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# ANATOMY, HISTOCHEMISTRY AND PHENOLIC COMPOUNDS CONTENT OF LEAVES FROM *Omphalea oleifera* Hemsl. (EUPHORBIACEAE) IN RESPONSE TO DAMAGE BY *Urania fulgens* WALKER

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## SUMMARY

Leaves of *Omphalea oleifera* Hemsl. damaged by *Urania fulgens* Walker were examined. Leaves were anatomically described, histochemical tests were performed and the content of lignin and phenolic acids estimated. Morpho-anatomical features were similar in damaged leaves and the control, but minor histochemical differences were observed. The tissues adjacent to the damage showed lignin deposits and calcium oxalate

crystals. The estimated amounts of lignin and caffeic, ferulic and chlorogenic acids were respectively 11% and 109.65, 16.58 and 0.082 $\mu\text{g}\cdot\text{g}^{-1}$  dry wt in the damaged leaves, whereas for the intact were 7%, 97.65, 5.48 and 0.051 $\mu\text{g}\cdot\text{g}^{-1}$  dry wt. The results suggest that the insect damage triggers induced responses in *O. oleifera* including production and accumulation of phenolic compounds and calcium oxalate crystals on the leaf tissues.

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## Introducción

*Omphalea* L. (Euphorbiaceae) is a genus of canopy lianas, shrubs and trees, comprised of ~20 tropical species with centers of diversity and endemism in the Caribbean and Madagascar (Gillespie, 1997; Radcliffe-Smith, 2001). The genus is represented in Mexico by *Omphalea oleifera* Hemsl.; individuals are trees 25-30m tall that form part of the canopy and sub-canopy of the high evergreen tropical rainforest of the states of Oaxaca and Veracruz (Dirzo and Mota-Bravo, 1997). The larval stage of diurnal moths of the genera *Urania* Fabricius, *Chrysidia* Hübner and *Alcides* Hübner, all belonging to the subfamily Uraniinae (Lepidoptera: Ura-

niidae) primarily feed on *Omphalea* leaves (Lees and Smith, 1991). At the Estación de Biología Tropical Los Tuxtlas, Veracruz, the larvae of *Urania fulgens* Walker 1854 feed on *O. oleifera* to an extent that has been considered by some authors as an uncommon case of high magnitude defoliation (Dirzo and Mota-Bravo, 1997).

Studies of *Omphalea* have focused on chemistry and taxonomy. Kite *et al.* (1991, 1997) reported the presence of alkaloidal glycosidase inhibitors in the species *O. diandra* and *O. queenslandiae*, and other authors have described features considered common in the genus such as white or red latex, nonarticulated laticifers, extrafloral nectaries, liana habit with tendril-like climbing

stems, mushroom-shaped androecia, and large fruits (Rudall, 1994a, b; Gillespie, 1997; Gillespie and Ambruster, 1997). Regarding *O. oleifera*, the seedlings content of some secondary metabolites (Del Amo *et al.*, 1986), its massive defoliation by *U. fulgens* and the presence of peduncular extrafloral nectaries, have been reported (Dirzo and Mota-Bravo, 1997) and the character afterwards confirmed and described (Aguirre *et al.*, 2013). Though it is known that plants as well as herbivores have developed molecular, physiological or behavioral adaptations to cope with the deleterious effects in their relationship (Konno, 2011), the effects of *O. oleifera* on the larvae of *Urania fulgens* or viceversa are unknown.

Plants exhibit a wide gamut of induced responses to the damage caused by pathogens and herbivores. Particularly, the induced responses that currently decrease the negative fitness consequences of attacks on plants are termed 'induced defenses' (Karban and Balwind, 1997). These responses can be morphological, chemical or a combination of both. There are numerous examples of constitutive (always expressed in the plant) and chemical plant responses (see Karban and Balwind (1997) and references therein). The majority of plants produce phenolic acids or their derivatives such as phytoalexins, flavonoids and lignin (Harborne, 1988). In addition to a structural role, lignin confers protection

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## KEYWORDS / Calcium Oxalate / Histochemistry / *Omphalea* / Phenolic Acid /

Received: 01/08/2015. Modified: 06/20/2016. Accepted: 06/23/2016.

