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Shifts in biomass and resource allocation patterns following defoliation in *Eucalyptus globulus* growing with varying water and nutrient supplies

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Summary In woody species, potential mechanisms to compensate for tissue loss to herbivory and diseases have been related to post-event shifts in growth, biomass and internal resource allocation patterns, as modulated by external resource limitations. We examined the interactive effects of belowground resource limitations by varying nutrient and water availability, and aboveground carbon limitation imposed by a single defoliation event (40% leaf removal) on stem growth, whole-tree and within-tree resource allocation patterns (total non-structural carbohydrate and nitrogen) and below- and aboveground biomass allocation patterns in 8-month-old, field-grown *Eucalyptus globulus* Labill. saplings. Two months after treatments were imposed, the direction of the stem growth response to defoliation depended on the abiotic treatment. Five months after defoliation, however, we found little evidence that resource availability constrained the expression of tolerance to defoliation. With the exception of the combined low-nutrient and low-water supply treatment, saplings grown with (1) adequate water and nutrient supplies and even with (2) low-water supply or (3) low-nutrient supply were able to compensate for the 40% foliage loss. The observed compensatory responses were attributed to the activation of several short- and longer-term physiological mechanisms including reduced biomass allocation to coarse roots, mobilization of carbohydrate reserves, robust internal N dynamics and increased ratio of foliage to wood dry mass.

Keywords: carbon partitioning, compensatory growth, nitrogen, non-structural carbohydrate, tolerance.

Introduction

The negative effects of defoliation on tree performance can be mitigated through the expression of direct and indirect host tolerance; that is, through regrowth (Karban and Baldwin 1997, Haukioja and Koricheva 2000). At the whole-plant level, tolerance to defoliation can be mediated by a variety of compensatory mechanisms including up-regulation of photosynthetic rates in remaining leaves (Bassman and Dickmann 1982, Houle and Simard 1996, Pinkard and Beadle 1998*a*, Ayres et al. 2004, Pinkard et al. 2004, Turnbull et al. 2007*a*), alteration in growth patterns to favour leaf area development (Strauss and Agrawal 1999, Mediene et al. 2002) and post-defoliation shifts in internal resource allocation patterns within the plant's above- and belowground organs (Houle and Simard 1996, Pinkard et al. 2004, Katjiua and Ward 2006, Frost and Hunter 2008, Stevens et al. 2008).

The degree of tolerance expressed is related to several variables including frequency, severity and pattern of damage, inherent tree growth and the availability of water and nutrients (Houle and Simard 1996, Wise and Abrahamson 2005, Pinkard et al. 2007). Contrary to the widely accepted compensatory continuum hypothesis, which predicts lower tolerance when the availability of resources is low (Maschinski and Whitham 1989), most studies have shown that woody plants growing in high-resource conditions are less tolerant to defoliation than woody plants growing in low-resource conditions (Hawkes and Sullivan 2001, Wise and Abrahamson 2005). For example, under low-nutrient conditions, aspen tolerance was positively correlated with the proportion of biomass in stems just before defoliation, whereas under high-nutrient conditions it was correlated with greater allocation to stems in response to the damage (Stevens et al. 2008).

For woody tree species, which have a proportionately large capacity for storage of carbon (C) and nutrient reserves compared with herbaceous species (Kozlowsski 1992), the allocation and accumulation of these reserves within the tree following defoliation is of particular interest because it may provide insights into why defoliation sometimes has little or no effect on growth (Bassman and Dickmann 1982, Anttonen et al. 2002). For example, young

eucalypts (< 4 years old) can compensate for removal of up to 50% of their leaf area without long-term growth reductions, especially when defoliation is applied before canopy closure (Pinkard and Beadle 1998*b*, Pinkard et al. 2004, Alcorn et al. 2008). Evidence suggests that reserves of total non-structural carbohydrate (TNC) and nitrogen (N) enable defoliated trees to uncouple their growth from the reduced C assimilation, thereby allowing them to maintain growth at or near pre-defoliation rates (e.g., Gleason and Ares 2004, Myers and Kitajima 2007). The ability of trees to support regrowth through TNC and N reserves is likely to be influenced by environmental conditions such as soil nutrient and water availability (e.g., Esparza et al. 2001 but see Gholz and Cropper 1991). Unfortunately, little is known about the interactive effects of environment and defoliation on TNC and N reserves following defoliation in woody tree species.

We selected the evergreen woody tree species *Eucalyptus globulus* Labill. to investigate if patterns of resource allocation (TNC and N), and below- and aboveground biomass allocation serve as compensatory mechanisms. *Eucalyptus globulus* can reach heights of up to 55 m and is recognized for its high growth rates. To date, studies on this commercially important plantation species and its closely related species *Eucalyptus nitens* (Deane & Maiden) Maiden have focused on photosynthetic capacity and growth processes in response to defoliation or environmental stress (Pinkard and Beadle 1998*a*, 1998*b*, Pinkard et al. 1998, 2004, 2007, Pinkard 2003, Turnbull et al. 2007*a*). By comparison, there are many fewer studies on biomass and resource allocation patterns (Pinkard and Beadle 1998*a*, Pinkard et al. 2004); however, shifts in these plant traits may affect the degree of compensation that occurs following defoliation in these species (Pinkard et al. 2007).

We established a field experiment to examine the morphological and eco-physiological effects of artificial defoliation (40% leaf removal) on growth and biomass allocation in 8-month-old *E. globulus* saplings grown in two nutrient and two water regimes. Specifically, we determined whether (1) whole-tree and within-tree growth and biomass allocation patterns are influenced by the interactive effects of abiotic and defoliation treatments; (2) whole-tree and within-tree TNC and N allocation patterns are influenced by the interactive effects of abiotic and defoliation treatments and (3) stored TNC and N reserves meet the energy demands during periods of negative carbon balance caused by defoliation.

