Reproductive biology, seed germination and regeneration of *Flourensia* DC. species endemic to Central Argentina (Asteraceae)

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Published on 30 June 2017

Delbón N., Cosa M. T. & Bernardello G. 2017. — Reproductive biology, seed germination and regeneration of *Flourensia* DC. species endemic to Central Argentina (Asteraceae). *Adansonia*, sér. 3, 39 (1): 71-85. https://doi.org/10.5252/a2017n1a6

ABSTRACT
Our objective was to study reproductive biology, seed germination and regeneration, through morphoanatomical and field observations and controlled experiments, to assess reproductive strategies in six rare *Flourensia* endemic to Central Argentina (*F. campestris, F. hirta, F. leptopoda*, *F. niederleinii*, *F. oolepis*, *F. tortuosa*). Structure of capitula, flowers, and achenes was described. Capitula were visited by a variety of insects. Achenes required 30-45 days to mature. Fruit set varied significantly among species. *Flourensia campestris* and *F. oolepis* were self-incompatible. Seed viability decreased after 19 months and was lost after 32 months. *Flourensia oolepis* and *F. campestris* had the highest germination percentages (>60%); the addition of gibberellic acid in 2-months old seeds did not influence germination. The remaining species had lower germination percentages (<30%). All species had xylopodia that were root and stem modifications. Burned individuals of *F. campestris* actively regenerated from underground buds of xylopodia, being suitable for restoration of degraded or burned areas. *Flourensia campestris* and *F. oolepis* had better reproductive success, but the remainder species can be considered at risk. Strategies should be implemented to protect them, such as to preserve its habitat together with attempts to increase their population sizes and maintain their pollinators.

KEY WORDS
Conservation, floral visitors, germination, morphoanatomy, fruit set, xylopodium.

RÉSUMÉ
Biologie reproductive, germination des semences et régénération des espèces endémiques de Flourensia *d'Argentine centrale (Asteraceae).*
Notre objectif est d'étudier la biologie de la reproduction, la germination des semences et la régénération, par des observations morphoanatomiques et de terrain, ainsi que par des expériences contrôlées, afin d'évaluer les stratégies de reproduction de six rares *Flourensia* endémiques d'Argentine centrale (*F. campestris, F. hirta, F. leptopoda, F. niederleinii, F. oolepis* et *F. tortuosa*). La structure et la morphoanatomie des capitules, des fleurs et des akènes sont décrites. Les capitules ont été visités par une variété d'insectes. Les akènes mûrissent en 30-45 jours. La production des fruits varie considérablement entre les espèces. *Flourensia campestris* et *F. oolepis* sont auto-incompatibles. La viabilité des semences a diminué après 19 mois et a été perdue après 32 mois. *Flourensia oolepis* et *F. campestris* ont les pourcentages de germination les plus élevés (> 60 %) ; l'addition d'acide gibbérellique aux semences vieilles de deux mois n'a pas influencé la germination. Les autres espèces présentaient des pourcentages de germination plus faibles (< 30 %). Toutes les espèces ont des xylopodes qui sont des modifications de racines et de tiges. Les individus brûlés de *F. campestris* régénèrent activement à partir des bourgeons souterrains des xylopodes, et conviennent à la restauration des zones dégradées ou incendiées. *Flourensia campestris* et *F. oolepis* ont mieux réussi à se reproduire, mais les autres espèces peuvent être considérées comme à risque. Des stratégies doivent être mises en œuvre pour les protéger, de manière à préserver leur habitat, et des essais entrepris pour augmenter la taille de leur population et maintenir leurs pollinisateurs.

MOTS CLÉS
Conservation, visiteurs floraux, germination, morphoanatomie, production de fruit, xylopode.

INTRODUCTION

As it is well known, human activity has produced major impacts on natural ecosystems, causing reduction and fragmentation of the habitat, as well as changes in the composition of flora and fauna (Pimm *et al.* 1995; Primack *et al.* 2001). Particularly in Central Argentina, Zak *et al.* (2008) studying changes in land cover that have occurred during the last decades of the 20th century in their Chaco forests showed that *c.* 80% of the area that was originally undisturbed forest is now occupied by crops, pastures, and secondary scrub. The main proximate cause of deforestation has been agricultural expansion, soybean cultivation in particular. Additional problems are the high frequency of natural and induced fires that provoke forest loss in the area (Gurvich *et al.* 2005; Giorgis *et al.* 2013) and the invasion of exotic species that produce profound changes in the structure and function of natural ecosystems (Charles & Dukes 2007; Hoyos *et al.* 2010).

