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Secretory Structures in *Flourensia campestris* and *F. oolepis*: Ultrastructure, Distribution, and (-)-Hamanasic Acid A Secretion

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Abstract

In this work, the localization, density, morphology and ultrastructure of secretory structures in aerial organs of *Flourensia campestris* (FC) and *F. oolepis* (FO) (Asteraceae) by means of a combination of light, fluorescence, transmission (TEM) and scanning electron microscopy (SEM) were examined. The possible role of secretory structures in the production and secretion of the phytotoxic sesquiterpene (-)-hamanasic acid A ((-)HAA) in both species was also assessed. Capitate glandular trichomes were found in all reproductive organs of FC and FO, and were being reported for the first time. These glandular trichomes, typically associated to edges and veins, were of the same type as those already described for vegetative organs, and were abundant in involucral bracts and corolla of tubulose and ligulate flowers. Their density in reproductive organs of both species was similar (ca. 30/mm^2) and lower than that found in leaves (ca. 100/mm^2) and stems (ca. 160/mm^2 in FC, and up to 650/mm^2 in FO). Glandular trichomes in vegetative organs followed a species-

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specific pattern of distribution. TEM and SEM observations suggest that each species differs in the way in which secretory materials are released to the outside: through cracks or pores in FC, or through a loose cuticle in FO. Similar inspections of the secretory ducts revealed lipophilic vacuoles localized in subepithelial and epithelial cells, in which secretions accumulated before being transferred to the duct. The presence of wall ingrowths in subepithelial cells suggests that granulocrine secretion operates in these species. Secretory ducts varied in density and diameter among the organs in both species, with the combination being maximal in woody stems. (-)HAA was only detected in surface secreted resins of both species, and its concentration (2D-TLC, GC-FID) was intimately associated with the distribution and density of glandular trichomes in each organ (capitula, leaves, and stems with primary or secondary growth). In addition, no (-)HAA was detected internally in the resins collected from secretory ducts. The composition of these resins showed distinctive profiles for FC and FO, and only four from ca. 30 compounds detected (GC/MS) were shared by both species. In addition to the elucidation of ultrastructural traits, distribution and density of secretory structures in aerial organs of FC and FO, present findings suggest a functional role for glandular trichomes in the secretion of the putative phytotoxic allelochemical (-)HAA.

Keywords

***Flourensia campestris*, *Flourensia oolepis*, Glandular Trichomes, Secretory Ducts, Reproductive Organs, Ultrastructure, (-)-Hamanasic Acid A, Resins**

1. Introduction

The Asteraceae are among the largest families of flowering plants with ca. 1700 genera and 23,000 - 26,000 species in all continents except for Antartica [1], and many species are well known for their different and important uses [2]. In Argentina, it is the largest family with ca. 1400 species, 382 being endemics [3]. *Flourensia* DC. (Subfamily Asteroideae, tribe Heliantheae) is endemic from America with 32 species; twelve species live in Argentina and six of them are endemic of the central region: *F. campestris*, *F. hirta*, *F. leptopoda*, *F. niederleinii*, *F. oolepis*, and *F. tortuosa* [4] [5].

Flourensia campestris (FC) and *Flourensia oolepis* (FO) are easily distinguished by the characteristic glossy aspect of their leaves due to resinous exudates of unknown composition, which represent ca. 40% and 20% of the biomass in young and adult leaves, respectively (Silva and Scopel, Personal communication, 2005). Plant resins, typically constituted by mixtures of volatile and non-volatile phenolic and/or terpenoids secondary compounds, are usually secreted in specialized structures located either internally or on the surface of the plant [6]. These resins, according to their constituents, are known to play important ecological functions, ameliorating or preventing damage from abiotic and biotic stresses in their natural environments (high irradiance, UVB, dissecation, herbivory, competition), whose properties have been used for the management of harmful diseases, insects, pests and weeds [7]-[9].

FC has been shown to produce a phytotoxic compound identified as (-)-hamanasic acid A {(-)HAA; 7-carboxy-8-hydroxy-1(2), 12(13)-dien-bisabolene} [10]. This bisabolanoid accumulates at exceptionally high concentrations in some FC organs (e.g., 1.6% of leaf dry weight). There is growing evidence that metabolites that accumulate on plant surfaces are largely produced and secreted by glandular trichomes [11]-[14]. This might be the case of FC, since short dips of fresh and dry leaves and flowers in water or ethyl acetate (EtOAc) showed high concentrations of (-)HAA, accounting for almost 20% - 30% of the total metabolites extracted [10]. In a recent paper, we have demonstrated that its natural occurrence is restricted only to some *Flourensia* species, strictly FC, FO and *F. fiebrigii*, being absent in *F. hirta*, *F. riparia* and *F. niederleinii*, and in 37 of the most representative co-occurring species of FC and FO [15]. The composition of the resins from the genus *Flourensia* has revealed the presence of other phytotoxic compounds, as in *F. cernua* [16] and in FO [17]-[19]. Other resin components in *Flourensia* spp. have also been reported to have insect antifeedant [20]-[22], antifungal [23], antibacterial [24] [25], antialgal and antitermite properties [26], and herbicidal [10] [15] [20], and to act as tyrosinase inhibitors [27]. To our knowledge, the link between resins secretion and secretory structures in the genus

Flourensia has never been addressed before.

In Asteraceae, two secreting systems occur: glandular trichomes at the surface and secretory ducts inside the organs [28]-[30]. Glandular trichome characteristics have been used to classify species within the Asteraceae [31]. Particularly in the tribe Heliantheae, most of the taxa have capitate glandular trichomes that apparently store sesquiterpene lactones [32]-[35], which have been shown to present biological activities and toxic properties. A high density of glandular trichomes, together with the presence of secretory ducts, was revealed by light microscopic observations in leaves and stems of FC and FO [36] [37], and of other *Flourensia* species (*F. hirta*, *F. leptopoda*, *F. nierderleinii* and *F. tortuosa*) [38]. However, data of secretory structures in flowers of *Flourensia*, as well as ultrastructural studies, are not available for the genus, and are still limited in the Asteraceae [29] [39] [40] [41].

In this work, we examined the localization, morphology, density and ultrastructure of secretory structures—in vegetative and reproductive organs—of FC and FO by means of a combination of light, fluorescence, transmission and scanning electron microscopy. We also assessed the possible role of secretory structures in the production and secretion of (-)HAA in FC and FO. The presence and distribution of (-)HAA in different organs were studied by means of 2D-TLC, GC-FID and GC-MS.

2. Materials and Methods

2.1. Plant Material and Study Sites

Plant materials were collected in natural areas corresponding to the Punilla Valley, Córdoba province, Argentina. The study areas were located in shrub communities (total plant cover 70% - 90%), dominated by the evergreen shrubs *Flourensia campestris* Griseb. (FC) and *F. oolepis* S.F. Blake (FO). Specimens of FC were collected in "El Dragón" (La Falda), and FO plants were collected in "Dique El Cajón" (Capilla del Monte), in summer (February) of 2009 and 2010. The voucher specimens (BAA 26.498-FC- and BAA 26.499-FO-) are deposited at the Herbario "Gaspar Xuárez" of the Facultad de Agronomía, Universidad de Buenos Aires, Argentina.

2.2. Structural and Ultrastructural Analyses

2.2.1. Light and Fluorescence Microscopy

For anatomical and morphological studies, cross and longitudinal section samples of fully developed capitula (common receptacle, phyllaries, paleae, and ligulate and tubular flowers), young and mature leaves, and stems from each species were obtained. Three developmental stages were considered in stems: primary growth (PG)-corresponding to the early growth of stems during the growing season; secondary growth (with early phellogen activity (SG_{ph})-corresponding to stems between 3 months to one year old, and woody stems (WS)-corresponding to stems of 2 + years. The three categories were processed separately. The materials for light microscopy (LM) were fixed in FAA and embedded in paraffin [42]. Sections (7 - 10 µm thick) were cut and stained with safranin combined with fast green (**Figure 1A**, **Figure 2A** and **Figure 2B**), with safranin (**Figure 2C** and **Figures 3A-D**),

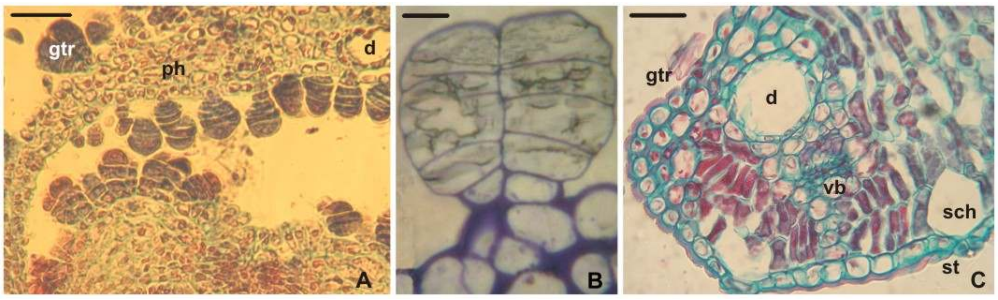

Figure 1. Secretory structures in capitula and leaves of FC and FO (LM). **A** FC, longitudinal section of capitula showing a young phyllary (ph) and the corolla of a tubular flower with glandular trichomes (gtr) and secretory ducts (d); **B** FC, detail of leaf glandular trichome formed by three basal cells, two short peduncle cells and a secretory head composed by six to eight cells; **C** FO, cross section of a leaf showing the epidermis unistrata with thin cuticle (0.97 µm) and a glandular trichome (adaxial surface); the abaxial surface showed large substomatal chamber (sch) and over level stomata (st); secretory schizogenous ducts are commonly found attached to the vascular bundle (vb), and included in the palisade chlorenchyma. Scale bars: **A** 75 µm, **B** 10 µm, and **C** 50 µm.