Materials and methods

Site and experimental design

The experiment was conducted in an *E. globulus* plantation located 20 km east of Hobart, Australia (42°49.4′ S and 147°30.6′ E). Soil at the site comprises an Aeolian-derived sandy A-horizon of 1–2 m depth overlying a sandy clay B-horizon. Bulk density of the A-horizon is 1.4 g cm^{-3}. Mean annual rainfall at the site is 500 mm, and mean pan evaporation is in excess of 1300 mm (Australian Bureau of Meteorology). Mean daily maximum and minimum temperatures are 22.5 and 12.5 °C in summer and 12.0 and 4.0 °C in winter. In December 2006, *E. globulus* seedlings of 0.25 m height were planted at the site at a spacing of 2 × 2 m. Seedling survival was 100%. There were six seedlings per plot with a single row of seedlings surrounding each plot as a buffer and all seedlings selected for measurements were surrounded by eight living seedlings. The small seedling size precluded the shading of adjacent seedlings. The seedlings were irrigated with municipal water every second day to provide the rainfall equivalent of 3 mm until the abiotic treatments (ATs) were applied. Seedlings were fertilized (100 kg N ha^{-1} $year^{-1}$ and 60 kg P ha^{-1} $year^{-1}$ plus trace elements; O'Grady et al. (2005)) at 2-week intervals until planted at the study site, and at 3-month intervals following planting. In February 2007, the ATs were applied in a completely randomized split-plot design with three replicates of four plot-level treatments: (1) TOT = adequate water and N (watered every second day with the rainfall equivalent of 1.5 mm daily + rainfall plus a full fertilizer dose comprising 100 kg N ha^{-1} $year^{-1}$ + trace elements, applied quarterly; irrigation was doubled in December 2007); (2) Low N&W = low water and low N (rain-fed plus fertilized with 25% of the full fertilizer dose); (3) Low N = limiting N only (irrigated as in TOT plus fertilized with 25% of the full fertilizer dose) and (4) Low W = limiting water only (rain-fed plus a full fertilizer dose). The ATs were applied to the plot and buffer seedlings. Because the study site has a sandy soil with low organic matter and low nutrition, the Low-N&W and Low-N treatments included fertilization with 25% of the full fertilizer dose to ensure that all treatments received a baseline amount of N.

In March 2007, we removed all whole leaves from the upper 50% of each sapling for half of the saplings (three saplings per AT), excluding the apical foliage, with long-nosed secateurs. The defoliation process was completed within a day. Based on the allometric equations developed by O'Grady et al. (2006) for saplings growing at the same site, we estimated that about 40% of the leaf area was removed.

Growth responses

Height (*h*; m) and diameter at 15 cm above the ground (*d*; cm) of each sapling were measured in March, May and August 2007. We measured the height from the ground to the apical meristem and measured the stem diameter with callipers. Mean height and diameter of all plot saplings (a total of 72 saplings) at the start of the experiment were 0.76 m and 1.16 cm, respectively. At the time of defoliation, there were no significant differences between the mean heights and diameters of undefoliated saplings (1.05 m and

1.78 cm) and defoliated saplings (1.01 m and 1.78 cm). Stem volume (V; m^3) was calculated as: $V = 0.333(\pi(d/2)^2)h$.

Biomass harvesting

In May 2007, there were significant effects of the ATs and defoliation on the growth responses of the saplings (Table 1A). To investigate these effects, about 5 months after defoliation (August 2007), one sapling per defoliation treatment per plot (total of two saplings per plot = 24 saplings) was destructively harvested for measurements of above- and belowground biomass. Saplings of similar size per plot were selected. Stem diameters were measured at 0.15, 0.5 and 1.3 m height. The length and the diameter (measured at 4 cm from the base) of all branches (ranging from 27 to 51 branches per tree) were measured and total sapling height was recorded.

For each sapling, the crown was divided into three height zones to facilitate detection of any within-tree variation, particularly as a function of old and new growth. These zones were: (1) lower crown zone (L-CZ) = the undefoliated half of the initial tree height when treatments commenced; (2) middle crown zone (M-CZ) = the initial sapling height when treatments commenced minus the lower zone and (3) upper crown zone (U-CZ) = all new height growth subsequent to imposing treatments (Figure 1). The defoliation treatment was applied in the M-CZ. Aboveground biomass from each zone was separated into leaves, main stems and branches and oven-dried to constant mass at 65 °C. Before drying, the leaf areas of a stratified random sample of 10 leaves per L-CZ and U-CZ were measured with a planimeter (Delta-T Devices, Cambridge, UK) for determination of specific leaf area (SLA; $m^2\ kg^{-1}$). Although all the branches sampled in each crown zone originated from within that zone, many spanned two and sometimes three zones. When this occurred, the branches were separated into various zones, so that biomass could be apportioned to the appropriate crown zone for total zone biomass analysis (Figure 1). Belowground biomass was separated into four root classes (2–10 mm, 10–20 mm, > 20 mm and rootball) and oven-dried to constant mass at 65 °C. Because of the presence of dead weed roots in the surface soil, fine roots (< 2 mm) were not sampled but the biomass was estimated based on the allometric relationships developed by O'Grady et al. (2006). Biomass of a given organ from each crown zone was pooled and then subsampled for N and TNC analyses. We focused on aboveground resource allocation patterns and one root class (2–10 mm), which accounted for ~ 50% of the total root biomass (Table 2).

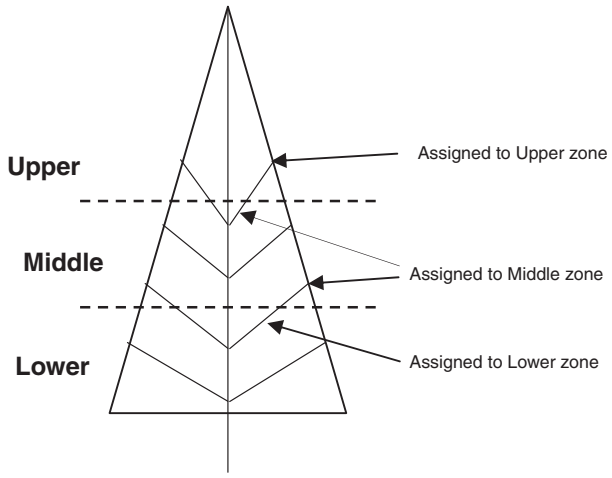

Figure 1. Apportioning of biomass between zones for total biomass analysis per zone.

Chemical analyses

About 160 mg of tissue (leaves, stems and branches from L-, M- and U-CZ) was ground to powder in liquid nitrogen

Table 1. Effects of AT × defoliation interaction on mean diameter and height increments of *E. globulus* saplings (A) 2 months and (B) 5 months after abiotic and defoliation treatments were imposed. Values are means of nine replicates with SE in parentheses. Abbreviations: Undef., undefoliated; Def., defoliated; TOT, adequate water and N; Low N&W, low water and low N; Low N, limiting N only and Low W, limiting water only. Treatment groups followed by the same letter did not differ significantly from each other at $\alpha > 0.05$, LSD test following ANOVA. If no letters are shown, results did not differ significantly.

