RESEARCH ARTICLE

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Roles of mucilage in *Emilia fosbergii*, a myxocarpic Asteraceae: Efficient seed imbibition and diaspore adhesion[1]

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PREMISE OF THE STUDY: Several angiosperm families have myxodiaspory, such as the Asteraceae in which cypselae are frequently wind-dispersed. The roles of mucilage in cypselae remain misunderstood, and the route of water uptake from substrate to embryo remains unknown. In this work, we analyze the fruits of *Emilia fosbergii* aiming to clarify how the water is absorbed and how the structure of the pericarp can be related to the processes of diaspore adhesion and seed imbibition.

METHODS: The anatomy and ultrastructure of the cypselae of *Emilia fosbergii* were analyzed with histochemical tests and light, scanning and transmission electron microscopy. We assessed the roles of mucilage in seed imbibition using apoplasmic tracing with Lucifer yellow and epifluorescence microscopy and in adhesion with a sand assay.

KEY RESULTS: We describe structural and ultrastructural aspects of the exocarpic cells, especially the mucilaginous twin hairs. Lucifer yellow was absorbed only by the twin hairs, the cells where water primarily enters the seed during seed imbibition. In the sand assay, the mucilage was adhesive.

CONCLUSIONS: The twin hairs on the surface of the cypselae can play a dual role in the establishment of new plants of this species. First, these trichomes constitute the main passage for water intake, which is essential for seed imbibition and germination, and after imbibition, they release mucilage that can adhere the diaspore. Therefore, the presence of myxocarpy in Asteraceae could be important in anemochoric species to avoid secondary dispersal.

KEY WORDS apoplasmic tracing; cypsela; *Emilia fosbergii*; mucilaginous trichome; plant establishment; seed dispersion; seed imbibition

Water uptake is a fundamental requirement for the initiation and completion of seed germination (Manz et al., 2005). In permeable seeds, water can be absorbed by the entire seed coat. However, when the seed coat is impermeable, absorption can only be performed in certain regions such as the hilum, chalaza, or micropyle. According to Werker (1997), some extreme conditions can weaken random or predetermined regions in the seed coat, allowing water absorption. Besides the barrier of the seed coat, the pericarp of indehiscent fruits can also restrict absorption or present structures that favor water uptake by the embryo. Many mechanisms for breaking impermeability and promoting imbibition are mentioned in the literature, but little has been published that actually shows the way water passes from the substrate to the embryo.

Species of Asteraceae produce cypselae, dry indehiscent fruits that originate from inferior ovaries, with a single seed attached only by the funiculus (Mirbel, 1813); these fruits are named achenes by some authors (see Marzinek et al., 2008) and are mainly wind-dispersed. These cypselae are usually elongate fruits and have appendages such as enlarged ribs, wings, and hooks, or certain types of epidermal outgrowths (Roth, 1977). The epidermis can have papillae, glandular and nonglandular trichomes, giant cells, and rarely, stomata (Roth, 1977). The trichomes are of several types (see Marzinek and Oliveira, 2010), often biseriate (Robinson, 2009) and have been called twin hairs. The cell walls can be thin or thick, and the protoplast can sometimes have mucilage or crystals (Roth, 1977). Slime or mucilage cells and slime trichomes frequently occur on the surface of cypselae and, as speculated by Roth (1977), may fasten the fruits to the substrate.

Seeds or fruits of many species of angiosperms produce a pectinaceous mucilage (myxospermy) that has multiple functions in seed germination as well as in seedling establishment (Yang et al.,

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2012a). Mucilage has been reported for seeds or fruits of species in 110 families and at least 230 genera of angiosperms (Yang et al., 2012b). On the basis of published and unpublished data, Grubert (1974) recognized slime-producing trichomes in 33 genera of Asteraceae, but *Emilia* was not mentioned. Western (2012) described the occurrence of myxocarpy in Asteraceae, in which fruits can be surrounded by mucilage when put in the water. According to Western, mucilage is deposited in the apoplast of the epidermal cells of the pericarp and is released in response to hydration, thus playing multiple ecological roles. One of these important roles is to promote successful seedling establishment in a low precipitation regime (Yang et al., 2012a).

The twin hairs occur in both aerial and underground organs of species of Asteraceae (Appezzato-da-Glória et al., 2012), and they are especially common in fruits (Grubert, 1974; Marzinek and Oliveira, 2010). In a comparative study of the twin hairs of Asteraceae, Hess (1938) suggested that these trichomes facilitate water absorption by the fruit. Because the seed is retained in an indehiscent pericarp, the fruit wall serves a similar function to the seed coat, mediating water relations between substrate and embryo. Therefore, we asked, how is water absorbed by this type of fruit and does the structure of the pericarp function in the processes of dispersion, diaspore fixation, and seed imbibition?

In this work, we analyzed the structure of the pericarp and followed each step of water entry into the fruits of *Emilia fosbergii*, with the aim of answering the previously stated questions. The study species, *E. fosbergii,* originated in the Old World (Cabrera, 1950), but has a widespread distribution, reproducing only by seeds (Lorenzi, 1991) and occurring as a pioneer in disturbed areas in tropical and subtropical regions (Holm et al., 1997). Species of *Emilia* can have oblong, ribbed, and pubescent cypselae with a pappus of many fine bristles (Bremer, 1994), features that frequently occur in wind-dispersed Asteraceae. We chose *E. fosbergii* as our study object since it is representative of these wind-dispersed Asteraceae.

