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Lignotubers in Mediterranean basin plants

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Abstract Lignotubers are swollen woody structures located at the root-shoot transition zone and contain numerous dormant buds and starch reserves. This structure enables the plant to resprout prolifically after severe disturbances that remove the aboveground biomass. These are considered adaptive traits in ecosystems with highly frequent and severe disturbances—such as fire-prone ecosystems. In this paper, we aim to contribute to the knowledge of lignotubers in the Mediterranean basin and highlight the evolutionary implications. We first summarise existing knowledge on lignotuber species in the Mediterranean basin. We then provide a detailed morpho-anatomical description of early lignotubers in two common woody species (*Arbutus unedo* L. and *Phillyrea angustifolia* L.). Finally, we compare our anatomical results with those obtained in studies conducted with other lignotuberous species from different Mediterranean regions. Lignotubers were verified in 14 species in the Mediterranean basin; all being from lineages with origins dating to the Tertiary and thus pre-dating the Mediterranean climate. In *A. unedo* and *P. angustifolia*, lignotubers are macroscopically discernible in 4- and 2-year-old saplings, respectively. In these two species, the lignotubers have numerous buds protected by hypertrophied scales, and have a contorted xylem containing abundant starch. Our results challenge the traditional idea that pre-Mediterranean lineages suffered evolutionary inertia; instead, lignotuberous species may be considered examples of plants that adapted to the increased fire activity that occurred throughout the Tertiary and Quaternary. We also highlight the use of morpho-anatomical traits to unambiguously distinguish between lignotuberous and non-lignotuberous resprouting species.

Keywords Morpho-anatomy · Bud bank · Fire adaptations · Fire regime · Resprouting · Tertiary Madrean–Tethyan vegetation

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Introduction

The ability of plants to resprout is a functional trait that enables the regeneration of their aboveground biomass from a set of protected dormant buds. This is a geographically widespread trait, occurring in very different lineages and ecosystems worldwide (Vesk and Westoby 2004a). There are different types of resprouting, depending on the location and amount of buds and their degree of protection (Bellingham and Sparrow 2000; Clarke et al. 2013). In some woody species, basal resprouting occurs from a woody swelling at the root-shoot transition zone that conceals numerous buds and starch; this structure is called a lignotuber (James 1984 and references therein).

Lignotubers are not induced by repeated disturbances or infections, as was formerly thought, but they are ontogenetically determined (Kerr 1925). Hybridisation experiments in Myrtaceae species indicate that lignotubers are dominantly inherited and are under multigenic control (Pryor and Byrne 1969; Shepherd et al. 2008). Accordingly, intraspecific comparisons of the progeny of lignotuberous and non-lignotuberous forms of *Eucalyptus* and *Erica* species indicate that lignotuber development depends on the presence of these structures in the parent plants (Mullette and Bamber 1978; Verdaguer and Ojeda 2005). Contrarily, the swollen root crown that appears in some other resprouting species (named stump or burl, and sometimes incorrectly called lignotuber) is induced by multiple resprouting events and is not genetically determined (James 1984). Therefore, lignotuberous species are best distinguished from those forming other woody swellings by detailed observations during the early stages of the ontogeny.

Lignotuber buds initially appear in the cotyledonary region (sometimes also at the first few leaf nodes) just a few months after germination (Kerr 1925; Chattaway 1958; Carr et al. 1983; Molinas and Verdaguer 1993a; Graham et al. 1998; del Tredici 1999; Mibus and Sedgley 2000). The buds progressively proliferate and arrange in clusters; they become very abundant (even in the order of thousands) in more advanced developmental stages (Wildy and Pate 2002). In early lignotubers, buds are protected by a tanniniferous parenchymatic tissue in some *Eucalyptus* species (Chattaway 1958), by tannin-rich hypertrophied scales in *Quercus suber* (Molinas and Verdaguer 1993a), or by the extended base of the cotyledons (also tannin rich) in *Banksia* (Mibus and Sedgley 2000). In *Eucalyptus* and *Banksia*, these buds are enfolded by further development of the lignotuber that becomes macroscopically evident as a swelling at the cotyledonary node (Chattaway 1958; Graham et al. 1998; Mibus and Sedgley 2000). This swelling is not apparent in *Q. suber* seedlings; instead, this species shows an elongated hypocotyl with abundant cluster buds enfolded by hypertrophied scales, most of which are below ground (Molinas and Verdaguer 1993a). Therefore, lignotubers are characterised by a large bud bank where meristematic tissues are protected by means of a wide diversity of mechanisms.

As in leaf axillary buds, lignotuber bud growth is funded by carbohydrates stored as starch (James 1984). Although the starch sustaining lignotuber bud growth is mainly stored in the roots (see Clarke et al. 2013), the lignotuber starch might also contribute to energetically supporting resprouting (Canadell and López-Soria 1998). In fact, starch grains are very abundant in the lignotuber, and normally stored in the parenchymatous cells of the contorted xylem, but can also be found in the lignotuber cortex or in the stem pith—depending on the species (Carrodus and Blake 1970; Bamber and Mullette 1978; Molinas and Verdaguer 1993a).

The ecological meaning of lignotuber resprouting differs from that of other resprouting types. Basal resprouting (i.e. from the lignotuber or root crown) enables post-disturbance regeneration and is not related to resource exploitation, space colonisation, or clonal recruitment as in other below-ground resprouting mechanisms (i.e. from rhizomes or roots; see van Groenendael et al. 1996; Clarke et al. 2013). In addition, species resprouting from lignotubers typically have a larger bud bank than those resprouting from root crowns (Clarke et al. 2013). Very large bud banks are costly not only in terms of large construction and maintenance investments, but especially due to costs related to bud protection and reserve storage (Vesk and Westoby 2004b). Therefore, these large bud banks should only be selected when they have a significant effect on survival, such as in disturbance-prone environments. That is, the large amount of strongly protected buds of the lignotubers, their high starch content, and their ontogenetic development suggests that lignotubers are adaptations to frequent and severe disturbances

such as fires (James 1984; Kummerow 1989; Canadell and Zedler 1995). In fact, although resprouting occurs in many biomes and after different disturbance types (Vesk and Westoby 2004a), resprouting from lignotubers is mostly restricted to fire-prone ecosystems, and especially in those characterised by crown fires (Lloret et al. 1999; Montenegro et al. 2003; Knox and Clarke 2004; Paula et al. 2009; Keeley et al. 2012). That is, although resprouting occurs in many ecosystems worldwide and after diverse disturbances, specific information on the type of resprouting provides valuable insights on the evolutionary pressures to which lineages were subjected. For instance, the traditional view is that postfire seeders in the Mediterranean basin are adapted to the Quaternary fire and drought regimes, while resprouters are relicts from Tertiary lineages that suffered a phenotypic stability (evolutionary inertia; Herrera 1992). However, the presence of specialised structures such as lignotubers in Tertiary lineages would challenge this idea as they are strongly related to fire-prone ecosystems (see above). Currently, quality information on the presence and characteristics of lignotubers in the Mediterranean basin is scarce and fragmented (Paula et al. 2009), thus limiting our ability to fully understand the ecology and evolution of resprouting.

In this paper, we aim to contribute to the knowledge of lignotubers in the Mediterranean basin, and highlight the evolutionary implications. To do so, we provide a detailed morphological and anatomical description of lignotuber formation in two common woody species: *Arbutus unedo* L. and *Phillyrea angustifolia* L. This provides evidence for an ontogenetical origin of the lignotubers. To frame this anatomical analysis, we first summarise existing knowledge on the lignotuber species in the Mediterranean basin. We also compare our anatomical results with those obtained in similar studies conducted with other lignotuberous species from different Mediterranean regions. In this way, we aim to identify the diagnostic morpho-anatomical traits of early lignotubers.

Methods

Data compilation and field observations

The presence of lignotubers in Mediterranean basin species was initially compiled from the BROT database (Paula et al. 2009; Paula and Pausas 2013). Original sources were then consulted to determine the criteria for lignotuber assignment. We were conservative in the sense that we excluded those species cited as having a lignotuber if there was no clear anatomical evidence or if there was little support for a proper lignotuber (i.e. ontogenetically programmed woody swelling). Additionally, we searched for field evidence of lignotubers by excavating adult plants of species that were candidates for forming a lignotuber. Field surveys were conducted in Serra de la Murta (Valencia, Spain; 39°4′N, 0°12′W, ca. 450 m asl) and Serra d'Espadà (Castelló, Spain; 39°52′N, 0°16′W, 430 m asl). For each lignotuberous species, we also compiled the growth form, the post-fire regeneration strategy (from Paula et al. 2009), and the age of the lineage (i.e. Tertiary or Quaternary-evolved lineage, cf. Herrera 1992). Two of the species were chosen for a detailed morpho-anatomical study of the root-shoot transition zone in early stages of plant development (see below).