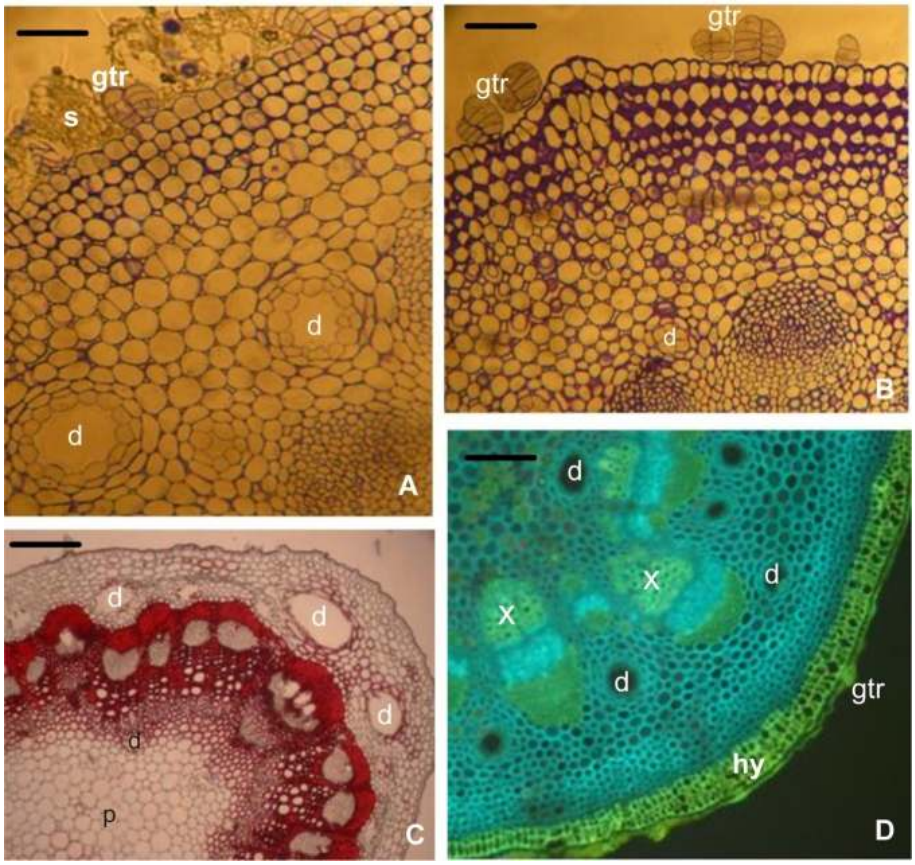

Figure 2. Secretory structures observed in transversal sections of primary stems from FC and FO (LM and FM). **A** FO, young stem showing the epidermis with glandular trichomes (gtr) and their secretions (s), and secretory ducts (d); **B** FC, young stem with glandular trichomes and ducts; **C** FC, initial secondary growth showing conspicuous groups of fibers (stained red) at the protophloem; secretory ducts were found near to the primary xylem (pith = p) and in the cortex; **D** FC, young stem under UV excitation (FM) showing autofluorescence of glandular trichomes, peridermis (hy, 2 - 3 cell layers) and xylem vessels (x); secretory ducts do not autofluoresce (darker green). Scale bars: **A** and **B** 100 µm; **C** and **D** 250 µm.

or with aqueous toluidine blue (**Figures 1B** and **Figures 1C**) [42], and observed with a Wild M20 microscope. For fluorescence microscopy (FM), similar sections of fresh, unstained materials were examined and photographed using ultraviolet and blue light excitation with a Universal Microscope Axioplan Zeiss. Images were recorded by a digital camera Canon PowerShot A650 IS.

Quantitative Analysis

Density of glandular trichomes (number of secretory structures per tissue surface unit area) in aerial organs was measured in fresh material previously submerged in EtOAc for 20 s in order to remove surface-secreted compounds. Epidermal layers were manually removed, mounted in 50% glycerine, and trichomes were counted under LM, using a digital videomicroscopic camera with the corresponding software (MotiCam 1.3 MP). Density and diameter of secretory ducts were measured in cross sections of common receptacle and stems (PG, WS) while ducts length was measured in longitudinal sections of tissues.

Quantitative density analysis of described parameters was performed in 5 different individuals, in 5 different samples of 1 mm^2 from each one. Diameter and length of the ducts were measured in 5 different individuals, in 5 different secretory ducts from each one.

2.2.2. Transmission Electron Microscopy (TEM)

For TEM studies, plant materials were pre-fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.2) overnight and then post-fixed in OsO_4 at 2˚C in the same buffer for 3 h. Following dehydration in a graded ethanol series,

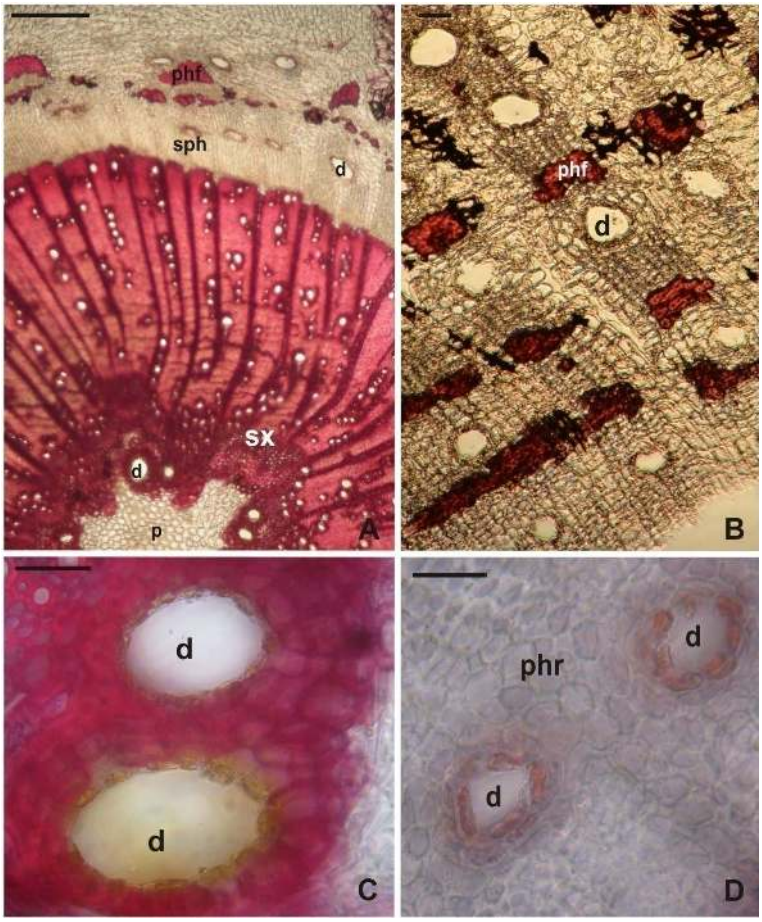

Figure 3. Secretory structures observed in transversal sections of secondary stems of FC and FO (LM). **A** FO, secondary stem showing secretory ducts (d) in the cortex near the protophloem fibers (phf) (stained red); the most internal ducts found in the secondary phloem (sph) are products of the cambial activity; pith (p), secondary xylem (SX); **B** Secondary phloem with the presence of fibers (phf) and ducts; **C** secretory ducts in the pith, attached to the protoxylem with abundant fibers; yellowish resin was found closed to the epithelial cells; **D** Two young secretory ducts included in the secondary phloem, and close to the phloematic rays (phr). Scale bars: **A** 150 µm, **B** 25 µm, **C** 75 µm, and **D** 35 µm.

the material was embedded in Spurr's resin. Thin sections (1 µm) were made with a Reichert-Jung ultramicrotome and stained with toluidine blue for observation with light microscope. Ultrathin sections (750 - 900 nm) were made with the same ultramicrotome and then stained with uranyl acetate and lead citrate [43]. These sections were observed and photographed with a JEOL 1200 EX II.

2.2.3. Scanning Electron Microscopy (SEM)

Plant materials were fixed with FAA (2.2.1), dehydrated in a graded ethanol series and coated with a thin layer of gold. Observations were carried out on a Zeiss scanning electron microscope at 15 kV.

2.3. Phytochemical Analyses

Based on the results of type, distribution and density of secretory structures in the plant organs studied (capitula, leaves and stems), different extraction methods were performed in order to investigate the possible role of the two different secretory structures in (-)HAA storage and secretion.

The presence and content of (-)HAA in FC and FO was assessed in whole tissues, on the tissues surface (surface secreted resins), and in the resins collected directly from secretory ducts in pooled materials harvested from 5 different individuals.

2.3.1. Tissue Extractions

Whole Tissue: For whole organs, tissue extracts were obtained from grinded fresh material (50 mg), extracted twice using EtOAc (1.5 mL and 0.5 mL), vortexed for 2 min and centrifuged (10 min, 3000 g). The EtOAc phases were pooled, dried at 30˚C under N_2 flow and weighed [10].

Surface Secreted Resins: Fresh organs were washed with gentle agitation in cold EtOAc during 20 s (short dips) [10] [13] [14]. Special care was taken for dipping intact organs in order to avoid contamination from endogenous sources. This procedure did not damage the surface of the tissues as revealed by LM inspection.

Duct Resins: Resins produced in secretory ducts were collected from fresh stems of FC and FO, after peeling the external tissues and allowing the products to exude for ca. 10 min at ambient temperature. Resins were picked up using a micro syringe, weighed, and stored at −20˚C until analysis.

2.3.2. (-)HAA Analysis

(-)HAA was identified and measured by means of GC-FID and 2D-TLC [10] [15], yielding comparable results. For the 2D-TLC, (-)HAA concentration was determined by digital analysis (ImageJ http://rsb.info.nih.gov/ij/index.html). The integrated area (relative density) of a spot corresponding to (-)HAA was compared to that of a standard (-)HAA (purified from plant tissue, not commercially available). The relation between the concentration of the standard (-)HAA and the integrated areas of the spots (relative density values) proved to be linear between 0.5 $mg \cdot mL^{-1}$ and 2 $mg \cdot mL^{-1}$ ($r^2 = 0.94$). Inter batch spot (I_2) variations were minimized by loading the same volume of sample (3 µL) against a fixed concentration of standard (-)HAA (1 $mg \cdot mL^{-1}$), and running the 2D-TLC as already described by Silva *et al*. [10]. Means from triplicates were expressed as mg (-)HAA per g dry weight ($mg \cdot g^{-1}$ DW) in the case of whole tissue extractions, and as mg (-)HAA per 100 mg of total surface secreted or duct resins (mg % w/w).

(-)HAA was also investigated in duct resins by GC-MS on a Perkin Elmer Clarus 600, and TurboMass 5.4.2 for data acquisition. The GC column was DB5 (60 m, 0.25 mm ID, 0.25 µm particle size) (Perkin Elmer) and the carrier gas used was He (49.6 psi). The initial temperature of 60˚C was gradually increased after 2 min to 300˚C by a ramp of $5^{\circ}C \cdot min^{-1}$ and held for 10 min at 300˚C. The injector was used in split mode ($20\ mL \cdot min^{-1}$) with the inlet temperature set to 250˚C. Samples were diluted in acetone or in EtOAc as follows: in 100 µL of solvent and 1/50 µL of solvent, the injection volume was 1 µL. Some peaks were identified by comparison of mass spectra using NIST MS Search 2.0., and using previous own data from volatiles in the same species [10]. Pure (-)HAA in this system showed a retention time of 23.5 min and m/z (%): 234 (12)[M^+H_2O], 219 (2), 191 (6), 164 (3), 149 (14), 123 (9), 109 (100), 105 (9), 79 (22), 69 (65), 55 (20), 43 (35), 39 (6).

