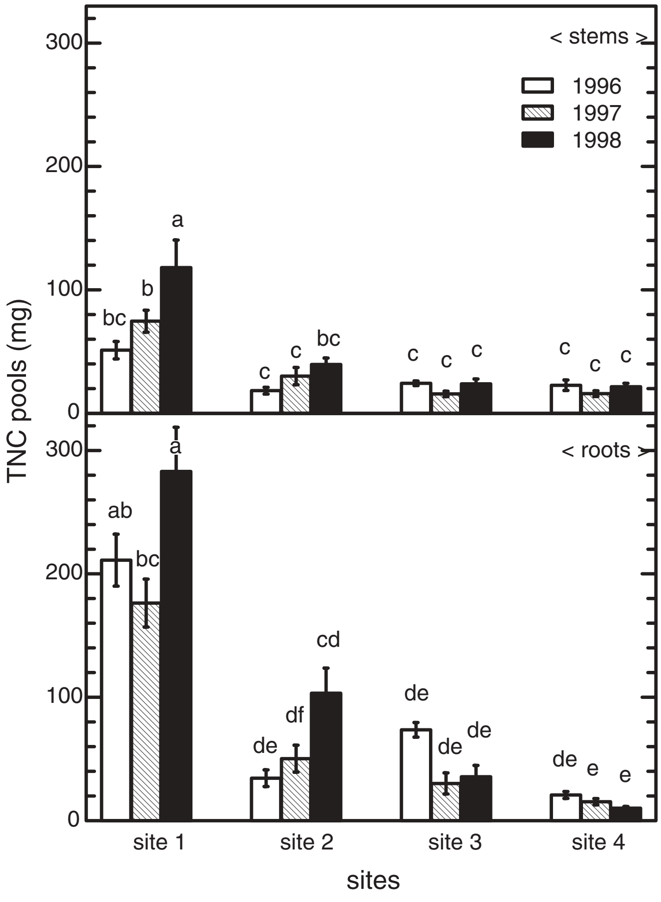

Fig. 4. Mean TNC pools in the stems and roots of the seedlings (*error bars* show standard errors, $n = 10$). The results of ANOVA are shown in Table 2. Columns with different *letters* are significantly different at the $P < 0.05$ level according to the Tukey-Kramer test

(Table 2). However, the differences in stem TNC concentrations between the sites were smaller than the differences in root TNC concentrations (Fig. 3). When seedlings of all years were pooled, the TNC concentration in the roots was higher than in stems, except at site 4 (Wilcoxon's signed-rank test at $P < 0.05$).

The root and stem TNC pools were also affected by experiment Year and Site, and these factors interacted (Table 2). The seedlings at site 1 had the highest dry mass and root TNC concentrations in all three years, so the root TNC pools were highest in those seedlings (Fig. 4). In 1997 and 1998, the stem TNC pools were also higher at site 1 than at the other three sites (Fig. 4). Root TNC pools in seedlings growing at sites 3 and 4 tended to decrease in successive years, although this decline was not statistically significant (Fig. 4).

Table 5. Correlation coefficients (r) of the relationships between the resprouting parameters (the resprouting ratio and the length, the number of leaves, and the maximum lamina length of resprouting shoots) in the clipping treatments and the initial mean TNC levels (TNC concentrations and TNC pools) in roots of the control seedlings, which were collected at the time of shoot clipping. Correlation coefficients between resprouting ratio and initial TNC concentration (pools) were calculated after log transformation of both values

	r (probability)			
	Initial TNC concentrations		Initial TNC pools	
Resprouting ratio	0.71	(0.010)	0.65	(0.022)
Shoot length	0.71	(0.010)	0.84	(<0.001)
Number of leaves	0.65	(0.023)	0.89	(<0.001)
Maximum lamina length	0.83	(<0.001)	0.86	(<0.001)

Relationship between TNC in roots and resprouting ability

Across the combinations of 12 treatments (four sites and 3 years), the resprouting ratio increased with increases in both initial TNC concentrations and TNC pools in the roots (Table 5). The resprouting shoot length, number of leaves, and maximum lamina length were also positively correlated with the initial TNC concentrations and pools. Correlation coefficients of the relationships between sprout-size parameters and the initial TNC pools were higher than those of the relationships between sprout-size parameters and the initial TNC concentrations.