Morpho-anatomical study

Selected species

We studied the morphology and anatomy of early lignotubers in two Mediterranean species: *Arbutus unedo* L. (Ericaceae) and *Phillyrea angustifolia* L. (Oleaceae). They are long-lived evergreen sclerophyllous species that grow as large shrubs or small trees, and their lineages pre-date the onset of the Mediterranean climate in the Mediterranean basin (Verdú and Pausas 2013). *Arbutus unedo* occurs around the Mediterranean basin, although its distribution extends along the Atlantic coast of Europe (Sealy 1949a). The distribution of *P. angustifolia* is limited to the western Mediterranean basin (Valdés et al. 1987). Both species are common in Mediterranean shrublands and forests. The early development of the lignotuber in *A. unedo* has been described in plants from populations outside the Mediterranean region (in Ireland; Sealy 1949b). In the case of *P. angustifolia* L., the presence of a lignotuber in adult plants was previously mentioned in the BROT database based on non-systematic observations (Paula et al. 2009); no information exists regarding the ontogeny of the lignotuber in this species.

Plant material

Saplings of *A. unedo* ($n = 26$) and *P. angustifolia* ($n = 34$) at various developmental stages were obtained from a forest nursery (Banc de Llavors, Valencia; 39°28′N; 0°31′W, 78 m asl; mean annual rainfall 454 mm; mean annual temperature 22.3 °C). The seeds of both species were originally collected from the Serra Calderona and Sierra de Chiva, respectively (Valencia, eastern Spain). *Arbutus unedo* seeds were stratified during 3–4 months at 4 °C. Germination of *P. angustifolia* seeds was stimulated by incubation in a germination chamber under a 12 h cycle of alternate temperature and light regime (10 °C in dark-20 °C in light). Seeds were sown in 1-L pots with a substrate composed of peat, coir, and perlite (3:1:1). Additionally, because lignotuber development might be shaped by the substrate conditions (Mullette and Bamber 1978), four saplings of *A. unedo* and three of *P. angustifolia* grown in the wild were also collected (Serra de la Murta, Valencia, Spain; 39°4′N, 0°12′W, 450 m asl; mean annual rainfall 633 mm; mean annual temperature 17.4 °C). For each plant (including those grown in the nursery as well as those in the wild), the root-shoot transition zone was separated (ca. 2 cm above and below the root crown) and fixed in FAA (formalin-acetic acid-alcohol) for two days and then preserved in 70 % ethanol.

Morphological study

Many saplings had one to several visible swellings at the root-shoot transition zone. All swellings were counted and the three largest diameters of each swelling (perpendicular to each other) were measured to estimate their volumes. For plants with several swellings, the lignotuber volume was measured as the sum of the individual swelling volumes. All distinguishable buds appearing in the swellings were then counted. The age of each sapling was determined by counting the annual rings on the root just below the cotyledonary node or the main lignotuber (when present). To understand the ontogenetic trajectory of the enlargement of the bud bank, for each species, we regressed the number of buds against the sapling age and the lignotuber volume. For this analysis, we only considered saplings grown under nursery conditions. To meet normality and homoscedasticity assumptions,

the variables were log-transformed prior to the analyses.

Anatomical study

The anatomical study of the root-to-shoot transition zone was conducted on 24 saplings of *A. unedo* and 30 saplings of *P. angustifolia*. Longitudinal sections (20–25 μm) were obtained from non-embedded lignotuber samples using a sliding microtome (Leica Microsystems, Nussloch, Germany). The sections were stained with safranin (alcohol-50 to 1 % solution) and iodine (I_2KI; 1 % aqueous solution) and were then permanently mounted with glycerinated gelatin. For cross and tangential sections, the samples were prepared following Johansen (1940). Specifically, the samples were dehydrated using an increasing ethanol gradient and finalised with xylol—and then embedded in paraffin. Fine (15–17 μm) cross and tangential sections were obtained with a rotatory microtome (Wetzlar, Germany). The sections were attached to glass slides using Mayer adhesive, and stained with tannic acid (1 % aqueous solution), ferric chloride (3 % aqueous solution), safranin (alcohol-50 to 1 % solution) and/or Fast-Green (triarylmethane alcoholic solution-95 % to 0.5 %). Finally, the sections were permanently mounted with Eukitt® and observed under light microscopy.

Results

Data compilation

We retrieved reliable information of the presence of lignotuber for 14 species—including 12 angiosperms and two gymnosperms (Table 1). From these species, we verified the existence of lignotubers in the field by excavating adult plants of *Juniperus oxycedrus* subsp. *oxycedrus*, *Viburnum tinus*, *Olea europaea* subsp. *europaea*, and *Phillyrea angustifolia*. Previous studies reported anecdotal and/or contradictory data regarding the existence of lignotuber in these species (Paula et al. 2009; Paula and Pausas 2013). For *Quercus suber*, lignotubers have been reported from anatomical studies in the first stages of plant development (Molinas and Verdaguer 1993a, b), but we found no obvious swelling in the root-shoot transition of adult plants (Fig. 1d). The presence of lignotuber in

Table 1 State-of-the-art in lignotuber knowledge in Mediterranean basin species. Selected references are included in brackets (see table footnotes)

Species	Ontogenetic stage	Observations
Cupressaceae		
Juniperus oxycedrus L. subsp. *oxycedrus*	Adult	Field evidence of lignotuber resprouting after fire and clipping (1). Lignotuber formation is variable within this species (1)
Tetraclinis articulata (Vahl) Mast.	Adult	Large lignotubers are traditionally appreciated for furniture-making (2)
Caprifoliaceae		
Viburnum tinus L.	Adult	Field observation (1). Evidence of basal resprouting after wildfire (3)
Ericaceae		
Arbutus unedo L.	Adult	Field observation (1). Evidence of lignotuber resprouting after experimental fire and clipping concomitant with lignotuber starch consumption (4, 5)
Arbutus unedo L.	Sapling	Anatomical evidence of protected cluster buds, contorted xylem and starch in lignotubers (1, 6)
Arbutus andrachne L.	Adult	Field observation (7, 8)
Erica arborea L.	Adult	Evidence of lignotuber resprouting after fire and clipping (1). Root and lignotuber starch consumption after resprouting has been reported (4, 9)
Erica australis L.	Adult	Evidence of lignotuber resprouting after fire and clipping (1). Root and lignotuber starch consumption after resprouting has been reported (9, 10)
Erica multiflora L.	Adult	Evidence of lignotuber resprouting after fire and clipping (1, 11)
Erica lusitanica Rudolphi	Adult	Field observation of lignotuber resprouting (12)
Erica scoparia L.	Adult	Evidence of lignotuber resprouting after fire and clipping (1). Root starch consumption after resprouting has been reported (9)
Rhododendron ponticum L.	Adult and juvenile	Field observation of lignotuber resprouting (13)
Fagaceae		
Quercus suber L.	Adult	No clear basal swellings are observed in the field (1). Evidence of basal resprouting after wildfire (1, 14)
Quercus suber L.	Seedling and sapling	Anatomical evidence of protected cluster buds and starch in lignotubers (15) Resprouting after clipping below the cotyledonary node has been reported (16)
Oleaceae		
Phillyrea angustifolia L.	Adult	Evidence of lignotuber resprouting after wildfire and clipping (1)
Phillyrea angustifolia L.	Sapling	Anatomical evidence of protected cluster buds, contorted xylem and starch in lignotubers (1)
Olea europaea L. subsp. *europea*	Adult	Field observation (1). Evidence of basal resprouting after fire (17)

References: 1: This study (including observations after wildfires by JG Pausas and S Paula); 2: Charco (1999); 3: Quevedo et al. (2007); 4: Canadell and López-Soria (1998); 5: Canadell et al. (1991); 6: Sealy (1949b); 7: Keeley (2012); 8: Ç. Tavsanoglu (pers. com.); 9: Paula and Ojeda (2009); 10: Cruz et al. (2003); 11: Lloret and López-Soria (1993); 12: Silva et al. (2002); 13: Çolak et al. (2009); 14: Pausas (1997); 15: Molinas and Verdaguer (1993a); 16: Verdaguer et al. (2001); 17: Catry et al. (2010)

Tetraclinis articulata and in resprouting *Erica* species is well-known in the local culture as they were used for making furniture (*T. articulata*) and tools (*Erica* spp.; smoking pipes, spoons, etc.); the lignotuber in *Arbutus* species has also been known since long ago (e.g. Sealy 1949b; Keeley 2012). All of the lignotuberous species belonged to Tertiary-evolved lineages; they are all evergreen small trees or large shrubs, and all are obligate resprouters, except for the *Erica* species, for which postfire recruitment has been reported—although not as profusely as in typical seeder species.