2.4. Data Analysis

One way ANOVA or Mann-Whitney nonparametric test for the significance of the difference between the distributions of independent samples for ordinal data were applied.

3. Results

3.1. Structural and Ultrastructural Analyses

3.1.1. Light and Fluorescence Microscopy

Secretory structures in the capitula of both species were composed by glandular trichomes and secretory ducts (**Figure 1A**), similar to those observed in leaves (**Figures 1B** and **Figures 1C**), and stems (**Figure 2** and **Figure 3**). The capitates glandular trichomes are biseriate with 6 - 8 head celled, a two celled short-stalk (with cutinized lateral walls) and three basal cells (**Figure 1B**). No significant differences in the density and distribution of glandular trichomes between the capitula of both species could be found (**Table 1**). As shown in **Figure 1A**, glandular trichomes are very abundant in paleae and phyllaries primordia. In fully developed structures, the density of glandular trichomes in the capitula was higher in the paleae, followed by the phyllaries, tubular and ligulate flowers (ca. 2.5× higher in paleae cf. to ligulate flowers, **Table 1**). In the bracteae, most of the glandular trichomes were found along the edges and veins, while in the corollas of ligulate and tubular flowers ca. 80% of the trichomes were located in the distal portion of these structures and associated to veins. For both species, the density of trichomes in capitula was significantly smaller than that found in leaves and stems (**Table 1**). No other type of glandular trichomes was detected in the capitula from both species.

Table 1. Density of glandular trichomes in capitula, leaves and stems of FC and FO.

Species	Bracteae		Flowers		Leaves		Stems		
	Phyllaries	Paleae	Corolla of ligulate flowers	Corolla of tubular flowers	Adaxial surface	Abaxial surface	PG	SG_{ph}	WS
FC	25 ± 2**e**	40 ± 4**d**	14 ± 1**f**	26 ± 2**e**	123 ± 8**b**	93 ± 5**c**	174 ± 12**a**	162 ±12**a**	ND
FO	31 ± 4**d**	45 ± 4**d**	19 ± 1**e**	20 ± 2**e**	95 ± 3**c***	85 ± 4**c**	656 ± 22**a***	344 ± 16**b***	ND

Data (media ± SE, n = 25) represent the number of glandular trichomes per mm^2. PG: stems with primary growth; SG_{ph}: stems with secondary growth and incipient phellogen activity; WS: woody stems; Letters (a - f) indicate significant differences among tissues; asterisks (*) show differences between species ($p < 0.05$); ND: not detected.

In leaves, glandular trichome density on the adaxial surfaces of FC was higher than in FO (**Table 1**). Stems (PG and SG_{ph}) in both species, showed significantly higher glandular trichome densities than the other organs; between 1.5 (FC) and 7.3 (FO) times higher than those found in the leaves and capitula (**Table 1**). Densities of glandular trichomes in PG and SG_{ph} stems were significantly higher in FO as compared to FC (**Table 1**). Glandular trichomes were absent in WS of both species (**Table 1**).

Secretory ducts were observed in all the floral pieces of both species and were similar in shape to those observed in the cross section of the common receptacle (data not shown). Density and diameter of secretory ducts present in the common receptacle were significantly different in FC as compared to FO (**Table 2**). Density was 4× smaller in FC than in FO, while diameter was ca. 1.7× larger in FC relative to FO (**Table 2**). In stems, the distribution and localization of secretory ducts in both species are shown and described in **Figure 2** and **Figure 3**. The density of secretory ducts showed significant differences between developmental stages in both species; the smaller values corresponded to stems with primary growth (PG), and the larger to stems with secondary growth (WS) (**Table 2**, **Figure 2** and **Figure 3**). Duct diameters followed a similar pattern, spanning from 17 µm to 165 µm. Ducts in stems with SG (cortex and secondary phloem) were significantly larger than those in PG stems; the highest values corresponded to FC (**Table 2**). In the longitudinal plane, these ducts could be seen as elongated spaces, between 225 µm to 1237 µm long -for older ducts located in the cortex-, and 50 µm to 275 µm for ducts present in the secondary phloem. Estimations based on the diameters and densities of FC ducts in SG stems at both phases indicate that at transversal sections ducts occupy between 0.025% (pith) and 2.4% (cortex) of the surface. Microscopical inspections of the ducts performed since early stages of development suggested a schizogenous origin (**Figure 2B** and **Figure 2C**), partially disintegrated cells being never observed in the periphery of the space. The presence of an epithelium surrounding the duct confirmed the schizogenous origin.

3.1.2. Transmission and Scanning Electron Microscopy

There were no ultrastructural differences between the glandular trichomes of FC and FO, with the exception of a broken cuticle commonly observed in FC. The secretory cells of the trichome head have a dense cytoplasm and small vacuoles (**Figure 4**). The outer tangential cell wall of these cells has two layers, the inner with a more electron-dense fibrillar structure than the external translucent structure pecto-cellulosic layer (**Figure 4A** and **Figure 4B**). This wall is coated by a detached cuticle that creates a subcuticular chamber (**Figures 4A-C**). The cytoplasm shows the presence of abundant mitochondria (**Figure 4A**), dictyosomes (**Figure 4E**), endoplasmic reticulum (**Figure 4F**), chloroplasts with numerous starch grains (**Figure 4G**), and leucoplasts (not shown). The lipophilic vacuoles have an elaborated system of inner membranes and numerous lipidic globules (**Figure 4F**). The dictyosomic vesicles are abundant; some are observed in contact with the plasmalemma (**Figure 4B**). In active glands, the outer tangential wall of the head cells presents cavities filled with substances of moderate to high electron-density (**Figure 4C** and **Figure 4D**). Substances with the same electron-density are also observed in the subcuticular chamber where secretions accumulate, giving the trichome head a spherical shape. Scanning electron images of the abaxial surface of mature leaves of FC (**Figure 5A**) and FO (**Figure 5B**) show glandular and non-glandular trichomes on the veins. A detailed inspection of the images in FC allowed to observe the presence of glandular trichomes with a ruptured or completely removed cuticle (**Figure 5D** and **Figure 5E**). In contrast, no pores or ruptures could be observed in FO (**Figure 5C**). These observations, together with the TEM images described (**Figure 4**), suggest that each species would differ in the way they release the products that accumulate in the head cells. In FC, the products would accumulate temporarily in the interfibrillar spaces of the cell wall, and transferred to the subcuticular chamber where they would accumulate until the cuticle fully distends

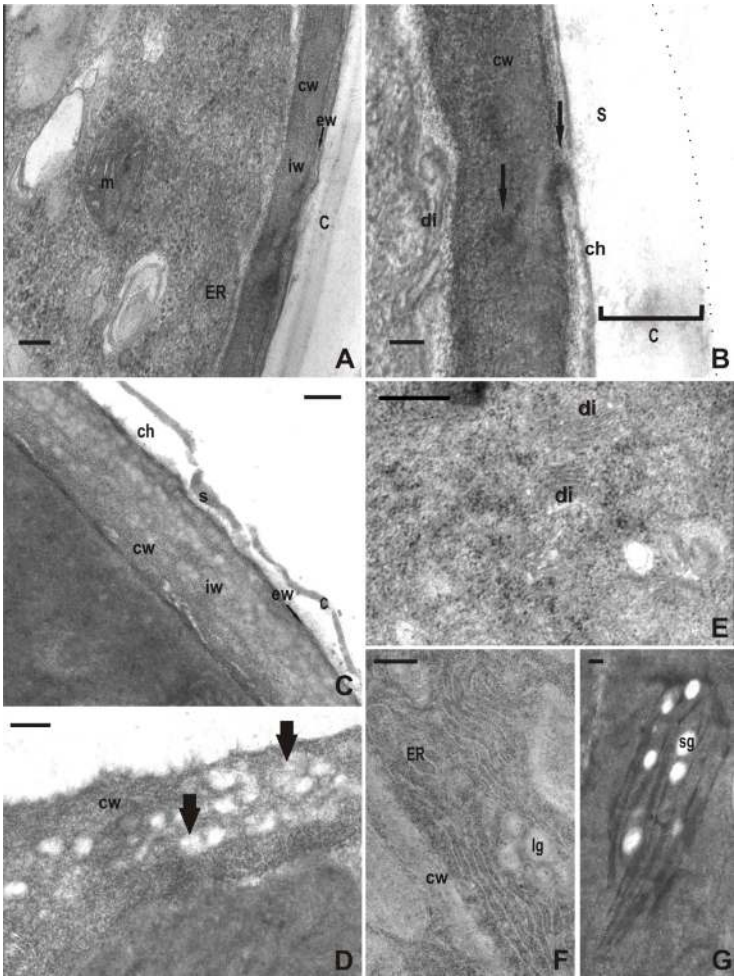

Figure 4. Ultrastructure (TEM) of glandular trichome cells in FC and FO. **A** FO, secretory cell, outer tangencial cell wall (cw) showing an inner layer with more electron-dense fibrillar structure (iw) and an external layer (ew) with a thick loose and detached cuticle (c) an electron-dense cytoplasm content, presence of mitochondria (m) and endoplasmic reticulum (ER); **B** Detail of previous micrograph, the arrows indicates a possible pathway of the secreted substances (s) across the cell wall and their accumulation in the subcuticular chamber (ch); dotted line indicates the external limit of the cuticle; **C** FC, secretory cell wall with interfibrillar spaces filled with secretions; secretions should be released through a ruptured cuticle; **D** FC, arrows indicate lipophilic substances in the interfibrillar spaces of the cell wall; **E** FC, secretory cell showing dense cytoplasm with dictyosomes (d); **F** FO, a view of the cell content with endoplasmic reticulum (ER) attached to the cell wall, and lipid globules (lg); **G** FC, detail of a chloroplast in the secretory cell with starch grains (sg). Scale bars: **A** 0.3; **B**, **C**, **D** and **F** 0.1 µm; **E** 0.2 µm; **G** 0.5 µm.

Table 2. Density and diameter of secretory ducts in capitula and stems of FC and FO.