Plant species with restricted ranges, such as most endemics, may be especially vulnerable to extinction under these pressures, including climate change (Myers *et al.* 2000; Malcolm *et al.* 2006). The study of reproductive biology of these rare or endangered species is critical to founding effective conservation programs if they have few populations that can provide propagules for future generations; in addition, it is important to understanding the evolution and systematic relationships of species (e.g. Richards 1997; Stuessy *et al.* 2014).

Endemics are infrequent in the Chaco forests of Central Argentina. Among them, there are several Asteraceae, the largest family in the country, both in total number and in number of endemics (Zuloaga *et al.* 1999; Katinas *et al.* 2007). Six of them that grow in these hills with poor soils belong to *Flourensia* DC., an amphitropical American shrubby genus with 32 resinous species (Blake 1921; Dillon 1984; Ariza Espinar 2000). This genus is included in tribe Heliantheae, one of the largest, most diverse, and derived of the family, and in subtribe Enceliinae that encompasses five genera: *Flourensia* DC., *Encelia* Adans., *Enceliopsis* A. Nelson, *Geraea* Torr. & A.Gray and *Helianthella* Torr. & A.Gray (Panero 2005).

These six species are endangered according to PlanEAr (Villamil *et al.* 2000), as follows. *Flourensia hirta* S.F. Blake, *F. leptopoda* S.F. Blake and *F. niederleinii* S.F. Blake are in the 5th category, which corresponds to plants of restricted distribution with small populations under threat factors, such as habitat destruction, overexploitation, and biological invasions. *Flourensia oolepis* S.F. Blake is in the 4th category corresponding to species restricted to a single or to confined areas in two or more neighbor political provinces. Finally, *F. campestris* Griseb. and *F. tortuosa* Griseb. are in the 3rd category because they are common but not abundant.

Previous data on these species pointed out that they have anatomical adaptations allowing them to thrive in xeric environments, such as secretory ducts and glandular trichomes producing resins (Delbón *et al.* 2007a, b, 2012; Silva *et al.* 2015). In *F. campestris* and *F. oolepis* essential oils with bactericidal, antifungic and insecticidal effects were identified (e.g. Joray *et al.* 2011; Silva *et al.* 2012; López *et al.* 2014).

Upon this background, the objective of this research was to describe the reproduction of six *Flourensia* species at various stages from flower production to seed germination, though field observations and controlled experiments. We studied the reproductive biology (phenology, capitula, flower and fruit morphoanatomy, floral visitors, fruit set and spontaneous autogamy), seed germination, and regeneration ability of these rare species, to understand the implications of these data in the assessment