There were other species in which some authors mentioned the presence of lignotubers (cf. Paula et al. 2009; Paula and Pausas 2013), but we cannot confirm
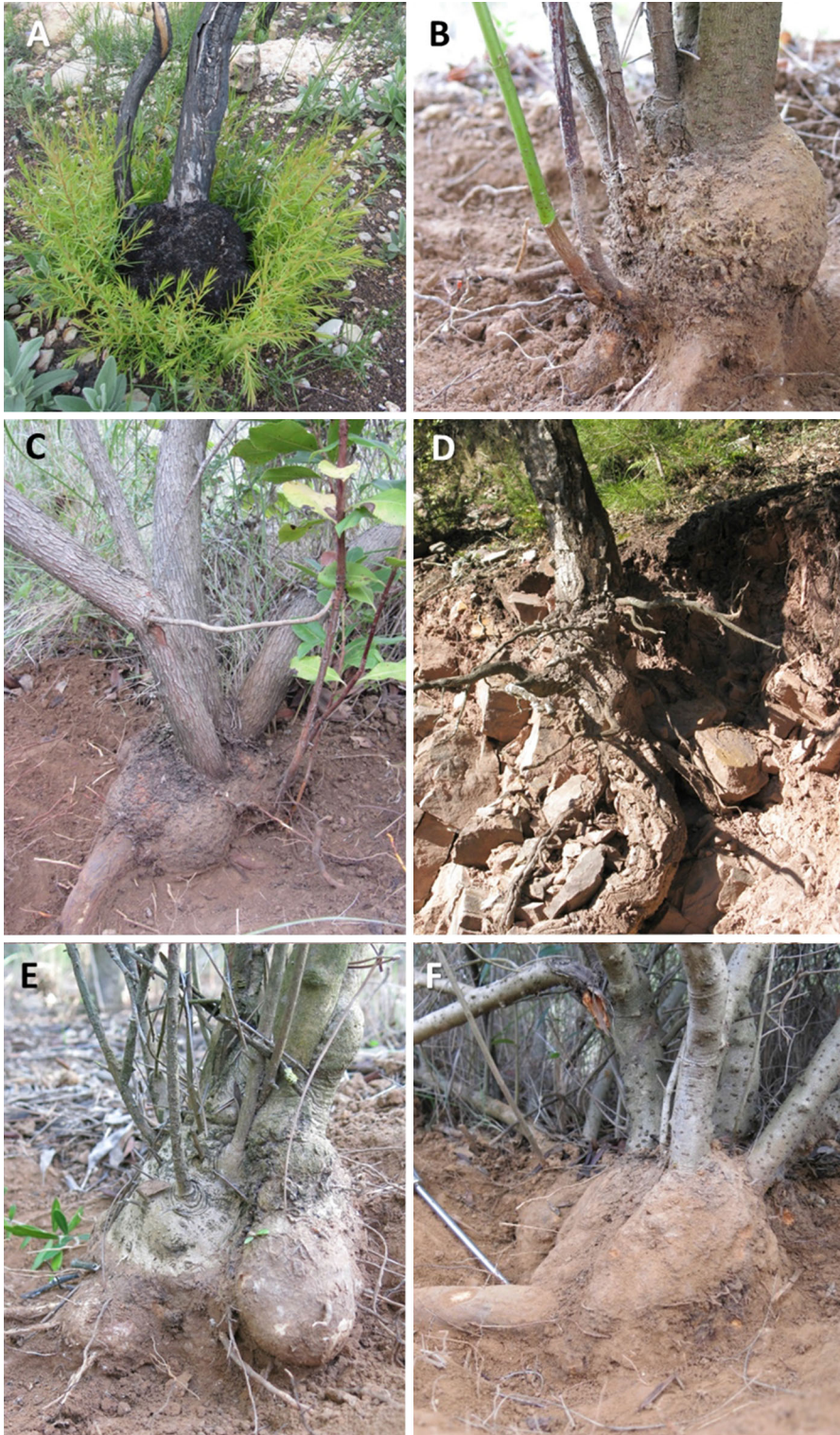
◀ **Fig. 1** Examples of lignotubers for Mediterranean basin species. **a** *Juniperus oxycedrus* subsp. *oxycedrus* (resprouting after fire). **b** *Viburnum tinus*. **c** *Arbutus unedo*. **d** *Quercus suber*. **e** *Olea europaea* subsp. europaea. **f** *Phillyrea angustifolia*. In *V. tinus* (**b**), *A. unedo* (**c**) and *P. angustifolia* (**f**) the lignotuber is only evident after excavating the root-shoot transition zone, whereas a conspicuous lignotuber is more easily discernible in *J. oxycedrus* (even in undisturbed plants). Note that there is not a clear basal swelling in *Q. suber* (**d**). Lignotuber resprouts might emerge from different depths (see for instance **b**), suggesting different degrees of bud protection from fire

these reports. For instance, we discarded the existence of lignotuber in *Pistacia lentiscus* L., based on studies conducted by Ladd et al. (2005), who illustrated a rhizome-like structure from which post-fire resprouts emerge. Similarly, anatomical studies of the cotyledonary region of *Q. ilex* concluded that this species does not develop a lignotuber (Pascual et al. 2002). Excavated *Rhamnus lycioides* L. juveniles did not show a clear lignotuber (see Fig. S1 in Supplementary Materials).

Morphological study

The age of *A. unedo* saplings ranged from 2.5 to 5 years, whereas *P. angustifolia* saplings were between 1 and 3 years old. In the early stages of development, the lignotuber can be distinguished with the naked eye as a small swelling on the stem at each side of the cotyledonary node. In some individuals, this swelling can also be seen at the first few succeeding leaf nodes. At later stages, these co-occurring swellings merged into a single lignotuber (Fig. 2a, b). The youngest saplings of *A. unedo* with swellings was 4-year old for those growing under nursery conditions, and 3-year old for plants from wild populations. In the case of *P. angustifolia*, these swellings were not visible in most saplings younger than 2 years of age (rarely observed in 1.5-year-old saplings).

In plants with a conspicuous lignotuber, cluster buds were visible on the lignotuber surface, although the buds appeared protected by the hypertrophied scales. The scales were arranged according to the phyllotaxis of each species: alternated in *A. unedo* and decussated in *P. angustifolia* (Fig. 2c, d). The number of externally visible buds tended to increase with age, although the relationship between these two variables

was significant only for *P. angustifolia* ($R^2 = 0.21$, $p = 0.009$; $R^2 = 0.14$, $p = 0.098$ for *A. unedo*). Considering only the saplings within the age range shared by the two species (i.e. 2–3 year-olds), *P. angustifolia* had more buds (29 ± 39) than *A. unedo* (13 ± 9 buds, mean $\pm$ SD). The number of buds was positively and significantly correlated to the lignotuber volume for the two species (*A. unedo*: $R^2 = 0.42$, $p = 0.002$; *P. angustifolia*: $R^2 = 0.81$, $p < 0.001$). Data collected on saplings from wild populations fell within (or close to) the confidence intervals of such relationships (see Fig. S2 in the Supplementary Materials).

Anatomical study

In 2.5-year-old saplings of *A. unedo*, an irregular protuberance was observed at the scar of the cotyledons (Fig. 3a). This protuberance enclosed several buds under the periderm (Fig. 3b). An earlier ontogenetic stage of lignotuber development was studied in *P. angustifolia* saplings. In 1-year-old saplings, an axillary bud was found at the scar of each cotyledon insertion (Fig. 3c). Axillary buds were accompanied by two accessory buds (Fig. 3c) and connected to the pith by a strand of parenchyma tissue (hereafter, bud trace; Fig. 3d).