MATERIALS AND METHODS

Fertile individuals of *Emilia fosbergii* Nicolson were collected in ruderal areas in Botucatu (São Paulo State) and Belo Horizonte (Minas Gerais State) in Brazil. For anatomical studies, 100 mature cypselae were fixed in formaldehyde–acetic acid–50% ethanol for 48 h (Johansen, 1940) and then kept in 70% ethanol (Jensen, 1962). This material was subsequently dehydrated in an ascending ethanol series, embedded in (2-hydroxyethyl)-methacrylate (Leica Microsystems, Heidelberg, Germany), and sectioned at 6–10 μm using a rotary microtome. Sections were stained with 0.05% toluidine blue (O'Brien et al., 1964, modified) in acetate buffer at pH 4.7, mounted with synthetic resin, examined with light microscopy (LM, Primo Star bright field, Zeiss, Oberkochen, Germany), and imaged as digital photomicrographs with a Canon PowerShot A650 (Melville, New York, USA).

Considering the inferior origin of the ovary, we adopted a sensu lato definition of the pericarp, considering the exocarp as derived from the outer epidermis of the inferior ovary, the endocarp from the inner epidermis, and the mesocarp from the ground and vascular regions, according to Martins and Oliveira (2007).

Experimental and control sections of fresh, hand-cut, fully expanded, unripe cypselae were treated with an aqueous solution of ruthenium red (prepared with three crystals of the dye and distilled water added drop by drop until the solution became reddish pink) to detect acidic polysaccharides and a solution of iodine in potassium iodide (prepared with 0.3 g iodine, 1.5 g potassium iodine, 100 mL water) plus a drop of 75% sulphuric acid to detect cellulose (Johansen, 1940).

The hydrophilic character and conformation modifications of mucilage were tested using mature cypselae. Paradermal sections of larger ribs from 20 cypselae were immersed in an aqueous solution of ruthenium red and evaluated with LM to verify the entrance of the reagent into the twin hairs. Assays ended when the entire trichome exhibited a positive reaction. For testing mucilage expansion and the consequent release from the twin hairs, 20 cypselae were soaked in distilled water at room temperature (approximately 25°C), and 20 were soaked in boiled water (approximately 95°C). Mucilage release was assessed by LM observations every 2 h for the room temperature treatment and during the first minute after the immersion of cypselae in the boiled water treatment because of the rapid response.

Adhesive properties of the mucilage were tested in dry sand. Mature cypselae were separated in two groups: 20 dry cypselae and 20 cypselae after mucilage release (boiling water method), which were placed in contact with the sand and immediately evaluated. If sand had adhered, the cypselae were dehydrated to verify the maintenance of the adhesive properties of the mucilage.

For scanning electron microscopy (SEM) analyses of fruit surfaces, cypselae were fixed in 2.5% v/v glutaraldehyde in phosphate buffer (0.1 M, pH 7.2) for 24 h and dehydrated in an ascending ethanol series followed by dehydration to critical point using CO_2. Samples were then mounted on aluminum stubs and coated with gold (Robards, 1978) using a MED010 Balzers Union apparatus. Some cypselae were collected and kept in an incubator at 60°C for 48 h; after drying, they were adhered to stubs and gold-coated as previously described. Cypselae of the sand assay were adhered to stubs and gold-coated. Samples were examined using a Quanta 200 SEM (FEI Company, Eindhoven, Netherlands), and the images were captured digitally.

For transmission electron microscopy (TEM), samples of ovaries from flowers at anthesis, cypselae 48 h postanthesis, and fully expanded unripe cypselae were prepared using conventional methods (Roland, 1978). Samples were fixed in Karnovsky solution (Karnovsky, 1965) for 24 h, postfixed in 1% osmium tetroxide (0.1 M phosphate buffer, pH 7.2) and dehydrated in a graded acetone series for embedding in Araldite resin. Ultrathin sections (50 nm) were contrasted with a saturated solution of uranyl acetate and lead citrate. The sections were examined using a Tecnai G2-Spirit transmission electron microscope (Philips/FEI Co., Eindhoven, Netherlands) at 80 kV.

We followed the methods of Briggs et al. (2005) to observe the apoplasmic pathway. To ensure genetic variability among samples, approximately 1000 intact, randomly chosen mature fruits were immersed in 0.1% (w/v) aqueous Lucifer yellow (LY; Sigma, St. Louis, Missouri, USA) and kept in the dark. Among these 1000 samples, 10 cypselae were taken at 15-min intervals over 8 h (320 total), washed in distilled water, and hand-sectioned with a razor blade. The sections were mounted in water, immediately observed using an Olympus epifluorescent microscope (Olympus, Southall, UK) under blue excitation (filter 490 nm, dichroic mirror 500nm, barrier 515 nm), and digitally imaged with 200 ISO and exposure of 10 s. Ten more samples were embedded in distilled water at similar time periods to assess autofluorescence in the sections.

RESULTS

***Cypselae structure*—**In mature cypselae of *Emilia fosbergii*, the pappus has several thin bristles (Fig. 1A). Each cypsela is elliptical and elongate, with evident ribs; the larger ribs are covered by trichomes and intercalated by intercostal regions with smaller, glabrous ribs (Fig. 1A). When desiccated, the trichomes become twisted (Fig. 1B); these are biseriate claviform trichomes (Fig. 1B–D) that are called twin hairs. The twin hairs accumulate mucilage, which stained pink by ruthenium red (Fig. 1C). The mucilage was released when the cypselae were exposed to water for more than 24 h (compare Fig. 1A with Fig. 1E), thus making the surface of the larger ribs sticky.

