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LEAF ANATOMY OF THE GOETZEACEAE

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ABSTRACT

A comparative study of the leaf anatomy of three species of three genera of Goetzeaceae (Henoonia, Goetzea, and Espadaea) revealed a number of characteristics common to all genera, viz. anomocytic stomata, sinuous anticlinal epidermal walls, both glandular and uniseriate nonglandular trichomes, crystal sand and druses in the mesophyll (often in the same cell), and intraxylary phloem. The midveins of these species exhibit secondary growth and parenchymatous rays. The unifying anatomical features of this family are, however, not uncommon in the Solanaceae. The results of this study support placement of the Goetzeaceae in or very near Solanaceae. The ecology of the species studied is reflected in the anatomy of the leaves. The leaves of Henoonia exhibit a number of xeromorphic features; the leaves of Goetzea are mesomorphic; while those of Espadaea are intermediate.

Key words: Espadaea, Goetzea, Goetzeaceae, Henoonia, herbivory, leaf anatomy, Solanales, Solanaceae, xeromorphy.

INTRODUCTION

The Goetzeaceae comprise a small Antillean family of four genera of evergreen trees or shrubs: Goetzea Wydler, Henoonia Griseb., Espadaea A. Rich., and Coeloneurum Radlk. The family is poorly known anatomically, phytochemically, and ecologically. Lithophytum Brandg., formerly placed in the Goetzeaceae (Airy Shaw 1973), has been shown to be congeneric with Plocospermum Benth., a member of the Loganiaceae (Chiang and Frame 1987). The genus Bissea Fuentes was established in 1985 to replace Henoonia, but as Carlquist (1988) pointed out, Fuentes has not yet shown Henoonia to be a mere orthographic variant of the legitimate, earlier Henonia Moq. (Amaranthaceae).

Questions about the taxonomic affinities of this family have led several previous workers to study its vegetative anatomy. Radlkofer (1888) studied the vegetative anatomy of Henoonia and concluded that the genus should be placed in the Solanaceae on the basis of the presence of crystal sand and intraxylary phloem, and absence of laticifers. Kramer (1939) and Record (1939) concluded on the basis of anatomical features of the wood that Henoonia instead resembled Bombax and was clearly sapotaceous. D’Arcy and Keating (1973), studying the leaves of Goetzea and other members of the family, also concluded that the Goetzeaceae were unrelated to the Solanaceae. However, Carlquist (1988) suggested, on the basis of wood anatomy, that the family shows affinity to Solanaceae. Miers (1869) was first to argue in favor of recognizing the Goetzeaceae as a distinct family.

The results presented herein serve both to document the range of variation in anatomical features of Goetzea, Henoonia, and Espadaea, and to interpret those features in light of what is known of the ecology of the species. Although not the original purpose of the study, systematic conclusions also can be drawn from the anatomical findings.
MATERIALS AND METHODS

Dried material from herbarium specimens was first rehydrated in glycerine-70% ethanol-10% Aerosol OT as described by Martens and Uhl (1980) for 24 h at 60 °C. Liquid-preserved and rehydrated leaf samples were then prepared for anatomical study by dehydrating in a standard tertiary butanol series and embedding in paraffin. Sections were cut 12–13 μm thick on a rotary microtome, stained with a safranin-fast green combination, and mounted in synthetic resin (Coverbond). Epidermal peels were prepared using Jeffrey’s Solution (10% chromic acid and 10% nitric acid, 1:1). Samples were placed in the solution until the cutinized epidermis fell away from the mesophyll. The epidermis was washed, dehydrated in ethanol, stained with safranin, and mounted in synthetic resin. Leaf clearings were prepared with 10% NaOH, bleached with 10% Clorox, dehydrated, stained with safranin, and mounted.

Air-dried specimens were sputter-coated with gold and examined with an ISI WB6 scanning electron microscope.

Measurements were made using an ocular micrometer. Lamina thickness, palisade layer thickness, vessel diameter (inside diameter at widest point), and stomatal density and dimensions were based on averages of ten measurements. Drusé diameter and epidermal wall thickness were based on averages of fewer than 10 measurements. As only one leaf per collection was sampled, primary vein thickness and petiole diameter were both single measurements. Measurements from separate collections comprise the ranges reported below.