At more advanced developmental stages (i.e. when the lignotuber was macroscopically discernible), most of the lignotuber was composed of xylem which was arranged in a contorted fashion (Figs. 4a, c, 5a, e). Abundant amyloplasts were revealed in the parenchyma cells and in the tracheary elements, especially in *A. unedo* (Fig. 4c; see also Fig. S3 in the Supplementary Materials). In *A. unedo*, tannins were also frequent in the xylem parenchyma cells (Fig. 4c–e). Embedded into the lignotuber xylem were several structures of parenchymatic nature surrounded by a sheath of promeristem; in some cases, it is possible to discern tracheary elements within this sheath (Figs. 4c–e, 5d–f). These structures, circular when viewed in a cross section, corresponded to the bud traces observed in earlier stages. Cluster buds disrupted a tannin-rich periderm (Figs. 4d, 5c, d). The lignotuber periderm tends to be thicker than that of the stem. Specifically, from measures conducted in one 2.5-year-old sapling per species (each one gathering ca. 60 externally visible buds) we obtained that, in *P. angustifolia*, the periderm was 247 ± 32 μm in the lignotuber and
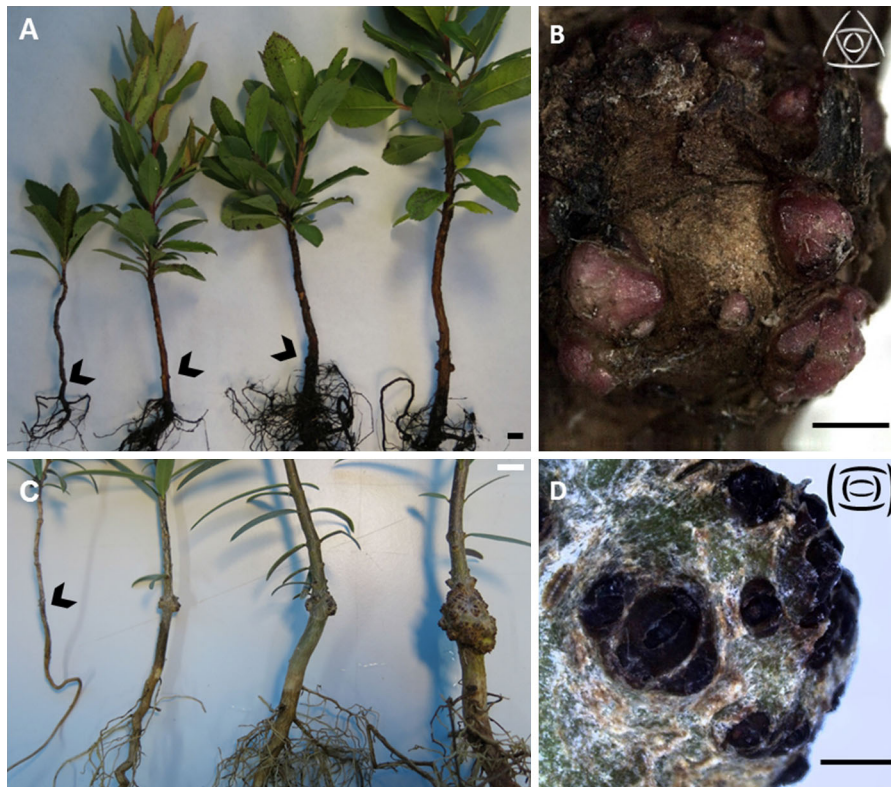
Fig. 2 Ontogenetic development of the lignotuber and detail of the cluster buds in *Arbutus unedo* (**a**, **c**) and *Phillyrea angustifolia* (**b**, **c**). On the right side of the figure, the position of the lignotuber is indicated with an *arrow* (when needed). Saplings ages were (from left to right) 2.5, 4.5, 4.5 and 4 years for *A. unedo* (**a**) and 1, 1.5, 2.5 and 2.5 years for *P. angustifolia* (**b**). Note that lignotuber size is more tightly related with sapling size than with the plant age (see also Supplementary Materials). Scale arrangement is alternate in *A. unedo* and decussate in *P. angustifolia* (see also diagrams in **b** and **d**). *Scale bar* 1 cm in **a** and **b**; 1 mm in **c** and **d**

189 ± 16 μm in the stem, whereas in *A. unedo* the periderm thickness was 175 ± 66 μm in the lignotuber and 145 ± 28 μm in the stem (mean ± SD). Notice that the lignotuber periderm tends to be thicker in older plants (see Figs. 4a, 5a). In *P. angustifolia*, it is possible to observe clusters of sclereids between the cortex and phloem (Fig. 5b). The buds were partially hidden by hypertrophied scales (100–200 μm in radial longitudinal section) with abundant tannins, being particularly tannin-rich in *A. unedo* (Figs. 4d, 5c, d).

Discussion

Lignotuberous species in the Mediterranean basin

Overall, we found reliable evidence for the presence of lignotubers in 14 woody species in the Mediterranean basin (Table 1), and Ericaceae is the family with most lignotuberous species (at least 8 species, Table 1). This review includes the first report of lignotuber existence in *Olea europaea* subsp. *europaea* and *Viburnum tinus*, as well as its confirmation in *Phillyrea angustifolia* and *Juniperus oxycedrus* subsp. *oxycedrus* after previous anecdotal observations (Paula et al. 2009; Paula and Pausas 2013). For *J. oxycedrus*, our observations in the studied population match well with other observation in different coastal populations in Spain; however, there is evidence of inland populations without lignotuber that fail to resprouting after fire (Pausas, pers. obser.). These 14 species comprise a small proportion of the woody resprouting species of the Mediterranean basin flora (ca. 6 % considering data compiled in Paula et al. 2009). A higher proportion of lignotuberous species was reported for other Mediterranean regions such as the Chilean *matorral* (Montenegro et al. 2003) or Western
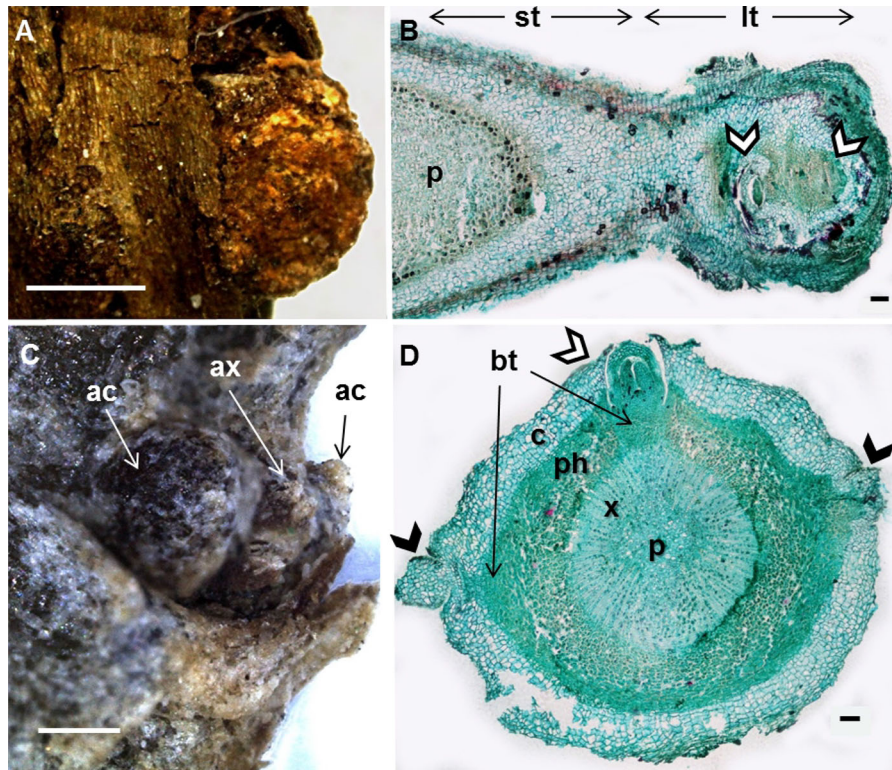
Fig. 3 Cotyledonary region in young saplings of *Arbutus unedo* (2.5 year-old; **a**, **b**) and *Phillyrea angustifolia* (1 year-old; **c**, **d**). **a** Details of the cotyledonary node, showing an incipient cluster bud; *scale bar* 500 µm. **b** Cross section at the level of the cluster bud, showing two buds (*white arrows*); *scale bar*: 100 µm. **c** Detail of the cotyledonary node, showing the axillary bud accompanied by two accessories buds; *scale bar* 500 µm. **d** Cross section of the cotyledory region, showing a developed cotyledonary bud connected to the bud trace (*white arrow*), and two buds (tangential section) corresponding to the first leaf node after the cotyledonary insertion (*black arrows*); *scale bar*: 100 µm. *ac* accessory bud, *ax* axillary, *bt* bud trace, *c* cortex, *lt* lignotuber, *p* pith, *ph* phloem, *st* stem, *x* xylem

Australian forests (Burrows and Wardell-Johnson 2003). However, the prevalence values of lignotubers in these floras are not necessarily comparable considering that (i) we were conservative (i.e. unconfirmed or anecdotal evidence was not considered); (ii) the paucity of studies on resprouting available for the south and east of the Mediterranean basin; and (iii) the difficulty in unambiguously differentiating lignotubers from other basal resprouting structures (i.e. post-disturbance burls). It is likely that the Mediterranean basin flora includes more species with lignotubers; however, currently there are no unambiguous published evidence for other species, and further research is needed (e.g. other resprouting *Erica* species).