AT	Diameter increment (mm)		Height increment (cm)	
	Undef.	Def.	Undef.	Def.
A				
Low N	7.8 (2.3) a	3.8 (0.9) b	38.6 (11.5)	34.9 (4.2)
Low W	3.7 (1.1) a	6.2 (1.7) b	22.6 (4.4)	35.7 (10.0)
Low N&W	2.9 (1.3)	3.2 (1.6)	17.9 (3.7)	23.3 (5.8)
TOT	9.7 (1.8)	8.0 (1.7)	49.8 (11.7)	46.4 (8.5)
B				
Low N	13.5 (3.5)	10.6 (2.7)	58.9 (14.2)	55 (3.0)
Low W	7.8 (2.1)	10.0 (2.5)	38.6 (5.8)	48.8 (5.9)
Low N&W	10.3 (2.1)	4.5 (1.7)	39.2 (8.4)	33.8 (3.7)
TOT	14.4 (1.9)	13.1 (2.5)	65.9 (9.2)	63.8 (5.6)

Table 2. Summary of a two-way, split-plot ANOVA the effects of AT (df = 3), defoliation (D) (df = 1) and their interaction (df = 3) on whole-tree biomass and resource parameters of *E. globulus* saplings 5 months after treatments were imposed.

	AT		Defoliation		AT × *D*	
	F	*P*	*F*	*P*	*F*	*P*
SLA (m^2 kg^{-1})	0.26	0.85	0.02	0.89	1.07	0.42
Leaf area (m^2)	0.78	0.55	2.47	0.15	1.04	0.43
Biomass (g tree^{-1})						
Leaf	1.09	0.42	4.01	0.08	0.92	0.47
Stem	1.77	0.25	0.10	0.76	1.02	0.43
Branch	0.30	0.82	1.91	0.21	1.02	0.43
Stem and branch	0.82	0.53	1.26	0.30	1.27	0.35
Total aboveground	0.91	0.49	2.42	0.16	1.12	0.40
Root 2–10 mm class	0.45	0.73	7.24	0.03	3.09	0.09
Root 10–20 mm class	0.52	0.68	0.43	0.53	0.67	0.60
Root 20–30 mm class	0.98	0.46	7.87	0.02	2.30	0.16
Rootball	0.77	0.55	0.02	0.90	1.80	0.23
Belowground (> 2 mm)	0.40	0.76	8.56	0.02	3.37	0.01
Estimated fine roots (< 2 mm)[1]	1.24	0.38	2.08	0.19	0.72	0.57
Total above- and belowground[2]	0.75	0.56	3.42	0.10	1.41	0.31
New foliar growth	1.27	0.37	0.99	0.35	0.40	0.76
Shoot:root ratio	1.47	0.32	5.11	0.05	4.15	0.05
Relative biomass (%)						
Leaf	1.19	0.39	0.52	0.49	1.20	0.37
Stem	2.89	0.12	4.28	0.07	0.36	0.78
Branch	0.34	0.80	0.16	0.70	0.26	0.85
Stem and branch	1.19	0.39	5.12	0.05	1.60	0.27
Coarse root	1.78	0.25	4.20	0.08	4.45	0.04
Resource pool (g tree^{-1})						
Leaf nitrogen	0.88	0.50	4.41	0.07	1.15	0.39
Stem nitrogen	2.46	0.16	0.56	0.48	1.05	0.42
Branch nitrogen	0.55	0.67	5.19	0.05	1.54	0.28
Leaf SS	2.23	0.19	1.19	0.31	0.73	0.56
Stem SS	1.78	0.25	0.07	0.79	1.05	0.42
Branch SS	0.48	0.71	1.15	0.31	0.39	0.76
Leaf starch	4.71	0.05	0.01	0.93	0.34	0.80
Stem starch	2.44	0.16	2.78	0.13	0.50	0.69
Branch starch	1.45	0.32	5.05	0.06	0.30	0.83
Leaf TNC	3.10	0.11	0.70	0.43	0.56	0.66
Stem TNC	2.19	0.19	1.06	0.33	0.79	0.53
Branch TNC	0.72	0.57	2.15	0.18	0.27	0.84
Root SS	0.86	0.51	4.83	0.06	1.93	0.20
Root starch	1.24	0.37	4.80	0.06	1.61	0.26
Root TNC	1.23	0.38	5.29	0.05	1.81	0.22

[1] Based on allometrics (O'Grady et al. 2006).
[2] Total biomass not including the estimated biomass of fine roots.

with a mortar and pestle, and analyzed for N by the single acid hydrogen peroxide technique (Lowther 1980). Total N (mg g^{-1}) was measured with a continuous flow colorimetric auto-analyzer (QuikChem 8000, Lachat Instruments).

Following the method of Palacio et al. (2007), soluble sugars (SS) were extracted from 50 mg of dried tissue (leaves, stems and branches from L-, M- and U-CZ, and roots (2–10 mm class)) in 10 ml of 80% (v/v) ethanol at 60 °C. Starch and complex sugars remaining in the pellet after ethanol extraction were digested to glucose with amyloglucosidase (Fluka-10115, Sigma-Aldrich). The concentrations of SS and starch were determined by a phenol–sulfuric acid colorimetric assay (DuBois et al. 1956) as modified by Buysse and Merckx (1993). Non-structural carbohydrates measured after the ethanol extraction are referred to as SS, carbohydrates measured after the enzymatic digestion are referred to as starch and the sum of SS and starch measured in glucose equivalents is referred to as TNC. The pool sizes of SS, starch and TNC (g glucose equivalents) for a given organ type were calculated as the product of an individual organ's biomass and either SS, starch or TNC concentration (mg g^{-1}).