The relationships between the re-establishment ratio and the initial TNC concentrations and between the re-establishment ratio and the the initial TNC pools follow saturation curves (Fig. 5). The re-establishment ratio was strongly influenced by both the initial TNC concentrations ($R^2 = 0.78$) and the initial TNC pools ($R^2 = 0.79$).

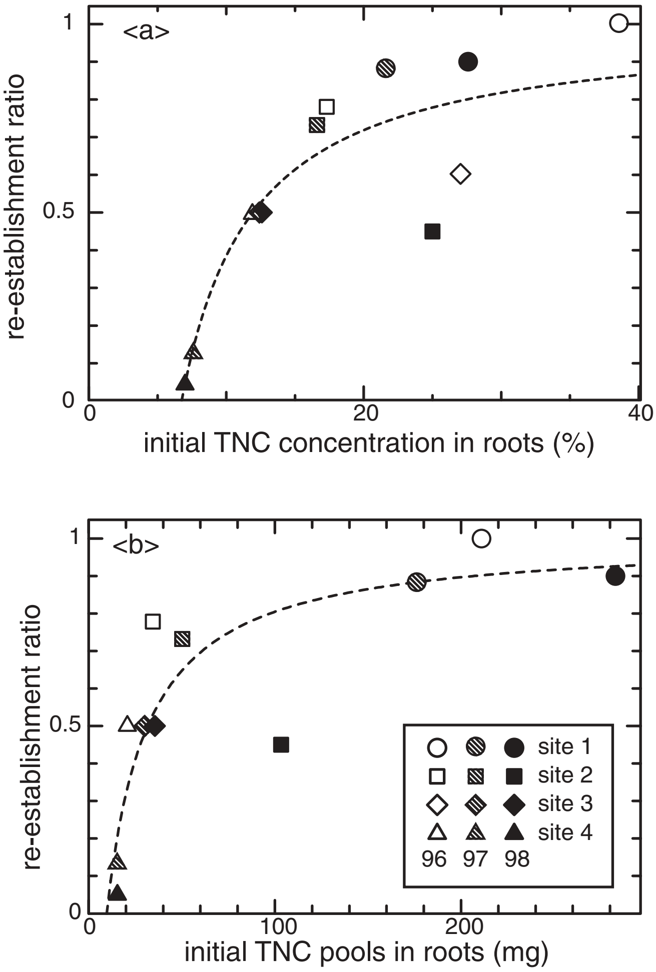

Fig. 5. **a** Relationship between the re-establishment ratio and initial TNC concentration. **b** Relationship between the re-establishment ratio and initial TNC pools. Data are for years 1996–1998. Regressions were calculated across the 12 treatment combinations (4 sites and 3 years). For **a**, the function of the regression curve is $y = 1/(1 + 5.19/(x–6.75))$ ($R^2 = 0.78$). For **b**, the function of the regression curve is $y = 1/(1 + 21.9/(x–9.74))$ ($R^2 = 0.79$)

Discussion

The resprouting abilities of *Quercus crispula* seedlings after shoot destruction were enhanced by release from shaded conditions, probably because levels of stored carbohydrate in the roots rose following the improvement in light conditions. In support of this hypothesis, *Q. crispula* seedlings stored a high level of carbohydrates in their roots, and the level of carbohydrates was negatively affected by the degree of vegetation cover. Furthermore, there were positive relationships between the level of carbohydrate in roots and resprouting in this species.