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## ANATOMÍA, HISTOQUÍMICA Y CONTENIDO DE COMPUESTOS FENÓLICOS DE HOJAS DE *Omphalea oleifera* Hemsl. (EUPHORBIACEAE) EN RESPUESTA AL DAÑO POR *Urania fulgens* WALKER

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### RESUMEN

Se examinaron hojas de *Omphalea oleifera* Hemsl. dañadas por acción de *Urania fulgens* Walker. Se describió la anatomía foliar, se practicaron pruebas histoquímicas y se estimó el contenido de lignina y ácidos fenólicos. Las características morfo-anatómicas fueron similares en las hojas dañadas y control, pero se observaron pequeñas diferencias histoquímicas. Los tejidos adyacentes al daño mostraron depósitos de lignina y cristales de oxalato de calcio. El contenido estimado de

lignina y ácidos caféico, ferúlico y clorogénico fue respectivamente 11%; 109,65; 16,58 y 0,082  $\mu\text{g}\cdot\text{g}^{-1}$  peso seco en las hojas dañadas, mientras que en las intactas fue de 7%; 97,65; 5,48 y 0,051  $\mu\text{g}\cdot\text{g}^{-1}$  peso seco. Los resultados sugieren que el daño causado por la larva de *Urania fulgens* desencadena respuestas inducidas en *O. oleifera* que incluyen la producción y acumulación de compuestos fenólicos y cristales de oxalato de calcio en los tejidos de las hojas.

## ANATOMIA, HISTOQUÍMICA E CONTEÚDO DE COMPOSTOS FENÓLICOS DE FOLHAS DE *Omphalea oleifera* Hemsl. (EUPHORBIACEAE) EM RESPOSTA DANO POR *Urania fulgens* WALKER

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### RESUMO

Folhas de *Omphalea oleifera* Hemsl danificadas por *Urania fulgens* Walker foram examinadas. A anatomia da folha foi descrita, testes histoquímicos realizados e estimado o conteúdo de lignina e ácidos fenólicos. As características morfoanatômicas foram similares nas folhas danificadas e no grupo controle, entretanto foram observadas pequenas diferenças histoquímicas. Os tecidos adjacentes ao dano evidenciaram depósitos de lignina e cristais de oxalato de cálcio. O conteúdo estimado

de lignina e ácidos caféico, ferúlico e clorogénico foi respectivamente 11%; 109,65; 16,58 e 0,082  $\mu\text{g}\cdot\text{g}^{-1}$  peso seco nas folhas danificadas, enquanto que, nas folhas intactas foi 7%; 97,65; 5,48 e 0,051  $\mu\text{g}\cdot\text{g}^{-1}$  peso seco. Os resultados sugerem que os danos causados pela larva de *U. fulgens* desencadeia respostas induzidas em *O. oleifera* incluindo a produção e acumulação de compostos fenólicos e cristais de oxalato de cálcio nos tecidos das folhas.

against pathogens and insects (Swain, 1979; El Modafar and El Boustani, 2004), while caffeic, p-coumaric, ferulic and sinapic acids participate in cell wall composition, singly or in a range of sterified forms (Harborne, 1988). Another defense-related adaptations are the calcium oxalate crystals in any of its forms (Hanley *et al.*, 2007).

This study is a preliminary exploration of the relationship between *O. oleifera* and *U. fulgens* from the plant perspective. The aim was to evaluate the plant responses after insect damage by comparing morphological, anatomical and chemical features of damaged leaves (consumed by the larvae) and controls. The leaves were examined by light microscopy and scanning electron microscopy (SEM) techniques, and its anatomical and histochemical features described. The acid detergent fiber procedure was used

to estimate the lignin content, whilst phenolic acids (caffeic, coumaric, ferulic, chlorogenic) were analyzed by HPLC.

### Materials and Methods

#### Plant material

Twenty five mature *Omphalea oleifera* leaves were randomly collected before (control) and 25 after (damaged) the arrival of *Urania fulgens*. Leaves were considered damaged when they showed signs of the larval attack (Figure 1A, B). The sampling was done at the Estación de Biología Tropical Los Tuxtlas-UNAM, Veracruz, México, located at 18°34'-18°36'N and 95°04'-95°09'W (García-Guzmán and Dirzo, 2001).

#### Histochemistry

Transversal sections of leaves were fixed in FAA (formal-

dehyde, acetic acid, 96% ethanol, water; 2:1:10:7) for 24h, then dehydrated through an ethanol series until 100% ethanol and finally embedded in Paraplast® blocks (Ruzin, 1999). Transverse sections (8-10  $\mu\text{m}$ ) were cut with an AO 810 rotatory microtome and placed on slides used for the following histochemical tests: periodic acid-Schiff reagent to detect non soluble polysaccharides, naphthol blue-black for proteins, oil red O for lipids, phloroglucinol-HCl for lignin, vanillin-HCl for hydrosoluble tannins and lugol for starch (Jensen, 1962; López *et al.*, 2005). Observations and micrographic records were made with an Olympus Provis AX70 light microscope.

