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ROOT STARCH STORAGE AND ALLOCATION PATTERNS IN SEEDER AND RESPROUTER SEEDLINGS OF TWO CAPE *ERICA* (ERICACEAE) SPECIES[1]

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Post-fire sprouting of dormant buds in resprouter plants is facilitated by stored carbohydrate reserves, with starch being the critical reserve. Starch is mainly stored in xylem parenchyma ray tissue of woody underground organs, such as burls, lignotubers, and roots. We carried out a comparative analysis of the pattern of starch storage and the proportion of parenchymatic ray tissue in the upper root or cotyledonary region of seedlings from seeder and resprouter forms within two Cape *Erica* (Ericaceae) species: *E. coccinea* L. and *E. calycina* L., which were raised in the greenhouse under controlled irrigation. We also explored the root-to-shoot allocation patterns of seeder and resprouter seedlings in these two species. Resprouter seedlings of both species showed higher relative amounts of upper-root starch and upper-root storage tissue as well as a higher root-to-shoot allocation than their seeder counterparts. Pronounced swelling of the upper root region suggests ontogenetic development of a lignotuber in the resprouter forms of the two *Erica* species. The distinct allocation of starch in roots seems to be genetically determined and would account for the apparent differences in the root-to-shoot allocation patterns between both regeneration forms from the early seedling stage.

Key words: Cape Floristic Region; *Erica*; Ericaceae; fynbos; lignotuber; post-fire regeneration; root : shoot ratio; starch storage tissue.

Fire seems to have played a crucial role as a selective pressure in determining regeneration modes of mediterranean woody plants (Naveh, 1975; Keeley, 1986; Cowling, 1987; but see Mesléard and Lepart, 1989; López-Soria and Castell, 1992; and Ojeda, 2001 for a critical view from the Mediterranean Basin). Generally, woody species are grouped into two categories according to their post-fire response: seeders and resprouters (Bond and van Wilgen, 1996). Seeder plants are killed by fire, with stands being reestablished by a massive seedling recruitment from a preexisting, soil- or canopy-stored seed bank. Resprouter plants, by contrast, survive the complete removal of all the aerial parts by fire, and recover relatively quickly by growth of dormant buds protected from the action of fire, either by thick bark or by location near the soil surface, irrespective of their effectiveness on post-fire seedling emergence. Although both seeder and resprouter post-fire responses imply resilience to recurrent wildfires at the population level, their effects in the dynamics of populations are altogether different. Each fire means a complete generation turnover for seeder populations, being nonoverlapping generations (assuming that seed germination is induced by fire, e.g., see Keeley and Bond, 1997). Besides, seeder populations will be prone to dramatic size fluctuations (i.e., bottlenecks) between fires, whereas resprouter populations are somewhat buffered from such fluctuations because of the ability of resprouter adult individuals to survive fire. These differences in the population dynamics have crucial consequences in the long-term diversification patterns of seeder and resprouter lineages (Wells, 1969; Ojeda, 1998).

Post-fire sprouting of dormant buds is boosted by stored nutrient and carbohydrate reserves. The major critical carbohydrate reserve in resprouter plants is starch (Miyanishi and Kellman, 1986; Pate et al., 1990; Verdaguer et al., 2001). Several authors have claimed the role of swollen underground structures such as root-crowns, burls, or lignotubers as specialized organs for starch storage (e.g., Bamber and Mullette, 1978; Canadell and López-Soria, 1998) and a placement for dormant buds (James, 1984; Molinas and Verdaguer, 1993a, b). However, other authors argue that these structures are no more efficient than woody roots as tissues for starch storage (e.g., Cruz and Moreno, 2001) and that the selective advantage of developing a swollen underground structure is mainly related to the large amount of concealed buds found on its surface in a protected position close to the ground (Carrodus and Blake, 1970).

Starch is mainly stored within xylem parenchyma ray tissue of underground organs (Loescher, McCamant, and Keller, 1990; Pate et al., 1990; Bell, Pate, and Dixon, 1996; Bell and Ojeda, 1999). This type of storage tissue can be considered to be expensive in terms of resource allocation as ray parenchyma cells of wood are living and non-photosynthetic (Salisbury and Ross, 1992; Bowes, 1996) and require a high metabolic demand to be both created and maintained. In addition, the synthesis of starch to fill such cells detracts from the pool of carbon that could otherwise be invested in photosynthetic tissue. Therefore, allocation to underground starch storage would imply a physiological trade-off for the plant in terms of allocation to aboveground growth of photosynthetic parts (Bloom, Chapin, and Mooney, 1985; Iwasa and Kubo, 1997; Sakai, Sakai, and Akiyama, 1997).

