Trees
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SHORT COMMUNICATION

Unraveling the role of dragon's blood in the undisturbed growth of dragon trees

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Abstract

Key message **Dragon's blood secretion is an integral part of the normal development of the leaves and of the tissue protecting the trunk of dragon trees.**

Abstract Dragon's blood is a common name of a red resin produced in response to wounding by tree-like monocot species of the genus *Dracaena* (Asparagaceae), known as dragon trees. This resin has important medicinal uses and economic value. However, its ecological significance and mechanism of secretion are understudied. We specifically addressed this knowledge gap through the investigation of leaf shedding, a natural processes in plant development, associated with self-wounding. We aimed to characterize the form of the resin of the Macaronesian (*D. draco*, *D. tamaranae*) and Socotran (*D. cinnabari*) dragon trees, and to explain its role in the development of leaves and of the tissue covering the leafless mature trunks. Based on the NADI test and the analysis of large-area longitudinal sections, we show for the first time that the resin occurs in parenchyma cells in the form of terpene-filled vesicles which tend to aggregate. The resin is an anatomical marker of the area where the leaf's abscission zone will be formed. After leaf shedding, the resin containing leaf scars completely cover the trunk. This study highlights that dragon's blood is secreted not only following wounding caused by external biotic and/or abiotic factors, but also in the undisturbed growth of dragon trees.

Keywords Leaf scar · Red resin · Protective layer · Secretion · Wound

Introduction

In the course of evolution, plants have developed various mechanisms to facilitate adaptation to different environments. One of these is the chemical defense system directed against the effects of the attack of pathogens and herbivores. The effectiveness of toxic or repellent substances to fight invading organisms has been documented for many species (Wang et al. 2004; Krokene et al. 2013; Ramos et al. 2019), which may accumulate them in resin ducts, laticifers (Langenheim 2003; Franceschi et al. 2005; Pickard 2008; Prado and Demarco 2018), glandular trichomes (Schuurink and Tissier 2020), or non-secreting cells and tissues. Resin production and secretion is more common in gymnosperms and dicotyledons than in monocotyledons, where it only occurs in five families, i.e., Araceae, Arecaceae, Asparagaceae, Asphodelaceae, Xanthorrhoeaceae (Cabrita 2019).

In this paper, we focused on the red resin secreted by dragon trees (*Dracaena* spp., Asparagaceae), a group of threatened monocot species, endemic to different regions of the world such as Socotra Island (*D. cinnabari* Balf.), Canary Islands, Cape Verde Islands, and Morocco (with different subspecies of *D. draco* L. and the endemic from Gran Canaria *D. tamaranae* A. Marrero , R.S. Almeida and M.González-Martin), Southern Arabia (*D. serrulata* Baker), or Southern China, Vietnam and Laos (*D. cochinchinensis* Pierre ex Laness) (Durán et al. 2020; Maděra et al. 2020; Liu et al. 2021). Commonly known as dragon's blood, this resin is a commercially important product, used by traditional medicine worldwide (Gupta et al. 2008; Liu et al. 2021). As it typically appears in response to wounds in leaves (Wang et al. 2010), stem (Cui et al. 2013; Jura-Morawiec and Tulik 2015;

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Al-Okaishi 2020) and roots (Jura-Morawiec, unpublished data), our knowledge on dragon's blood mostly derives from studies of mechanical damage by incisions made to induce its secretion. Although the formation and secretion of dragon's blood are not yet fully understood, we know that dragon trees do not possess specialized structures such as resin ducts associated with its secretion (Jura-Morawiec and Tulik 2015; Liu et al. 2021) and that its production and accumulation are stimulated by fungal inoculation (Wang et al. 2011; Cui et al. 2013). Its composition is complex and species-specific (González et al. 2003; Vaničkova et al. 2020) and over two hundred chemicals have been extracted from it until now (mainly flavonoids, steroids and phenolic compounds; Liu et al. 2021).