Specimens examined.—Espadéa amoena A. Rich. CUBA: vicinity of Soledad, Las Villas, A. Gonzales s.n. (FLAS); FLORIDA, Miami, cultivated at the U.S.D.A. Plant Introduction Station from seed collected at the Harvard Botanical Garden, Soledad, Cuba, USDA PI 087511 (material pickled in FAA).—Goetzea elegans Wyd. PUERTO RICO: Guajataca Gorge, W. G. D’Arcy 1729 (FLAS); Gambalache Experimental Forest, E. L. Little 13463 (GH); without locality, Wydler 333 (GH).—Henoonia myrtifolia Griseb. CUBA: Oriente, Brasil, Cerro de Fraile (as H. brittonii [Small] Manachino), E. L. Ekman s.n. (A) and E. L. Ekman 3262 (A); Las Villas, Loma San Juan (as H. brittonii) C. E. Wood & E. A. Atchison 7408 (A); without locality, Wright 2930 (GH).

In addition, specimens of Coeloneurum ferrugineum (Spr.) Urb. of Hispaniola were examined in the herbarium of the Jardín Botánico Nacional “Dr. Rafael M. Moscoso” in the Dominican Republic. Specimens of Henoonia were examined in the herbarium of the Jardín Botánico Nacional de Cuba, Havana.

Goetzea ekmanii O. E. Schulz, of Hispaniola, was unavailable for study.

RESULTS

Henoonia myrtifolia Griseb. (Fig. 1, 4b).—The leaf margin of H. myrtifolia is slightly revolute, with 10–17 second-order veins, as seen in transection. The midvein, visible externally as a prominent midrib on the abaxial surface, is nearly circular in transection, 240–550 μm in diameter (Fig. 1a). Secondary growth in the xylem of the midvein produces a large bundle sheath of fibers oriented longitudinally, located abaxially to the primary xylem. Uniseriate rays of erect cells (i.e., whose long axis parallels the axis of the midvein) are present in the midvein. Tracheids, in radial files, measure 10–11 μm in diameter. Phloem is bicolateral,
Fig. 1. Anatomical features of Hennoonia myrtifolia (a, Wright 2930; b–d, Ekman 3262).—a. Transection through the midvein; adaxial phloem collapsed.—b. Leaf clearing; druses visible as dark spheroidal bodies scattered throughout the mesophyll.—c. Adaxial epidermis.—d. Abaxial epidermis. (Fig. 1a–d, scale with a = 0.2 mm)

represented by a band of apparently crushed sieve tube members and parenchyma cells on the abaxial side and surrounded by a discontinuous band of fibers. An apparently functional strand of phloem is present on the adaxial side (Fig. 1a). Second-order veins are likewise surrounded by a fibrous bundle sheath.

The adaxial epidermis consists of cells with sinuous anticlinal walls and highly cutinized, smooth outer walls, 9–14 μm thick (Fig. 1c). Short, single-celled trichomes are present over the midvein. The lamina is 74–170 μm thick between the veins. A single palisade layer, 18–36 μm thick, is present. The spongy layer contains large intercellular spaces and abundant, evenly distributed idioblastic cells containing druses and crystal sand. Druses are spherical and range up to 42 μm in diameter (Fig. 1b). The outer wall of the abaxial epidermis is likewise highly cutinized, ca. 9 μm thick. This epidermis (Fig. 1d) has sinuous anticlinal walls and bears two types of trichomes. On the abaxial surface, there are long (to 125 μm) two- to three-celled uniseriate trichomes with verrucose walls and long ter-
minal cells. On the adaxial surface above the midvein, shorter unicellular trichomes are occasionally found. Stomata are evenly distributed (185–395 stomata per mm²), and of the anomocytic type (Fig. 1d). Guard cells are 22–27 μm long by 6–7 μm wide, with a cutinized outer lip.

Dissecting microscope examination of a specimen (J. Bisse et al. 48172) in the herbarium of the Jardín Botánico Nacional de Cuba revealed the presence of small capitate-glandular trichomes on the very young shoots and abaxial surface of young leaves.