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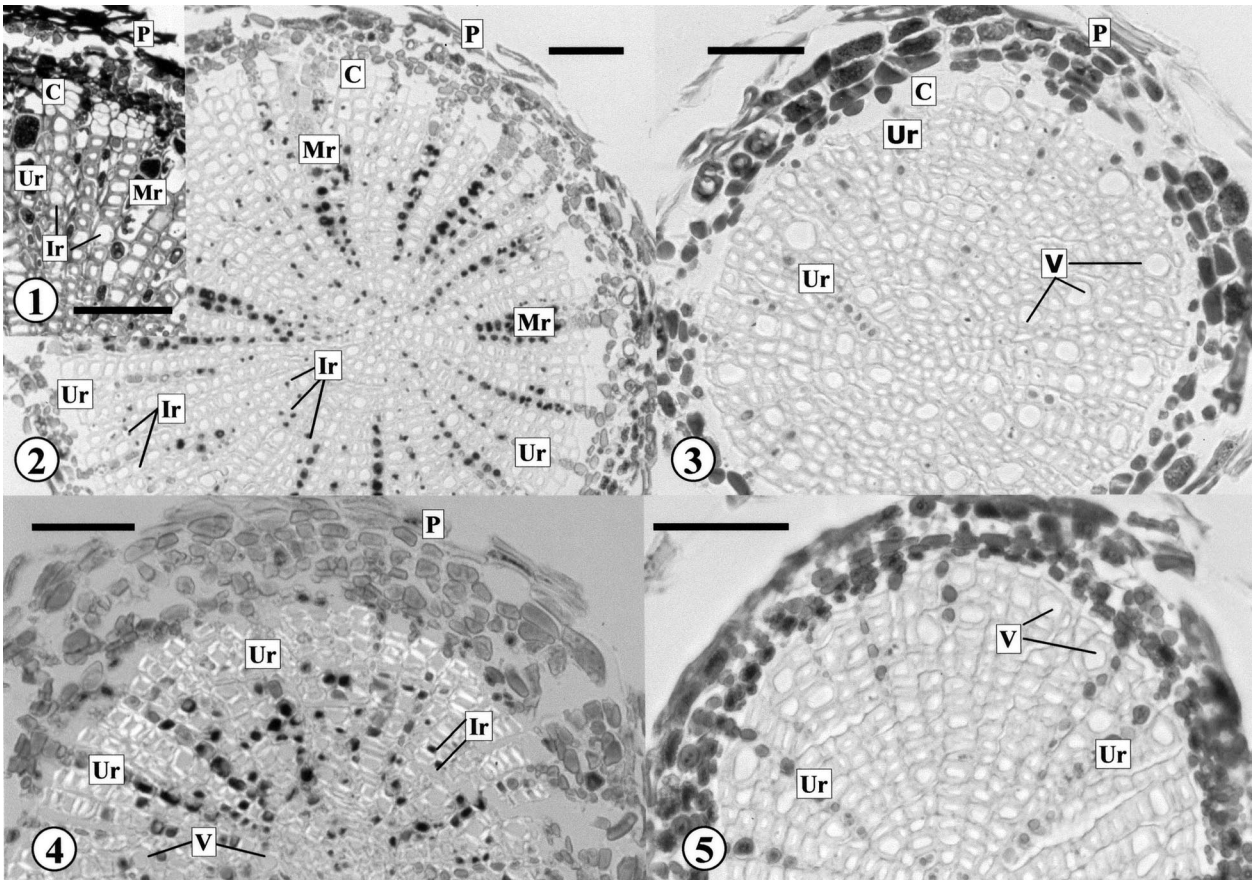
TABLE 1. Geographic location of the seeder and resprouter populations used for seed collection.

Species	Locality	City
Erica coccinea		
Seeder	Soetanysberg (Agulhas)	Bredasdorp
Resprouter	Marloth Nature Reserve	Swellendam
Erica calycina		
Seeder	Devil's Peak	Cape Town
Resprouter	Garcia's Pass	Riversdale

The South African Cape Floristic Region includes one of the five mediterranean regions of the world (Cody and Mooney, 1978). Despite its relatively small size (90 000 km^2), this region is one of the world's richest areas in plant diversity (Goldblatt, 1997). Most taxa show a pattern of high species concentration and endemism in fynbos, the most characteristic vegetation-type in this region. Poor soils, mediterranean or semi-mediterranean climate, and the recurrence of summer wildfires constitute the main selective regime of fynbos (Cowling, 1992). The genus *Erica*, with approximately 600 species, represents a paradigm amongst the highly speciated taxa of the fynbos (Goldblatt, 1997).

About 90% of Cape *Erica* species are seeders (Oliver, 1991; Ojeda, 1998). Only 6% are resprouters, capable of surviving fire and resprouting from developed underground lignotubers. The remaining 4% of species, termed ''mixed'' species by Ojeda (1998), includes distinct resprouter and seeder individuals, generally in well-separated populations. In a recent study, Bell and Ojeda (1999) found that adult resprouter individuals of three ''mixed'' species had consistently higher percentages of parenchymatic ray tissue and accumulated more starch in roots than their seeder counterparts. Are these phenotypic differences in allocation to root storage fixed attributes, happening from early seedling stages or are they a consequence of environmental differences experienced during development of the plant?

In this paper, we present the results of a comparative analysis of (1) the pattern of starch storage, (2) the proportion of parenchymatic ray tissue in upper regions of the root system, and (3) the root-to-shoot allocation pattern between seedlings



Figs. 1–5. Photomicrographs of transverse sections taken from 1 cm below the cotyledonary node of roots of young seedlings. Figs. 2–5. Sections stained with iodine/potassium iodide. **1.** Resprouter form of *Erica calycina* showing well-developed cambial cells and periderm. Some xylem rays can be seen. Section stained with thionin. **2.** Resprouter form of *E. calycina*; note the uniseriate and multiseriate xylem rays cells filled with starch and numerous starch-filled inter-ray parenchyma cells. **3.** Seeder form of *E. calycina* showing uniseriate rays with no starch-filled cells. **4.** Resprouter form of *E. coccinea* section viewed with polarized light showing uniseriate rays formed composed mostly of starch-filled cells. Observe also the abundance of inter-ray parenchyma cells containing starch. **5.** Seeder form of *E. coccinea*; note the uniseriate rays stand out due to accumulation of tannins rather than starch in ray parenchymatic cells. Bars = 50 μm.