In this investigation, we carry out a detailed analysis of leaf development in the rosette of several species of *Dracaena*. Using large-area longitudinal sections and histochemical tests on resin presence, we study leaf shedding (a natural process in plant development that is associated with wounding) to help elucidate the process of red resin secretion. We aim to (i) identify resin at the cellular level and (ii) assess its role in the normal, undisturbed growth of dragon trees.

Materials and methods

Plant material

The study was carried out with the age-diverse collection of dragon trees growing outdoors at the Botanical Garden "Viera y Clavijo"—Unidad Asociada al CSIC of the Cabildo de Gran Canaria, in Gran Canaria Island. Namely, we used selected specimens of *D. draco* (endemic to Macaronesia, Morocco and Cape Verde), *D. tamaranae* (endemic to Gran Canaria) and *D. cinnabari* (endemic to Socotra Island). These species are listed on the IUCN Red List of threatened species (Miller 2004; Silva et al. 2021) as critically endangered (*D. tamaranae*), endangered (*D. draco*), and vulnerable (*D. cinnabari*). We observed the morphology of 2–3 growing rosettes of each species to characterize the changes in the distribution of resin on the leaf surface at different stages of development. The two rosettes of ~ 50-year-old individuals of *D. draco* were cut out and their leaves were carefully separated leaf after leaf. The resin distribution was also confirmed in decapitated, unbranched *D. draco* plants obtained from a commercial nursery.

Anatomy and microscopy

To detect the place where the resin is produced during leaf formation, several samples of the *D. draco* shoot apex were fixed in FAA and stored in 70% ethanol. Subsequently, they were embedded in Epon resin according to the standard protocol (Meek 1976) and longitudinally cut to sections 3 µm thick with a Tesla 490A microtome. The resulting sections were then stained using the Periodic Acid Schiff reaction (PAS) and toluidine blue, mounted in Euparal, and examined under transmitted light using an Olympus BX41 microscope. Other shoot apex samples were used to obtain large-area longitudinal sections (60 µm thick) with a WSL microtome. Some of these sections were stained with Safranin 0 and Astra Blue [1:1; v/v], then dehydrated in ethanol series (50–100%) and mounted in Euparal (Roth). Pictures of these sections were taken with Telecentric Optical System Nikon 2x/0.09 and 1x/0.03, respectively. Unstained sections were observed with fluorescence microscopes Zeiss Axio. Lab1 (UV, 365 nm) and Nikon Microphot SA (VL, 405 nm).

Histochemical test

For the histochemical detection of resin, fresh samples of the leafy stems were longitudinally sectioned with a WSL microtome. Next, the sections were subjected to the NADI reagent (mixture of: 0.1% α-naphtol, 1% *N*,*N*-dimethyl-*p*-phenylenediamine and 0.1 M sodium phosphate buffer, pH 7.2) according to the procedure described by Demarco (2017). Monoterpenes and sesquiterpenes (essential oils) stain in blue, whereas diterpenes, triterpenes, tetraterpenes and derivatives stain in red, and mixtures of essential oils and resins stain in various shades of violet to purple, depending on the proportion of each compound. As reported by Vaničkova et al. (2020) twenty terpenoid compounds were identified from *Dracaena* resin.

Results

Morphological evidence of constitutive resin production

In the rosettes of dragon tree, the reddish pigmentation indicating the presence of resin was restricted to the base of the leaves (Fig. 1a, b), and its distribution changed in successive leaves of the rosette. Namely, there was a noticeable increase in the area of the leaf base covered with resin during the transition from young to mature stages, and until leaf senescence (Fig. 1b–e). After leaf shedding, the leaf scars, bundle scars and bud scars, completely cover the trunk surface (Fig. 1f). The leaf scars may have different shapes and sizes depending on the rate of plant growth. In the case of premature leaf abscission, the resin barrier zone was clearly visible after removing the leaf (Fig. 1g, h).