The lineage origin of all Mediterranean basin species identified as lignotuberous is pre-Mediterranean and tied to the Tertiary Madrean–Tethyan sclerophyllous vegetation that occupied a subhumid belt across much of North America-Eurasia in the middle Eocene (Axelrod 1975). This lineage has been considered to be relict taxa that suffered phenotypic stability for long periods of time (evolutionary inertia) without adapting to current Mediterranean conditions (dry ecosystems with recurrent fires) (Herrera 1992; Valiente-Banuet et al. 2006). Given the strong link between lignotubers and fire (see "Introduction" Section), our observations challenge the traditional view of the evolutionary stability of this 'fire-independent' vegetation, and suggest that some lineages with origins dating back to the Tertiary (or earlier) also adapted to the increased fire activity throughout the Tertiary and Quaternary (Keeley et al. 2012). Consequently, two pathways for the evolution of resprouting from ancestral lineages may be hypothesised. Firstly, some resprouting lineages acquired the capacity to form a permanent fire-resistant seed bank,
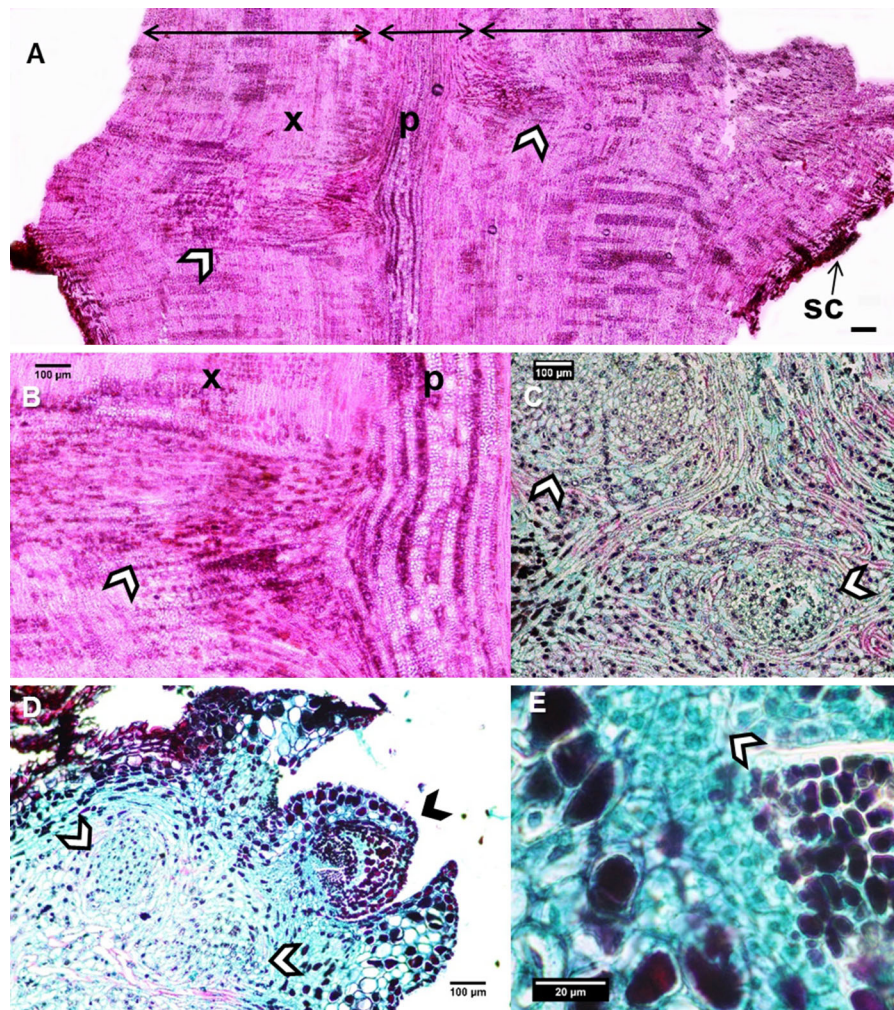
Fig. 4 Lignotuber anatomy in a 5-year-old sapling of *Arbutus unedo*. **a** Longitudinal section of the cotyledonary region, showing the connection of two bud traces (*white arrows*) to the stem pith. *Scale bar* 500 μm. **b** Detail of **a**, showing one bud trace (*white arrow*) connected to the stem pith, the latter with abundant starch. **c** Several bud traces in cross section (*white arows*) surrounded by contorted xylem. **d** Radial longitudinal section of a lignotuber bud with very thick scales rich in tannins (*black arrow*); two bud traces are indicated with white arrows. **e** Detail of **d**, showing the promeristem of the lignotuber bud. *lt* lignotuber, *p* pith, *st* stem, *x* xylem

and some even lost their resprouting ability to become obligate postfire seeders (Pausas and Verdú 2005; Pausas and Keeley 2014). Secondly, other lineages acquired mechanisms to ensure efficient resprouting (obligate resprouters), such as having very thick bark for epicormic resprouting (Pausas 1997, 2015) or by developing a lignotuber. The species studied here are examples of the latter. It would be interesting to test this hypothesis in a phylogenetic framework, although more information regarding post-fire resprouting mechanisms is still needed.