		Capitula	Stems			
		Common receptacle	PG		WS	
			Cortex	Pith	Cortex	Secondary phloem
FC	Density (N°/mm^2)	8.8 ± 1.3**a***	13.2 ± 1.2**a**	5.9 ± 0.9**b**	13.8 ± 0.8**a**	57.1 ± 4.6**c**
	Diameter (µm)	131.4 ± 12.1**a***	39.5 ± 2.9**b**	25.6 ± 1.3**c**	165.5 ± 18.6**a***	58.9 ± 9.5**b**
FO	Density (N°/mm^2)	34.7 ± 2.2**a**	15.5 ± 0.4**b**	5.5 ± 0.3**c**	12.2 ± 1.0**b**	45.2 ± 2**a**
	Diameter (µm)	78.6 ± 1.6**a**	31.3 ± 2.8**b**	17.5 ± 1.8**c**	89.1 ± 7.2**a**	53.9 ± 3.1**d**

Data are expressed as mean ± SE, n = 25. PG: stems with primary growth; WS: woody stems. Letters (a - d) indicate significant differences among tissues; asterisks (*) show differences between species ($p < 0.05$).

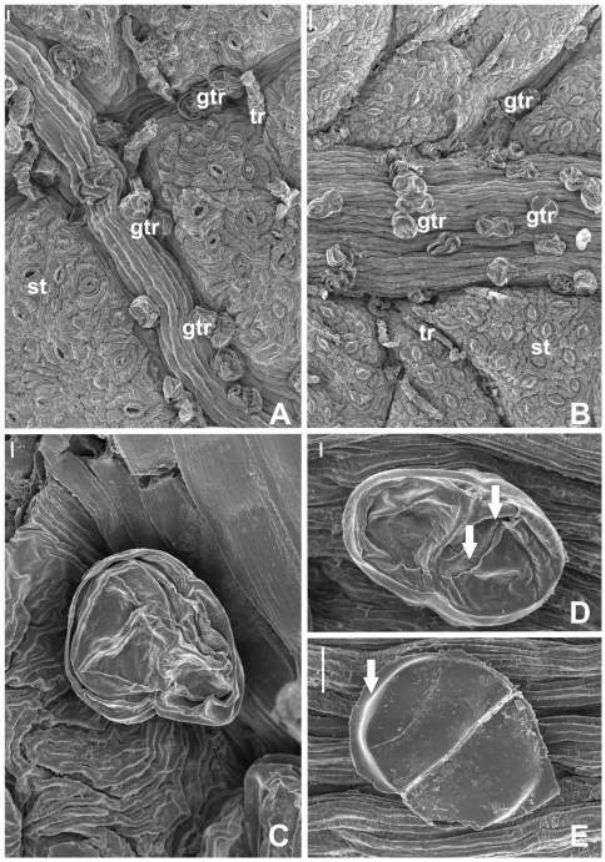

Figure 5. Scanning electron micrographs of leaf glandular trichomes in FC and FO. **A**-**B** FC and FO, respectively, abaxial epidermis showing the distribution of glandular (gtr) and e-glandular trichomes (tr) mostly on the main vein, and over-level stomata (st); **C** FO, head of a glandular trichome, no pores were observed; **D** FC, ruptured cuticle (arrows) in the head of a glandular trichome; **E** FC, glandular head with a totally removed cuticle (arrow). Upper left corner white vertical scale bars: **A** 15 µm; **B** 30 µm; **C** and **D** 3 µm; **E** 10 µm.

and ultimately breaks releasing the products (**Figure 4C**, **Figure 4D**, **Figure 5D** and **Figure 5E**). In contrast, in FO, the secretions of the accumulated products seem to be released through a loose cuticle, without showing any pores or cracks (**Figure 4A**, **Figure 4B** and **Figure 5C**).

In secretory ducts, the lumen is surrounded by a layer of specialized cells, the epithelium (**Figure 6A**). These cells have thin walls, conspicuous nucleus, vacuoles, abundant mitochondria, some plastids, rough endoplasmic reticulum and free ribosomes (**Figure 6A** and **Figure 6B**). The vacuoles have varied contents; some of them show an electron-dense material similar to lipid globules (**Figure 6A**). The plasmalemma is slightly invaginated and a fibrillar material is observed between it and the cell wall. This characteristic is more evident in the sub-epithelial cells, which have numerous plastids (including chloroplasts) and big vacuoles with contents that present moderate electron-density and some inclusions (**Figure 6A**).

3.2. Phytochemical Analyses

Whole tissue concentrations of (-)HAA in all aerial organs of FO were significantly lower than those originally reported in FC (**Table 3**); 14× to ca. 20× lower in capitula and leaves, respectively, and 2× lower in PG stems. (-)HAA distribution among organs differed between species (**Table 3**). While in FC the higher levels of (-)HAA were detected in capitula and leaves, in FO the highest concentration corresponded to stems with PG (**Table 3**). In whole stems (-)HAA content showed an age-dependent distribution, with highest levels being present in the young stems (PG) followed by SG_{ph}, and by negligible levels of (-)HAA detected in WS (**Table 3**).

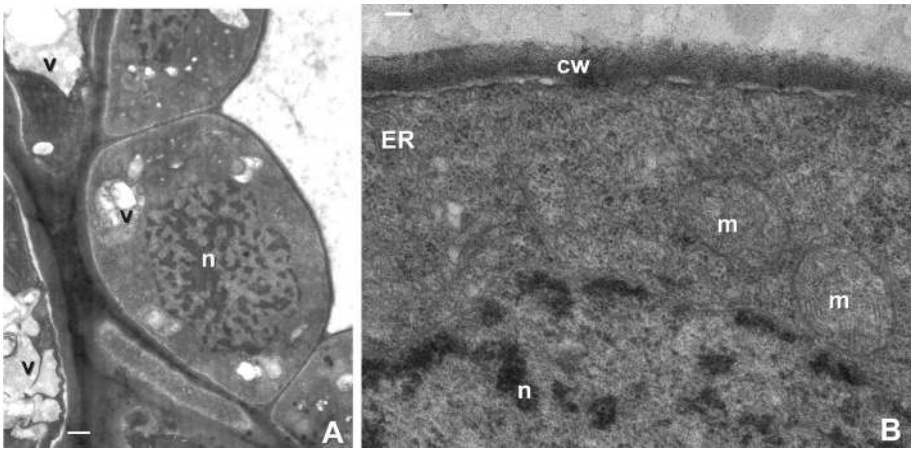

Figure 6. Ultrastructure (TEM) of leaf secretory duct cells in FC and FO. **A** FC, epithelial cells from ducts showing vacuoles (v) and a conspicuous nucleus (n), and sub-epithelial cells showing wall ingrowths and vacuoles; **B** FO, duct epithelial cell showing the nucleus, abundant mitochondria (m), and granular endoplasmic reticulum (ER); secretions (resins) are released across the cell wall (cw) into the duct lumen. Lower **A** and upper **B** left corner white scale bars: **A** 2 µm; **B** 0.2 µm.

Table 3. Content of (-)HAA in whole tissues and resins of FC as compared to FO.

	(-)HAA					
	FC			FO		
	Whole tissue mg·g^{-1} DW	Surf. secreted resins mg %	Duct resins mg %	Whole tissue mg·g^{-1} DW	Surf. secreted resins mg %	Duct resins mg %
Capitula	17.0 ± 0.6**a***	21.0 ± 0.6**b***	NA	1.2 ± 0.1**c**	1.02 ± 0.03**b**	NA
Leaves	17.9 ± 0.1**a***	26.6 ± 0.7**a***	NA	0.90 ± 0.01**c**	0.76 ± 0.02**c**	NA
Stems						
PG	10.5 ± 0.5**b***	21.4 ± 0.6b*	ND	5.3 ± 0.2**a**	6.45 ± 0.19**a**	ND
SG$_{ph}$	4.9 ± 0.2**c***	17.3 ± 0.3**c***	ND	1.8 ± 0.1**b**	1.20 ± 0.03**b**	ND
WS	0.60± .03**d***	2.0 ± 0.1**d***	ND	0.30 ± 0.02**d**	0.65 ± 0.02**d**	ND

(-)HAA levels in whole tissues of FC and FO (mean ± SE; N = 3). Values in FC (Silva *et al*. 2012) are shown for comparison purposes. NA: not applicable; ND: not detected; PG: primary growth; SG$_{ph}$: secondary growth and incipient phellogen activity (last season); WS: woody stems (2 + yrs). Letters (a - d) indicate significant differences among tissues; asterisks (*) show differences between species ($p < 0.05$).

Surface secreted resins obtained from short dip extractions with EtOAc revealed the presence of (-)HAA in all aerial organs; concentrations being within the range of those found in whole tissue (**Table 3**). (-)HAA in whole tissues expressed as relative concentrations (mg % w/w) of the total resins extracted, tended to yield lower values than those found in surface secreted resins (data not shown), as could be observed in the 2D-TLC profiles (**Figure 7A**, PG stems). These differences may be accounted for a dilution effect in whole tissues, since the extractions included both, surface secreted and internal compounds (including duct resins). The presence of (-)HAA among the surface resins of the organs was also evidenced by GC-MS (**Figure 7C**, inset, PG stems). Chromatographic profiles of surface secreted resins differed between species, and slight differences were also observable among organs in each species (data not shown).

In duct resins collected from fresh stems of both species, (-)HAA was not detected by means of 2D-TLC or GC-MS (FC: **Figure 7B** and **Figure 7C**, respectively); confirming that (-)HAA only pertains to surface secreted resins.