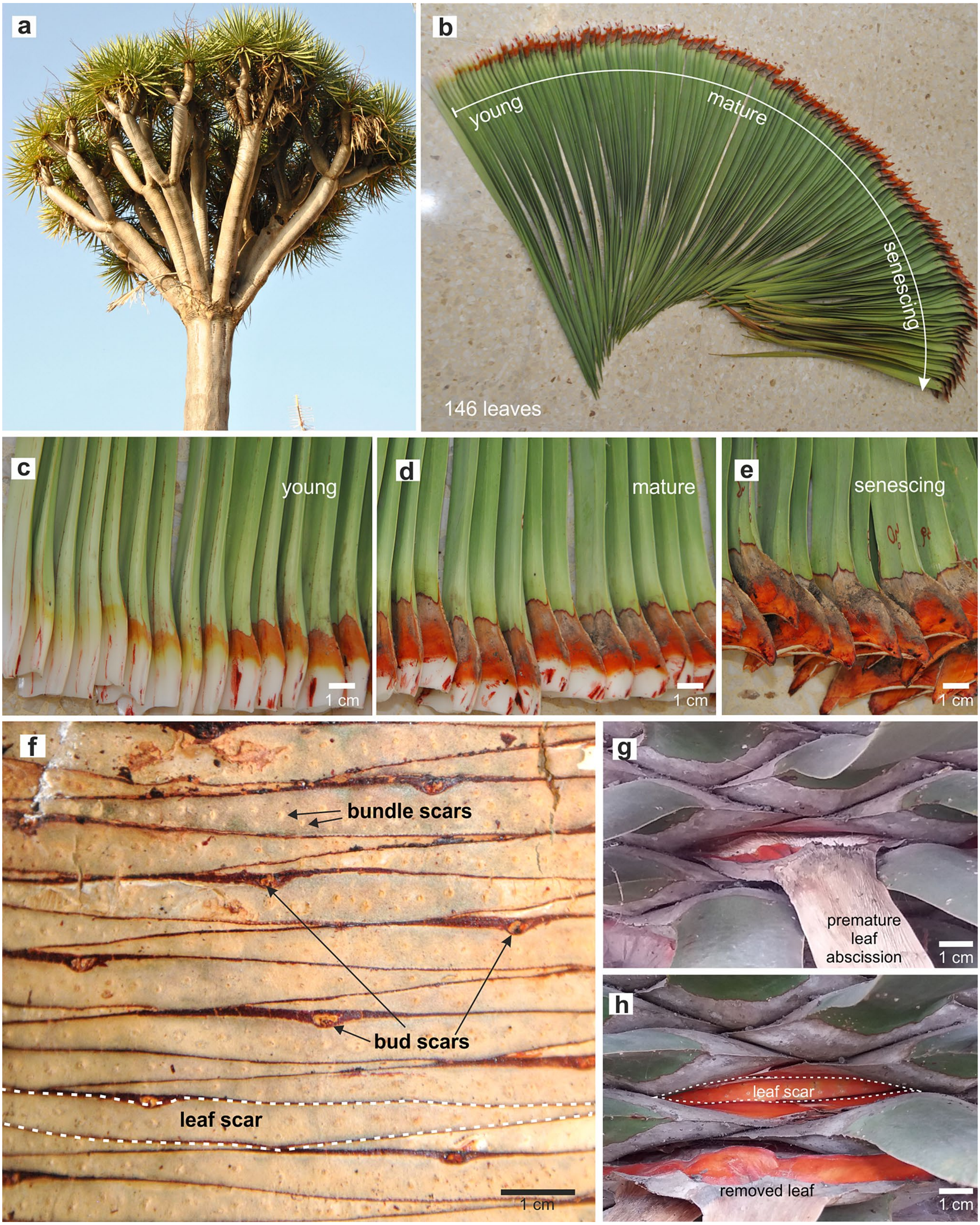
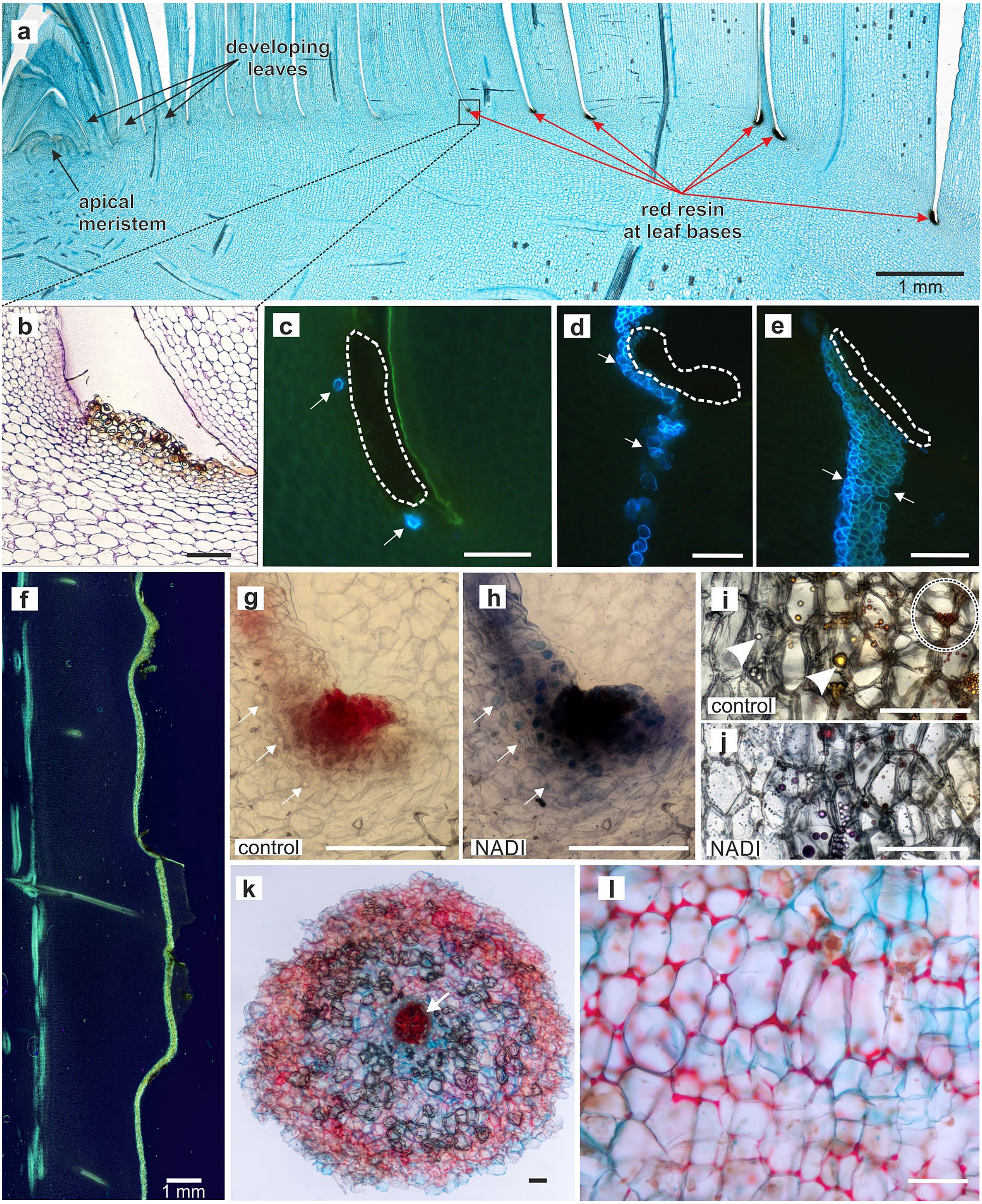