Statistical analyses

Whole-tree N, SS, starch and TNC pool sizes (g tree^{-1}) were calculated as the sum of the contents in each zone (L-, M- and U-CZ). New foliar biomass (defined as foliar material produced after defoliation) was calculated for undefoliated saplings as: biomass of M-CZ + U-CZ − biomass removed by defoliation (i.e., 50 ± 2 g, which was the mean biomass removed for 12 replicates) and for defoliated saplings as: biomass of M-CZ + U-CZ. Effects of ATs and defoliation and their interactions on whole-tree growth measurements (*h* and *d* increments), absolute biomass (g), relative biomass allocation (expressed as a percent of total biomass), new foliar biomass, branch diameters, SS, starch, TNC and N concentration and pool sizes (mg g^{-1}or g tree^{-1}) were examined by split-plot analysis of variance (ANOVA). The block and AT were the main plot factors and were tested by the block × plot mean square; the subplot factor was defoliation and was tested by the subplot error mean square. Total degrees of freedom (df) in the ANOVA model were 23. Block was treated as random factors, and AT and defoliation as fixed factors.

Effects of AT and defoliation on within-tree biomass, SS, starch, TNC and N concentration and pool (mg g^{-1}or g zone^{-1}) were examined by a split–split plot ANOVA. The pool sizes of N, SS, starch and TNC for each organ per zone were calculated as the product of an individual organ's biomass and either N, SS, starch or TNC concentration (mg g^{-1}). Block was treated as a random factor, and AT, defoliation and crown zone as fixed factors. Total df in the ANOVA model were 71. Branch diameters from each zone were averaged before performing the split–split plot ANOVA.

All analyses were performed using Genstat Version 10.1 (VSN International) followed by Fisher's protected least significant difference (LSD) post hoc tests to determine significant differences among treatment means. Values of the foliar starch and TNC pools, root starch and TNC concentrations (mg g^{-1}) and all relative biomass allocation percentages were arcsin-square-root transformed to meet assumptions of normality and homogeneity of variance in ANOVA. Relationships among stem volume and biomass and resource factors were examined by linear regression.

Results

Stem growth influenced by abiotic treatment and defoliation

Two months after defoliation, diameter ($P < 0.05$) and height ($P = 0.051$) increments in undefoliated saplings grown with low-water availability (alone and in combination with low-nutrient availability) were decreased by at least 55% compared with the corresponding increments in TOT saplings ($P < 0.05$) (Table 1). Defoliation halved the diameter increment of saplings in the Low-N treatment but stimulated diameter increment in the Low-W treatment by 41% ($P < 0.05$) (Table 1). The diameter increments of saplings in the other ATs were unaffected by defoliation (Table 1). The direction of these stem growth responses changed 5 months after defoliation. Neither AT nor defoliation nor their interaction significantly affected diameter or height increment (Table 1). There was a non-significant reduction in diameter increment of 56% in defoliated saplings in the Low-N&W treatment ($P < 0.069$) (Table 1).

Allocation to aboveground biomass unaffected by AT and defoliation

Aboveground biomass of all plant organs was unaffected by the ATs (Figure 2; Table 2). There were non-significant trends of reduced leaf area ($P > 0.05$), leaf biomass ($P = 0.08$) and branch biomass ($P > 0.05$) in defoliated saplings (Tables 2 and 3). Close examination of the new foliar biomass revealed no reduction in new leaf production

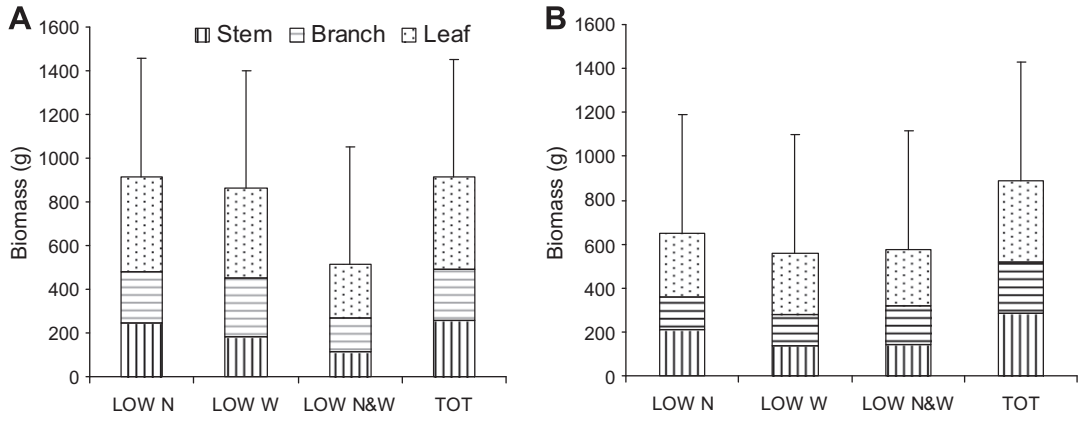

Figure 2. Mean biomass of whole-tree aboveground components (leaf, stem and branch) of (A) undefoliated and (B) defoliated *E. globulus* saplings 5 months after abiotic and defoliation treatments were imposed. Values are means of three replicates ±95% LSD bars. Abbreviations: TOT, adequate water and N; Low N&W, low water and low N; Low N, limiting N only and Low W, limiting water only.

Table 3. Effects of defoliation on whole-tree growth and biomass allocation of undefoliated and defoliated *E. globulus* saplings 5 months after treatments were imposed. Values are means of 12 replicates with SE in parentheses. Asterisk (*) indicates significant differences between defoliation treatments ($\alpha < 0.05$, LSD test following ANOVA) and ns indicates no significant difference.

Growth parameter/tree	Undefoliated	Defoliated	Significance
Height increment (m)	0.64 (0.07)	0.62 (0.06)	ns
Diameter increment (cm)	1.0 (0.2)	1.0 (0.2)	ns
SLA (m^2 kg^{-1})	10.0 (0.3)	10.0 (0.3)	ns
Leaf area (m^2)	3.75 (0.3)	3.1 (0.4)	ns
Total biomass (g tree^{-1})			
Leaf	377 (34)	300 (33)	ns
Stem	199 (25)	193 (29)	ns
Branch	224 (30)	176 (27)	ns
Stem and branch	423 (50)	369 (53)	ns
Total aboveground	800 (81)	668 (85)	ns
Root 2–10 mm class	107 (14)	74 (12)	*
Root 10–20 mm class	34 (5)	30 (5)	ns
Root 20–30 mm class	23 (6)	7 (2)	*
Rootball	64 (7)	74 (12)	ns
Belowground (total coarse root (> 2 mm)	233 (28)	175 (23)	*
Estimated fine roots (< 2 mm)[1]	334 (32)	280 (34)	ns
Total above- and belowground[2]	1033 (108)	843 (106)	ns
New foliar growth	167 (22)	148 (17)	ns
Shoot:root ratio	3.6 (0.2)	4.0 (0.3)	*
Relative biomass allocation (%)			
Leaf	37 (1)	37 (1)	ns
Stem	19 (1)	23 (1)	ns
Branch	21 (1)	20 (1)	ns
Stem and branch	40 (1)	43 (1)	0.052
Coarse root	22 (1)	21 (1)	ns