Relationships between the level of carbohydrate storage in the roots and resprouting abilities of the seedlings

We believe that carbohydrate storage in *Quercus crispula* seedlings is likely to affect several stages in resprouting and, thus, have a pronounced effect on overall resprouting success (i.e., re-establishment after shoot destruction). Firstly, seedlings' ability to produce new shoots following shoot destruction is likely to depend on their level of stored carbohydrate (Table 5). Bowen and Pate (1993) showed that, in *Stirlingia latifolia*, plants could not resprout after exhaustion of stored carbohydrate, even if they had a high potential to resprout (i.e., they had enough dormant buds). Secondly, seedlings with relatively low amounts of stored carbohydrate can only produce small shoots initially, if they are able to resprout at all. In previous studies, shading has been found to reduce the size of new shoots re-growing after partial defoliation of original shoots (Harmer 1999; Blundell and Peart 2001). In this study, it is further suggested that the smaller size of resprouting shoots in shaded conditions is due to the lower amounts of carbohydrate that they can store (Table 5). Other studies have shown that the amount of stored carbohydrate is important for the degree of regrowth and affects the size of resprouting shoots (e.g., Webb 1981; Kays and Canham 1991; Bowen and Pate 1993; McPherson and Williams 1998). Some studies have pointed out that stored resources only contribute to resprouting in early periods of regrowth since the role of stored carbohydrate is taken over by photosynthesis after the new shoots' leaves have expanded (e.g., Richards and Caldwell 1985; Danckwerts 1993; van der Heyden and Stock 1996). However, the current-year resprouting shoots have already grown close to their final size in *Q. crispula* before they begin to photosynthesize adequately, since the species has a flush-type shoot elongation pattern. Of course, photosynthates produced by resprouting shoots would strongly influence the shoots' sizes as from the next growth season.

Once functional shoots have developed, seedlings with destroyed shoots can photosynthesize for themselves. Following this, the degree of vegetation cover will affect the growth and survival of resprouting seedlings by limiting the rate of photosynthesis in the new shoots. At closed canopy sites, particularly sites 3 and 4, even undamaged *Quercus crispula* seedlings could not photosynthesize sufficiently to grow (Fig. 1). Under such conditions, resprouting shoots of the seedlings are likely to be so tiny that they will not be able to maintain individuals, and mortality after shoot destruction will be high (Fig. 2B). In addition to the photosynthetic ability of the resprouting shoots, high coefficients of determination of the relationships between the re-establishment ratio and initial carbohydrate levels suggest that the level of initial carbohydrate storage also affects the resprouting success (Fig. 5). Previous studies have suggested that the level of carbohydrate storage is one of the most important factors governing the survival of seedlings in shaded conditions (Kitajima 1994; Kobe 1997; Canham et al. 1999). Since stored carbohydrate acts as an important carbon source in shaded conditions, the amount stored might decide the survival of the seedlings in shaded sites. Low levels of carbohydrate storage may also limit resprouting success through increasing susceptibility to fungal infection. Plants with low reserves of carbohydrates show high sensitivity to infection by fungal pathogens (Wargo 1977; Carroll et al. 1983; Marson and Waring 1984). In undamaged seedlings of *Q. crispula* growing in shaded conditions, a very important cause of mortality is dieback, probably caused by pathogenic fungal infections (Kabeya 2001), and almost all clipped seedlings that did not resprout in this study were killed by dieback. Thus, seedlings that are growing in shaded conditions and store low amounts of carbohydrates might die due to fungal infection, even if they have sufficient levels of stored carbohydrate to resprout. These results collectively suggest that *Q. crispula* seedlings growing in shaded conditions with low levels of stored carbohydrate are likely to find it difficult to survive if their original shoots are destroyed.

The relationship between the level of root carbohydrate storage and the re-establishment ratio followed a saturation curve (Fig. 5). Thus, seedlings with high levels of stored carbohydrate had much more than the minimum needed for re-establishment. It is also possible that seedlings can resprout several times using excess stored carbohydrate, which would be advantageous in habitats where disturbances occur frequently (Hidaka 1973; Bowen and Pate 1993; McPherson and Williams 1998).