#### Scanning electron microscopy

Foliar morphology was observed with a Jeol JSM5310-LV SEM. Free-hand and microto-

me transverse sections of 25  $\mu\text{m}$  thick were dehydrated through an ethanol series to 100% ethanol. The dehydrated material was dried using an CPD 030 (Bal-Tec) critical point drier, mounted onto aluminum stubs using double-sided carbon tape, and gold coated using an Desk II (Denton Vacuum) sputter-coater. Leaf sections of 1  $\times$  1 cm were similarly processed for the observations of the abaxial and adaxial faces.

#### Phenolic compounds

Lignin: Content percentage was estimated by triplicate, using the Van Soest *et al.* (1991) method.

Phenolic acids: Dried and ground leaves (0,1g) were extracted at 60°C for 5min with 5ml of 80% aqueous methanol. The extracts were filtered and the solvent eliminated under reduced pressure. The residue was redissolved

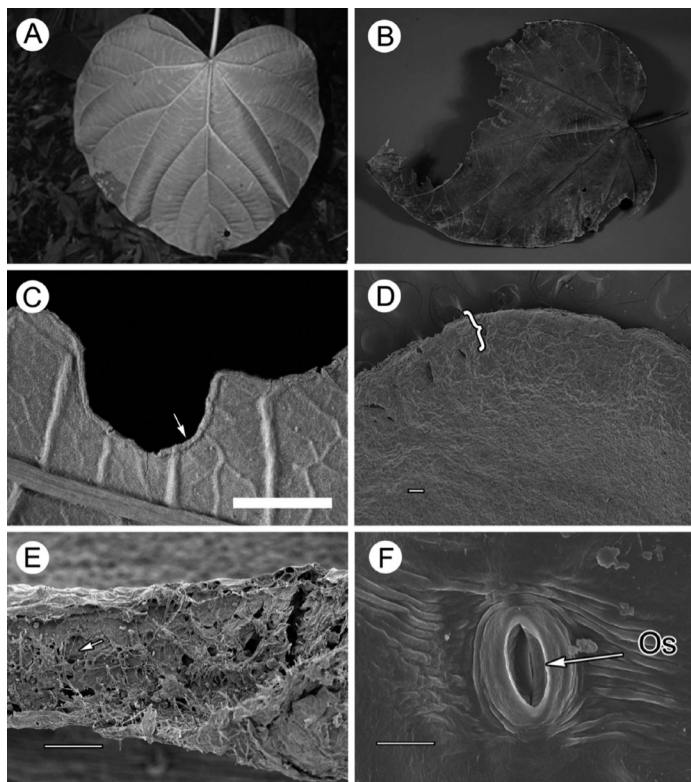


Figure 1. *Omphalea oleifera*. A: control leaf, B: leaf damaged by larvae of the diurnal moth *Urania fulgens* showing ash-colored zones, C-F: SEM micrographs except C, C: the border or scar formed by lignin deposits (arrow), D: width of the scar at the edge of a damaged leaf (bracket), E: the damaged zone with unorganized tissues with a druse section (arrow), F: anomocytic stoma with perpendicular cuticular striations (arrow), Os: ostiole. Scale bars are 3.17mm in C; 100µm in D and E; 10µm in G and F.

in 5ml MeOH and filtered through a nylon membrane (0.45µm pore). An aliquot of the filtrate (40µl) was analyzed with a HPLC apparatus (Merck-Hitachi LaChrom) equipped with a RP-18 column (250×4mm, 5µm particle size). The solvent system consisted of: A) methanol, and B) KH<sub>2</sub>PO<sub>4</sub>, pH 2.4 at a flow rate of 1.5ml·min<sup>-1</sup>, using the following gradient in both samples and standards: 15 to 55% A in 5min, 55 to 80% A in 5min, 80 to 100% A in 2min, 100% A in 8min and 100-15% A in 5min. Caffeic (Sigma-Aldrich) coumaric (Sigma-Aldrich), ferulic (Merck) and chlorogenic (Sigma-Aldrich) acids were used as standards at 1mg·ml<sup>-1</sup>. Retention times were 9, 15.2, 13.7 and 9.2min, respectively. The samples were analysed twice and the variation coefficient was <5%.