The 2D-TLC chromatographic profiles of whole tissue of PG stems also revealed the presence of a fast chromatographic fraction represented by less polar compounds relative to (-)HAA, which was also detected in duct produced resins (cf. **Figure 7A**—right vs. **Figure 7B**—left). GC-MS analysis of stem duct resins of FC and FO

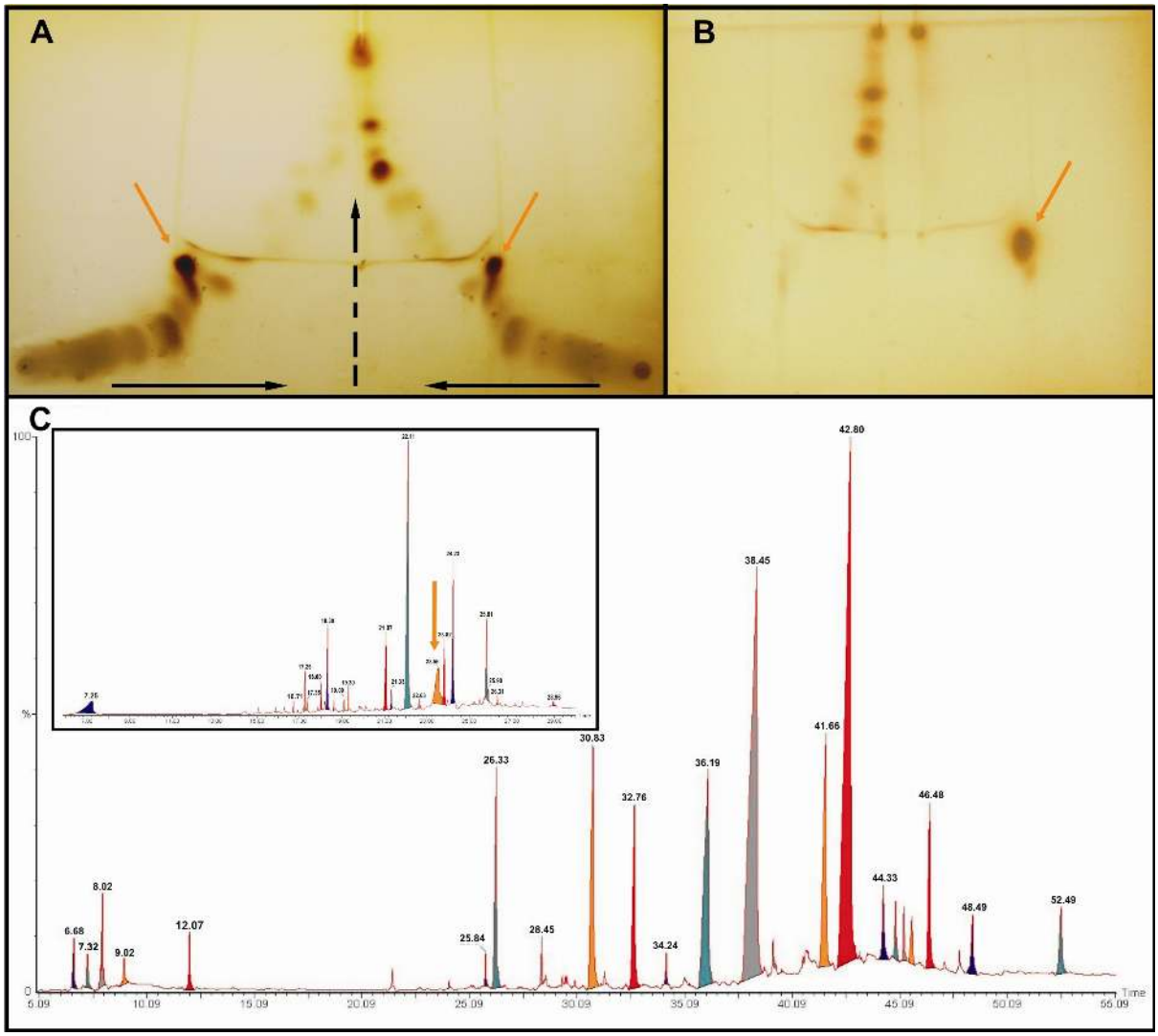

Figure 7. Identification of (-)HAA in FC young stems. **A-B** 2D-TLC, long broken and whole arrows indicate the first and second dimensional runs, respectively. **A** (-)HAA (small arrows) in surface resins (left) vs. whole tissue extract (right); additional compounds from the internal tissue can be observed in the latter; **B** Duct resins (left) vs. pure (-)HAA (right, small arrow); duct resins show no (-)HAA and the same compound profile observed in whole tissue extract; **C** GC of surface resins showing the presence of (-)HAA (small arrow) inset, compared to GC of duct resins showing a distinctive profile in which (-)HAA is absent.

showed distinctive profiles. From a total of 30 compounds found in each species only four (13%) were present in both; the volatiles santolinatriene and spathulenol could be identified by this analysis. Remarkably only 7 of these metabolites accounted for 75% (FC) and 90% (FO) of the total duct resins composition.

4. Discussion

Results showed that both species present two types of secretory structures: glandular trichomes-capitate wit3 h a biseriate-pluricelular head, and secretory ducts, confirming the observations of Delbón *et al*. [36] [37]. Biseriate glandular trichomes are typically found in Asteraceae, in different tribes and genera, such as *Ambrosia trifida* [44], *Helianthus annuus* [45] [46], *Sigesbeckia jorullensis* [41], *Grindelia pulchella*/Heliantheae [29], *Centrapalus pauciflorus* and *Chrysolaena* spp./Vernonieae [28] [47], respectively, *Helichrysum* [48] and *Inula*/Inuleae [49], *Artemisia annua* [50] and *Tanacetum cinerariifolium*/Anthemideae [51].

Present observations demonstrate, for the first time in the genus *Flourensia*, the existence of glandular tri-

chomes in reproductive organs. These were morphologically identical to those found in the vegetative organs of both species studied, and were also similar to those described in the vegetative organs of other species of the genus, like *F. hirta*, *F. tortuosa*, *F. niederleinii* and *F. leptopoda* [38]. Observations of *Plectranthus ornatus* (Lamiaceae) reproductive organs revealed the presence of an unusual kind of glandular trichome in respect to those found in vegetative organs [52]. In several species belonging to subfamily Lamioideae (Labiatae) a new type of capitate trichome, exclusive of calices and corollas, was described [53]. This are not the case for the species of *Flourensia* studied, and may constitute an important trait for the taxonomy in the genus. Similar studies in the reproductive organs of other species from the genus are needed to confirm these findings.

Despite the density of glandular trichomes found in capitula was lower than that found in leaves, and much lower than that found in stems, these trichomes may still play relevant ecophysiological functions in the reproductive organs. In this sense, secretions of glandular trichomes in floral structures have been shown to act as pollinator attractants and/or to be involved in the chemical defense against herbivore insects and microbes [52] [54].

The densities of glandular trichomes in the leaves of both species were very similar to those previously reported by Delbón *et al*. [36], while their densities in stems were never reported before. In contrast to reproductive organs, glandular trichomes in the vegetative organs of FC and FO exhibited a different pattern of distribution. While in FC leaves and stems with PG and SG_{ph} showed very similar densities, in FO, the higher densities were found in stems. Moreover, trichomes density in FO stems was between ca. 2× (SG_{ph}) to 4× (PG) higher than those found in FC. The observed striking difference, in addition to their differences in gland secretion compositions as observed in the present, may be involved in These unexpected results between two species that do not share populations [4], pose the question of whether the increased number of trichomes may be involved in organ-specific functions that may be relevant for their corresponding adaptations through habitat specialization [6]. The ultrastructure of the head cells of glandular trichomes revealed components typically found in cellular structures that produce and secrete metabolites [29] [55], although some glandular trichomes in Asteraceae only present smooth endoplasmic reticulum [28]. More specifically, the presence of abundant chloroplasts with visible starch grains in FC and FO is indicative of their functionality. Starch grains have been identified in leucoplasts and chloroplasts in the head cells of mature glandular trichomes of *Stevia rebaudiana* (Asteraceae) [56] [57].

Detailed observations of SEM and TEM images suggest that each species would differ in the way they release the secretions to the plant surface. In FC, cuticle rupture was commonly observed. The products would accumulate temporarily in the interfibrillar spaces of the cell wall, and would be transferred and stored in the subcuticular chamber until the cuticle fully distends and ultimately breaks releasing the products. In FO, instead, cuticle rupture or pore formation was not observed by SEM in any of the materials inspected. In this species, the secreted products seem to be released to the plant surface through a loose cuticle.

Storage of secretions in the subcuticular space and cuticle rupture has been reported for other Asteraceae species [28] [29] [41] [48] [49] [58]. On the other hand, Fahn [55] reports that secretion by the apical head cells continue as long as the cells on the peduncle remains functional, which might be the case for FO.

A growing body of experimental evidence shows that terpene biosynthesis takes place within glandular trichomes [11] [12] [59]-[63]. As an example, transcriptome analysis of *Artemisia annua* glandular trichomes revealed the presence of contigs corresponding to multiple enzymes for terpenoid and flavonoid biosynthesis suggesting their important metabolic activity [64]. In the tribe Heliantheae, most of the studied species seems to accumulate sesquiterpene lactones in their glandular trichomes [65]. The observations of FC and FO under UV revealed a green-yellow autoflorescence inside the trichomes and on the surface of the cuticle where the secretions are released, typical of phenilpropanoids and terpenoids as those found in *Lippias caberrima* (Verbenaceae) [66]. Our results show that the sesquiterpenoid (-)HAA concentration was positively associated with the distribution of glandular trichomes in the different organs of both species. In FC, the higher densities of glandular trichomes were found in capitula and leaves, followed by stems with PG, and (-)HAA concentration closely resembled this pattern (cf. FC, **Table 1** and **Table 3**). This was also the case for FO, in which the highest density of glandular trichomes in stems with PG corresponds to the highest levels of (-)HAA detected (cf. FO, **Table 1** and **Table 3**). Although glandular trichomes are still very conspicuous in stems with SG_{ph}, the lower concentration of (-)HAA may result from a lower number of functional trichomes. A developmental stage-specific expression in glandular trichomes has been reported in other Asteraceae as *Helianthus annuus* [46] and *Tenacetum parthenium* [12]. In *Artemisia annua*, trichome collapse has been proposed for the decrease in artemisinin con-

tent during later stages of leaf development [67]. It is worthwhile to note that concentration of (-)HAA was always higher in FC than in FO, regardless of the organ and trichomes density. This might be due to a differential composition of the metabolites produced by the trichomes in each species, in which (-)HAA may not be the main compound in FO.

Differences in the chemical composition of glandular trichomes, even at within-plant level, have also been reported. In this sense, Appezzato-da-Glória *et al*. [28] found that glandular trichomes from underground organs (rhizophorous) of *Chrysolaena platensis* and *C. obovata* (Asteraceae) produced a higher diversity of compounds compared to those from the leaves. In the present work, slight differences in surface secreted resins profiles among the organs of FC and FO were found (data not shown). This variation in the mixture of chemical compounds may play a central role in ecological interactions of resins as already reviewed [6].

The mild dipping extraction method of intact organs, which did not damage the epidermis or external tissues, proved that (-)HAA is only present in the surface secretions found in glandular trichomes bearing organs. The presence of small amounts of (-)HAA on the surface of WS may derive from the product being lixiviated by rain from the younger portions of the branch. The fact that (-)HAA is easily leached out by water [10] supports this notion.