Morpho-anatomical key traits in early lignotubers

Lignotubers of *Arbutus unedo* and *Phillyrea angustifolia* were macroscopically distinguishable later in ontogeny when compared to the Australian species *Eucalyptus cinerea* and *Banksia menziesii*, where the lignotuber is evident in ca. 6-month-old seedlings; at this age, lignotubers of the former species were shown to contain 17 and 35 developed buds, respectively (Graham et al. 1998; Mibus and Sedgley 2000). Similarly, in *Sequoia sempervirens*, cluster buds were
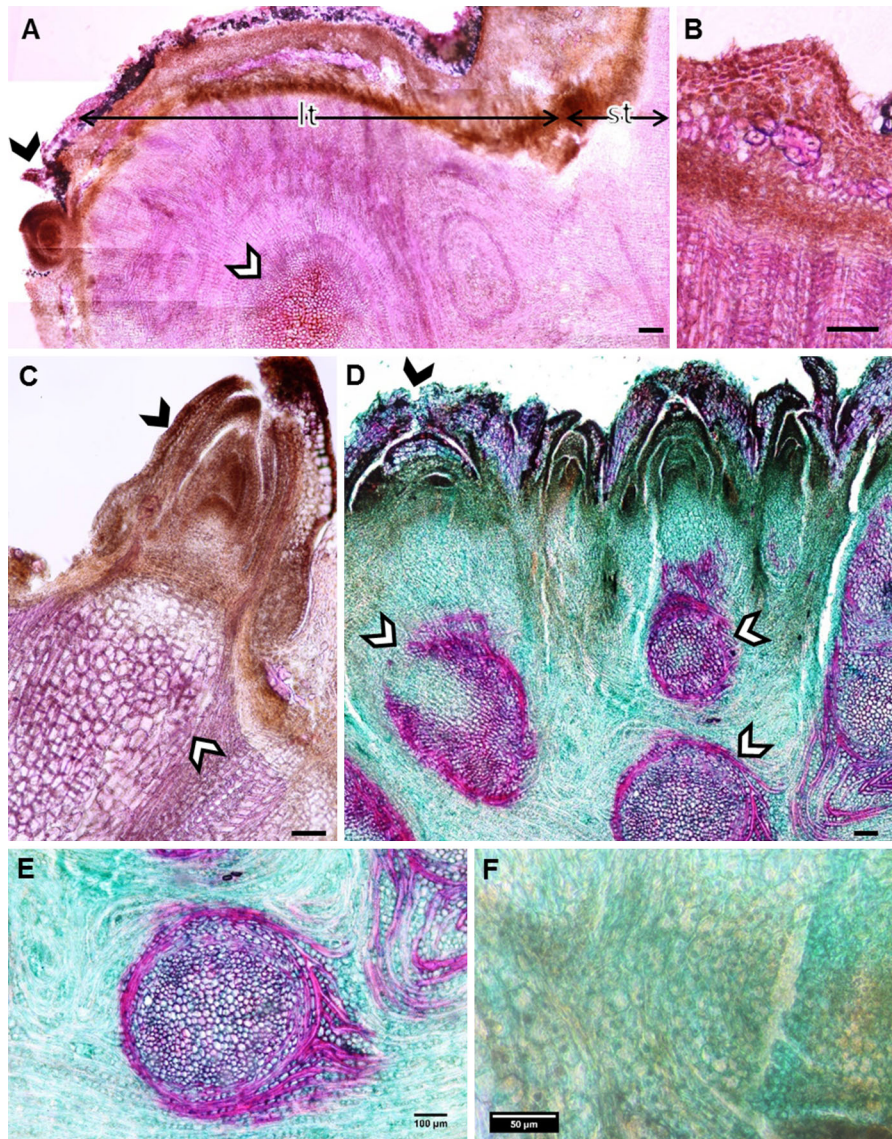
Fig. 5 Lignotuber anatomy in a 2.5-year-old sapling of *Phillyrea angustifolia*. **a** Longitudinal section of the cotyledonary region, including the main stem and the lignotuber. A lignotuber bud (*black arrow*) and a bud trace (*white arrow*) are observed; *scale bar* 200 μm. **b** Detail of **a**, showing a sclereid cluster between the cortex and the phloem; *scale bar* 100 μm. **c** Radial longitudinal section of a lignotuber bud (*black arrow*) connected to a bud trace (*white arrow*); *scale bar* 100 μm. **d** Several lignotuber buds (in longitudinal section; one marked with a *black arrow*) and bud traces (in cross section; *white arrows*) surrounded by contorted xylem; *scale bar* 100 μm. **e** Detail of **d**, showing a bud trace and the contorted xylem. **f** Detail of **d**, showing the promeristem of a lignotuber bud (that marked with a *black arrow* in **d**). *lt* lignotuber, *p* pith, *st* stem

discernible with the naked eye within the first 6 months of development (Del Tredici 1999). In central Chile, the lignotuber of the studied species is almost indistinguishable in 1-year-old saplings and no buds were observed in the root-shoot transition zone at this stage (Montenegro et al. 1983). These results

(summarised in Table 2) highlight the difficulty in discerning lignotubers at very early ontogenetic stages; they also highlight the existence of a temporal window in which resprouting species may be more sensitive to bud exhaustion from recurrent disturbances. This fire-sensitive window may be consistent

with the historical fire return interval, although further research is needed in this regard. The study of intraspecific variability in lignotuber development rates along a gradient of disturbance would shed light on the factors modulating lignotuber ontogeny.

For *Eucalyptus* species, the existence of two accessory buds (one adaxial and another abaxial to the axillary bud at the cotyledonary node) has been claimed to be a requisite for lignotuber formation (Chattaway 1958; Graham et al. 1998). Similarly, endogenous accessory buds are found in the cotyledonary node of the lignotuberous species *B. menziesii* during early development (Mibus and Sedgley 2000). As described for eucalypts, we found two accessory buds in the earlier stages of the lignotuber formation of *P. angustifolia* (1-year old; Fig. 3c). Following Sealy (1949b), the first two lignotuber buds of *A. unedo* were endogenously originated at the end of the cotyledonary traces which appeared early after the cotyledon abscission; these buds developed below the bark, and they rose toward the lignotuber surface in more developed stages. Therefore, the meristematic potential of the lignotuber of *A. unedo* is unlikely to be related to the accessory buds associated with the axillary bud complex. More research is needed to understand how the cotyledonary traces adventitiously generate the initial lignotuber bud. Similarly, lignotuber buds of *Quercus suber* are not originated at the cotyledonary axil, but between the cotyledon petioles and the embryonic axis in the fused (and enlarged) portion of the cotyledonary node (Molinas and Verdaguer 1993a). In lignotuberous *Erica* species, the meristematic potential of the lignotuber is probably generated by the accumulation of axillary buds of basal branches that progressively merge to a conspicuous lignotuber (Verdaguer and Ojeda 2005). Therefore, there is no unique ontogenetic program for the development of cluster buds.

In both *A. unedo* and *P. angustifolia*, lignotuber buds are connected to the stem pith by a parenchyma strand surrounded by a promeristem sheath in early developmental stages, and these subsequently become tracheary elements. These bud traces were described previously in *A. unedo* by Sealy (1949b). Analogously, in *Q. suber*, *B. menziesii*¸ and *Erica* species, lignotuberous buds are connected to the pith by means of a strand of vascular cells (Molinas and Verdaguer 1993a; Mibus and Sedgley 2000; Verdaguer and Ojeda 2005). Bud traces have also been described in *Eucalyptus*

species, although there is some controversy regarding their nature (see Graham et al. 1998 and references therein). For *E. cinerea*, it has been proposed that bud traces would provide the meristematic tissue to generate accessory buds (Graham et al. 1998). However, the presence of both parenchyma cells and tracheary elements in the bud traces of the studied species suggest that they would supply water and carbon to sustain bud metabolism and post-disturbance growth. Sealy (1949b) reported aleurone grains (which are typical of Poaceae) in the parenchyma cells of bud traces of *A. unedo*. However, we did not observe aleurone grains (nor other reserve compounds) within the bud traces. Abundant starch was found in the lignotuber xylem parenchyma, stem pith parenchyma and in some conducting cells of the lignotuber xylem in the two studied species (see Fig. S3 in Supplementary Materials). For *A. unedo*, Sealy (1949b) reported the presence of aleurone grains in the same cell types (including xylem vessels) and this suggests that the reserves described by Sealy were probably starch. A storage function of the tracheary elements of the lignotuber xylem has also been described for some eucalypts (Chattaway 1958), but it cannot be generalised (Carrodus and Blake 1970). Therefore, more research is needed to ascertain the potential storage function of the tracheary elements in the lignotuber.

In saplings with macroscopically distinguishable lignotubers, most of the root-shoot transition zone was occupied by xylem that was much more distorted and twisted than in the stem (Fig. 4c, 5e; see also Sealy 1949b). This has also been observed in many *Eucalyptus* species (Chattaway 1958; Carrodus and Blake 1970). Interestingly, burls without a bud bank in *Eucalyptus* species do not show this type of internal anatomy (Carrodus and Blake 1970). However, a contorted xylem was not observed in very young saplings of the studied species, which is consistent with the observations in seedlings ($\leq$1-year-old) of other lignotuberous species such as *Quercus suber, Eucalyptus cinerea* and *Banksia menziesii* (Molinas and Verdaguer 1993a; Graham et al. 1998; Mibus and Sedgley 2000; see Table 2). The fact that lignotuber xylem becomes more disorganised as lignotuber grows suggests that the irregular proliferation of the xylem could be associated with water and carbohydrate supply for bud metabolism and resprouting in well-developed lignotubers. Specifically, as bud clusters multiply, more bud traces cross the xylem in

Table 2 Key traits of early lignotubers in species from different Mediterranean climate-type regions (WA: Western Australia; SA: South Africa; CC: central Chile; MB: Mediterranean basin). References are included in brackets (see Table footnotes)