The pericarp has a uniseriate exocarp that is composed of the epidermis, which has different types of cells in the larger trichomatous ribs and in the intercostal regions (Fig. 1F–G). In the larger ribs with the twin hairs are large cells (called giant cells) that have lax walls; in the intercostal regions are ordinary cells that are small and quadrangulate in cross section (Fig. 1F–G). The larger ribs have an aerenchymatous and partially collapsed mesocarp; in the other regions, the mesocarp shows well-differentiated fibers (Fig. 1F–G). The endocarp is indistinct, composed of the residues of cell walls of compressed cells (Fig. 1G).

The single seed occupies the entire cypsela chamber (Fig. 1F). The seed coat is collapsed, and only residues of cell walls were observed juxtaposed to the pericarp (Fig. 1G). The endosperm persists as two layers of almost cubic cells with evident nuclei and dense cytoplasm (Fig. 1G). The embryo with two dense, voluminous cotyledons fills the seed (Fig. 1F–G).

Histochemical tests on samples of mucilage from the twin hairs of *E. fosbergii* indicate the presence of pectin (positive result with ruthenium red) and the absence of cellulose (verified by the test with iodine in potassium iodide plus 75% sulphuric acid, not shown).

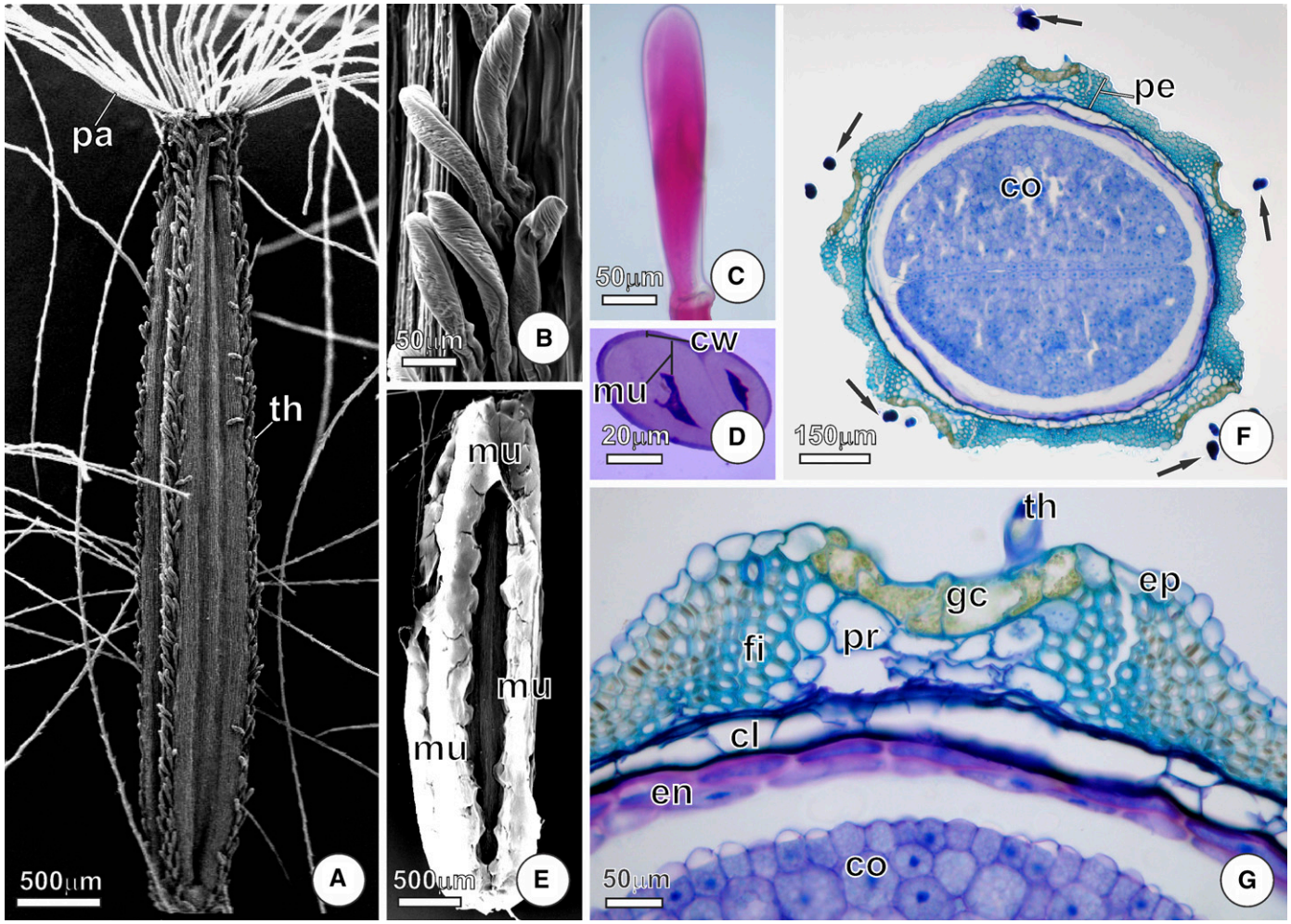

FIGURE 1 Cypsela structure of *Emilia fosbergii*. (A) General view of dry fruit. (B) Detail of (A), showing dried, twisted, and claviform trichomes. (C) Isolated trichome (twin hair) after 2 min in ruthenium red, showing positive reaction for mucilage. (D) Transverse section of a twin hair stained with toluidine blue; see mucilage accumulation in the periplasmic space. (E) Cypsela after mucilage release, showing the mucilage envelope over the larger ribs that hinders the trichomes. (F, G) Transverse sections of the cypsela. (F) General view; note the single seed filling the seed chamber (arrows: trichomes). (G) Detail of (F), showing a larger rib with trichomes. cl, crushed layers of inner pericarp and seed coat; co, cotyledon; cw, cell wall; en, endosperm; ep, ordinary cells of epidermis; fi, fibers; gc, giant cell; mu, mucilage; pa, pappus; pe, pericarp; pr, parenchyma; th, twin hair.