The effect of ambient light conditions on the level of carbohydrate reserves

The *Quercus crispula* seedlings in the gap site (site 1) stored large amounts of carbohydrates and developed the largest shoots (Figs. 1 and 3). However, in seedlings growing in gaps, it seems to be generally advantageous to enhance height growth by allocating as many of the available resources as possible to overcoming height competition with neighboring seedlings (Blundell and Peart 2001) rather than conserving resources. Why then do *Q. crispula* seedlings growing in gaps store considerable amounts of carbohydrates? Shoots of *Q. crispula* seedlings are often destroyed in the understory, and resprouting following this destruction was found to enhance seedling longevity in a previous study (Kabeya 2001). In addition, it is known that stems are gnawed by rodents (major agents of shoot destruction) at the open site without *Sasa* (Sato 2000). Hence, as suggested by previous authors (e.g., Bellingham 2000), it may be advantageous for the seedlings to allocate some resources to height growth while conserving some to counter possible future damage.

High levels of carbohydrate storage in the seedlings at the gap site may be interpreted according to C/N balance theory. It is known that carbohydrates often accumulate in leaves, stems, and roots when plants receive high light levels but low levels of nutrients and water, because carbohydrate production may then exceed requirements to satisfy the quantitative balance between carbohydrates and nutrients (Bloom et al. 1985; Chapin et al. 1990). Thus, in the site with a canopy gap but no *Sasa*, excess carbohydrates might be accumulated in the roots of the seedlings because the abundant light allows relatively large amounts of carbohydrates to be generated compared to the nutrient supplies available to the seedlings.

The role of carbohydrate storage in survival in heavily shaded conditions

Carbohydrate reserves in roots of *Quercus crispula* seedlings should contribute to survival in heavily shaded conditions. In site 4, the most heavily shaded site, the resprouting ratio decreased significantly with age. Moreover, the level of carbohydrate storage in roots and the reestablishment ratio both tended to decrease with age at this site, although there were no statistically significant differences in these parameters between years. The tendency for these variables to decrease with time at site 4 cannot be explained solely by annual fluctuations, since the annual patterns of these parameters varied among sites. They probably occurred because in these conditions the seedlings could not add further resources to their reserves (cf. Hashimoto and Shirahata 1995) and, instead, consumed reserves in order to survive. In such cases carbohydrate storage would decline as the seedlings aged. Where then had the initial reserves come from? We propose that they originated from seed reserves. *Quercus* spp. usually have large seeds with large amounts of carbohydrates (TNC accounts for approximately 60% of the weight of *Q. crispula* seeds). Some authors have pointed out that the reserve substances in seeds are important for survival in shaded conditions (e.g., Westoby et al. 1992). We further hypothesize that some of the seed reserves are translocated to roots and stored as initial carbohydrate reserves, regardless of the light conditions, as an adaptive mechanism allowing the seedlings to withstand environmental stresses, such as shoot destruction and heavy shading. These initial reserves are consumed for survival and decline with time in heavily shaded conditions, but when seedlings are released from such conditions, the level of reserves (and resprouting ability) should recover.

Conclusions

Quercus crispula seedlings growing under canopy gaps have greater resprouting abilities than seedlings growing under more shaded conditions, mainly because they store higher levels of carbohydrates, which play a very important role in resprouting. Because several types of disturbance, such as herbivory and shoot dieback, often cause shoot destruction, storage of resources to withstand such disturbances and periods of stress would be of considerable adaptive value in this species, despite their potential growth costs.

Acknowledgments We would like to thank Kouki Hikosaka for providing thoughtful comments and helpful suggestions on this study.