[1] Based on allometrics (O'Grady et al. 2006).
[2] Total biomass not including the estimated biomass of fine roots.

following defoliation (Tables 2 and 3). There was no significant defoliation × AT interaction on total aboveground biomass across all plant organs (Table 2). There were, however, non-significant trends of reduced total biomass in defoliated saplings in the Low-N and Low-W treatments, whereas there was no apparent change in total biomass in the TOT and Low-N&W saplings ($P = 0.16$) (Figure 2; Table 2). In the absence of defoliation, mean total biomass was about 40% less ($P > 0.05$) in Low-N&W saplings than in saplings in the other ATs (Figure 2).

Analysis of relative biomass allocation indicated that defoliation did not affect stem and branch biomass when considered individually, however when combined, there was a 6% ($P = 0.052$) increase in total aboveground woody tissue (Tables 2 and 3). There were significant abiotic × defoliation effects on relative biomass allocation to new leaves. In the Low-N&W treatment, defoliated saplings allocated 37% less to new foliage compared with undefoliated saplings ($P < 0.05$) (Figure 3A). In the absence of defoliation, Low-W saplings allocated 38% less to new foliage that Low-N&W saplings (Figure 3A). Branch numbers per zone were not significantly affected by AT or defoliation. There was, however, a significant defoliation × zone interaction with mean (±SE) branch diameter in M-CZ being 22% smaller in defoliated saplings (4.3 ± 0.2 mm) (mean number of branches = 11) than in undefoliated saplings (5.6 ± 0.4 mm, mean number of branches = 12) ($P < 0.01$).

Allocation to belowground biomass influenced by abiotic treatment and defoliation

Defoliation reduced belowground biomass (defined as total coarse root > 2 mm in diameter) by 25% ($P < 0.05$) (Table 2). Among the four root classes, defoliation reduced the biomass of roots in the 2–10 and 20–30 mm classes by 30% and 71%, respectively ($P < 0.05$) (Table 3). For undefoliated saplings, relative biomass allocation to coarse roots was unaffected by any AT (Figure 3B). In contrast, in defoliated saplings, relative biomass allocation to coarse roots was about 30% greater in Low-N&W saplings than in saplings in the other AT ($P < 0.05$) (Figure 3B). This change in root biomass was reflected in a 13% increase in shoot:root biomass ratio from 3.6 ± 0.2 to 4.0 ± 0.3, indicating that defoliation resulted in increased allocation to aboveground stems, branches and leaves ($P < 0.05$) (Table 3). The ATs influenced the effect of defoliation on this ratio ($P = 0.055$) (Figure 3C). For example, the shoot:root biomass ratio of

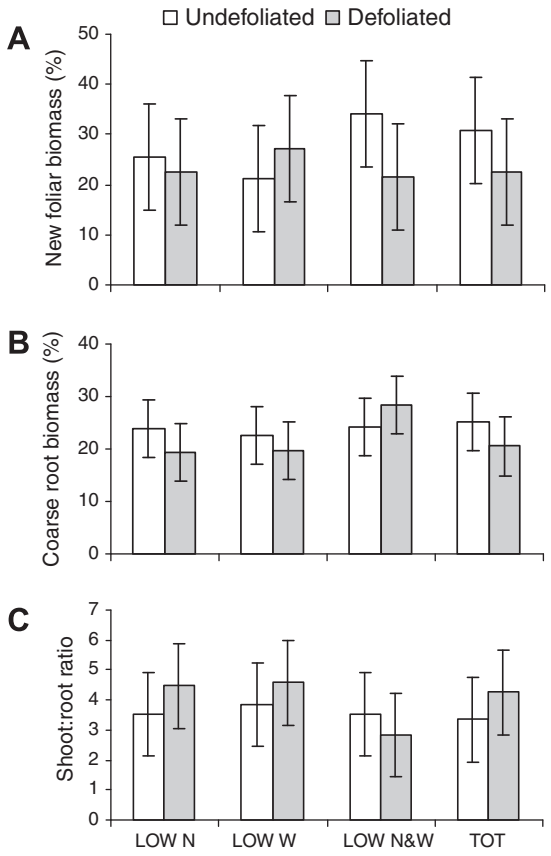

Figure 3. The AT × defoliation interaction effects on whole-tree (A) relative biomass allocation to new foliage, (B) relative biomass allocation to coarse roots (> 2 mm in diameter) and (C) shoot-to-root biomass ratio of *E. globulus* saplings 5 months after abiotic and defoliation treatments were imposed. Values are means of three replicates ±95% LSD bars. Abbreviations: TOT, adequate water and N; Low N&W, low water and low N; Low N, limiting N only and Low W, limiting water only.

defoliated saplings was 33% lower in the Low-N&W treatment than in the TOT treatment (Figure 3C).

Whole-tree carbohydrate dynamics influenced by abiotic treatment and defoliation

In general, total carbohydrate pools for each plant organ were unaffected by the AT and the AT × defoliation interaction (Table 2). Total SS and starch pools were the highest in leaves (33.4 ± 2.7 and 15.1 ± 1.4 g tree^{-1}) followed by branches (11.9 ± 1.1 and 6.1 ± 0.7 g tree^{-1}) and stem (4.85 ± 0.5 and 5.6 ± 0.7 g tree^{-1}). Roots (2–10 mm class) had a larger pool of starch (4.3 ± 0.6 g tree^{-1}) than of total SS (3.08 ± 0.4 g tree^{-1}). Defoliation tended to decrease the total branch starch pool (7.2 ± 1.0 versus 5.0 ± 0.9 g; $P = 0.055$).

In roots of the 2–10 mm class, defoliation decreased the pools of SS (3.6 ± 0.7 versus 2.6 ± 0.5 g) and starch (5.0 ± 0.8 versus 3.7 ± 0.8 g) by 28% ($P = 0.059$) and 27% ($P = 0.06$), respectively. This resulted in a 28% reduction in the TNC pool in response to defoliation (8.6 ± 1.4 versus 6.2 ± 1.2 g; $P = 0.05$).