***Exocarpic cell ultrastructure*—**The exocarpic cells differ in thickness and in ultrastructural aspects of the cell walls (Fig. 2), mainly in the outer periclinal face. In the twin hairs, there is a pectocellulosic wall, thicker in the proximal region where it reaches about 2 μm; the cuticle is extremely thin (Fig. 2A, C–D), reaching only 10–20 nm. The giant cells show pectocellulosic cell walls, 0.4–1.0 μm thick in the outer periclinal face; the cuticle is approximately 50 nm thick (Fig. 2E–F). In the ordinary exocarpic cells, the walls are also pectocellulosic and have a sinuous contour on the outer periclinal face; their cell walls are 0.9–1.3 μm thick, and the cuticle is almost 0.250 μm thick (Fig. 2G–I).

The twin hairs have a dense and organelle-rich cytoplasm (Fig. 2A–C), with mitochondria and dictyosomes being particularly abundant (Fig. 2A, B). In these cells, the endoplasmic reticulum is well developed (Fig. 2B), and infrequent plastids contain many starch grains (not shown). In samples at anthesis, mucilage synthesis appears to be in its initial stage in the twin hairs of ovaries, and vesicles are in various stages of budding from the dictyosomes and distributed throughout the cytoplasm (Fig. 2B). Vesicles have also fused with the plasma membrane, and some have opened to secrete the contents into the periplasmic space. The accumulation of secretions in the periplasmic space made it quite difficult to distinguish between the cell wall and the mucilage, especially at the end of the secretion process at about 48 h after anthesis (Fig. 2C–D). Mucilage had accumulated in the periplasmic space, thus compressing the protoplast, which had pulled away from the cell wall and collapsed by the end of the process, leaving the cell filled with mucilage (Fig. 2A–D).

The giant cells have an organelle-poor cytoplasm with a large vacuole that has compressed the cytoplasm against the plasma membrane and the cell wall (Fig. 2E).

The ordinary epidermal cells that cover the intercostal regions have a large vacuole, and plastids predominate among organelles in the cytoplasm (Fig. 2G). The plastids present a dense stroma and oil droplets; such oil droplets were also observed as free oil deposits in the cytoplasm (Fig. 2H). Mitochondria are scarce indicating low metabolic activity.

***Apoplasmic tracing*—**When mature cypselae were kept in water, only the cuticle of the exocarp and seed coat, the cell walls of giant cells in the exocarp, and the mesocarpic fibers autofluoresced (greyish green, Fig. 3A). In the cypselae kept in the LY solution, the entire trichomes fluoresced lemon-yellow from LY by the first evaluation at 15 min (Fig. 3B). By 6 h, LY fluorescence was seen in cells to the interior of the exocarpic giant cell (Fig. 3C); by 7 h, the LY fluorescence was farther inward, reaching the parenchyma in the mesocarp (Fig. 3D). By 8 h, LY fluorescence was recognized in the crushed layers of the pericarp, in the seed coat, and in the residual endosperm (Fig. 3E), reaching the embryo.

***Imbibition and adhesion assays*—**In the paradermal sections of the cypselae treated with ruthenium red, the reagent was detected inside the twin hairs 1 min after exposure, and stain was visible throughout the trichome. After 2 min, all the mucilage within the twin hair had been stained (see Fig. 1C).

When cypselae were immersed in water at room temperature, the mucilage expanded slowly between 24 and 48 h of imbibition. By 48 h, when our evaluation was finished, the content of the twin hairs had not been completely released.

Cypselae immersed in boiled water release mucilage abruptly. Practically all the content of the twin hairs was released 1 min after immersion. Immediately after release, the mucilage exhibited a strong adhesive character, firmly fastening to sand grains that remained adhered even after complete dehydration of the mucilage (Fig. 4A–D). After mucilage release, the twin hairs exhibited typical features, with structured and apparently intact cell walls. The released mucilage was hyaline and appeared sticky, without any evidence of cellulose microfibrils, becoming an amorphous mass when completely dehydrated (Fig. 4D). When dried cypselae were exposed to sand, we did not note any adhesion of sand to the diaspore surface.

DISCUSSION

***Cypselae structure and mucilage composition*—**According to Robinson (2009), Asteraceae trichomes are often biseriate and apparently common in most genera in this family aside from Barnadesioideae, a basal clade. In *E. fosbergii*, these trichomes accumulate mucilage, which is released to the surface when the cypsela is exposed to water.

In this work, we showed that the epidermis of the fruits of *E. fosbergii* has a cuticle of varying thickness, depending on the cell type; the cuticle becomes inconspicuous on the twin hairs. One of the major functions of the plant cuticle is water repellence (Riederer, 2006), so the thin cuticle on trichomes is a feature that facilitates water uptake, making absorption possible.