Species	Family	Region	Age with cluster buds	Bud initiation (proposed location)	Bud trace	Bud protection	Contorted xylem	Starch location	Shape
Eucalyptus cinerea F. Muell. ex Benth. (1)	Myrtaceae	WA	6 months (17 buds)	Cotyledonary axil, lignotuber cortex and xylem	Yes	Lignotuber cortex and xylem	No (6 months)	–	Swollen
Banksia menziesii R. Brown (2)	Proteaceae	WA	6 months (35 buds)	Cotyledonary axil and fused base of cotyledons	Yes	Extended base of the cotyledons	No (6 months)	–	Swollen
Erica coccinea L. (3)	Ericaceae	SA	>9 months	Cotyledonary and leaf axil	Yes	–	No (≤ 9 months)	–	Elongated
Erica calycina L. (3)	Ericaceae	SA	>9 months	Cotyledonary and leaf axil	Yes	–	No (≤ 9 months)	–	Elongated
Cryptocaria alba (Mol.) Looser (4)	Lauraceae	CC	>1 year	Lignotuber cortex	–	–	–	Cortex	Elongated
Sequoia sempervirens (D. don) Endl. (5)	Cupressaceae	CA	6 months	Cotyledonary axil	–	–	–	–	Swollen
Quercus suber L. (6)	Fagaceae	MB	5 months	Fused base of cotyledons	Yes	Hypertrophied scales	No (4 years)	Pith & xylem	Elongated
Arbutus unedo L. (7, 8)	Ericaceae	MB	2–3 years (13 buds)	Cotyledonary trace within the lignotuber xylem	Yes	Hypertrophied scales	Yes (4 years)	Pith & xylem	Swollen
Phillyrea angustifolia L. (8)	Oleaceae	MB	2–3 years (29 buds)	Cotyledonary axil	Yes	Hypertrophied scales	Yes (2.5 years)	Pith & xylem	Swollen

The symbol ">" in the fourth column denotes that, at the indicated age, no cluster buds were yet observed. Bud protection refers to the mechanism conferring additional bud resistance to biotic and abiotic stress; in all cases, soil probably confers most of the isolation from fire temperatures

References: 1: Graham et al. (1998); 2: Mibus and Sedgley (2000); 3: Verdaguer and Ojeda (2005); 4: Montenegro et al. (1983); 5: Del Tredici (1999); 6: Molinas and Verdaguer (1993a); 7: Sealy (1949b); 8: This study

multiple directions, thus probably forcing the xylem to grow in a contorted fashion.

The strong relationship between lignotuber size and the number of buds explains why plants with large lignotubers show more vigorous resprouting and are more likely to survive recurrent disturbances (e.g. Rundel et al. 1987; Paula and Ojeda 2006), although this relationship might be lost or even inverted in very old and/or repeatedly disturbed plants (Zammit 1988; Paula and Ojeda 2006). In fact, the high number of buds is one of the key traits that indicates the adaptive role of lignotubers to frequent and severe disturbances (Canadell and Zedler 1995). Less attention has been paid to the role of bud protection. For the two species studied, the buds are protected by hypertrophied scales, which have a very thick tannin-rich periderm. These scales have been also reported for *Quercus suber* (Molinas and Verdaguer 1993a; for epicormic bud scales in this species, see Burrows and Chisnall 2015). Scaled-bud formation in branches of evergreen broad-leaf trees is related to resistance to disturbances such as wind, drought or herbivory (Nitta and Ohsawa 1998). In fact, the high tannin content in the scales would add protection to the buds against biological damage, since tannins are a herbivory deterrent (Bernays et al. 1989) and an inhibitor of microorganism activity (Field and Lettinga 1992). However, the role of fire in shaping this kind of protection cannot be discarded. The fact that postfire resprouts in lignotuberous plants emerge mostly from below ground level (e.g. see Fig. 1a; in some cases the whole lignotuber is below ground, see Fig. 1b, c, f) suggests that soil confers most of the isolation to fire temperatures. In these conditions of reduced fire temperature, a slight protection (e.g. from scales) may significantly increase bud survival. In any case, bud protection by lignotuber tissues increases bud longevity and so favours resprouting after fire.

Conclusions

Although much effort has been made in recent years to understand the functional role and evolution of resprouting (Clarke et al. 2013, Pausas and Keeley 2014, Pausas et al. 2015), the present review emphasises the need to increase our knowledge of

the different types of resprouting. Even though resprouting from the lignotuber is considered to be one of the mechanisms of vegetative regeneration most tightly linked to fire (Keeley et al. 2011), little is known about its prevalence in many Mediterranean ecosystems. The shortage of reliable information may be due to the fact that in many species, lignotubers are underground and not evident without excavation (Fig. 1). Confusing information regarding lignotubers in Mediterranean basin species could also be partially explained by their relatively late development during ontogeny (compared to species from other Mediterranean-type climate regions; Table 2).

All Mediterranean basin species identified as lignotuberous belong to Tertiary-evolved lineages related to the Madrean–Tethyan sclerophyllous vegetation. Considering the prevalence of lignotubers in fire-prone ecosystems (e.g. Keeley et al. 2012), our results challenge the traditional view of the evolutionary stability of this 'fire-independent' vegetation (Herrera 1992), and suggest that lineages with origins dating back to the Tertiary (or earlier) also adapted to increased fire activity throughout the Tertiary and Quaternary (Keeley et al. 2012).

Morpho-anatomical studies provide the key traits that permit early identification of lignotubers. Although the origin of the initial meristematic source of the lignotuber probably differs between *A. unedo* and *P. angustifolia*, both share a series of anatomical traits including a plethora of protected buds connected to the stem pith by a bud trace. We propose using these key traits to standardise the identification of lignotubers in other species. It is also probable that contorted xylem would be a diagnostic trait in well-developed lignotubers, although more studies are needed in this regard.

References

Axelrod DI (1975) Evolution and biogeography of Madrean-Tethyan sclerophyll vegetation. Ann Mo Bot Gard 62:280–334

Bamber RK, Mullette KJ (1978) Studies of lignotubers of *Eucalyptus gummifera* (Gaertn. and Hochr.). II anatomy. Aust J Bot 26:15–22

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

Bernays EA, Cooper-Driver GA, Bilgener M (1989) Herbivores and plant tannins. In: Begon M, Fitter AH, Ford ED, MacFadyen A (eds) Advances in ecological research, vol 19. Academic Press Ltd, London, pp 263–302

Burrows GE, Chisnall LK (2015) Buds buried in bark: the reason why *Quercus suber* (cork oak) is an excellent post-fire epicormic resprouter. Trees 1–14

Burrows N, Wardell-Johnson G (2003) Fire and plant interactions in forested ecosystems of south-west Western Australia. In: Abbott I, Burrows N (eds) Fire in ecosystems of south-west Western Australia: impacts and management. Backhuys Publishers, Leiden, pp 225–268

Canadell J, López-Soria L (1998) Lignotuber reserves support regrowth following clipping of two mediterranean shrubs. Funct Ecol 12:31–38

Canadell J, Zedler PH (1995) Underground structures of woody plants in Mediterranean Ecosystems of Australia, California, and Chile. In: Arroyo MTK, Zedler PH, Fox MD (eds) Ecology and biogeography of Mediterranean ecosystems in Chile, California, and Australia. Springer, New York, pp 177–210

Canadell J, Lloret F, López-Soria L (1991) Resprouting vigour of two Mediterranean shrub species after experimental fire treatments. Vegetatio 95:119–126

Carr DJ, Jahnke R, Carr SGM (1983) Development of the lignotuber and plant form in Lehmannianae. Aust J Bot 31:629–643

Carrodus BB, Blake TJ (1970) Studies on lignotubers of *Eucalyptus obliqua* L'Heri. I. Nature of lignotuber. New Phytol 69:1069–1072

Catry F, Rego F, Moreira F, Fernandes P, Pausas JG (2010) Post-fire tree mortality in mixed forests of central Portugal. For Ecol Manage 260:1184–1192

Charco J (1999) El bosque mediterráneo en el norte de África. Agencia Española de Cooperación Internacional, Madrid

Chattaway MM (1958) Bud development and lignotuber formation in eucalypts. Aust J Bot 6:103–115

Clarke PJ, Lawes M, Midgley J, Lamont B, Ojeda F, Burrows G, Enright N, Knox K (2013) Resprouting as a key functional trait: how buds, protection and resources drive persistence after fire. New Phytol 197:19–35

Çolak AH, Rotherham ID, Spethmann W (2009) The Importance of lignotubers in arid-zone and humid-zone ecosystems with particular reference to *Rhododendron ponticum* L. Arboric J 32:67–82

Cruz A, Pérez B, Moreno JM (2003) Resprouting of the mediterranean-type shrub *Erica australis* with modified lignotuber carbohydrate content. J Ecol 91:348–356

Del Tredici P (1999) Redwood burls: immortality underground. Arnoldia 59:14–22

Field J, Lettinga G (1992) Toxicity of tannic compounds to microorganisms. In: Hemingway RW, Laks PE (eds) Plant polyphenols. Synthesis, properties, significance. Plenum Press, New York, pp 673–692