References

Abe M, Miguchi H, Nakashizuka T (2001) An interactive effect of simultaneous death of dwarf bamboo, canopy gap, and predatory rodents on beech regeneration. Oecologia 127:281–286

Abrams MD (1996) Distribution, historical development and ecophysiological attributes of oak species in the eastern United States. Ann Sci For 53:487–512

Bellingham PJ (2000) Resprouting as a life history strategy in woody plant communities. Oikos 89:409–416

Bloom AJ, Chapin FS, Mooney HA (1985) Resource limitation in plants – an economic analogy. Annu Rev Ecol Syst 16:363–392

Blundell AG, Peart DR (2001) Growth strategies of a shade-tolerant tropical tree: the interactive effects of canopy gaps and simulated herbivory. J Ecol 89:608–615

Bond JW, Midgley JJ (2001) Ecology of sprouting in woody plants: the persistence niche. Trends Ecol Evol 16:45–51

Bowen BJ, Pate JS (1993) The significance of root starch in post-fire shoot recovery of the resprouter *Stirlingia latifolia* R. Br. (Proteaceae). Ann Bot 72:7–16

Canham CD, Kobe RK, Latty EF, Chazdon RL (1999) Interspecific and intraspecific variation in tree seedling survival: effects of allocation to roots versus carbohydrate reserves. Oecologia 121:1–11

Carroll JE, Tattar TA, Wargo PM (1983) Relationship of root starch to decline of sugar maple. Plant Dis 67:1347–1349

Chapin FS, Schulze E-D, Mooney HA (1990) The ecology and economics of storage in plants. Annu Rev Ecol Syst 21:423–447

Crow TR (1988) Reproductive mode and mechanisms for self-replacement of northern red oak (*Quercus rubra*) – a review. For Sci 34:19–40

Crow TR (1992) Population dynamics and growth patterns for a cohort of northern red oak (*Quercus rubra*) seedlings. Oecologia 91:192–200

Danckwerts JE (1993) Reserve carbon and photosynthesis: their role in regrowth of *Themeda triandra*, a widely distributed subtropical graminaceous species. Funct Ecol 7:634–641

Danckwerts JE, Gordon AJ (1987) Long-term partitioning storage and re-mobilization of carbon-14 assimilated by *Lolium perenne* Cultivar Melle. Ann Bot 59:55–66

Danckwerts JE, Gordon AJ (1989) Long-term partitioning, storage and remobilization of 14C assimilated by *Trifolium repens* (cv. Blanca). Ann Bot 64:533–544

Dubois M, Gilles KA, Hamilton PA, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356

Fircks Y von, Sennerby-Forsse L (1998) Seasonal fluctuations of starch in root and stem tissues of coppiced *Salix viminalis* plants grown under two nitrogen regimes. Tree Physiol 18:243–249

Frazer GW, Canham CD, Lertzman KP (1999) Gap light analyzer (GLA), version 2.0. Imaging software to extract canopy structure and gap light transmission indices from true-colour fisheye photographs. Users manual and program documentation. Simon Fraser University, Burnaby, British Columbia, Canada

Garcia W, Nygren P, Desfontaines L (2001) Dynamics of nonstructural carbohydrates and biomass yield in a fodder legume tree at different harvest intensities. Tree Physiol 21:523–531

Harmer R (1999) Survival and new shoot production by artificially browsed seedlings of ash, beech, oak and sycamore grown under different levels of shade. For Ecol Manage 116:39–50

Hasegawa S (1984) Basic studies on the conservation of natural coastal forests in Hokkaido. Res Bull Coll Exp For Hokkaido Univ 41:312–422

Hashimoto R, Shirahata M (1995) Comparative study of leaf carbon gain in saplings of *Thujopsis dolabrata* var. *hondai* and *Quercus mongolica* var. *grosseserrata* in a cool-temperate deciduous forest. Ecol Res 10:53–64

Herrera J (1995) Acorn predation and seedling production in a low-density population of cork oak (*Quercus suber* L.). For Ecol Manage 76:197–201

Heyden F van der, Stock WD (1996) Regrowth of a semiarid shrub following simulated browsing: the role of reserve carbon. Funct Ecol 10:647–653

Hidaka M (1973) Effect of cutting on the total nonstructural carbohydrate (TNC) contents in the roots and crowns of *Rumex obtusifolius* L. J Jpn Grassl Sci 19:313–317