In order to further investigate the possible role of secretory structures specifically related to the storage and secretion of (-)HAA, we compared the concentrations of (-)HAA found internally (secretory ducts) with those found on the surface of the plant (glandular trichomes). In organs such as leaves and capitula, in which resins from internal tissue were very difficult to obtain without surface contamination, whole tissue extractions were compared against surface resins. Results from 2D-TLC and GC-MS (as shown in **Figure 7**) demonstrate that (-)HAA is more concentrated in the surface resins of glandular trichome-bearing organs. The absence of (-)HAA inside organs like stems, where resins could be collected internally, demonstrate that this sesquiterpenoid is not produced by the secretory ducts. These facts indicate that (-)HAA is not translocated from the interior to the plant surface, but strongly suggest that it is synthesized, stored and secreted by glandular trichomes.

Based on the above, we propose that glandular trichomes in FC and FO are the secretory structures involved in the production of this putative phytotoxic allelochemical. These results are also consistent with those originally described by Hashidoko *et al*. [68], where he found precursors of the (-)HAA isomer ((+)HAA) in the glandular trichomes of *Rosa rugosa*.

In respect to secretory ducts, the importance of resin production in Asteraceae has been long recognized by Carlquist [69] in an extensive revision of the wood anatomy of Asteraceae. Robinson [70] used morphological characteristics and resin composition of secretory ducts in phylogenetic studies to define the subtribes of the tribe Heliantheae, including the genus *Flourensia* in the subtribe Ecliptinae. The morphology and distribution of secretory ducts in vegetative tissues (leaves and stems) of FC and FO coincided with those already described by Delbón *et al*. [37], and were similar to the ones found in reproductive organs. Using LM inspections, Delbón *et al*. [37] classified these ducts as shcizogenous. The presence of conserved epithelial cells, with no damaged membranes, and a well-defined clear lumen, as well as the observations at early stages of development strongly suggest the schizogenous origin of the ducts.

Albeit the importance and widespread existence of secretory ducts in woody shrubs of arid environments, morphometric data is rarely presented. In both *Flourensia* species, the highest density of secretory ducts was observed in stems with SG, specifically in the secondary phloem, while the larger diameters corresponded to ducts in the cortex of SG stems. Estimations based on the diameters and densities of ducts indicate that ducts in SG stems may represent as much as 2.6% per unit of volume (1 μm^3), emphasizing the importance of this secretory system in FC and FO. In two varieties of *Senecio filaginoides* (Asteraceae), Feijóo *et al*. [71] reported higher density and diameter of secretory ducts in PG stems, relative to leaves. Densities (4 - 10 mm^{-2}) were similar to those found in PG stems of FC and FO, while diameters were larger (47 µm to 203 µm, spring and winter, respectively) in *S. filaginoides* compared to those found in *Flourensia.* Diameter of secretory ducts is the most important trait that determines resin yield in conifers [72], and also in *Boswellia papyrifera* (Burseraceae), a resinous tree native to Ethiopia [73]. In the latter, average diameter of axial resin ducts (113 µm) was similar to those found in FC and FO in SG stems; a significant trade-off between density and average diameter of secretory ducts was also found in *B. papyrifera*.

In FC and FO, the localization of the ducts in the secondary phloem, close to the cork, would facilitate the secretion of products to the surface of stems through the lenticels or cracks (by the new phellogen activity near the surface). In fact, in plants growing in their natural environment, stems usually present sticky depositions (bulk

resins) that have been secreted to the surface as resin drops of different colors (yellowish to reddish) and viscosity, probably due to different degrees of oxidation.

The ultrastructural analysis of secretory ducts of FC and FO revealed the presence of chloroplasts with starch accumulation in the epithelial and subepithelial cells. The presence of abundant lipid globules in the dense cytoplasm strongly suggests their involvement in the synthesis of resins. Wall ingrowths, typically associated with transfer processes [74], were observed in the epithelial and subepithelial cells of secretory ducts in both *Flourensia* species. According to Herrero and Dickinson [75], the cell wall ingrowths associated to the plasmodesmata assures a great efficiency in the cellular exchange. Bartoli *et al*. [29] also described the presence of a special tissue associated to secretory ducts in *Grindelia pulchella* (Asteraceae).

Preliminary results from GC-MS analysis of resins collected directly from stem ducts of FC and FO showed very distinct profiles for each species, and no (-)HAA was detected as discussed above. Only some monoterpenic and sesquiterpenic volatile compounds already reported (FC: [10]; FO: [22]) could be recognized (data not shown). Despite the matching of most compounds with those from GC-MS libraries was generally higher than 75%, their complete identification was not possible and is under research. In their natural environment these compounds would serve as defense weapons against biotic stressors such as microbes and insects. The complete characterization of the copious resins present in the different organs deserves further investigation in order to link composition to biological activities in nature, and to explore potential sources for new biopesticides and other industrial applications.

5. Conclusions

Reproductive organs of FC and FO presented the same type of secretory structures (glandular trichomes and secretory ducts) described in vegetative organs. Our observations demonstrate, for the first time in the genus *Flourensia*, the presence of glandular trichomes in reproductive organs. These capitate glands were morphologically identical to those found in vegetative organs of all *Flourensia* species studied so far, and might constitute an important trait for the taxonomy of the genus.

Glandular trichome's density was very similar in reproductive organs of both species. In contrast, densities of glandular trichomes in vegetative organs were significantly different between the two species; the highest density corresponded to FO stems.

Secretory ducts varied in density and diameter among the organs of both species, with the combination being maximal in stems with SG. TEM and SEM observations of glandular trichomes suggested that each species differed in the way in which secretory materials were released to the outside: through cracks or pores in FC, or through a loose cuticle in FO. In secretory ducts, the localization of lipophilic vacuoles and the presence of wall ingrowths in subepithelial cells suggest the existence of a granulocrine mechanism of secretion. Similar inspections performed since early stages of ducts development revealed a schizogenous origin.

Our studies are the first to report the distribution of the sesquiterpenoid (-)HAA in different organs of FO. Concentration of (-)HAA in all organs was significantly lower than in FC, as measured in whole tissues, with the highest contents being present in stems with primary growth. In both species, (-)HAA was only detected in surface secreted resins from those organs where glandular trichomes were also present (capitula, leaves and stems). The concentration of (-)HAA was closely and positively related to the density of the glandular trichomes. In addition, no (-)HAA was detected in the resins collected from secretory ducts. These findings strongly suggest that glandular trichomes are the sites of accumulation and secretion of (-)HAA. Biochemical investigations on the biosynthetic capacity of isolated glandular trichomes to produce (-)HAA are under way. Preliminary data on resins composition—from resins extracted directly from secretory ducts in stems— show distinctive profiles for FC and FO; only 13% of the compounds detected are shared by both species.

In addition to the elucidation of ultrastructural traits, distribution and density of secretory structures in reproductive and vegetative organs of FC and FO, our findings suggest a functional role for glandular trichomes in the secretion of the putative phytotoxic allelochemical (-)HAA.

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References

[1] Funk, V.A., Susanna, A., Stuessy, T.F. and Robinson, H. (2009) Classification of Compositae. In: Funk, V.A., Susanna, A., Stuessy, T.F. and Bayer, R.J., Eds., *Systematics*, *Evolution*, *and Biogeography of Compositae*, International Association for Plant Taxonomy (IAPT), Vienna, 171-192.

[2] Simpson, B. (2009) Economic Importance of Compositae. In: Funk, V.A., Susanna, A., Stuessy, T.F. and Bayer, R.J., Eds., *Systematics*, *Evolution*, *and Biogeography of Compositae*, Sheridan Books, Ann Arbor, 45-58.

[3] Katinas, L., Gutiérrez, D.G., Grossi, M.A. and Crisci, J.V. (2007) Panorama de la familia Asteraceae (Compositae) en la República Argentina. *Bol. Soc. Argent. Bot.*, **42**, 113-129.

[4] Dillon, M.O. (1984) A Systematic Study of *Flourensia* (Asteraceae, Heliantheae). *Fieldiana*, *Botany*, *New Series*, **16**, 1-67.

[5] Ariza Espinar, L. (2000) Familia Asteraceae. Tribu Heliantheae. *Prodromo de la Flora Fanerogámica de Argentina Central*, **2**, 1-111.

[6] Langenheim, J.H. (2003) Plant Resins: Chemistry, Evolution, Ecology and Ethnobotany. Timber Press, Portland.

[7] Abrahim, D., Braguini, W.L., Kelmer-Bracht, A.M. and Ishii-Iwamoto, E.L. (2000) Effects of Four Monoterpenes on Germination, Primary Root Growth, and Mitochondrial Respiration of Maize. *Journal of Chemical Ecology*, **26**, 611-624. http://dx.doi.org/10.1023/A:1005467903297

[8] Dayan, F.E. and Duke, S.O. (2006) Clues in the Search for New Herbicides. In: Reigosa, M.J., Pedrol, N. and González, L., Eds., Allelopathy: A Physiological Process with Ecological Implications. Springer, Dordrecht, 63-81. http://dx.doi.org/10.1007/1-4020-4280-9_3

[9] Romagni, J.G., Allen, S.N. and Dayan, F.E. (2000) Allelopathic Effects of Volatile Cineoles on Two Weedy Plant Species. *Journal of Chemical Ecology*, **26**, 303-313. http://dx.doi.org/10.1023/A:1005414216848

[10] Silva, M.P., Piazza, L.A., López, D., López Rivilli, M.J., Turco, M.D., Cantero, J.J., Tourn, G.M. and Scopel, A.L. (2012) Phytotoxic Activity in *Flourensia campestris* and Isolation of (-)-Hamanasic Acid A as Its Active Principle Compound. *Phytochemistry*, **77**, 140-148. http://dx.doi.org/10.1016/j.phytochem.2011.09.020

[11] Croteau, R.B., Davis, E.M., Ringer, K.L. and Wildung, M.R. (2005) (-)-Menthol Biosynthesis and Molecular Genetics. *Naturwissenschaften*, **92**, 562-577. http://dx.doi.org/10.1007/s00114-005-0055-0

[12] Majdi, M., Liu, Q., Karimzadeh, G., Malboobi, M.A., Beekwilder, J., Cankar, K., De Vos, R., Todorović, S., Simonović, A. and Bouwmeester, H. (2011) Biosynthesis and Localization of Parthenolide in Glandular Trichomes of Feverfew (*Tanacetum parthenium* L. Schulz Bip.). *Phytochemistry*, **72**, 1739-1750. http://dx.doi.org/10.1016/j.phytochem.2011.04.021

[13] Urzúa, A. (2004) Mono- and Sesquiterpenes in the Trichome Secreted Exudates from *Pseudognaphalium cheiranthifolium*, *P. heterotrichium*, *P. vira vira* and *P. robustum*. *Biochemical Systematics and Ecology*, **32**, 211-214. http://dx.doi.org/10.1016/S0305-1978(03)00158-3

[14] Wagner, G.J., Wang, E. and Shepherd, R.W. (2004) New Approaches for Studying and Exploiting an Old Protuberance, the Plant Trichome. *Annals of Botany*, **93**, 3-11. http://dx.doi.org/10.1093/aob/mch011

[15] López, D., Piazza, L.A., Silva, M.P., López Rivilli, M.J., Cantero, J.J., Tourn, G.M. and Scopel, A.L. (2014) Distribution of (−)-Hamanasic Acid A in South American Species of *Flourensia* and Phytotoxic Effects of Leaf Aqueous Extracts. *Natural Product Communications*, **9**, 341-345.