A transection through the middle of the petiole reveals that the petiole is roughly oval in shape, ca. 700 μm by 450 μm, and that the single, circular vascular strand has intraxylary phloem. The parenchymatous ground tissue is rich in idioblasts containing crystal sand. Druses are also present, and often the druses, composed of angular crystals of varying sizes, are found embedded in crystal sand. The epidermis is covered with uniseriate trichomes of 1 to 4 cells.

**Espadaea amoena** A. Rich. (Fig. 2).—The leaves of this species are broad and lack revolute margins. The arc-shaped fiber sheath of the midvein, 320–435 μm thick, nearly encircles the intraxylary phloem. Thin, uniseriate rays are present in the fibrous bundle sheath, and a thin sheath of fibers is present just outside the abaxial phloem (Fig. 2a). Tracheids, in radial files, are 11–14 μm in diameter. The abaxial phloem appears partially crushed and may be nonfunctional (Fig. 2a). A layer of parenchyma approximately four cells thick is found between this outer fibrous bundle sheath and the abaxial epidermis. Bundle sheaths are also present around second-order veins.

The lamina is 200–255 μm thick between the veins. The palisade layer, a single layer of cells, is 28–38 μm thick and clearly differentiated. Druses and a few crystal sand idioblasts (to 42 μm in diameter) are abundant and are distributed evenly throughout the leaf (Fig. 2b) The adaxial epidermis is ca. 25 μm thick (lumen height), composed of isodiametric cells with highly sinuous anticlinal walls (Fig. 2d); outer walls are weakly cutinized and are 5–6 μm thick. Epidermal cells are elongate over veins. The abaxial epidermis likewise is composed of cells with sinuous anticlinal walls. Trichomes, to 175 μm long, are present; they are uniseriate, composed of 2–4 cells, with an elongate terminal cell. Anomocytic stomata are present at a density of 235–580 per mm². The guard cell pair is ovate-circular in outline. Each guard cell is 20–24 μm long and 7–8 μm wide.

The petiole is oval to semicircular in transsectional view, ca. 730 μm by 980 μm. The single vascular bundle contains intraxylary phloem (Fig. 2c). Druses are found in the internal phloem, as well as throughout the parenchymatous tissue of the petiole. Cells containing crystal sand and druses embedded in crystal sand are numerous. The epidermis is covered with uniseriate trichomes consisting of 2–4 cells.

**Goetzea elegans** Wydler. (Fig. 3, 4a).—The leaf margins of *G. elegans* are only slightly revolute. Approximately 30 second-order veins are visible in the transection. The midvein exhibits secondary growth, with a bundle sheath of fibers completely surrounding the intraxylary phloem (Fig. 3c). The sheath, round in transection, is approximately 290 μm in diameter. Uniseriate rays of erect cells are present among the fibers. Ray cells measure 50 × 12 μm in radial section. Tracheids are arrayed in radial files and measure 13–18 μm in diameter. Numerous
fibers are present in the crushed abaxial phloem, forming a discontinuous sheath (Fig. 3c). Smaller veins are also surrounded by a bundle sheath of fibers. The lamina is 140 μm thick. The palisade layer, only weakly differentiated, is ca. 39 μm thick. Large (to 60 μm) spherical crystal sand idioblasts and druses are scattered along the midvein in the basal third of the leaf (Fig. 3a). They are less frequent near the leaf margin (Fig. 3b). The outer wall of the adaxial epidermis is 7 μm thick, smooth, and heavily cutinized. The abaxial cutinized epidermis is 5 μm thick. The abaxial epidermis is composed of isodiametric cells with highly sinuous anticlinal walls (Fig. 3d). Over first- and second-order veins, the epidermal cells are elongate and the anticlinal walls less sinuous. Three-celled trichomes, with the terminal cells elongate and cutinized, are present along the midrib. Stomata, of the anomocytic type, are distributed evenly across the abaxial surface of the leaf at a density of 180–320 per mm². Each guard cell is ca. 22 μm long by 6 μm wide.
The transectional shape of the petiole is roughly reniform, 1000 μm by 625 μm. The vascular arc is surrounded by phloem. The xylem is composed of tracheids in radial chains and a few fibers. The parenchymatous ground tissue is rich in druses and crystal sand idioblasts. Druses can often be found embedded in crystal sand. The epidermis is covered by uniseriate multicellular trichomes. Examination by scanning electron microscopy of the external surface of the calyx of *G. elegans* revealed the presence of glandular trichomes mixed with more common uniseriate trichomes (Fig. 4a). *Henoonia myrtifolia* illustrates the verrucose walls of the nonglandular trichomes common to the genera examined here (Fig. 4b).