Figure Abbreviations: C = cambium, CN = cotyledonary node, Ir = inter-ray parenchyma cell, Mr = multiseriate ray parenchyma, P = periderm, U = upper root, Ur = uniseriate ray parenchyma, V = vessel element.

of seeder and resprouter forms of two ''mixed'' Cape *Erica* species: *E. coccinea* L. and *E. calycina* L., raised under controlled glasshouse conditions. This common garden approach is aimed to determine if the source of phenotypic variation in allocation to root storage in *Erica* species is genetically determined or, by contrast, may be better viewed as an example of environmentally driven phenotypic plasticity (see Futuyma, 1998). Moreover, this study will reveal important information about the ontogenetic formation of the lignotuber in the resprouter forms of these two species. Although caution must be taken, the intraspecific approach of this seeder versus resprouter comparison implemented in two different species allows us to make inferences about patterns of root starch storage in seeder and resprouter *Erica* lineages at the seedling stage. The implications of the results of this study are of paramount importance to developing a better understanding of the biogeography and the evolutionary history of the genus *Erica* in the Cape Floristic Region (Ojeda, 1998).

MATERIALS AND METHODS

Plant material and growth conditions—Mature seeds of seeder and resprouter forms of *Erica calycina* and *E. coccinea* were collected in March–April 1998 from randomly selected individuals in four distinct localities within the South African Cape region (see Table 1 for geographic location). Seeder and resprouter *Erica* plants are easily authenticated in the field since resprouters are usually multistemmed and have lignotubers, i.e., conspicuous woody swellings at the base of the shoot system, belowground or just at the ground level, containing adventitious buds. Seeders never have lignotubers nor signs of a swollen stem base and are very seldom multistemmed. Seeds were air-dried and stored in darkness at room temperature with silica-gel sachets to keep them dry. In April 1999, seeds were sown by scattering in seedlings trays in a soil mixture of siliceous grit sand and sphagnum moss peat (3 : 1 volume/volume). A 400-g sachet of mycorrhizal medium recommended for growing Cape *Erica* species (not commercial; kindly supplied by A. Hitchcock, from Kirstenbosch Botanic Garden, Cape Town, South Africa) was added to the soil mixture to ensure that germinating seedlings were provided with appropriate mycorrhizal fungi. The pH of the soil mixture, measured in a soil saturated paste, was 4.0, a pH value similar to those of acid, sandy fynbos soils, where most Cape *Erica* species are found (Oliver, 1991). Trays were housed under greenhouse conditions at the University of Sevilla (Seville, Spain) and were kept moist by controlled irrigation. Germination started about 1 mo after sowing, but was not synchronous, since seeds were not smoke-treated (see Brown, Kotze, and Botha, 1993). Harvesting of seedlings took place in January 2000, 9 mo after sowing, while some seedlings were still germinating. Harvested seedlings were classified into two age classes: young (1.0 cm $<$ shoot length $<$ 3.0 cm) and old (3.0 cm $<$ shoot length $<$ 9.0 cm) seedlings, using shoot length as a approximate surrogate of seedling developmental stage.

Root anatomy and starch determination—A fragment of main root located 1 cm below the cotyledonary node was taken from each seedling and fixed in 4% formaldehyde in 0.1 mol/L phosphate buffered saline (pH 7.5) and held in a vacuum at room temperature for a minimum of 48 h. Root fragments were then dehydrated through an isopropyl alcohol series and embedded in glycol-methacrylate (GMA) (Pascual, Molinas, and Verdaguer, 2002). Transverse sections 3–5 μm thick were obtained by using a rotatory microtome Autocut 1150 (Reichert-Jung, Wien, Austria) and mounted on glass slides. Sections were stained with thionin or 2% iodine solution (I_2KI) for detection of starch granules. Samples were viewed with an Olympus-Vanoxlight microscope (Olympus Optical, London, UK).

For anatomical purposes, ray parenchyma cells, inter-ray parenchyma cells, and wide vessel elements of root material were readily distinguishable even in young seedlings. Ray parenchyma cells were those living cells disposed radially, regardless of whether they contained starch or not (Figs. 1–5). Xylem parenchyma rays were classified as being uniseriate when they remained one cell wide from close to the center of the root to the outermost region of the root to just below the phloem. Rays were classified as being multiseriate when they were two or more cells wide at the outermost region of the root. Inter-ray parenchyma cells were those lying between xylem rays (1) having very wide lumina with or without a secondary wall, (2) having no secondary cell wall, and/or (3) filled with starch grains (see Figs. 1, 2, 4). Xylem vessels were considered to be those cells more than 8 μm in diameter and never filled with starch (see Figs. 3–5). Finally, cells with small lumens and secondary cell walls that were never filled with starch constituted the remainder of vascular tissue, narrow vessel elements, tracheids and fibers, all of which were often difficult to distinguish from each other owing to the presence of transitional forms.

For quantitative determination of starch and relative amount of xylem ray parenchyma tissue, image analysis was used. The image analysis system consisted of a Sony CCD-IRIS color video camera (Model DXC-107AP, Tokyo, Japan) connected with an Olympus BX40 light microscope (Olympus Optical, London, UK) and a PC computer equipped with an Imaging software package (Scion Corporation, Frederick, Maryland, USA). The video camera was connected to the light microscope and images were captured directly. Eight young seedlings were randomly selected from within each species and regeneration form and transverse root sections were taken from each. Depending on the diameter of the root section, between 4 and 23 image fields were captured from within the boundary of the vascular tissue, accounting for at least 90% of the whole cross-sectional area. The relative amounts of starch in root tissues were calculated in each image field as percentage area having starch grains compared to whole area of the image. The total number of xylem elements per image field was determined and the relative amount of xylem ray parenchyma was calculated as the percentage of ray parenchyma cells. As ray parenchyma cells were clearly distinguishable regardless of whether they were filled with starch or not, we also calculated the percentage of starch-filled ray parenchyma cells in each image field. Since our aim was to ascertain whether differences in root starch storage and the proportion of starch storage tissue between regeneration forms happened from earliest seedling stages, old seedlings were not included in this comparative analysis.