Aboveground N dynamics unaffected by abiotic treatments and defoliation

Whole-tree N pool (Table 2) and concentration (data not presented) for all plant organs were largely unaffected by the ATs, defoliation and their interaction. Defoliation, however, decreased total branch (1.8 ± 0.2 and 1.3 ± 0.2 g, $P = 0.052$) and leaf N pools (9.8 ± 0.9 and 7.6 ± 0.9 g, $P = 0.069$) by 29% and 22%, respectively. The total N pool ranged from 8.7 ± 0.6 g tree^{-1} in leaves to 0.77 ± 0.07 g tree^{-1} in stem and 1.5 ± 0.1 g tree^{-1} in branches.

Table 4. Effects of defoliation × zone interaction on within-tree (A) absolute biomass and relative biomass allocation (% of total biomass within each zone) and (B) resources allocation of undefoliated and defoliated *E. globulus* saplings 5 months after abiotic and defoliation treatments were imposed. Values are means of 12 replicates with SE in parentheses. Abbreviations: Undef., undefoliated and Def., defoliated. Treatment groups followed by the same letter did not differ statistically significantly from each other ($\alpha > 0.05$, LSD test following ANOVA). If no letters are shown, results did not differ significantly.

Zone	Foliar biomass (g zone^{-1})		Relative foliar biomass (%)		Relative branch biomass (%)	
	Undef.	Def.	Undef.	Def.	Undef.	Def.
A						
Upper	72 (14)	68 (9)	62 (2) a	67 (1) b	28 (2) a	21 (1) b
Middle	145 (13) a	80 (10) b	55 (2) a	45 (2) b	29 (2)	33 (2)
Lower	160 (24)	151 (22)	38 (3)	39 (2)	26 (3)	24 (2)

Zone	Branch N concentration (mg g^{-1})		Foliar SS pool (g zone^{-1})		Branch SS concentration (mg g^{-1})		Foliar TNC pool (g zone^{-1})	
	Undef.	Def.	Undef.	Def.	Undef.	Def.	Undef.	Def.
B								
Upper	15.2 (0.8) a	13.3 (0.9) b	6.77 (1.1)	7.48 (1.4)	80.4 (6.0)	73.8 (3.3)	10.3 (1.5)	10.5 (1.9)
Middle	7.90 (0.5)	8.13 (0.4)	13.6 (1.2) a	7.91 (1.2) b	57.4 (4.2) a	70.9 (5.6) b	18.4 (1.7) a	10.7 (1.7) b
Lower	6.27 (0.5)	5.71 (0.2)	15.9 (2.2)	15.2 (2.6)	58.5 (4.0)	51.1 (2.2)	22.7 (3.2)	22.9 (3.3)

Within-tree biomass and resource allocation patterns influenced by abiotic treatments and defoliation

Defoliation reduced foliar biomass in the M-CZ – the zone of defoliation – by 46% ($P < 0.05$) (Table 4A). In the L-CZ, SLA was reduced in defoliated Low-N (9.1 ± 1.4 m^2 kg^{-1}) and defoliated Low-W (9.5 ± 0.4 m^2 kg^{-1}) saplings compared with defoliated Low-N&W (12.1 ± 0.8 m^2 kg^{-1}) and defoliated TOT (10.5 ± 1.8 m^2 kg^{-1}) saplings ($P < 0.05$). In defoliated Low-N&W saplings, SLA in the U-CZ decreased by 32% (8.2 ± 0.7 m^2 kg^{-1}) ($P < 0.05$), indicating the production of thinner leaves following defoliation. In contrast, the SLA of undefoliated saplings was unaffected by the AT × zone interaction. Mean SLA per tree was not significantly affected by the ATs (ranging from 9.6 to 10.6 m^2 kg^{-1}) or by defoliation (10.0 ± 0.3 versus 10.0 ± 0.3 m^2 kg^{-1}) or their interaction ($P > 0.05$).

In the U-CZ, defoliation caused an 8% increase in relative biomass allocation to foliage ($P < 0.001$), whereas it decreased relative biomass allocation to branches by 27% ($P < 0.05$) (Table 4A). This result is supported by a strong negative linear relationship between leaf and branch relative biomass not only in the M-CZ but also in the U-CZ in both defoliated and undefoliated saplings (Figure 4A). However, the slope of this linear relationship differed significantly between undefoliated saplings ($y_{Undef.} = -0.937x + 86.4$) and defoliated saplings ($y_{Def.} = -0.315x + 42.0$) (Figure 4B), indicating that foliage produced after defoliation was more densely arranged on a smaller branch framework (Table 4A).

Defoliation caused a 12.5% reduction in branch N concentration only in the U-CZ ($P < 0.05$) (Table 4B); however, this reduction was significant ($P < 0.05$) only in the Low-N (12.4 ± 2.5 mg g^{-1}) and TOT (10.2 ± 1.3 mg g^{-1}) saplings and not in the Low-N&W (15.1 ± 0.7 mg g^{-1}) and Low-W (15.4 ± 0.6 mg g^{-1}) saplings. Defoliation decreased the foliar SS pool by 42% ($P < 0.05$) and increased branch SS concentration by 20% but only in the M-CZ ($P < 0.05$) (Table 4B). Overall, the foliar TNC pool in the M-CZ was 41% lower in defoliated saplings than in undefoliated saplings ($P < 0.05$) (Table 4B).