Fig. 1 Morphological details of red resin distribution during leaf development of dragon trees. **a** *D. draco*, general view of the crown with rosettes at the tips of the branches. **b** *D. draco*, successive leaves of a single rosette with red resin at leaf bases. **c–e** *D. draco*, age-dependent changes in the distribution of resin at the young stage, mature stage, and leaf senescing. **f** *D. draco*, surface of the trunk, below the rosette, entirely covered with leaf scars, bud scars, and bundle scars that form a characteristic pattern. **g, h** *D. cinnabari*, premature leaf abscission and red resin visible after removing the leaf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◂**Fig. 2** Anatomical details of resin occurrence during leaf development and after leaf shedding in *D. draco*. **a** Longitudinal section of the apical part of the rosette. **b** High magnification view of resin at the base of the developing leaf. **c–e** Successive stages of abscission zone (arrows) formation, lignified cell walls are visible under UV light, location of resin layer is marked by the dotted line. **f** Longitudinal section along the abscission zone of a few successive leaves (VL). **g, h** Fresh, longitudinal sections through the base of the developing leaf before and after staining with NADI reagent; a layer of red resin and terpene-filled resin vesicles (arrows) in parenchymatous cells stain in blue. **i, j** Fresh, longitudinal sections of the abscission zone; colorless and red–orange vesicles (arrowheads) in untreated section stain with NADI in different colors depending on the content of terpene compounds, note that resin vesicles tend to aggregate (encircled with dotted line). **k** Paradermal section of the bundle scar, the vascular elements are occluded with resin (arrow). **l** Paradermal section of the leaf scar, red resin is present in cells walls and intercellular spaces. Scale bar = 100 µm, valid for sections **b–e**; **g–l**