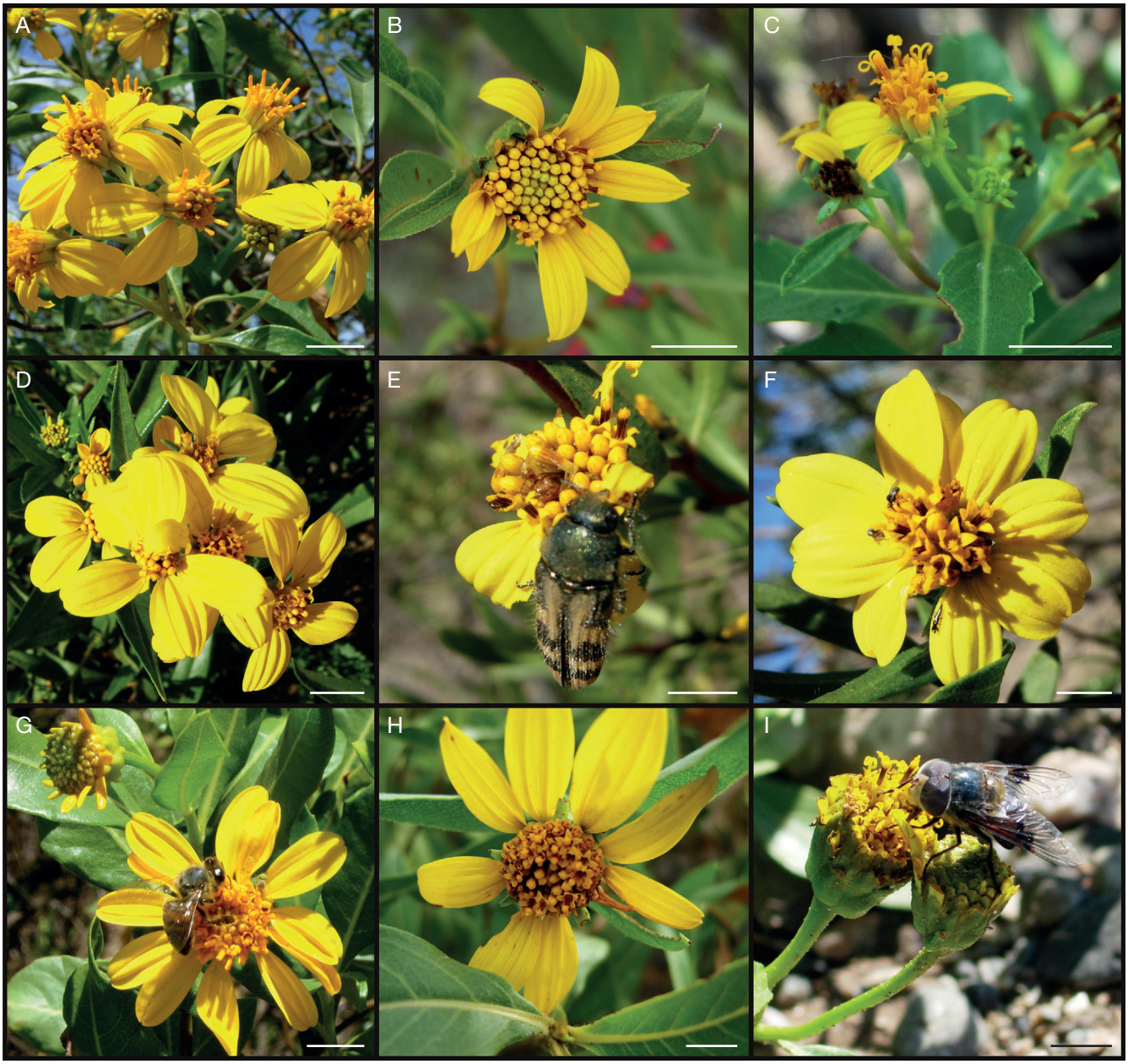

Fig. 1. — Inflorescences and flower visitors in *Flourensia* DC.: **A**, *F. campestris* Griseb.; **B**, *F. hirta* S.F. Blake; **C**, *F. leptopoda* S.F. Blake; **D-F**, *F. niederleinii* S.F. Blake; **G**, *F. oolepis* S.F. Blake; **H-I**, *F. tortuosa* Griseb.; **E**, Coleoptera; **F**, Thysanoptera; **G**, Hymenoptera; **I**, Diptera. Scale bars: A-D, G-I, 1 cm; E, F, 0.5 cm.

of their reproductive strategies and future conservation. Except a few data on seed germination in *F. campestris* and *F. oolepis* (Delbón & Eynard 2006; Galíndez *et al.* 2009b) and on nectar composition and floral visitors of *F. campestris* (Torres & Galetto 2002, 2008), little is known about the reproductive biology of *Flourensia* species.

MATERIALS AND METHODS

Flower and fruit

Collection data of *Flourensia* species were included in Table 1. In 2008-2009 and 2009-2010, field macroscopic observations of individual plants and flowering time were done between December and April. From 15 to 30 capitula at different maturation stages from five individuals (five replicates) of one population per species and per year were collected.In each capitulum, the number of ligulate and tubular flowers and full fruits were registered. Not parasitized achenes with developed embryos were considered full fruits; fruits that looked empty were opened and observed with a stereoscope to confirm they did not have embryo. Fruit set was calculated as the ratio between the numbers of full fruits and tubular flowers.