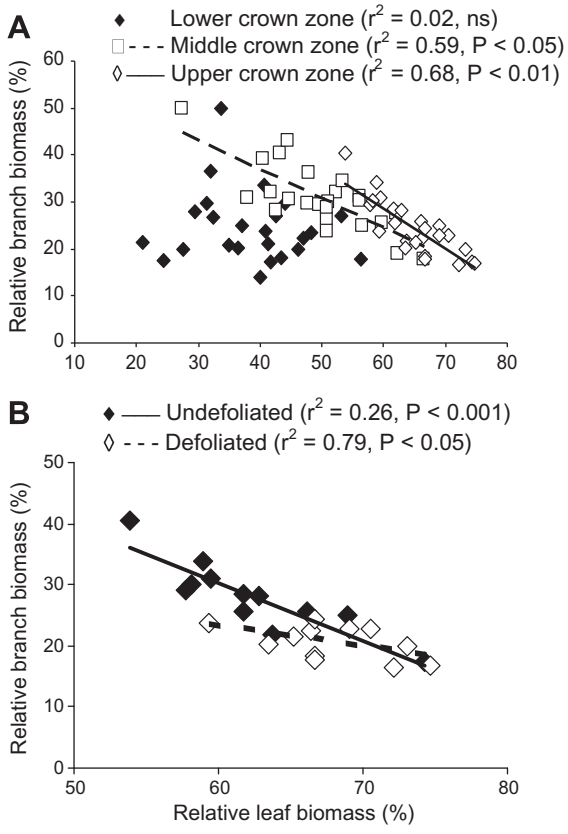

Figure 4. Relationships between within-tree relative branch biomass (%) and relative leaf biomass (%) for (A) all crown zones ($n = 24$ saplings) and (B) U-CZ only of undefoliated and defoliated *E. globulus* saplings 5 months after abiotic and defoliation treatments were imposed ($n = 12$ replicates per treatment). Relationships are shown only wherever significant ($P < 0.05$).

Relationship between stem volume and carbohydrate pools affected by defoliation

Although there were significant relationships between final stem volume and various biomass and resource factors, the majority of the relationships were unaffected by the defoliation treatment. The positive relationship between final stem volume and whole-tree stem starch pool was affected by defoliation (Figure 5A), indicating a greater depletion of stem starch reserves to maintain stem growth in defoliated saplings than in undefoliated saplings. For a given stem volume, defoliated saplings had a smaller stem starch pool than undefoliated saplings. Likewise, stem volume was positively related to branch starch pool but only in defoliated saplings (Figure 5B). This relationship suggests that the branch starch pool may offer an indirect means of evaluating stem volume in response to defoliation. There was no clear relationship between stem volume and total foliar starch pool in either undefoliated or defoliated saplings (Figure 5C).

Discussion

We found that limited resource availability, defined as low-nutrient and low-water conditions, did not constrain the expression of tolerance to defoliation. Defoliated *E. globulus* saplings grown with an adequate supply of nutrients and water conditions and even with a limited supply of nutrients or water, but not limited supplies of both nutrients and water, were able to compensate for a 40% foliage loss within 5 months after defoliation. Our findings contrast with other studies showing that the effect of defoliation on stem growth is more severe on low-productivity sites than on high-productivity sites (Pinkard and Beadle

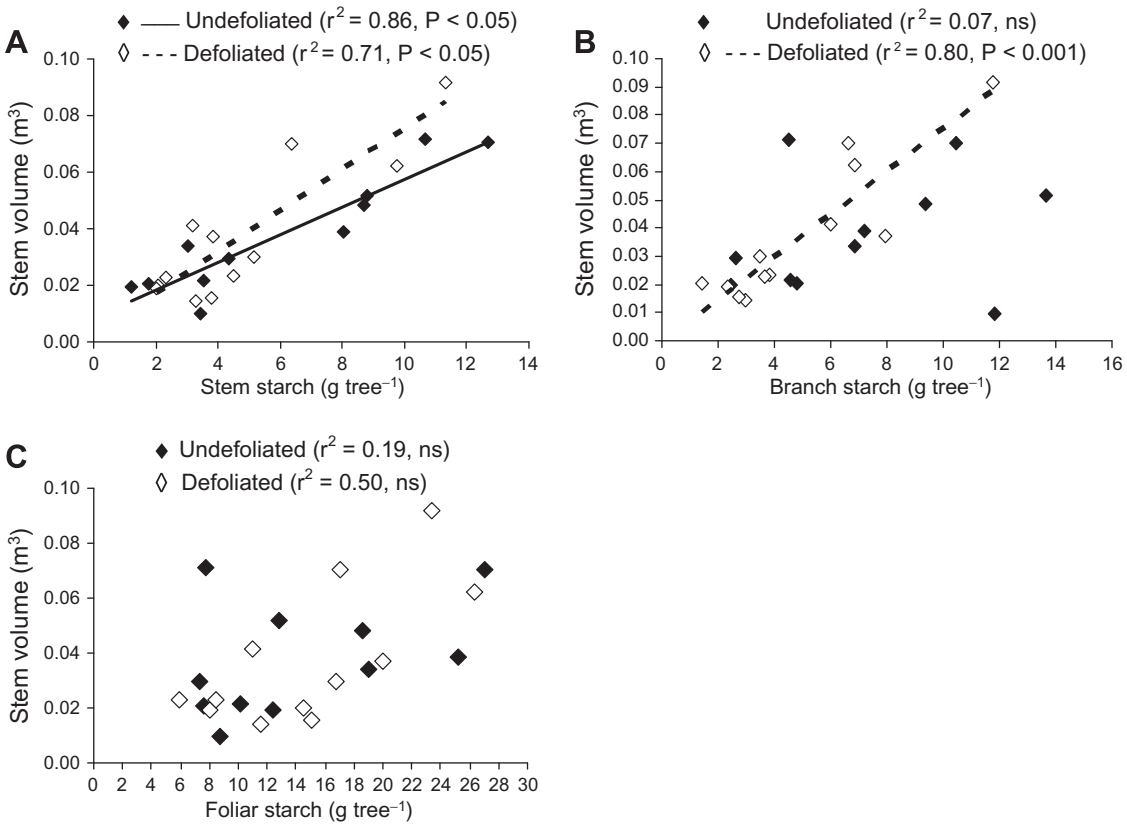

Figure 5. Relationships between stem volume and (A) stem starch, (B) branch starch and (C) foliar starch pools of undefoliated and defoliated *E. globulus* saplings 5 months after abiotic and defoliation treatments were imposed (n = 12 replicates per treatment). Relationships are shown only wherever significant ($P < 0.05$).

1998*c*, Anttonen et al. 2002, Pinkard et al. 2007), suggesting that environmental conditions influence the degree of tolerance to defoliation (Stamp 2003, Wise and Abrahamson 2005, Valladares et al. 2007). Although there were significant AT and defoliation effects on diameter and height increments 2 months after defoliation, by 5 months, these effects had largely disappeared across all ATs with the exception of the defoliated Low-N&W treatment (Table 1). Furthermore, 5 months after defoliation, height increment was unaffected by defoliation across all ATs. Previous studies have also demonstrated that diameter increment is more sensitive to pruning and defoliation than height increment (Pinkard and Beadle 1998*a*, Pinkard 2002, Smith et al. 2006, Alcorn et al. 2008), but this difference may depend on various factors including species, severity of damage and recovery time (Anttonen et al. 2002, Thomas et al. 2006). Our finding that *E. globulus* saplings were able to compensate for a 40% loss in foliage within 5 months even when grown with a limited supply of water or nutrients, reflects the very strong apical dominance exhibited by young eucalypts.