Root-to-shoot allocation—Aboveground (shoot) dry biomass and root thickness (cross-section diameter) at 1 cm below the cotyledonary node were recorded in young and old seedlings. We established allometric relationships between these two variables in order to explore possible differences in the root-to-shoot allocation pattern between seeder and resprouter forms (see below).We used root thickness as an indicator of root dry biomass as it was not possible to harvest seedlings for anatomical purposes and not damage their root system.

Statistical analyses—We tested the differences between regeneration forms in (1) the relative amount of root starch, (2) the proportion of ray parenchyma root tissue, and (3) the proportion of starch-filled ray parenchyma root tissue, for each species. Because the response variable, in the three instances, was measured repeatedly in a number of image fields from each individual plant root cross-section (see above), we used the following model to describe the data: $X_{ijk} = \mu + \alpha_i + \beta_{j(i)} + \varepsilon_{ijk}$. This model corresponds to a hierarchically nested ANOVA design. X_{ijk} is any replicate image field quantitative determination (k) of the response variable in any of the eight replicate seedling cross-sections (j) in any of the two regeneration forms (i); the term μ denotes the population grand mean, i.e., the mean value of all image field observations in all the individual seedlings sampled; α_i is the ith level of the main effect (*regeneration*, fixed factor); the $\beta_{j(i)}$ term stands for the mean of k image field observations in the jth seedling cross-section (*seedling*, random factor) nested in the ith regeneration form; finally, the ε_{ijk} term is the random error, representing the individual variability from one image field observation to another within a particular seedling cross-section. It should be made clear that the $i \times j$ individual seedling cross-sections, not the $i \times j \times k$ image fields, are the experimental units in this nested design, thus overcoming pseudoreplication (see Underwood, 1997). Probability levels of $P < 0.05$ were regarded as significant throughout the analyses.

We also estimated the relative contribution of individual factors to the response variables in the ANOVAs by determining their magnitude effects (ω^2) through calculating their variance components (Graham and Edwards, 2001). Unfortunately, methods detailed by Graham and Edwards (see also Underwood, 1997) only consider balanced designs, whereas our data were strongly unbalanced. To overcome this difficulty, we obtained the average sample size (n_0) within each *ij* level using the computations described in Sokal and Rohlf (1995) and replaced n by n_0 in Graham and Edwards' (2001) formulae for nested ANOVAs.

Size (root diameter) of the seedlings used in this study was not constant nor homogeneous across species; whereas seeder and resprouter *E. calycina* seedlings did not show significant differences [seeder (S): 0.4 ± 0.1 mm (mean ± SD); resprouter (R): 0.6 ± 0.2 mm; $t = -1.87$, df = 14, $P = 0.08$), seeder *E. coccinea* seedlings were larger than resprouters and had slightly but significantly thicker root diameters than resprouter ones (S: 0.5 ± 0.1 mm; R: 0.3 ± 0.1 mm; $t = 3.46$, df = 14, $P < 0.01$). However, considering root diameter as a covariate did not qualitatively change the results, so it was not included in the ANOVAs. Over-parameterized model estimation and type III sums of squares were used to fit the data, using the STATISTICA visual GLM module (StatSoft, 1999). Data, given in percentages, were arcsine-transformed prior to analyses to deal with heteroscedasticity and nonnormality (Sokal and Rohlf, 1995).

The relationship between shoot dry biomass and root thickness was determined for each species and regeneration form using simple linear regression analysis. We tested the existence of different regression slopes between seeder and resprouter forms, for each species, by means of analysis of covariance (Underwood, 1997). This analysis allowed us to ascertain whether seeder and resprouter seedlings differed in their root to shoot patterns of biomass allocation.

RESULTS

Root anatomy and starch storage—Sections of upper roots of young (i.e., 1.0 cm < shoot length < 3.0 cm) seeder and resprouter seedlings from both species of *Erica* showed the presence of secondary growth with differentiation of the cambium and phellogen (Figs. 1 and 5). The secondary xylem was constituted by a horizontal transport system of mainly uniseriate xylem rays (Figs. 3–5) and by a vertical transport system composed of inter-ray parenchyma, tracheary elements, and fibers (Figs. 1–5).

Young resprouter seedlings of both species of *Erica* showed a higher relative amount of starch in roots than their seeder counterparts (Table 2; Fig. 6). This starch was mostly stored in parenchymatic xylem rays (Figs. 2, 4), although some inter-ray parenchyma cells were also found to contain starch, particularly in resprouter seedlings of *E. coccinea* (Fig. 4).

A higher proportion of ray parenchyma cells was found in the root xylem of young resprouter seedlings than in that of seeder seedlings (Fig. 7). Whereas these differences were highly significant between seeders and resprouters of *E. calycina*, however, the analysis failed to detect such significant differences for *E. coccinea* (Table 3). Notwithstanding, a significantly higher proportion of starch-filled ray parenchyma cells was found in root tissue of resprouter young seedlings of both *Erica* species compared to their seeder counterparts (Table 4; Fig. 8).