According to Western (2012), mucilage is a generic term for plant-secreted polysaccharides (mainly pectin and hemicelluloses) or proteoglycans that undergo substantial swelling upon hydration. Besides pectin and hemicelluloses (the true slime), seed mucilage can have an additional cellulosic component; this cellulosic mucilage contains dispersed cellulose microfibrils (Kreitschitz, 2012; Western, 2012). Among 33 genera of Asteraceae, Grubert (1974) reported cellulosic material only in the mucilage of species of *Trixis* and *Senecio*. The results of our histochemical tests clearly show that mucilage in *E. fosbergii* cypselae does not contain cellulose fibrils but has pectins (see Fig. 1C), and thus constitutes true slime. In our assays, this type of mucilage proved to be very sticky and highly efficient at adhering diaspores to the substrate. It is important to emphasize that the water solubility and physical properties of mucilages are dependent upon their chemical composition (see Roshchina and Roshchina, 1993). Due to the physical nature and slow expansion of the mucilage found in *E. fosbergii* cypselae, the presence of hemicellulose is expected, but additional tests are needed for verification.

***Exocarpic cell ultrastructure*—**In the analysis of the three cell types of the exocarp of *E. fosbergii*, the ordinary small cells have the thickest cuticle, and giant cells and twin hairs have thin cuticles (Fig. 1G). In addition, the twin hairs served as the main entrance for water due to the large accumulation of mucilage.

The protoplast structure of the twin hair cells of *E. fosbergii*, primarily the dictyosome structures (Fig. 2B), seems to be related to the synthesis of noncellulosic polysaccharides (see Paiva and Martins, 2011). Young et al. (2008) also associated dictyosomes with the secretion of pectin-rich mucilage. During the stage of intense secretion, the ultrastructure of the protoplast of the twin hair cells, with vesicles budding from dictyosomes and fusing with the plasma membrane, was typical of cells that are metabolically active and involved in secretion (Paiva, 2009). The accumulation of mucilage in