Resin production throughout the development of leaves and in the tissue protecting the trunk

To determine when the resin appears within the rosette, we cut the rosette tip longitudinally and analyzed the bases of developing, mature and senescing leaves. The resin layer was visible at a short distance from the rosette tip, above and below the base of each developing leaf (Fig. 2a, b). It was located close to the parenchyma cells whose walls undergo lignification, and started to form the abscission zone that finally extended across the leaf base (Fig. 2c–e). The abscission zone was clearly visible in the longitudinal section of the leafy part of the stem that encompassed different parts of the bases of the successive leaves (Fig. 2f). We observed many red–orange and translucent vesicles (Fig. 2g, i). The vesicles tended to aggregate (Fig. 2i), and the histochemical test with the NADI reagent revealed the presence of a mixture of terpenes in their contents (Fig. 2h, j). After shedding the leaves, resin plugged the vascular elements of the leaf traces within the bundle scars, and filled the cell walls and intercellular spaces in the leaf scars (Fig. 2k, l).

Discussion

Our results suggest that resin secretion in the three investigated *Dracaena* species is an integral part of the normal development of the dragon tree's leaves and of the tissue protecting the leafless mature trunks. This finding highlights an overlooked aspect of the function of the resin, because the investigations carried out thus far have mainly focused on (i) its chemical contents and medicinal importance (reviewed by Al-Awthan and Bahatabb 2021; Liu et al. 2021), or on (ii) its secretion after mechanical wounding of tissues and/or following inoculation by fungi (e.g., Wang et al. 2011; Cui et al. 2013; Jura-Morawiec and Tulik 2015, 2016).

Resin in leaf development

In many plants, during the early stages of leaf development, the abscission zone has morphologically distinct cells at the leaf base, whose position indicates the point of separation from the stem when leaves are shed (Addicott 1982; Tucker and Kim 2015; Olsson and Butenko 2018). Importantly, our study shows that resin occurrence can be used as an anatomical marker to predict the formation of the abscission zone during leaf development, as it assists in the shedding of leaves and in the formation of a protective layer for the underlying tissues. The resin appears quite early (close to the shoot apex), but the whole process of leaf separation proceeds slowly because the leaves of dragon trees may live several years before they senesce, die, and finally shed, similar to palm leaves (e.g., *Cocos nucifera*, Krishnakumar et al. 2019).

We have also noted that the size of the basal leaf surface that becomes covered with resin increases with age. Given that the basal parts of dragon tree leaves are water reservoirs (Jura-Morawiec and Marcinkiewicz 2020), we envision at least three potential explanations for this pattern that should be clarified with more detailed studies. First, that resin is a visual, mechanical and chemical barrier that protects the leaf water reservoirs against desiccation, and hampers access by insects. Indeed, the orange and red colors of the toxic resin have a potential as aposematic (warning) coloration (Lev-Yadun 2014, 2021). Therefore, the older the leaves, the wider their bases grow, and the bigger the water reservoir to be protected becomes. Second, the resin may result from injuries that happen during the shrinkage of the leaf water reservoirs in case of low water content (Jura-Morawiec and Marcinkiewicz 2020). Finally, the surface covered with resin may be simply connected with the increasing production of resin in the area of abscission zone, as a preparation for leaf shedding.