The analyses also detected significant differences in relative amount of root starch between individual seedlings within regeneration forms for both species (Table 2) and differences between the proportion of ray parenchyma cells containing starch (Table 4). However, the seedling factor contributed little ($\omega^2 < 15\%$) to the total sums of squares of these two response variables. Most of the variation of these two variables, as well as the variation in the proportion of ray parenchyma tissue in

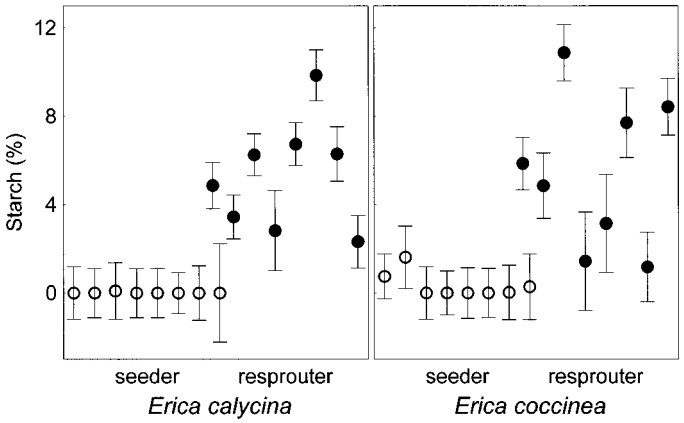


Fig. 6. Mean percentage (± standard error) of ray parenchyma cells containing starch in transverse sections of roots of young seeder ($n = 8$) and resprouter ($n = 8$) seedlings of *Erica calycina* and *E. coccinea*.

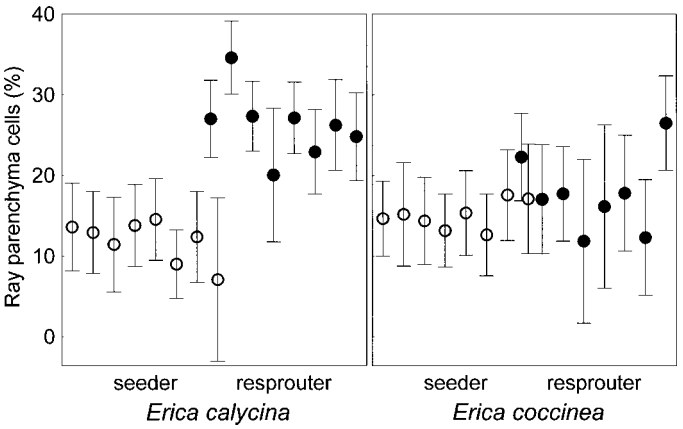


Fig. 7. Mean percentage (± standard error) of ray parenchyma cells (with and without starch) in transverse sections of roots of young seeder ($n = 8$) and resprouter ($n = 8$) seedlings of *Erica calycina* and *E. coccinea*.

TABLE 2. Nested ANOVA results of the comparisons between seeder and resprouter forms of *Erica calycina* and *E. coccinea* (''regeneration,'' fixed factor) for the percentage area containing starch in image fields taken from root transverse sections of young seedlings of seeder ($n = 8$) and resprouter ($n = 8$) forms (''seedling,'' random factor).

	Erica calycina					*Erica coccinea*				
Source of variation	df	MS	*F*	*P*	ω^2	df	MS	*F*	*P*	ω^2
Regeneration	1	2.264	119.5	<0.0001	80.8%	1	1.371	42.5	<0.0001	75.1%
Seedling (regeneration)	14	0.024	6.5	<0.0001	6.1%	14	0.039	24.5	<0.0001	14.9%
Error	227	0.003			13.1%	171	0.002			10.0%

TABLE 3. Nested ANOVA results of the comparisons between seeder and resprouter forms of *Erica calycina* and *E. coccinea* for the percentage of ray parenchyma cells (with or without starch) in image fields from root transverse sections of young seedlings of seeder (n = 8) and resprouter (n = 8) forms.

	Erica calycina					*Erica coccinea*				
Source of variation	df	MS	F	P	ω^2	df	MS	F	P	ω^2
Regeneration	1	2.063	67.9	<0.0001	38.9%	1	0.062	2.7	0.11	2.5%
Plant (regeneration)	14	0.031	1.2	0.27	0.8%	14	0.024	1.5	0.12	4.1%
Error	227	0.026			60.3%	171	0.016			93.5%

TABLE 4. Nested ANOVA results of the comparisons between seeder and resprouter forms of *Erica calycina* and *E. coccinea* for the percentage of starch-filled ray parenchyma cells in image fields from root transverse sections of young seedlings of seeder (n = 8) and resprouter (n = 8) forms.

	Erica calycina					*Erica coccinea*				
Source of variation	df	MS	F	P	ω^2	df	MS	F	P	ω^2
Regeneration	1	6.613	296.4	<0.0001	81.0%	1	2.651	64.3	<0.0001	75.0%
Plant (regeneration)	14	0.024	2.0	0.019	1.2%	14	0.049	8.3	<0.0001	9.7%
Error	227	0.012			17.9%	171	0.006			15.3%

upper roots, was explained by the regeneration effect (see Tables 2–4).

Transverse sections from old seedlings (i.e., 3.0 cm < shoot length < 9.0 cm) showed contrasting patterns of xylem parenchymatic rays in relation to width and starch distribution between seeder and resprouter regeneration forms of both species (Figs. 9–12). Old seedlings of resprouter *E. calycina* consistently had both broad (multiseriate) and thin (uniseriate) rays with most of the cells in this tissue type having large amounts of starch grains. This seedling type also had some starch-filled inter-ray parenchyma cells (Fig. 9). Comparable seedlings of seeder *E. calycina* did not possess any broad parenchyma rays and most rays were uniseriate or a maximum of two cells wide. Cells of rays and inter-ray parenchyma did not generally contain starch (Fig. 10).