[16] Mata, R., Bye, R., Linares, E., Macías, M., Rivero-Cruz, I., Pérez, O. and Timmermann, B.N. (2003) Phytotoxic Compounds from *Flourensia cernua*. *Phytochemistry*, **64**, 285-291. http://dx.doi.org/10.1016/S0031-9422(03)00217-6

[17] Diaz Napal, G.N. and Palacios, S.M. (2013) Phytotoxicity of Secondary Metabolites Isolated from *Flourensia oolepis* S.F. Blake. *Chemistry & Biodiversity*, **10**, 1295-1304. http://dx.doi.org/10.1002/cbdv.201200204

[18] Palacios, S.M., Maggi, M.E., Bazán, C.M., Carpinella, M.C., Turco, M., Muñoz, A., Alonso, R.A., Nuñez, C., Cantero, J.J., Defago, M.T., Ferrayoli, C.G. and Valladares, G.R. (2007) Screening of Argentinian Plants for Pesticide Activity. *Fitoterapia*, **78**, 580-584. http://dx.doi.org/10.1016/j.fitote.2007.03.023

[19] Seminara, C., Bollati, L., Avalos, S., Diaz Napal, G., Palacios, S. and Defagó, M. (2014) Antifeedant and Toxic Effect of Crude Extract from *Flourensia oolepis* and Their Impact on Nutritional Parameters of *Helicoverpa gelotopoeon*. *Journal of Applied Sciences and Environmental Management*, **18**, 577-581.

[20] Diaz Napal, G.N., Carpinella, M.C. and Palacios, S.M. (2009) Antifeedant Activity of Ethanolic Extract from *Flourensia oolepis* and Isolation of Pinocembrin as Its Active Principle Compound. *Bioresource Technology*, **100**, 3669-3673. http://dx.doi.org/10.1016/j.biortech.2009.02.050

[21] Faini, F., Labbe, C., Salgado, I. and Coll, J. (1997) Chemistry, Toxicity and Antifeedant Activity of the Resin of *Flourensia thurifera*. *Biochemical Systematics and Ecology*, **25**, 189-193.

http://dx.doi.org/10.1016/S0305-1978(96)00100-7

[22] García, M., Gonzalez-Coloma, A., Donadel, O.J., Ardanaz, C.E., Tonn, C.E. and Sosa, M.E. (2007) Insecticidal Effects of *Flourensia oolepis* Blake (Asteraceae) Essential Oil. *Biochemical Systematics and Ecology*, **35**, 181-187. http://dx.doi.org/10.1016/j.bse.2006.10.009

[23] de Rodríguez, D.J., Hernández-Castillo, F.D., Solís-Gaona, S., Rodríguez-García, R. and Rodríguez-Jasso, R.M. (2012) *Flourensia cernua* DC: A Plant from Mexican Semiarid Regions with a Broad Spectrum of Action for Disease Control. In: Larramendy, M.L. and Soloneski, S., Eds., *Integrated Pest Management and Pest Control*, *Current and Future Tactics*, InTech, Rijeka, 639-650. http://dx.doi.org/10.5772/31783

[24] Joray, M.B., Del Rollán, M.R., Ruiz, G.M., Palacios, S.M. and Carpinella, M.C. (2011) Antibacterial Activity of Extracts from Plants of Central Argentina—Isolation of an Active Principle from *Achyrocline satureioides*. *Planta Medica*, **77**, 95-100. http://dx.doi.org/10.1055/s-0030-1250133

[25] Reyes, M.G., Torres, M.J., Maggi, M.D., Marioli, J.M., Gil, R.R., Sosa, V.E., Uriburu, M.L. and Audisio, M.C. (2013) *In Vitro* Inhibition of *Paenibacillus larvae* by Different Extracts and Pure Compounds from *Flourensia* spp. *Industrial Crops and Products*, **50**, 758-763. http://dx.doi.org/10.1016/j.indcrop.2013.07.062

[26] Tellez, M., Estell, R., Fredrickson, E., Powell, J., Wedge, D., Schrader, K. and Kobaisy, M. (2001) Extracts of *Flourensia cernua* (L): Volatile Constituents and Antifungal, Antialgal, and Anti-Termite Bioactivities. *Journal of Chemical Ecology*, **27**, 2263-2273. http://dx.doi.org/10.1023/A:1012283005014

[27] Chiari, M.E., Joray, M.B., Ruiz, G., Palacios, S.M. and Carpinella, M.C. (2010) Tyrosinase Inhibitory Activity of Native Plants from Central Argentina: Isolation of an Active Principle from *Lithrea molleoides*. *Food Chemistry*, **120**, 10-14. http://dx.doi.org/10.1016/j.foodchem.2009.09.061

[28] Appezzato-da-Glória, B., Da Costa, F.B., Da Silva, V.C., Gobbo-Neto, L., Garcia Rehder, V.L. and Hayashi, A.H. (2012) Glandular Trichomes on Aerial and Underground Organs in *Chrysolaena* Species (Vernonieae: Asteraceae): Structure, Ultrastructure and Chemical Composition. *Flora*, **207**, 878-887. http://dx.doi.org/10.1016/j.flora.2012.10.003

[29] Bartoli, A., Galati, B.G. and Tortosa, R.D. (2011) Anatomical Studies of the Secretory Structures: Glandular Trichomes and Ducts, in *Grindelia pulchella* Dunal (Astereae, Asteraceae). *Flora*, **206**, 1063-1068. http://dx.doi.org/10.1016/j.flora.2011.07.012

[30] Pagni, A.M. and Masini, A. (1999) Morphology, Distribution, and Histochemistry of Secretory Structures in Vegetative Organs of *Santolina leucantha* Bertol. (Asteraceae). *Israel Journal of Plant Sciences*, **47**, 257-263. http://dx.doi.org/10.1080/07929978.1999.10676782

[31] Castro, M.M., Leitão-Filho, H.F. and Monteiro, W.R. (1997) Utilização de estruturas secretoras na identificação dos gêneros de Asteraceae de uma vegetação de cerrado. *Revista Brasileira de Botânica*, **20**, 163-174. http://dx.doi.org/10.1590/S0100-84041997000200007

[32] Ambrósio, S.R., Oki, Y., Heleno, V.C., Chaves, J.S., Nascimento, P.G., Lichston, J.E., Constantino, M.G., Varanda, E.M. and Da Costa, F.B. (2008) Constituents of Glandular Trichomes of *Tithonia diversifolia*: Relationships to Herbivory and Antifeedant Activity. *Phytochemistry*, **69**, 2052-2060. http://dx.doi.org/10.1016/j.phytochem.2008.03.019

[33] Schorr, K., Merfort, I. and Da Costa, F.B. (2007) A Novel Dimeric Melampolide and Further Terpenoids from *Smallanthus sonchifolius* (Asteraceae) and the Inhibition of the Transcription Factor NF-*κ*B. *Natural Product Communications*, **2**, 367-374.

[34] Spring, O., Zipper, R., Conrad, J., Vogler, B., Klaiber, I. and Da Costa, F.B. (2003) Sesquiterpene Lactones from Glandular Trichomes of *Viguiera radula* (Heliantheae; Asteraceae). *Phytochemistry*, **62**, 1185-1189. http://dx.doi.org/10.1016/S0031-9422(02)00747-1

[35] Stefani, R., Eberlin, M.N., Tomazela, D.M. and Da Costa, F.B. (2003) Eudesmanolides from *Dimerostem mavestitum*. *Journal of Natural Products*, **66**, 401-403. http://dx.doi.org/10.1021/np020304u

[36] Delbón, N., Cosa, M.T., Dottori, N. and Stiefkens, L. (2007) Análisis comparativo de los caracteres epidérmicos en *Flourensia campestris* y *F. oolepis* (Asteraceae). *Boletin de la Sociedad Argentina de Botanica*, **42**, 245-250.

[37] Delbón, N., Cosa, M.T. and Dottori, N. (2007b) Anatomía de órganos vegetativos en *Flourencia campestris* y *F. oolepis* (Asteraceae), con especial referencia a las estructuras secretoras. *Arnaldoa*, **14**, 61-70.

[38] Delbón, N., Cosa, M.T. and Bernardello, G. (2012) Exomorfología y anatomía de órganos vegetativos aéreos en especies de *Flourensia* DC. (Asteraceae) con importancia fitoquímica. *Acta Botanica Brasilica*, **26**, 2-10. http://dx.doi.org/10.1590/S0102-33062012000100002

[39] Amrehn, E., Heller, A. and Spring, O. (2014) Capitate Glandular Trichomes of *Helianthus annuus* (Asteraceae): Ultrastructure and Cytological Development. *Protoplasma*, **251**, 161-167. http://dx.doi.org/10.1007/s00709-013-0534-7

[40] Appezzato-da-Glória, B., Hayashi, A.H., Cury, G., Soares, M.K.M. and Rocha, R. (2008) Occurrence of Secretory Structures in Underground Systems of Seven Asteraceae Species. *Botanical Journal of the Linnean Society*, **157**, 789-

796. http://dx.doi.org/10.1111/j.1095-8339.2008.00823.x

[41] Heinrich, G., Pfeifhofer, H.W., Stabentheiner, E. and Sawidis, T. (2002) Glandular Hairs of *Sigesbeckia jorullensis* Kunth (Asteraceae): Morphology, Histochemistry and Composition of Essential Oil. *Annals of Botany*, **89**, 459-469. http://dx.doi.org/10.1093/aob/mcf062

[42] Zarlavsky, G.E. (2014) Histología Vegetal: Técnicas simples y complejas. Sociedad Argentina de Botánica, Buenos aires, 1-198.