## Results

Morpho-anatomical features were similar in the damaged and control leaves; however, the histochemical tests for lignin and the druse deposits

were more notorious in the damaged ones (Table I).

## Morphology

*O. oleifera* leaves are alternate and unifoliate. Leaf texture is coriaceous. The lamina is complete, simple and ovate-cordiform, 9-44.5cm long and 11-39cm wide when intact. The apex is acute to acuminate, the base is cordiform and the margin complete. Venation is reticulate, including veinlets (Figure 1A). The petiole apex shows two glands. The damaged leaves show a superficial off-white region circumscribed to the edges of damaged regions (Figure 1B). When observed in the SEM the region appears as a darkened border of 200 to 300µm wide (Figure 1C) that corresponds to lignin deposits (Figure 1D). The tissues of this region are morphologically disorganized (Figure 1E). Leaves are amphistomatic and possess anomocytic stomata with perpendicular (Figure 1F) or parallel (Figure 2G) cuticular striations surrounding the stoma. The cuticle can be smooth (Figure 2H) or covered with epicuticular wax (Figure 2I).

## Calcium oxalate crystals (COC)

Damaged and control leaves show druses in the epidermal cells of both surfaces (Figure 2J). The druses are projected through the external

periclinal wall of the epidermis, giving the leaf a glandular appearance in the SEM (Figure 2K). The wall can also appear broken and the crystals expelled (Figure 2I). Traverse sections near the damaged regions show accumulations of druses (Figure 3M).

## Trichomes

Simple uniseriate non-glandular trichomes are mainly present in the midrib and secondary veins, and scarcely in the lamina of both leaf sides, going from one or two to five, or absent, in a 1cm<sup>2</sup> area. The cuticle of the trichomes is papillose (Figure 3N). Big sized secretory glands (12-18) are present in the abaxial face, along and parallel to the margin (Figure 3O). In mature leaves, the glands appear to have lost their contents, leaving only remnants (Figure 3P). Big glands are surrounded by 5-6 smaller glands, not visible to the naked eye (Figure 3Q).

## Tissues

The transverse sections of the abaxial and adaxial surfaces show a single-layered epidermis formed by thin walled cells and an evident cuticle that reacted positively to oil red O, indicating presence of lipids (Figure 3R). The leaf is bifacial according to the mesophyll arrangement. In transversal sections the adaxial face shows

TABLE I  
MORPHOLOGICAL, ANATOMICAL AND HISTOCHEMICAL  
CHARACTERS OF THE LEAVES OF *Omphalea oleifera*

| Character                   | Character state   |
|-----------------------------|---|
| Mesophyll type              | Bifacial  |
| Stomata disposition         | Amphistomatic   |
| Stomata type                | Anomocytic  |
| Cuticular ornamentation     | Absent or with striations parallel or perpendicular to the stoma  |
| Epidermis                   | Single-stratified, on both leaf sides   |
| Collenchyma                 | Lacunar   |
| Adaxial parenchyma          | Palisade, two or three cellular strata  |
| Abaxial parenchyma          | Spongy, three or four strata, with intercellular space  |
| Lipids                      | Present in the cuticle  |
| Non-soluble polysaccharides | In cell walls and laticifers  |
| Protein bodies              | In cytoplasm of tissues and laticifers  |
| Lignin                      | Present in damaged zones  |
| Calcium oxalate crystals    | Druses distributed in epidermal cells, spongy and palisade parenchyma and parenchyma of the vascular tissue. Mainly in damaged areas. |
| Tannins                     | In idioblasts   |