Parenchyma rays in older seedlings of the resprouter form of *E. coccinea* were not as broad as those in similarly aged seedlings of the resprouter form of *E. calycina* rays, being uni- to triseriate. Nevertheless, they were composed mostly of starch-filled parenchyma cells, and inter-ray parenchyma cells with abundant starch grains were also detected (Fig. 11). In old seedlings of the seeder form of *E. coccinea*, as in those of the seeder form of *E. calycina*, only uniseriate rays were observed, and most ray and inter-ray parenchyma cells did not contain starch (Fig. 12).

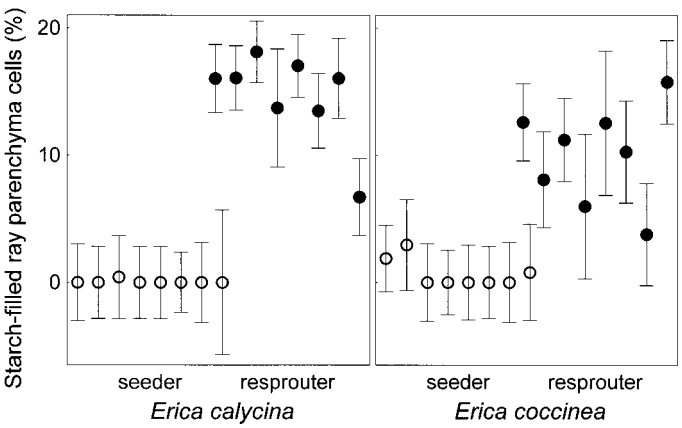


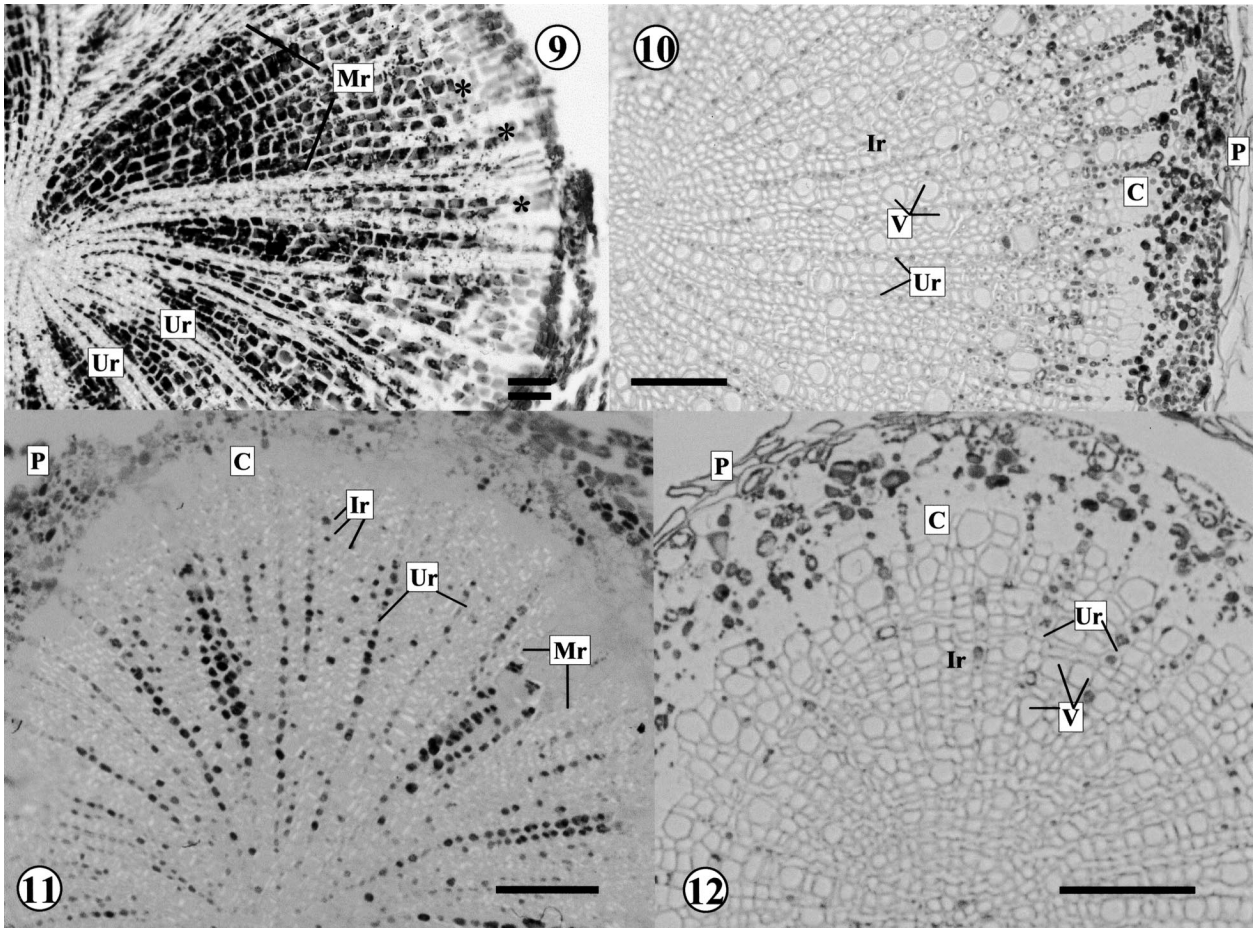
Fig. 8. Mean percentage (± standard error) of starch-filled ray parenchyma cells intransverse sections of roots of young in seeder (n = 8) and resprouter (n = 8) seedlings of *Erica calycina* and *E. coccinea*.