Many woody species, including *E. globulus*, are able to initiate multiple compensatory physiological mechanisms to offset the reduction in total photosynthetic capacity following defoliation. We suggest that the accompanying stem growth responses following defoliation were achieved by a complex suite of short- and longer-term shifts in growth, biomass and resource allocation patterns, which were modulated by resource availability. Across all ATs, defoliated saplings were able to produce similar amounts of aboveground biomass in all plant organs as undefoliated saplings (Figure 2). The biomass of new foliar growth was adversely impacted by defoliation in the Low-N&W saplings, but not in the Low-W, Low-N and TOT saplings (Figure 3A). The reduction in new foliar growth in the defoliated Low-N&W saplings may have contributed to the observed reduction in stem growth in these saplings. Of the new foliar growth in the U-CZ, defoliated saplings allocated a larger proportion to leaves and less to branches than undefoliated saplings (Figure 4B; Table 4A). Additionally, in the M-CZ, defoliation decreased branch size. An increased ratio of foliage to wood dry mass at the whole-tree level has also been reported in similar defoliation trials in both *E. nitens* and *E. globulus* (Pinkard and Beadle 1998*a*, Pinkard et al. 2004) and can be explained on the basis that shorter branches have more efficient use of carbon per unit of leaf area (Causton 1985). However, we also observed a small increase (P = 0.052) in the relative biomass of total aboveground woody tissue in response to defoliation. Because stem biomass was unaffected by defoliation, saplings

appeared to maintain stem biomass at the expense of branch biomass (Pinkard et al. 2004) or at the expense of leaves (Anttonen et al. 2002).

Notwithstanding the importance of an adequate root system for water and nutrient uptake and storage of resources (Canham et al. 1999), our defoliated saplings sacrificed absolute coarse root biomass (independent of AT) in favour of aboveground biomass (Table 3). An increased allocation of resources to shoots is a recognized compensatory response associated with tolerance to herbivory (Strauss and Agrawal 1999). However, our results contrast sharply with the majority of published data on the effect of biotic or abiotic stress on the allocation of root biomass patterns (Esparza et al. 2001, Hermans et al. 2005, Thomas et al. 2006, Snyder and Williams 2007). For example, in response to 2 years of severe water stress, 7-year-old *Prunus dulcis* (Mill.) D.A. Webb cv. Nonpareil trees maintained the same total root biomass (both fine and coarse) as controls (Esparza et al. 2001). Similarly, the root biomass of 4-month-old *Eucalyptus grandis* W. Hill ex Maiden seedlings was unaffected by 42% pruning (Thomas et al. 2006). Conversely, in 2-year-old *Quercus rubra* (L.) seedlings, defoliation reduced belowground carbon allocation to fine roots by 63% but this response was recorded only 7 days after defoliation (Frost and Hunter 2008). We did not measure fine root biomass, but based on allometric relationships developed by O'Grady et al. (2006), the estimated fine root biomass was not adversely affected by defoliation.

A link between amounts of non-structural carbohydrate reserves and tolerance to defoliation or pruning has been reported in both tree seedlings and saplings (e.g., Canham et al. 1999, Myers and Kitajima 2007) and adult trees (e.g., Webb 1981, Fang et al. 2006). In our study, 5 months after defoliation, leaves were the dominant aboveground organ for storage of carbohydrates, accounting for 64% of TNC. Nevertheless, carbohydrate dynamics were largely unaffected by the ATs and defoliation. Defoliation did, however, result in the depletion of whole-tree foliar SS and TNC pools (Table 4B), and reduced the TNC pool in roots (diameter class 2–10 mm), suggesting that defoliated saplings depended on carbohydrate reserves to overcome the negative carbon balance imposed by defoliation (Myers and Kitajima 2007). Similarly, although defoliation reduced both branch N concentration in the U-CZ (Table 4B) and stem N pool in both low-water treatments, N dynamics were unresponsive to ATs and defoliation. Our field site was characterized by deep Aeolian sand with suboptimal soil N concentrations, as indicated by the greater height of the defoliated TOT saplings compared with saplings in the other treatments (Table 1). Therefore, the apparent lack of significant results was unexpected and inconsistent with the previous studies (Mediene et al. 2002, Ayres et al. 2004, Turnbull et al. 2007*a*). For example, in young *E. globulus* saplings, foliar N concentration increased with defoliation but that was only 5 weeks after defoliation (Turnbull et al. 2007*a*). In 1-year-old peach trees (*Prunus persica* (L.)), transient changes in internal N concentrations occurred in response to 60% pruning but returned to control values by 2.5 months after pruning (Mediene et al. 2002). Turnbull et al. (2007*b*) demonstrated that the within-canopy N gradient in 4-year-old *E. globulus* was unaffected by fertilization, and Esparza et al. (2001) demonstrated that internal N concentration across all plant organs in 7-year-old *P. dulcis* was unaffected by severe water stress. It would appear that, even when subjected to external N constraints, defoliated *E. globulus* saplings by unknown mechanisms are able to maintain internal N dynamics comparable with those of undefoliated saplings.

In conclusion, saplings grown with a low-water supply, or a low-nutrient supply or with adequate water and nutrient supplies were able to compensate for foliage loss by the activation of several short- and longer-term physiological mechanisms. These mechanisms included (1) elevated photosynthetic rates (Pinkard et al. unpublished data), (2) reduced biomass allocation to coarse roots, (3) mobilization of carbohydrate reserves, (4) robust internal N dynamics and (5) increased ratio of foliage to wood dry mass. In contrast, saplings grown with limited water and nutrients were severely affected by defoliation that was manifest in a reduction in stem diameter increment that was linked to reduced new foliar growth. Our results also suggest that the effect of defoliation on biomass and resource allocation patterns had not been fully realized after 5 months of recovery time. In the previous studies of the effect of recovery time, mostly on growth rather than on biomass responses, woody tree species including eucalypt species required at least 1–2 years to recover from the removal of up to 50% of the crown (Anttonen et al. 2002, Alcorn et al. 2008).

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