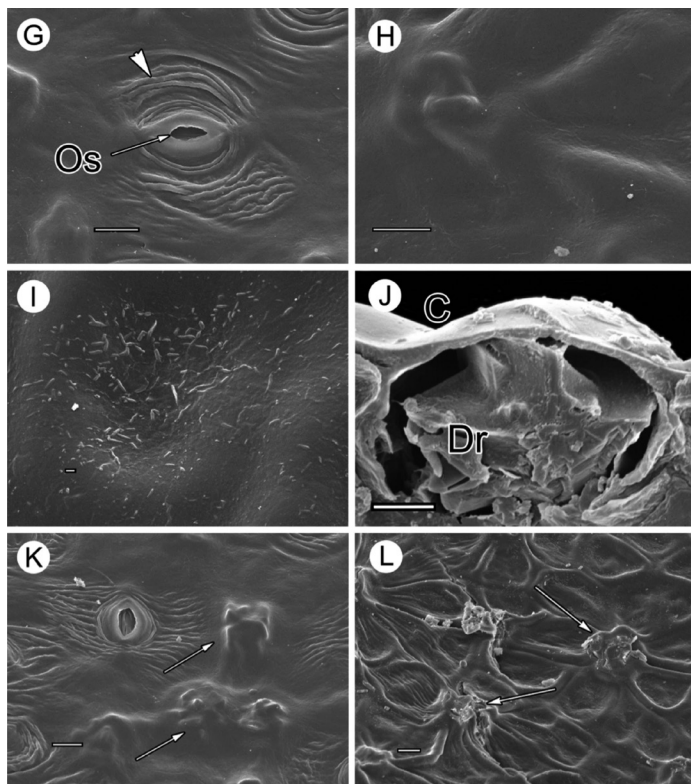


Figure 2. SEM micrographs. G: anomocytic stoma with parallel cuticular striations (arrowheads), H: smooth cuticle on the abaxial face of the leaf, I: epicuticular waxes on the cuticle from the adaxial face of the leaf, J: druse inside the epidermic cells from the adaxial face, K: external periclinal wall of the epidermis where druses are notorious (arrows), L: druses breaking the external periclinal wall of the epidermic cells (arrows). Os: ostiole, C: cuticle, Dr: druse. Scale bars are 10µm in F, G, H, K and L; 1µm in I; 5µm in J.

subepidermal lacunar collenchyma and two or three cellular strata of palisade parenchyma (Figure 4S). On the other hand, the abaxial face has three or four strata of spongy parenchyma formed by cells of variable size and shape and with intercellular spaces (Figure 4T). The midrib and secondary veins of damaged and control leaves characteristically feature druses in the parenchyma and idioblasts with tannins (Figure 4U, V). Epidermal cell walls, alongside collenchyma, spongy and palisade parenchymata, reacted positively to tests for the presence of non-soluble polysaccharides; protein bodies were noticed in the cytoplasm content, and starch was not detected. The lignin test was positive in the epidermis of damaged leaves, appearing as a thick border in damaged regions (Figures 1C and 4W); the same sites sho-

wed accumulations of druses (Figure 3M).

#### Phenolic compounds

The estimated content of phenolic compounds was higher in damaged leaves (Table II), but there were no statistical difference between leaf groups (Mann-Whitney  $U=94.5$ ,  $n=15$ ,  $p=0.4553$ ).

#### Discussion

The lack of studies on *O. oleifera* remarks the importance of the foliar characters herein observed and described. The presence of druses that nearly fill the cell lumen of the diverse mature tissues is a character reported for the first time in the genus *Omphalea*, but previously observed in *Conceveiba guianensis* (Roth, 1981) and species from the *Conceveibinae* subtribe (Murillo,