Specimens of *Coeloneurum ferrugineum* examined in the herbarium of Jardín Botánico Nacional “Dr. Rafael M. Moscoso” greatly resemble *Henoonia myrtifolia*; however, the leaves were not examined anatomically.

**Fig. 3.** Anatomical features of *Goetzea elegans* (a–b, Wyder 335; c–d, D’Arcy 1729).—a. Leaf clearing, basal portion of leaf, near the midvein; note druses (dark spheroidal bodies).—b. Leaf clearing, distal portion of leaf, near margin; note absence of druses.—c. Transection through midvein; adaxial phloem collapsed. Rays are readily visible among the dark-staining fibers of the bundle sheath.—d. Adaxial epidermis. (a–c, scale with a = 0.1 mm; d, scale = 0.1 mm)

Several species and varieties have been described from this plant. Species such as *G. elegans* have been studied in depth to understand their ecological and evolutionary relationships. The habitat of *G. elegans* is typically found in rocky, well-drained soils in dry, calcareous forests (to 600 meters above sea level), soils in dry, well-drained, limestone areas, and calcareous soils in dry, calcareous forests. The contrast is evident in the presence of druses, which range from 60 to 440 in *G. elegans*, as compared with 3 to 60 in *Henoonia myrtifolia*. However, the druse content of *G. elegans* does not exceed 100 in any sample.
DISCUSSION

Several anatomical features unify the Goetzeaceae, including: bifacial leaves, midveins with secondary growth and parenchymatous rays (Fig. 1a, 2a, 3c), thick adaxial epidermis, sinuous anticlinal walls in the epidermis (Fig. 1c, 2d, 3d), single palisade layer, anomocytic stomata (Fig. 1d), intraxylary phloem (Fig. 1a, 2a, 3c), and crystal sand and druses composed of angular crystals, often in the same cell. The presence of both glandular trichomes and uniseriate trichomes with long terminal cells is probably characteristic of these genera (Fig. 4a, b). The glandular trichomes reported here for Goetzea elegans and Henoonia are apparently not common or are perhaps quickly deciduous; further examination of Espadaea may reveal their presence in that genus too.

Ecological Aspects of the Leaf Anatomy

According to Fuentes (1985), Henoonia is found on calcareous or serpentine soils in dry coastal spinescent vegetation ("matorral xeromórfico espinoso costero"), from 0 to 300 m in elevation. Coeloneuron is a shrub of dry forests on limestone or serpentine, from 0 to 400 m. Likewise, Espadaea is found in dry forests (to 700 m) in association with Jacquinia sp. (Fuentes 1982). In stark contrast is Goetzea elegans, which grows in moist limestone and coastal forests from 60 to 90 m on the northern coast of Puerto Rico (Little, Woodbury, and Wadsworth 1974).

Henoonia exhibits most clearly several anatomical adaptations to arid habitats. They include: inrolled leaf margin, thick cuticle, and vascular bundles surrounded by fibrous bundle sheaths (Fig. 1a). Espadaea also shows elaboration of these same characteristics, although to a lesser extent. Not unexpectedly, Goetzea elegans does not exhibit strongly developed anatomical adaptations to aridity: its
margins are not inrolled, and by comparison with *Henoonia* and *Espadaeae*, the lamina is thinner, the cutinized outer wall of the adaxial epidermis is thinner, the stomata frequency range is broader, the diameter of the tracheids of the midvein is generally larger, and the midvein is smaller in diameter (Fig. 3a). In addition, *Henoonia* (and *Coeloneurum*) have narrow, spinescent leaf morphology more typical of a xerophyte, in contrast with the broad leaves of *E. amoena* and the still broader leaves of *G. elegans*. The patterns of xeromorphy shown here for *Espadaeae* and *Henoonia* closely parallel with those shown for the wood of these two species by Carlquist (1988).