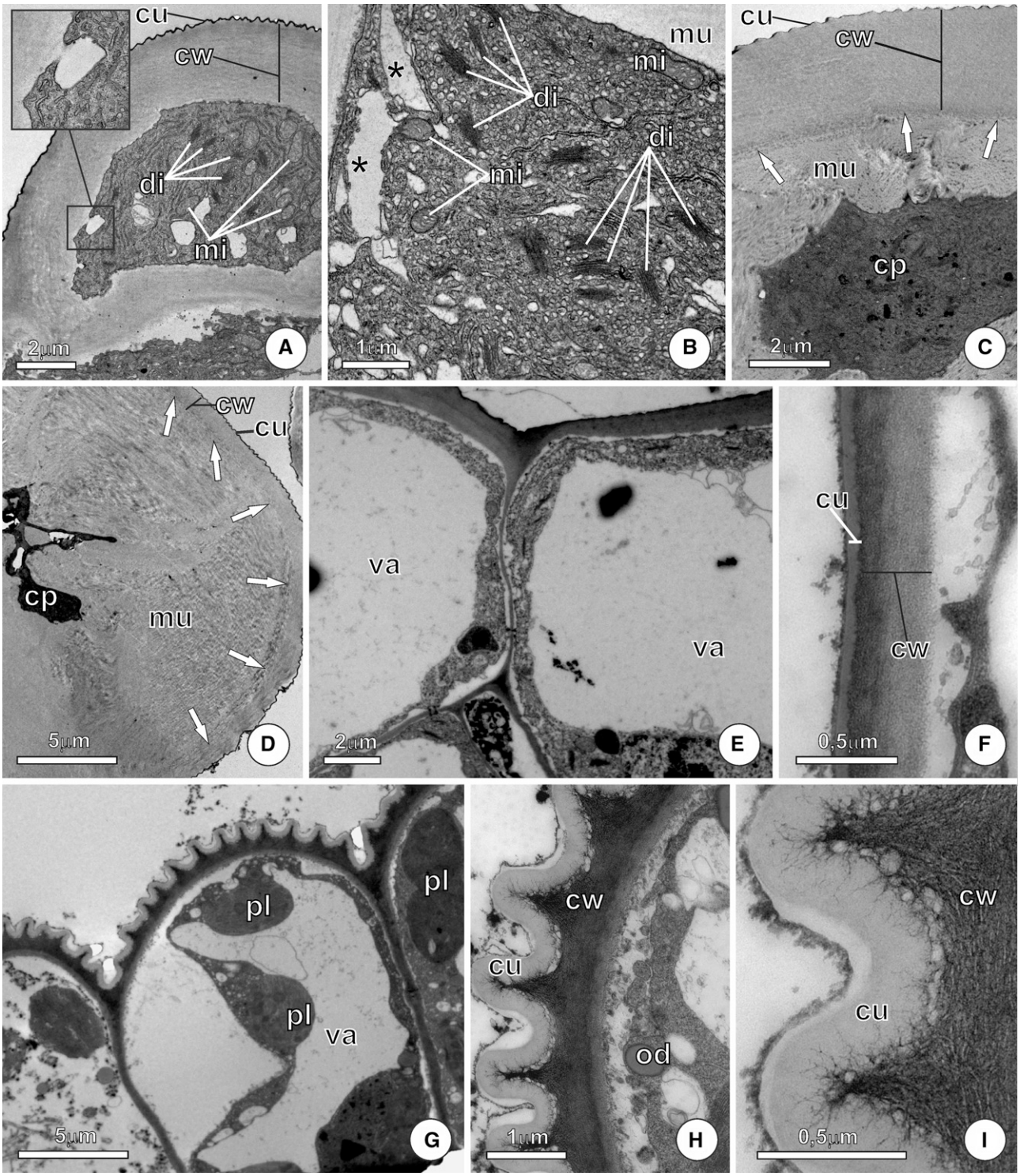

FIGURE 2 Ultrastructure of the epidermis of the fruit of *Emilia fosbergii*. (A–D) Biseriate trichomes (twin hairs). (A, B) At anthesis. (A) Median portion of the trichome showing dictyosomes and mitochondria prevalence in an organelle-rich cytoplasm; note the cell wall covered by an extremely thin cuticle. (B) Detail of the cytoplasm showing dictyosomes and budding vesicles; note mitochondria with well-developed cristae and vacuoles with mucilage (asterisks). Mucilage can be also seen in periplasmic space. (C, D) At 48 h postanthesis; arrows indicate the boundary between the cell wall and the accumulated mucilage. (C) Detail of the cell showing mucilage accumulation in the periplasmic space; note the darkened cytoplasm. (D) Cell with large amount of mucilage in periplasmic space; note the darkened and strongly compressed cytoplasm. (E, F) Giant cells; note a large central vacuole and the organelle-poor cytoplasm. In (F), note the cell wall juxtaposed to the cuticle. (G–I) Ordinary cells of the intercostal region; note the vacuolated cells and a striated cell wall with a thicker cuticle. cp, collapsed protoplast; cu, cuticle; cw, cell wall; di, dictyosome; mi, mitochondria; mu, mucilage; od, oil droplet; pl, plastid; va, vacuole.