Resin identification at the cellular level

In previous investigations, dragon's blood was presented solely as a red layer that covers the wounds or occludes injured groups of cells (e.g., Wang et al. 2011; Cui et al. 2013; Jura-Morawiec and Tulik 2015, 2016; Al-Okaishi 2020; Liu et al. 2021). Specialized structures such as resin ducts were not detected (Jura-Morawiec and Tulik 2015), and therefore the mechanism of red resin secretion was unclear. A significant finding of this study is the localization of dragon's blood at the cellular level with a NADI reagent commonly used for resin detection (Demarco 2017). In *D. draco*, resin occurs in the parenchyma cells as terpene-filled vesicles which tend to aggregate. The resin vesicles have different sizes, numbers and colors (i.e., they have heterogeneous components). This finding relates to a previous study of

Fig. 3 Partly abscised leaf of *D. tamaranae*. Note a very wide base of the leaf (dotted line) that contributes to the species–specific leaf scar pattern on the trunk surface

a wounded stem of *D. draco*, in which Jura-Morawiec and Tulik (2015) observed red–orange vesicles in the unstained section of the wounded area. However, in that work, the contents of these vesicles were described as polyphenolic-like inclusions, and not as a resin.

This vesicle-form of *D. draco* resin is very similar to the rubber granules (particles) reported for nearly all the parenchyma cells in the stem and roots of the guayule, a woody desert shrub (*Parthenium argentatum*, Asteraceae; Artschwager 1943; Backhaus and Walsh 1983; Rousset et al. 2021). Interestingly, the tissues of both species also behave similarly after the incisions: neither the rubber of guayule, nor the resin in the investigated dragon trees flow out immediately. In *D. draco*, the resinous material may plug the cells or be excreted into ordinary intercellular spaces and form a layer along the cell walls. Considering the similar behavior detected by us in *D. cinnanbari* and *D. tamaranae*, we propose that this way of resin secretion is common for all dragon tree species.

Resin on the trunk surface

After leaf shedding, leaf and bud scars of *D. draco*, *D. tamaranae* and *D. cinnabari* completely cover the surface of the trunk, forming species-specific patterns (Fig. 1f, 3). The outline of leaf scars is sometimes visible even in ~ 50-year-old individuals. Interestingly, the tissue covering the trunks is relatively thin. Therefore, it is reasonable to assume that resin presence on the surface of the trunk is an important adaptation to increase protection against the entry of pathogens and desiccation. Notably, dragon trees do not form compact communities in natural conditions and consequently, their trunks also need protection from UV radiation. The resin present in leaf scars may contribute to this protective function, as it is mainly composed of flavonoids (Liu et al. 2021), which are known to play a central role in screening and absorbing UV radiation (Khalid et al. 2019).

The results of our study also indicate a diagnostic value of the leaf scars. They may facilitate the recognition of remnants of dragon tree trunks. For instance, based on the length of leaf scars, it is possible to distinguish the two Macaronesian dragon tree species, as *D. tamaranae*'s basal leaf parts are substantially wider than those of *D. draco* (Marrero et al. 1998 and Fig. 3).

Overall, we conclude that red resin secretion is an integral part of the normal development of the leaves and of the tissue protecting the trunk of dragon trees. It represents an important adaptation that enables the efficient functioning of the thick, succulent trunk despite having only a very thin protective tissue. The form of red resin in the parenchyma cells of dragon trees is similar to that of the rubber of guayule. Our results raise further questions regarding the chemical content of the dragon's blood; for instance, we ignore whether the red resin associated with the normal development of dragon trees is chemically different from that formed in response to wounding. Thus, further studies are needed to clarify if the wounds or infection cause *de novo* resin synthesis, or if it is produced regardless of these factors.

Author contribution statement JJ-M conceived and designed research, conducted anatomical analysis, and wrote the manuscript draft. JM did the analysis of large-area longitudinal sections. JM and JCC revised critically the manuscript. All authors read and approved the manuscript.

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Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

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