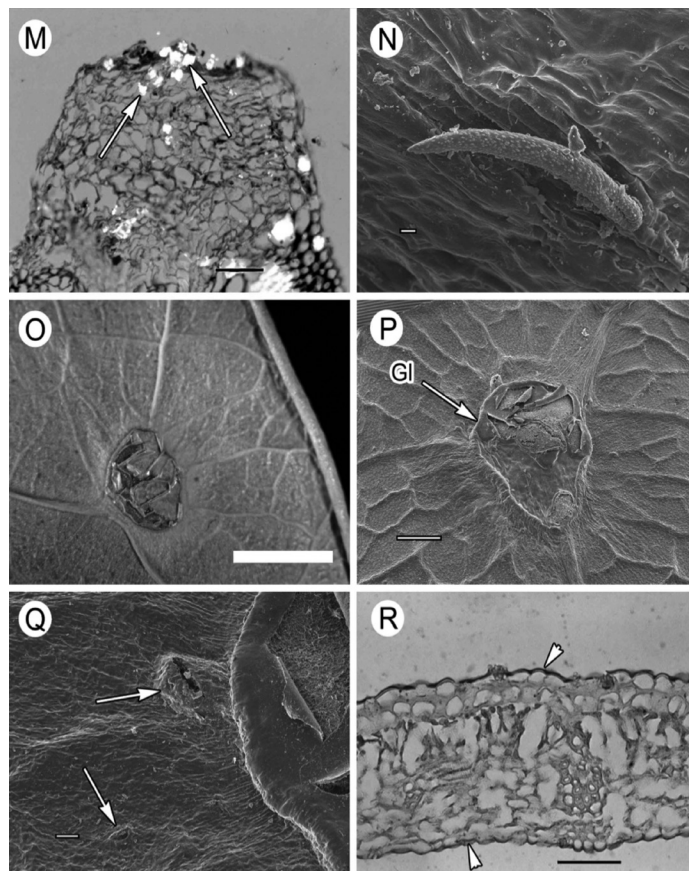


Figure 3. Light and SEM micrographs. M, druses (arrows) in the zone damaged by the herbivore, N: non-glandular simple unicellular trichome over the midrib with a papillose cuticle, O: secretory gland or extrafloral nectary on the abaxial face, P: closer view of the extrafloral nectary (arrow), Q: the smaller secretory glands (arrows) situated around the bigger secretory gland, R: bifacial leaf with unistratified epidermis on adaxial and abaxial faces with arrowheads pointing at cuticle (note the druses breaking the cell epidermis). Gl: gland. Scale bars are 20µm in M; 10µm in N, 3,17mm in O; 1mm in P; 100µm in Q and R.

2002). The druses were abundant in the epidermis of damaged leaves, contrasting with the controls, as evidenced by the SEM observations. Calcium oxalate crystals (COC) are a common trait of Euphorbiaceae and can be found as styloids that tear the epidermis giving the dehydrated leaf blade a rough surface, as in the genus *Claoxylon* and *Micrococca* (Kabouw *et al.*, 2008), as polygonal crystals within the mesophyll (Levin, 1986) or as druses in the palisade parenchyma, mesophyll and veins (Levin, 1986; Hussin *et al.*, 1996; Murillo, 2002; Kabouw *et al.*, 2008). Further studies should confirm the consistency of this character in *Omphalea* and determine its presence and possible variation along the leaf ontogeny.

The major functions proposed for COC in plants are bulk calcium regulation, metal detoxification and guard against herbivores, and the increased production of COC has been traditionally viewed as a defense response (Finley, 1999; Jáuregui-Zúñiga and Moreno, 2004; Franceschi and Nakata, 2005). The defensive role holds up in some plant species (Ward *et al.*, 1997; Molano-Flores, 2001; Ruiz *et al.*, 2002; Jáuregui-Zúñiga and Moreno, 2004; Korth *et al.*, 2006; Handley *et al.*, 2007) albeit not in others (Xiang and Chen, 2004; Nagaoka *et al.*, 2010) and some studies indicate that production of COC is increased even as a result of artificial herbivory, as reported for raphides in *Sida rhombifolia* (Molano-Flores, 2001).

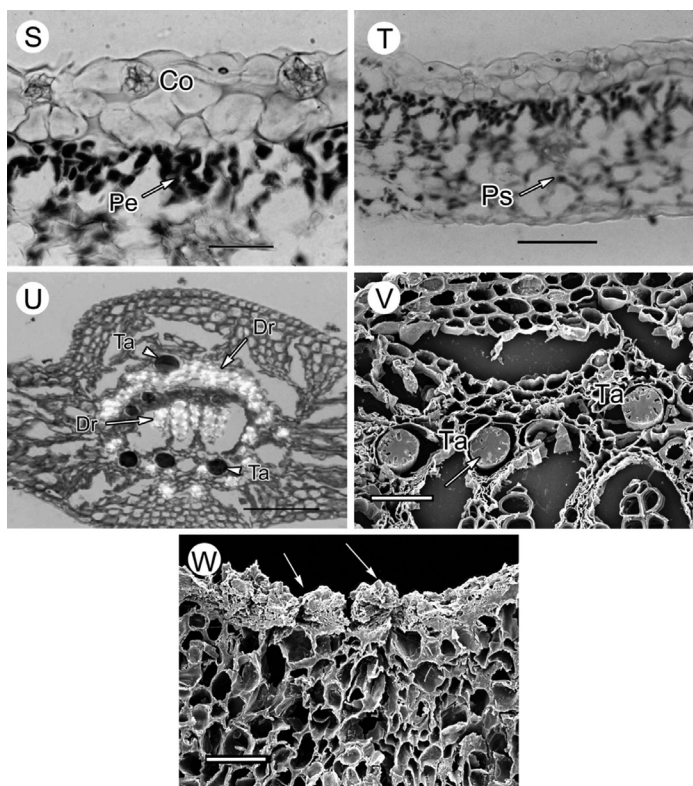


Figure 4. Light and SEM micrographs. S, the lacunar collenchyma and palisade parenchyma (arrow), note the druse on the epidermal cells; T, leaf cross section showing spongy parenchyma with irregular intercellular spaces (arrow); U, druses (arrows) and idioblasts with tannins (arrowheads); V, close up of idioblasts with tannins W, traverse section of a damaged leaf with evident lignin deposits (arrows). Co: collenchyma, Pe: palisade parenchyma, Ps: spongy parenchyma, Ta: tannins, Dr: druse. Scale bars are 6µm in S; 38µm in T; 70 µm in U; 50µm in V and W.