[43] O'Brien, T.P. and McCully, M.E. (1981) The Study of Plant Structure: Principles and Selected Methods. Termarcarphi Pty. Ltd., Melbourne.

[44] Lersten, N.R. and Curtis, J.D. (1988) Secretory Reservoirs (Ducts) of Two Kinds in Giant Ragweed (*Ambrosia trifida*; Asteraceae). *American Journal of Botany*, **75**, 1313-1323. http://dx.doi.org/10.2307/2444454

[45] Göpfert, J.C., Heil, N., Conrad, J. and Spring, O. (2005) Cytological Development and Sesquiterpene Lactone Secretion in Capitate Glandular Trichomes of Sunflower. *Plant Biology*, **7**, 148-155. http://dx.doi.org/10.1055/s-2005-837575

[46] Göpfert, J.C., MacNevin, G., Ro, D.K. and Spring, O. (2009) Identification, Functional Characterization and Developmental Regulation of Sesquiterpene Synthases from Sunflower Capitate Glandular Trichomes. *BMC Plant Biology*, **9**, 86. http://dx.doi.org/10.1186/1471-2229-9-86

[47] Favi, F., Cantrell, C.L., Mebrahtu, T. and Kraemer, M.E. (2008) Leaf Peltate Glandular Trichomes of *Vernonia galamensis* ssp. *galamensis* var. *ethiopica* Gilbert: Development, Ultrastructure and Chemical Composition. *International Journal of Plant Sciences*, **169**, 605-614. http://dx.doi.org/10.1086/533598

[48] Ascensão, L., Da-Silva, J.A.T., Barroso, J.G., Figuereido, A.C. and Pedro, L.G. (2001) Glandular Trichomes and Essential Oils of *Helichrysum stoechas*. *Israel Journal of Plant Sciences*, **49**, 115-122. http://dx.doi.org/10.1092/F85Y-YEN4-9VTF-B1AJ

[49] Werker, E. and Fahn, A. (1981) Secretory Hairs of *Inulaviscosa* (L.) Ait.—Development, Ultrastructure, and Secretion. *Botanical Gazette*, **142**, 461-476. http://dx.doi.org/10.1086/337247

[50] Kelsey, R.G. and Shafizadeh, F. (1980) Glandular Trichomes and Sesquiterpene Lactones of *Artemisia nova* (Asteraceae). *Biochemical Systematics and Ecology*, **8**, 371-377. http://dx.doi.org/10.1016/0305-1978(80)90039-3

[51] Ramirez, A.M., Stoopen, G., Menzel, T.R., Gols, R., Bouwmeester, H.J., Dicke, M. and Jongsma, M.A. (2012) Bidirectional Secretions from Glandular Trichomes of Pyrethrum Enable Immunization of Seedlings. *Plant Cell*, **24**, 4252-4265. http://dx.doi.org/10.1105/tpc.112.105031

[52] Ascensão, L., Mota, L. and Castro, M. de M. (1999) Glandular Trichomes on the Leaves and Flowers of *Plectranthus ornatus*: Morphology, Distribution and Histochemistry. *Annals of Botany*, **84**, 437-447. http://dx.doi.org/10.1006/anbo.1999.0937

[53] Giuliani, C. and Bini, L.M. (2008) Insights into the Structure and Chemistry of Glandular Trichomes of Labiatae, with Emphasis on Subfamily Lamioideae. *Plant Systematics and Evolution*, **276**, 199-208. http://dx.doi.org/10.1007/s00606-008-0085-0

[54] Jaime, R., Rey, P.J., Alcántara, J.M. and Bastida, J.M. (2013) Glandular Trichomes as an Inflorescence Defense Mechanism against Insect Herbivores in Iberian Columbines. *Oecologia*, **172**, 1051-1060. http://dx.doi.org/10.1007/s00442-012-2553-z

[55] Fahn, A. (2000) Structure and Function of Secretory Cells. In: Hallahan, D.L., Gray, J.C. and Callow, J.A., Eds., *Advances in Botanical Research*, *Incorporating Advances in Plant Pathology*, *Volume* 31, *Plant Trichomes*, Academic Press, London, 37-66. http://dx.doi.org/10.1016/S0065-2296(00)31006-0

[56] Monteiro, W.R., De Moraes Castro, M., Mazzoni-Viveiros, S.C. and Mahlberg, P.G. (2001) Development and Some Histochemical Aspects of Foliar Glandular Trichomes of *Stevia rebaudiana* (Bert.) Bert.—Asteraceae. *Revista Brasileira de Botânica*, **24**, 349-357. http://dx.doi.org/10.1590/S0100-84042001000300013

[57] Monteiro, W.R., Caldeira, W., De Moraes Castro, M., Mahlberg, P.G. and Cruz, M.V. (2003) Ultrastructural Observations on Foliar Glandular Trichomes of *Stevia rebaudiana*. *South African Journal of Botany*, **69**, 489-499.

[58] Werker, E., Putievsky, E., Ravid, U., Dudai, N. and Katzir, I. (1994) Glandular hairs, Secretory Cavities, and the Essential Oil in Leaves of Tarragon (*Artemisia dracunculus* L.). *Journal of Herbs*, *Spices & Medicinal Plants*, **2**, 19-32. http://dx.doi.org/10.1300/J044v02n03_04

[59] Duke, S.O., Canel, C., Rimando, A.M., Tellez, M.R., Duke, M.V. and Paul, R.N. (2000) Current and Potential Exploitation of Plant Glandular Trichome Productivity. In: Hallahan, D.L. and Gray, J.C., Eds., *Advances in Botanical Research*, *Incorporating Advances in Plant Pathology*, *Volume* 31, *Plant Trichomes*, Academic Press, London, 121-151.

[60] Hallahan, D.L. (2000) Monoterpenoid Biosynthesis in Glandular Trichomes of Labiate Plants. In: Hallahan, D.L. and Gray, J.C., Eds., *Advances in Botanical Research*, *Incorporating Advances in Plant Pathology*, *Volume* 31, *Plant Trichomes*, Academic Press, London, 77-120. http://dx.doi.org/10.1016/S0065-2296(00)31007-2

[61] Hashidoko, Y., Endoh, K., Kudo, T. and Tahara, S. (2001) Capability of Wild *Rosa rugosa* and Its Varieties and Hybrids to Produce Sesquiterpene Components in Leaf Glandular Trichomes. *Bioscience, Biotechnology and Biochemistry*, **65**, 2037-2043. http://dx.doi.org/10.1271/bbb.65.2037

[62] Hay, R.K.M. and Svoboda, K.P. (1993) Botany. In: Hay, R.K.M. and Waterman, P.G., Eds., *Volatile Oil Crops*: *Their Biology*, *Biochemistry*, *and Production*, Longman Scientific and Technical, Harlow, 5-22.

[63] Siebert, D.J. (2004) Localization of Salvinorin A and Related Compounds in Glandular Trichomes of the Psychoactive Sage, *Salvia divinorum*. *Annals of Botany*, **93**, 763-771. http://dx.doi.org/10.1093/aob/mch089

[64] Wang, W., Wang, Y., Zhang, Q., Qi, Y. and Guo, D. (2009) Global Characterization of *Artemisia annua* Glandular Trichome Transcriptome Using 454 Pyrosequencing. *BMC Genomics*, **10**, 465. http://dx.doi.org/10.1186/1471-2164-10-465

[65] Spring, O. (1989) Microsampling: An Alternative Approach Using Sesquiterpene Lactones for Systematic. *Biochemical Systematics and Ecology*, **17**, 509-517. http://dx.doi.org/10.1016/0305-1978(89)90093-8

[66] Combrick, S., Du Plooy, G.W., McCrindle, R.I. and Botha, B.M. (2007) Morphology and Histochemistry of the Glandular Trichomes of *Lippias caberrima* (Verbenaceae). *Annals of Botany*, **99**, 1111-1119. http://dx.doi.org/10.1093/aob/mcm064

[67] Lommen, W.J.M., Schenk, E., Bouwmeester, H.J. and Verstappen, F.W.A. (2006) Trichome Dynamics and Artemisinin Accumulation during Development and Senescence of *Artemisia annua* Leaves. *Planta Medica*, **72**, 336-345. http://dx.doi.org/10.1055/s-2005-916202

[68] Hashidoko, Y., Tahara, S. and Mizutani, J. (1994) Six Sesquiterpenoids from Glandular Trichome Exudates of *Rosa rugosa*. *Phytochemistry*, **35**, 325-329. http://dx.doi.org/10.1016/S0031-9422(00)94757-5

[69] Carlquist, S. (1966) Wood Anatomy of Compositae: A Summary, with Comments on Factors Controlling Wood Evolution. *Aliso*, **6**, 25-44.

[70] Robinson, H. (1981) A Revision of the Tribal and Subtribal Limits of the Heliantheae (Asteraceae). *Smithsonian Contributions to Botany*, **51**, 1-102. http://dx.doi.org/10.5479/si.0081024X.51

[71] Feijóo, M.S., Arce, M.E. and D'Ambrogio, A. (2005) Variación morfoanatómica en una población de *Senecio filaginoides* (Asteraceae). *Polibotánica*, **19**, 1-17.

[72] Baier, P., Führer, E., Kirisits, T. and Rosner, S. (2002) Defence Reactions of Norway Spruce against Bark Beetles and the Associated Fungus *Ceratocystis polonica* in Secondary Pure and Mixed Species Stands. *Forest Ecology and Management*, **159**, 73-86. http://dx.doi.org/10.1016/S0378-1127(01)00711-3

[73] Tolera, M., Menger, D., Sass-Klaassen, U., Sterck, F.J., Copini, P. and Bongers, F. (2013) Resin Secretory Structures of *Boswellia papyrifera* and Implications for Frankincense Yield. *Annals of Botany*, **111**, 61-68. http://dx.doi.org/10.1093/aob/mcs236

[74] Gunning, B.E.S. and Pate, J.S. (1969) "Transfer Cells" Plant Cells with Wall Ingrowths, Specialized in Relation to Short Distance Transport of Solutes—Their Occurrence, Structure, and Development. *Protoplasma*, **68**, 107-133. http://dx.doi.org/10.1007/BF01247900

[75] Herrero, M. and Dickinson, H.G. (1979) Pollen-Pistil Incompatibility in *Petunia hybrida*: Changes in the Pistil Following Compatible and Incompatible Intraspecific Crosses. *Journal of Cell Science*, **36**, 1-18.