Ida H, Nakagoshi N (1996) Gnawing damage by rodents to the seedlings of *Fagus crenata* and *Quercus mongolica* var. *grosserrata* in a temperate *Sasa* grassland-deciduous forest series in southwestern Japan. Ecol Res 11:97–103

Kabeya D (2001) Adaptive significance of carbohydrate storage on resprouting ability in seedlings of *Quercus crispula*, a deciduous broad-leaved tree. PhD dissertation, Tohoku University, Sendai, Japan

Kays JS, Canham CD (1991) Effects of time and frequency of cutting on hardwood root reserves and sprout growth. For Sci 37:524–539

Kikuzawa K (1983) Leaf survival of woody plants in deciduous broad-leaved forests. 1. Tall trees. Can J Bot 61:2133–2139

Kitajima K (1994) Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. Oecologia 98:419–428

Kobe RK (1997) Carbohydrate allocation to storage as a basis of interspecific variation in sapling survivorship and growth. Oikos 80:226–233

Kruger EL, Reich PB (1993) Coppicing affects growth, root:shoot relations and ecophysiology of potted *Quercus rubra* seedlings. Physiol Plant 89:751–760

Makita A, Makita H, Nishiwaki A (1995) Mass flowering of *Sasa kurilensis* to the south of Lake Towada, northern Japan in 1995. Bamboo J 13:34–41

Marson PA, Waring RH (1984) Effects of nutrient and light limitation on mountain hemlock: susceptibility to light. Ecology 65:1517–1524

Matsubara T, Hiroki S (1989) Ecological studies on the plants of *Fagaceae* V. Growth in the sapling stage and minimal participation of reserve materials in the formation of annual new shoots of the evergreen *Quercus glauca* Thunb. Ecol Res 4:175–186

McPherson K, Williams K (1998) The role of carbohydrate reserves in the growth, resilience, and persistence of cabbage palm seedlings (*Sabal palmetto*). Oecologia 117:460–468

Ono K, Terashima I, Watanabe A (1996) Interaction between nitrogen deficit of a plant and nitrogen content in the old leaves. Plant Cell Physiol 37:1083–1089

Richards JH, Caldwell MM (1985) Soluble carbohydrates, concurrent photosynthesis and efficiency in regrowth following defoliation: a field study with *Agropyron-sp.* J Appl Ecol 22:907–920

Sakai A, Sakai S (1998) A Test for the resource remobilization hypothesis – tree sprouting using carbohydrates from above-ground parts. Ann Bot 82:213–216

SAS (1989) SAS/STAT user's guide. SAS Institute, Cary, N.C.

Sato T (2000) Effects of rodent gnawing on the survival of current-year seedlings of *Quercus crispula*. Ecol Res 15:335–344

Shidei T (1974) Forest vegetation zones. In: Numata M (ed) The flora and vegetation of Japan. Kodansha, Tokyo and Elsevier, Amsterdam, pp 87–124

Sokal RF, Rohlf FJ (1995) Biometry, 3rd edn. Freedman, New York

Taylor TJB, Pharis RP (1982) The role of plant hormones and carbohydrates in the growth and survival of coppiced *Eucalyptus* seedlings. Physiol Plant 55:421–430

Wada N (1993) Dwarf bamboos affect the regeneration of zoochorous trees by providing habitats to acorn-feeding rodents. Oecologia 94:403–407

Wada N, Murakami M, Yoshida K (2000) Effects of herbivore-bearing adult trees of the oak *Quercus crispula* on the survival of their seedlings. Ecol Res 15:219–227.

Wargo PM (1977) Wound closure in sugar maple: adverse effects of defoliation. Can J For Res 7:410–414

Webb WL (1981) Relation of starch content to conifer mortality and growth loss after defoliation by the Douglas-fir tussock moth. For Sci 27:224–232

Westoby M, Jurado E, Leishman MR (1992) Comparative evolutionary ecology of seed size. Trends Ecol Evol 7:358–372