TABLE II  
PHENOLIC COMPOUNDS CONTENT OF  
*O. oleifera* LEAVES

| Compound         | Damaged ( $\mu\text{g}\cdot\text{g}^{-1}$ dry wt) | Control ( $\mu\text{g}\cdot\text{g}^{-1}$ dry wt) |
|------------------|---|---|
| Caffeic acid     | 109.65  | 97.65   |
| Coumaric acid    | 106.98  | 140.32  |
| Ferulic acid     | 16.58   | 5.48  |
| Chlorogenic acid | 0.082   | 0.051   |
| Lignin           | 11%   | 7%  |

The invertebrate herbivores are affected by the leaf structural traits at a fine scale. It was suggested that silica and COC are deterrents that affect the herbivores by the abrasion of its chewing mouthparts (Lucas *et al.*, 2000). This negative impact was later confirmed (Park *et al.*, 2009). Further, an increase of calcium also has been reported as a strategy to reduce the nutritional value of leaf tissues after an event of herbivory damage (Valentine *et al.*, 1983). For instance, Kovacevic (1956)

showed that an increase of COC in host oak foliage after defoliation may reduce the weight of gypsy moth pupae, producing a high rate of mortality. This suggests that chemical changes in the foliage due to natural herbivory represent an unfavorable nutritional value that causes physiological weakness in larvae.

Based on previous works mentioned above, our results may suggest that the presence of druses have an antiherbivore function; further, though druses are present in damaged and

control leaves, its accumulation in sites damaged by *U. fulgens* suggests a defense response from the plant. Although COC might be present, its quantity or size could have a threshold of effectiveness not met in the wholly defoliated plants. Moreover, the studies about COC in Mexico or with Mexican species have focused on anatomy-systematics (Bárcenas-Argüello *et al.*, 2014, 2015 and references therein), thus highlighting the *Omphalea-Urania* relationship as an opportunity to expand the knowledge of the COC role as an herbivory defense with a local species.

Non-glandular, unicellular, simple, slim and straight to slightly curved trichomes featuring a papilous cuticle were observed mainly in the leaf midrib and secondary veins. Similar trichomes have been described in other genera (Inamdar and Gangadhara, 1977; Raju and Rao, 1977; Martínez-Gordillo and Espinosa-Matías, 2005, Cervantes *et al.*, 2009) suggesting that this is a constant character in the family. The reported existence of extrafloral nectaries in *O. oleifera* (Dirzo and Mota-Bravo, 1997) was supported by the descriptions of those found in the petiole (Aguirre *et al.*, 2013), and is further confirmed by our observation of the big sized glands in the abaxial leaf margin, which are similar to those observed in *O. diandra* L. (Rudall, 1994b). Small glands surrounding large glands are a feature reported for the first time for *O. oleifera*. A thorough analysis is needed to confirm that extrafloral nectaries and the small glands are a constant character in species of *Omphalea*.

The fine deposits of epicuticular wax in *O. oleifera* contrast with the long filamentous structures observed in other Euphorbiaceae (Murillo, 2002; Elias *et al.*, 2008). The patterns and morphology of wax deposits have been used in plant systematics; nevertheless, similar structures could result from different compounds and the original shape can be modified by environmental factors, so caution should be exercised in using these deposits as taxo-