Root-to-shoot allocation—Differences between seeder and resprouter seedlings in the diameters of the upper portions of the root system and the cotyledonary node region were readily distinguishable in old seedlings of both species (Fig. 13). We also observed greater numbers of buds in the cotyledonary region of resprouter seedlings than in seeder seedlings, particularly for *E. coccinea* (Fig. 13). Strong positive correlations were detected between the diameters of the upper portions of the root system and the dry mass of the shoots for young and older seedlings of both species and regeneration forms (Table 5). Regression slopes in seeder seedlings were significantly steeper than those of resprouter seedlings in both *E. calycina* and *E. coccinea* (Table 5), with such differences being more marked in *E. calycina* (Fig. 14).

DISCUSSION

Gleeson and Tilman (1994) found that, contrary to expectation, seedlings of certain woody species that as adults had higher allocation to root biomass than others showed lower root allocations when grown individually in enriched conditions in the greenhouse. They concluded that root-to-shoot allocation patterns should be viewed as plastic responses to environmental conditions rather than fixed species attributes. In a recent study, Bell and Ojeda (1999) found that starch and storage tissue in roots in adult plants of resprouter lineages of Cape *Erica* species was consistently higher than in seeders. Could this within-species allocation to starch storage in roots be used as an example of environmentally driven phenotypic plasticity? The comparative study presented here has revealed that differences between seeders and resprouters in allocation to underground (root) starch storage can be detected in early seedling stages in two ''mixed'' *Erica* species, *E. calycina* and *E. coccinea*, when raised under common garden conditions.

Significant differences in the relative amount of xylem ray tissue (i.e., proportion of ray parenchyma cells) present in differing regeneration forms was detected in *E. calycina* but not in *E. coccinea* (see Table 3). The small sample size (eight



Figs. 9–12. Photomicrographs of transverse sections taken from 1 cm below the cotyledonary node of roots of older seedlings; sections stained with iodine/potassium iodide. **9.** Resprouter form of *Erica calycina* showing multiseriate rays composed of mainly starch-filled cells; numerous tannin-filled cells are also obvious. **10.** Seeder form of *E. calycina* showing uniseriate rays and inter-ray parenchyma cells filled with tannins rather than starch. **11.** Resprouter form of *E. coccinea*. Note that some multiseriate and uniseriate rays have numerous starch-filled cells and that there are some starch-filled cells in the inter-ray parenchyma. **12.** Seeder form of *E. coccinea*. Note that no multiseriate rays nor starch grains are observed. Bars = 100 μm in Figs. 9–11. Bar = 50 μm in Fig. 12.

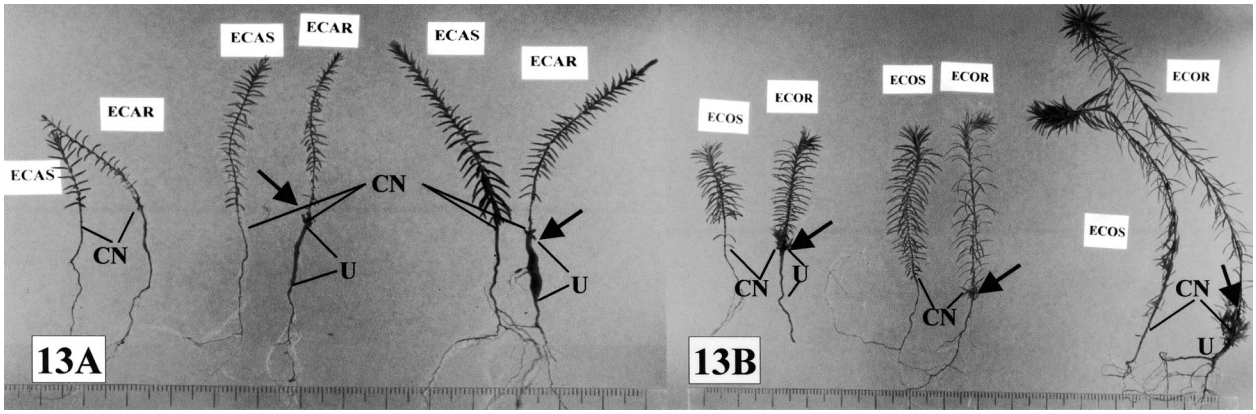


Fig. 13. Representative older seedlings of seeder and resprouter forms of three different shoot height classes in (A) *Erica calycina* and (B) *E. coccinea*. Note the large diameter of the hypocotyl-upper root region in resprouter forms of both species, particularly in *E. calycina* and the presence of buds (arrows) in the cotyledonary node of resprouter seedlings. ECAS = *E. calycina* seeder, ECAR = *E. calycina* resprouter, ECOS = *E. coccinea* seeder, ECOR = *E. coccinea* resprouter.

TABLE 5. Simple linear regressions between shoot dry mass and root diameter in seeder and resprouter seedlings of *Erica calycina* and *E. coccinea* and ANCOVA results comparing regression slopes of relationships within each species.

Analysis results	*Erica calycina*		*Erica coccinea*	
	Seeder (n = 30)	Resprouter (n = 33)	Seeder (n = 25)	Resprouter (n = 22)
Linear regression				
Pearson's r	0.80	0.85	0.81	0.91
P	<0.0001	<0.0001	<0.0001	<0.0001
ANCOVA				
F	28.1		8.6	
P	<0.0001		0.006	

individuals per species and regeneration form) as well as the fact that sampled young seedlings of the resprouter form of *E. coccinea* were slightly smaller and had significantly thinner root diameters than sampled seeder ones (see MATERIALS AND METHODS) might account for the lack of significant difference in this species. If old seedlings had been also included in the analysis we would have undoubtedly recorded significant differences, since wider parenchyma rays were obvious in these individuals (see Figs. 9–12). Notwithstanding, it must be borne in mind that ''old seedlings'' were never older than 9 mo, i.e., still certainly young.