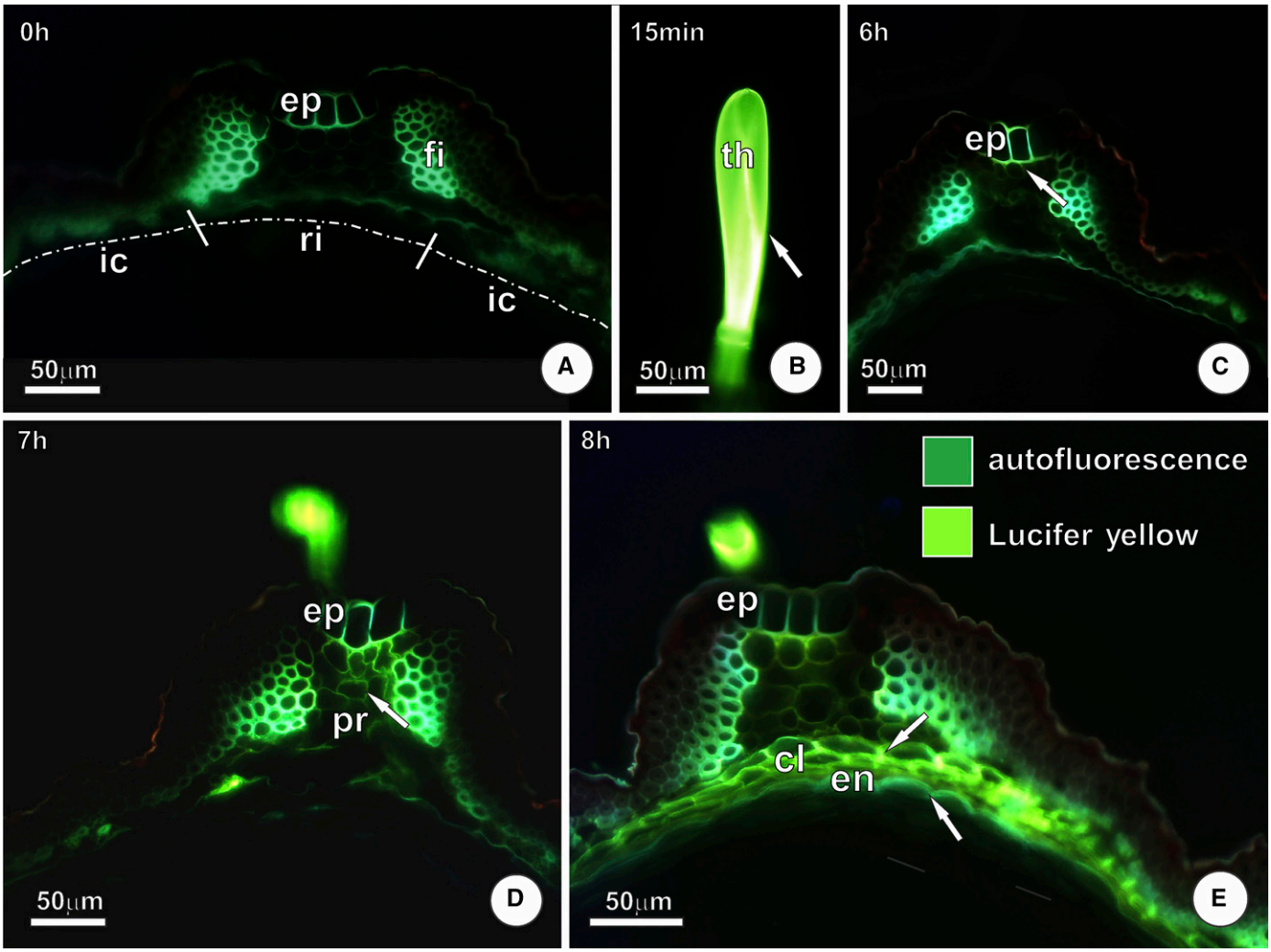

FIGURE 3 Cypselae of *Emilia fosbergii* showing apoplasmic route traced using Lucifer yellow (LY) (transverse sections). All images were taken using a 20× objective lens and an exposure time of 10 s (200 ISO). (A) Control (intact fruit immersed in distilled water without LY), showing greyish green autofluorescence only from the cuticle, giant cell walls, and mesocarpic fibers. For reference, compare with Fig. 1G. (B) Detail of twin hair on a fruit immersed in LY for 15 min; lemon-yellow fluorescence indicates LY inside the trichome. (C) Pericarp of a cypsela immersed in LY for 6 h; note that LY has begun to cross the epidermis. (D) Cypsela immersed in LY for 7 h; note LY fluorescence in the subepidermal parenchyma cells. (E) Cypsela immersed in LY for 8 h; note crushed layers (inner pericarp and seed coat) and the endosperm with LY fluorescence. The color scale allows distinguishing between autofluorescence and LY fluorescence in all images. Arrow, cell walls with LY fluorescence; cl, crushed layers; en, endosperm; ep, epidermis; fi, fiber; ic, intercostal region; pr, parenchyma; ri, rib; th, twin hair.

the periplasmic space (Fig. 2A-D) is a characteristic aspect of mucilage-producing cells, including those in seeds (Hyde, 1970; Van Caeseele et al., 1981; Young et al., 2008).

In *E. fosbergii*, mucilage occupies a large volume of the cell lumen of the twin hairs, greatly compressing the protoplast (Fig. 2D), as reported for other types of mucilage-secreting cells (Hyde, 1970; Trachtenberg and Fahn, 1981). In the mature twin hairs of *E. fosbergii* we observed, the protoplast was completely collapsed, as reported for the seed coat of canola (*Brassica campestris*) (Van Caeseele et al., 1981). When fruits are maturing and dessicating, mucilage remains inside the twin hairs and does not interfere with the typical anemochoric dispersion of this species. Our observation of mucilage in the cypselae of *E. fosbergii* permits us to characterize them, for the first time in the literature, as myxocarpic diaspores.

Myxodiaspory is a common feature in several families (Acanthaceae, Asteraceae, Brassicaceae, Lamiaceae, Linaceae, Plantaginaceae, Poaceae, and others), especially in species with ephemeral annual life cycles. Sun et al. (2012) showed that mucilage plays an important role in the dispersion of seeds of *Alyssum minus* (Brassicaceae) by aiding adhesion to soil, promoting seed hydration via increased surface contact, and serving as a water reservoir for germination. Since the report of Hanausek (1910), mucilaginous trichomes have been described in Asteraceae fruits, but only in the present work have their functions been experimentally tested, even though there has been speculation about their roles in water absorption (Hess, 1938) and adhesion to a substrate (Roth, 1977). Gutterman and Shem-Tov (1997) and Yang et al. (2012b) emphasized anchorage of seeds to soil particles as one of the functions of