nomic markers (Koch and Ensikat, 2008). The striate and smooth type of cuticular ornamentation of *O. oleifera* is common in Euphorbiaceae (Kulshreshtha and Ahmad, 1992). The striations, parallel or perpendicular to the stoma, have been observed in other genera of Euphorbiaceae: *Conceveiba* Aubl., *Claoxylon* A. Juss., *Micrococca* Benth., *Erythrococca* Benth., *Ricinus* L., *Sapium* P. Browne, *Alchornea* Sw., *Acalypha* L., *Manihot* Miller, *Jatropha* L., *Antidesma* L., *Bernardia* Miller (Kulshreshtha and Ahmad, 1992; Murillo, 2002; Kabouw *et al.*, 2008; Cervantes *et al.*, 2009). The functions of cuticle ornamentations are unclear. It has been proposed that they may favor colonization by fungi, moss or algae, which in turn hinder water drainage and diminishes the photosynthetic efficiency, or allow enhanced water drainage and light capture, particularly in plants growing under diffuse light (Murillo, 2002). The latter case might be occurring in *O. oleifera*, given the relatively high humidity of the environment in which the plants grow and the absence of microorganism growth evidence in the SEM observations.

The leaves of *O. oleifera* are bifacial and amphistomatic with anomocytic stomata profusely distributed in both leaf sides excluding over the veins. In Euphorbiaceae, bifacial leaves are a constant character (Metcalfe and Chalk, 1989); some genera have hypostomatic (Levin, 1986) or amphistomatic leaves (Aworinde *et al.*, 2009) and location of the stomata is restricted to the vein in the adaxial side (Kabouw *et al.*, 2008). Paracytic stomata are the most common in the Euphorbiaceae (Raju y Rao, 1977; Murillo, 2002), though anisocytic and anomocytic ones are also found (Levin, 1986; Murillo, 2002; Galeş and Toma 2006).

#### Phenolics

The damaged and control leaves are statistically similar, although the content of ferulic



acid content is higher in the damaged samples, and that of coumaric acid is higher in the controls. A study on *O. oleifera* seedlings found that damaged samples contained a greater amount of total phenols than healthy samples (Del Amo *et al.*, 1986). This partial contrast with our findings could be explained by the sensitivity and specificity of the quantification method, the collection season (Bernal *et al.*, 2013), and degree of damage (Del Amo *et al.*, 1986).

The content of phenolic compounds can rise after an insect attack (Coley, 1988; Morse *et al.*, 1991). Plants in general respond with morpho-anatomical and chemical changes that often involve mechanical/structural barriers that also turn difficult the contact or passage of opportunistic pathogens (Anderson-Prouty and Albersheim, 1975). The plants can also respond to defoliation with an increase in the photosynthetic rates, differential assignment of carbon, and creation of reserves of non-structural carbohydrates, as for example in the basal meristems of branches; these processes compensate the absence of synthesis produced by the defoliation and can be destined to post-event growth (Huss *et al.* 1996).

There were no morpho-anatomical changes between control and damaged leaves of *O. oleifera*. The evident response observed is the synthesis of lignin and druses in the cells near the damaged zone. Damaged leaves had, in general, a higher amount of the evaluated phenolics and contained druses mainly in the epidermal and parenchymal cells.

Morpho-anatomical and histochemical studies have an important taxonomic value but can also be useful to shed light on plant-insect interactions. Plants can recover after herbivore attack by compensatory growth that replaces the damaged organs, a commonly studied response after a damaging event that is associated to the term 'tolerance', defined as the capacity to reduce the negative

effects of damage on fitness, which is also related to resource allocation patterns, plant architecture, photosynthetic activity and phenological patterns (Fornoni, 2011, and references cited therein). Plants that are tolerant are expected to have a fast growth rate in relation to resource availability, and the *Omphalea-Urania* association is an interesting subject to study in this context. The defensive function of a trait can be evidenced if the relative fitness of the consumed plant is increased compared to a plant that lacks the trait and grows in the same environment (Karban and Baldwin, 1997). Under the present study settings, the existence of druses and phenolics are not enough to justify a defense response of the plant towards the herbivore, but given the reported magnitude of the defoliation of *O. oleifera* by *U. fulgens* (Dirzo y Mota-Bravo, 1997; Álvaro D. Campos, personal communication), a long term comprehensive study, comparing populations, before, during and after the defoliation, is necessary to characterize the plant conditions, evaluate the role of the COC and clarify whether the observed features or other traits (secondary metabolites, phenology, seasonality) influence interactions between *O. oleifera* and *U. fulgens*, as well as determine the biochemical, evolutionary and ecological implications of the herbivory event.

#### ACKNOWLEDGEMENTS

The authors thank Alejandro Martínez Mena for the micrographic records, Ana Isabel Bieler Antolin for the image layout, Fabiola Soto for the assistance in microtechniques, and Sonia Vázquez Santana and Angélica Hernández Guerrero for their comments to the manuscript.

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