The large, proximally indurate, sheathed midveins of the Goetzeaceae are noteworthy in possessing both secondary growth and uniseriate rays. The rays found in the midveins (Fig. 1a, 2a, 3c), though small, may be useful in storing water or photosynthates. The midveins, along with the sheathed vascular bundles of higher order veins, may also be herbivore deterrents (Potter and Kummer 1988), especially in areas where drought and nutrient deficit and slow rate of leaf replacement limit the number of leaves available to herbivores (Coley, Bryant, and Chapin 1985).

The scattered, basally distributed druses of *Goetzea elegans* (Fig. 3a) versus the more abundant and evenly distributed druses of the other genera (Fig. 1b, 2b) likely reflects a difference in defense resource allocation (Coley et al. 1983). In a moist forest habitat leaves are readily replaced and thus may not be as heavily defended. In contrast, *Henoonia* (and likely *Coeloneurum*), an inhabitant of dry, poorer soil may best utilize its limited resources in the defense of its leaves rather than their replacement. The leaves of *Goetzea* are probably short-lived relative to those of *Henoonia*; although ecological studies of this nature have not been undertaken. Field studies of these genera are sorely lacking, so conclusions regarding herbivory must be somewhat speculative.

Certain anatomical features, such as thickness of the lamina, thickness of the cuticle, and density of stomata, do exhibit quantitative variation. Comparisons of sun leaves vs. shade leaves or early season leaves vs. late season leaves may have contributed to this variation in quantitative traits. Further study may reveal to what extent this variation can be attributed to the influence of the habitat.

**Systematic Considerations**

The historical controversy regarding the placement of the genera now recognized as comprising the Goetzeaceae has been adequately reviewed elsewhere (Fuentes 1982; Carlquist 1988); alignments with the Sapotaceae (Kramer 1939; Record 1939) and Solanaceae (Radlkofer 1888; Carlquist 1988) have been suggested based on anatomical studies.

*Henoonia, Espadaeae, and Goetzea* display a number of solanaceous features, viz., glandular trichomes and uniseriate trichomes with long terminal cells, sinuous anticlinal epidermal walls, crystal sand and druses, and intraxylary phloem. None of these features singly would be unexpected for any member of the Tubiflorae (Metcalfe and Chalk 1950), but taken as a group, they suggest solanaceous affinities. As pointed out by Radlkofer (1888), the absence of laticifers, the presence of glandular trichomes, and the presence of interxylary phloem are characteristics that argue against the placement of *Henoonia* in the Sapotaceae. The absence of two-armed trichomes also weighs against alignment with the Sapotaceae. The
same suite of features is reported here for *Goetzea* and *Espadaea*, suggesting homogeneity within the Goetzeaceae and a placement within the Solanales close to Solanaceae.

The anatomical features of the Goetzeaceae also share much with the Convolvulaceae (also of the Solanales), but the absence of two-armed trichomes and secretory cells and the presence of crystal sand are more suggestive of Solanaceae. Carlquist (1988) has shown that wood anatomy supports Hunziker's (1979) treatment of Goetzeaceae as a family closely related to Solanaceae. Takhtajan (1986) placed the Goetzeaceae near Solanaceae in the Solanales; while Cronquist (1981) submerged Goetzeaceae within Solanaceae. Although the taxonomic rank of the Goetzeaceae may remain in question, its close relationship to the Solanaceae, as supported by the results of this study, does not.

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The major flavonol glycoside, and acetate, was found with other species. The 6-hydroxylutecin derived condition was studied.

Key words: *Keckiella*, cyanidin 3,5-diglucoside, flavonol, acetate

The genus *Keckiella* is recognized as a subgenus of *Hesperoceae*. The section *Hesperoceae* includes the genus *Keckiella* (Sapindaceae). The pigment, cyanidin 3,5-diglucoside, was studied, including its bioactivity in a new genus. Scogin et al. (1999) demonstrated the presence of the pigment in newly discovered species. The major flavonol glycoside and acetate were isolated from the genus *Keckiella*.

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