Graham AW, Wallwork M, Sedgley M (1998) Lignotuber bud development in *Eucalyptus cinerea* (F. Muell. ex Benth). Int J Plant Sci 159:979–988

Herrera CM (1992) Historical effects and sorting processes as explanations for contemporary ecological patterns—character syndromes in Mediterranean woody-plants. Am Nat 140:421–446

James S (1984) Lignotubers and burls—their structure, function and ecological significance in mediterranean ecosystems. Bot Rev 50:225–266

Johansen D (1940) Plant microtechnique. McGraw-Hill, New York

Keeley JE (2012) Fire in Mediterranean climate ecosystems—a comparative overview. Israel J Ecol Evol 58:123–135

Keeley JE, Pausas JG, Rundel PW, Bond WJ, Bradstock RA (2011) Fire as an evolutionary pressure shaping plant traits. Trends Plant Sci 16:406–411

Keeley JE, Bond WJ, Bradstock RA, Pausas JG, Rundel PW (2012) Fire in mediterranean ecosystems: ecology, evolution and management. Cambridge University Press, Cambridge

Kerr LR (1925) The lignotubers of eucalypt seedlings. Proceedings of the Royal Society of Victoria 37

Knox KJ, Clarke PJ (2004) Fire response syndromes of shrubs in grassy woodlands in the New England Tableland Bioregion. Cunninghamia 8:348–353

Kummerow J (1989) Structural aspects of shrubs in Mediterranean-type plant communities. Optìons Méditerranéennes 3:5–11

Ladd PG, Crosti R, Pignatti S (2005) Vegetative and seedling regeneration after fire in planted Sardinian pinewood compared with that in other areas of Mediterranean-type climate. J Biogeogr 32:85–98

Lloret F, López-Soria L (1993) Resprouting of *Erica multiflora* after experimental fire treatments. J Veg Sci 4:367–374

Lloret F, Verdú M, Flores-Hernández N, Valiente-Banuet A (1999) Fire and resprouting in Mediterranean ecosystems: insights from an external biogeographical region, the mexical shrubland. Am J Bot 86:1655–1661

Mibus R, Sedgley M (2000) Early lignotuber formation in *Banksia*—Investigations into the anatomy of the cotyledonary node of two *Banksia* (Proteaceae) species. Ann Bot 86:575–587

Molinas ML, Verdaguer D (1993a) Lignotuber ontogeny in the cork oak (*Quercus suber*, Fagaceae). 2. Germination and young seedling. Am J Bot 80:182–191

Molinas ML, Verdaguer D (1993b) Lignotuber ontogeny in the cork oak (*Quercus suber*, Fagaceae). 1. Late embryo. Am J Bot 80:172–181

Montenegro G, Avila G, Schatte P (1983) Presence and development of lignotubers in shrubs of the Chilean matorral. Can J Bot 61:1804–1808

Montenegro G, Gómez M, Díaz F, Ginocchio R (2003) Regeneration potential of Chilean matorral after fire: an updated view. In: Veblen TT, Baker WL, Montenegro G, Swetnam TW (eds) Fire and climatic change in temperate

ecosystems of the Western Americas. Springer, New York, pp 375–403

Mullette K, Bamber R (1978) Studies of the lignotubers of *Eucalyptus gummifera* (Gaertn. and Hochr.). III. Inheritance and chemical composition. Aust J Bot 26:23–28

Nitta I, Ohsawa M (1998) Bud structure and shoot architecture of canopy and understorey evergreen broad-leaved trees at their northern limit in East Asia. Ann Bot 81:115–129

Pascual G, Molinas M, Verdaguer D (2002) Comparative anatomical analysis of the cotyledonary region in three Mediterranean Basin *Quercus* (Fagaceae). Am J Bot 89:383–392

Paula S, Ojeda F (2006) Resistance of three co-occurring resprouter *Erica* species to highly frequent disturbance. Plant Ecol 183:329–336

Paula S, Ojeda F (2009) Belowground starch consumption after recurrent severe disturbance in three resprouter species of the genus *Erica*. Botany 87:253–259

Paula S., Pausas JG (2013) BROT: a plant trait database for Mediterranean Basin species. Version 2013.06. http://www.uv.es/jgpausas/brot.htm

Paula S, Arianoutsou M, Kazanis D, Tavsanoglu Ç, Lloret F, Buhk C, Ojeda F, Luna B, Moreno JM, Rodrigo A, Espelta JM, Palacio S, Fernández-Santos B, Fernandes PM, Pausas JG (2009) Fire-related traits for plant species of the Mediterranean Basin. Ecology 90:1420

Pausas JG (1997) Resprouting of *Quercus suber* in NE. Spain after fire. J Veg Sci 8:703–706

Pausas JG (2015) Bark thickness and fire regime. Funct Ecol 29:317–327

Pausas JG, Keeley JE (2014) Evolutionary ecology of resprouting and seeding in fireprone ecosystems. New Phytol 204:55–65

Pausas JG, Verdú M (2005) Plant persistence traits in fire-prone ecosystems of the Mediterranean basin: a phylogenetic approach. Oikos 109:196–202

Pausas JG, Pratt RB, Keeley JE, Jacobsen AL, Ramirez AR, Vilagrosa A, Paula S, Kanekua-Pia IN, Davis SD (2015) Towards understanding resprouting at the global scale. New Phytol. doi:10.1111/nph.13644

Pryor L, Byrne O (1969) Variation and taxonomy in *Eucalyptus camaldulensis*. Silvae Genetica 18:64–71

Quevedo L, Rodrigo A, Espelta JM (2007) Post-fire resprouting ability of 15 non-dominant shrub and tree species in Mediterranean areas of NE Spain. Ann Forest Sci 64:883–890

Rundel PW, Baker GA, Parsons DJ, Stohlgren TJ (1987) Postfire demography of resprouting and seedling establishment by *Adenostoma fasciculatum* in the California chaparral. In: Tenhunen JD, Catarino FM, Lange OL, Oechel WC (eds) Plant response to stress. Springer, Berlin, pp 575–596

Sealy JR (1949a) *Arbutus unedo*. J Ecol 37:365–388

Sealy JR (1949b) The swollen stem-base in *Arbutus unedo*. Kew Bull 4:241–251

Shepherd M, Kasem S, Lee DJ, Henry R (2008) Mapping species differences for adventitious rooting in a *Corymbia torelliana* × *Corymbia citriodora* subspecies *variegata* hybrid. Tree Genet Genomes 4:715–725

Silva JS, Rego FC, Martins-Loução MA (2002) Belowground traits of mediterranean woody plants in a portuguese shrubland. Ecol Mediter 28:5–13

Valdés B, Talavera S, Galiano EF (1987) Flora Vascular de Andalucía Occidental. Ketres, Barcelona

Valiente-Banuet A, Rumebe AV, Verdú M, Callaway RM (2006) Modern quaternary plant lineages promote diversity through facilitation of ancient tertiary lineages. Proc Natl Acad Sci USA 103:16812–16817

Van Groenendael J, Klimes L, Klimesova J, Hendriks R (1996) Comparative ecology of clonal plants. Philos Trans Royal Soc London B 351:1331–1339

Verdaguer D, Ojeda F (2005) Evolutionary transition from resprouter to seeder life history in two *Erica* (Ericaceae) species: insights from seedling axillary buds. Ann Bot 95:593–599

Verdaguer D, García-Berthou E, Pascual G, Puigderrajols P (2001) Sprouting of seedlings of three *Quercus* species in relation to repeated pruning and the cotyledonary node. Aust J Bot 49:67–74

Verdú M, Pausas JG (2013) Syndrome-driven diversification in a Mediterranean ecosystem. Evolution 67:1756–1766

Vesk PA, Westoby M (2004a) Sprouting ability across diverse disturbances and vegetation types worldwide. J Ecol 92:310–320

Vesk PA, Westoby M (2004b) Funding the bud bank: a review of the costs of buds. Oikos 106:200–208

Wildy DT, Pate JS (2002) Quantifying above- and belowground growth responses of the western Australian oil mallee, *Eucalyptus kochii* subsp. *plenissima*, to contrasting decapitation regimes. Ann Bot 90:185–197

Zammit C (1988) Dynamics of resprouting in the lignotuberous shrub *Banksia oblongifolia*. Austral Ecol 13:311–320