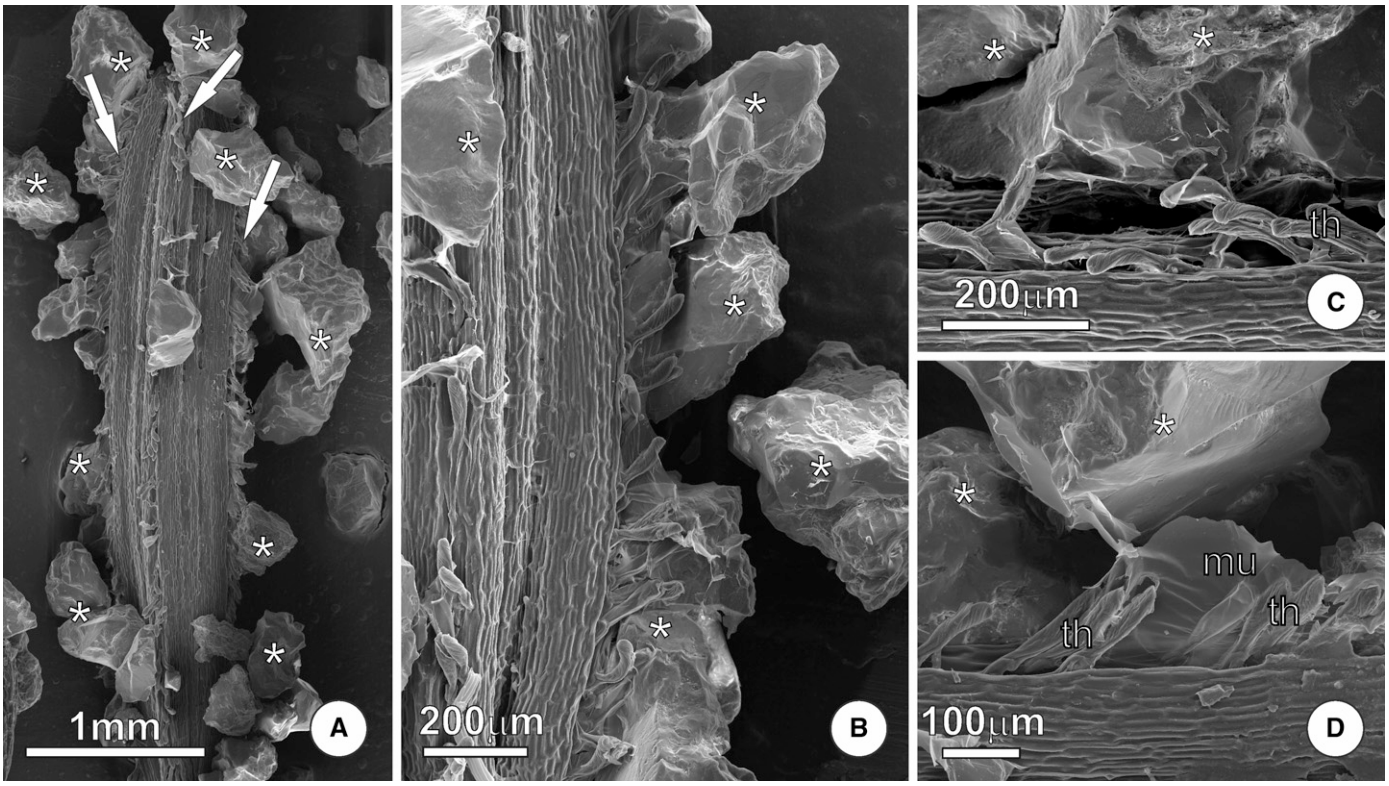

FIGURE 4 SEM adhesion assay of cypselae of *Emilia fosbergii*. (A) General view of a cypsela with sand particles adhered to the twin hairs (arrows). (B) Detail of a cypsela, highlighting sand particles over only the larger ribs. (C, D) Details showing the twin hairs and the released mucilage adhering to sand. Asterisk, sand particle; mu, mucilage; th, twin hair.

mucilaginous seed coats because of the adhesive qualities of mucilage, and our tests corroborate their opinion. We have not found any references to mucilaginous seeds for the Asteraceae, but in many species, the pericarp of the diaspores shows mucilaginous cells similar to the twin hairs we have described here for *E. fosbergii* (see Grubert, 1974; Mouradian, 1995; Kreitschitz and Vallès, 2007; Kreitschitz, 2012).

Myxodiaspory has been proposed to play a number of roles, including facilitation of seed hydration, regulation of germination by affecting oxygen entry into the seed, and mediation of seed dispersal through adhesion to soil or animal vectors (Western, 2012). The data obtained here indicate that the fruits of *E. fosbergii* have structural specializations that allow water absorption and, considering the adhesive properties of the mucilage, favor fixation to the substrate.

After wind dispersal of the cypselae, the twin hairs establish contact between the cypselae and the substrate, thereby absorbing and distributing water across the pericarp, the seed coat, and the endosperm, reaching the embryo and ensuring imbibition. Although Hess (1938) referred to expansion in the pericarpic trichomes in Asteraceae, we did not observe any expansion of the trichomes in our evaluations, only that the mucilage had been released outside of the trichome.

Imbibition and adhesion assays—The observation of LY fluorescence inside the twin hairs, the first place where water was registered in the diaspore, experimentally corroborated the suggestions of Hanausek (1910) and Hess (1938) about one of the roles of these trichomes. It is important to emphasize that the intense lemon-yellow fluorescence observed within the twin hairs after soaking is probably the result of the presence of mucilage. In these trichomes, the LY solution was not limited to the cell wall, but also occupied the entire lumen. In the other cells, autofluorescence is greyish green and limited to the cell wall, indicating an apoplasmic path of water entrance (see Fig. 3).

Our results show a slow, gradual release of mucilage from the twin hairs of *E. fosbergii* upon hydration. On the other hand, these substances are strongly hydrophilic, facilitating the entrance of water into the twin hairs within just a few minutes of water contact, as shown by the LY assay.

The light weight of the cypselae of *E. fosbergii* and the adhesive properties of the mucilage facilitated highly efficient adhesion to the substrate, even though the mucilage is limited to the twin hairs. The fast hydration of the trichomes and the slow release of mucilage suggest that adhesion could be a secondary role. The primary function of mucilage in *E. fosbergii* cypselae is probably to facilitate water entrance and the consequent seed imbibition. The assay using LY and sand with these diaspores demonstrated the properties of water uptake by twin hairs and the adhesive properties of mucilage, respectively. Our conclusions corroborate those of Gorai et al. (2014) who stated that mucilage on fruit enlarges the contact surface for the substrate, to ensure adherence and avoid any secondary dispersal caused by any wind over the pappus.

According to Young and Evans (1973), mucilaginous seeds do not require soil coverage for germination. In fact, our results allow the inference that quick water absorption provided by the presence of mucilage in the twin hairs ensures germination success. In this

way, our results show that in Asteraceae, myxocarpic diaspores are not necessarily restricted to species in dry habitats, as reported by Kreitschitz (2012) for *Artemisia*.

The adaptation of *E. fosbergii* to ruderal or disturbed environments and to quick colonization of new habitats explains the success of this species around the world (Holm et al., 1997). Its production of slime on the surface of its cypselae contribute significantly to its invasive character, as has been documented for other Asteraceae such as *Artemisia* and *Neopallasia* (Kreitschitz and Vallès, 2007) and *Matricharia* (Inceer, 2011), and for the grass *Eragrostis pilosa* (Kreitschitz et al., 2009). However, in all of these species, the mucilage envelope is produced by mucilaginous cells in the fruit coat instead of by mucilaginous twin hairs, as in *E. fosbergii*. Whether the mucilage is produced in mucilaginous cells or trichomes in a species, the ecological role is the same. Young and Evans (1973) also stated that seed mucilage is a common characteristic in weeds adapted to poor soil, and they appear to provide an ecological advantage to species that colonize disturbed habitats, as proposed by Yang et al. (2012b).

Since *E. fosbergii* represents features typical for the Asteraceae, such as anemochoric cypselae, we believe that the mechanism of seed imbibition we described could be present in other wind-dispersed species of the family, especially those that, similar to *Emilia*, are adapted to ruderal and disturbed environments.

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