Despite rendering significant, the seedling effect contributed very little to the variation in the relative amount of root starch and the percentage of starch-filled ray parenchyma cells compared to the regeneration effect. We can thus infer that the regeneration form consistently accounts for most of the within-species variability of allocation to underground (root) starch storage in *Erica* seedlings. Pate et al. (1990) described a pattern of differential allocation to root starch in seeder and resprouter pairs of co-generic shrub species at the seedling (2–4 yr old) stage. However, our study must not be seen merely as a further contribution to reinforce this pattern. We emphasize that the seeder–resprouter comparisons are within species and implemented in two different *Erica* species, which enables us to factor out possible ''phylogenetic constraints'' (Ackerly and Donoghue, 1995; Ricklefs, 1996; Silvertown and Dodd, 1996) and allows us to make generalizations to the genus *Erica* in the Cape region, although caution must be taken. Thus, the contrasted allocation to root starch storage between seeder and resprouter *Erica* lineages, previously detected in adult plants by Bell and Ojeda (1999), occurs from the earliest seedling stages. Moreover, the fact that seedlings were grown under controlled glasshouse conditions allows us to infer that it is a genetically determined and fixed attribute in this genus rather than an example of environmentally driven phenotypic plasticity.

The swelling of the upper root region and the observation of plentiful buds in the cotyledonary region of resprouter seedlings, presumably confering high sprouting capacity, suggest that not only are there differences in starch storage capacity and storage realization in roots, but that there is also the facility to develop a lignotuber early on in seedling development. Further studies are needed to confirm the presence of dormant buds in the cotyledonary region of resprouter and seeder forms and to follow the development of this region in later stages of seedling growth.

Higher allocation to underground starch storage and specialized storage tissue, which is expensive to create and maintain (Bloom, Chapin, and Mooney, 1985; Chapin, Schulze, and Mooney, 1990; Sakai, Sakai, and Akiyama, 1997), would imply a reduced aboveground biomass accumulation in resprouters (Pate et al., 1990; Bell and Pate, 1996). The different regression slopes relating root thickness to aboveground dry biomass between seeder and resprouter forms of both *Erica* species could be interpreted as a reflection of this root-to-shoot allocation trade-off from the earliest stages of seedling development.

The retarded aboveground growth of seedlings of resprouter lineages compared to those of seeders may render resprouter seedlings less able to withstand a seasonal (summer) soil moisture stress (see Taylor, 1989). Although it remains to be tested, this presumably higher sensitivity of resprouter *Erica* seedlings to summer drought in the first stages of establishment had been previously assumed by Ojeda (1998) in order to explain the selection of *Erica* seeder lineages, more prone to

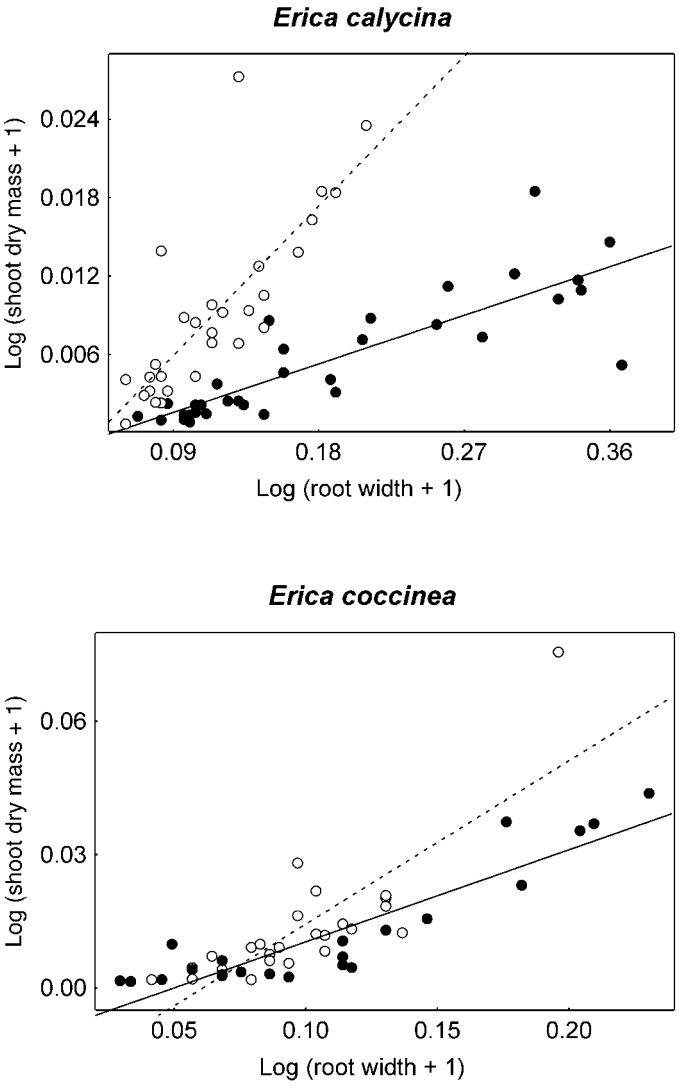


Fig. 14. Relationships between dry mass of shoot and width of root at 1 cm below the cotyledonary node in seeder (open circles) and resprouter (solid circles) forms of *Erica calycina* and *E. coccinea*. Both young and older seedlings were included in these analyses.

speciation (Wells, 1969), in the mild mediterranean southwestern Cape region.

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