Spontaneous autogamy tests were made in *F. campestris* and *F. oolepis*. In December 2008, capitula with flowers in preanthesis were covered with tulle fabric bags to prevent insect visits. Two

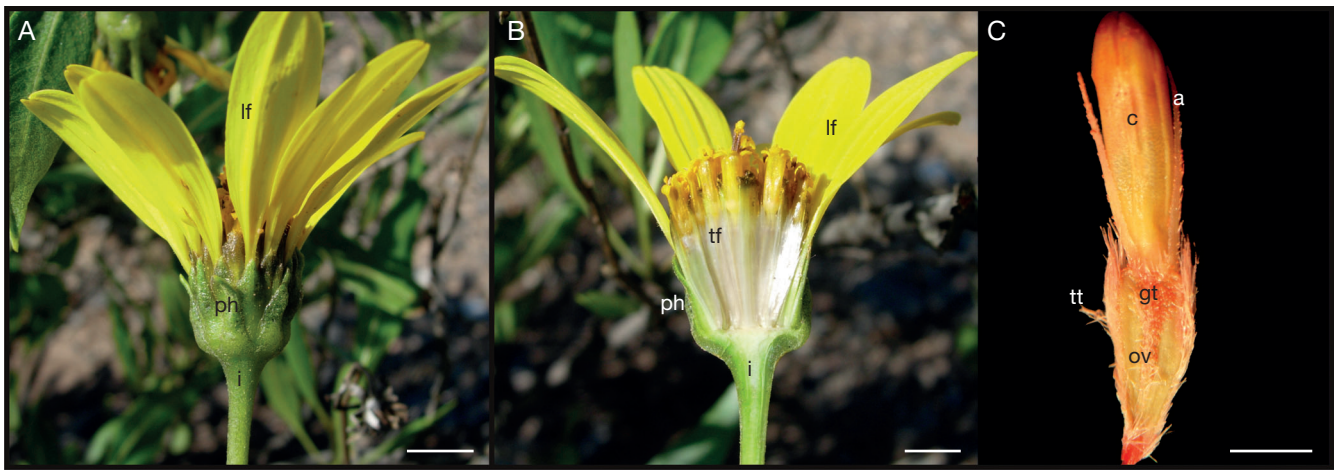

Fig. 2. — Capitulum and flower in *Flourensia* DC.: **A**, **B**, *F. tortuosa* Griseb.; **C**, *F. campestris* Griseb.; **A**, capitulum lateral view; **B**, capitulum longitudinal section; **C**, tubular flower in preanthesis. Abbreviations: **a**, awn; **c**, corolla; **gt**, glandular trichome zone; **i**, involucre; **lf**, ligulate flower; **ov**, ovary; **ph**, phyllary; **tf**, tubular flower; **tt**, twin trichome zone. Scale bars: A, B, 0.5 cm; C, 2 mm.

Table 1. — Collection data of *Flourensia* DC. species. All species from Argentina, vouchers were deposited at the herbarium of Museo Botánico de Córdoba (CORD).

Species	Province, department, place, collector and number, date
Flourensia campestris Griseb.	Córdoba, Punilla, Cerro El Cuadrado, La Falda, 31°0.5'S, 64°27'W, 1100 m asl; *Delbón 5*, 20.II.2009; *Delbón 7*, 21.III.2010
F. hirta S.F. Blake	La Rioja, Famatina, Campana, 28°33'S, 67°38'W, 1625 m asl; *Delbón 3*, 18.II.2009; *Barboza 2460*, 20.III.2010
F. leptopoda S.F. Blake	La Rioja, General San Martín, Ulapes, 31°34'S, 66°14'W, 734 m asl; *Delbón 4*, 19.II.2009; *Barboza 2438*, 18.III.2010
F. niederleinii S.F. Blake	La Rioja, Sanagasta, Sanagasta, 29°11'S, 67°0.3'W, 1212 m asl, *Delbón 1*, 16.II.2009; La Rioja, Chilecito, Sañogasta, 29°16'S, 67°36'W, 1318 m asl, *Barboza 2452*, 19.III.2010
F. oolepis S.F. Blake	Córdoba, Punilla, Capilla del Monte, Dique El Cajón, 30°51'S, 64°33'W, 970 m asl, *Delbón 6*, 20.II.2009; *Delbón 8*, 21.III.2010
F. tortuosa Griseb.	Catamarca, Andalgalá, Andalgalá, 27°32'S, 66°17'W, 1246 m asl, *Delbón 2*, 17.II.2009; Catamarca, Belén, Londres, 27°44'S, 67°10'W, 1233 m asl, *Barboza 2462*, 20.III.2010

months later, 155 capitula of *F. campestris* and 26 of *F. oolepis* from five individuals (five replicates) of each species were collected to count the number of tubular flowers and achenes produced and to calculate the fruit set.

Anatomy

Plant material was preserved in 70 FAA (formalin-acetic-alcohol mixture), was dehydrated in a series of ethyl alcohol/xylene and was included in paraffin. A minimum of three individuals and three capitula per species were examined through permanent slides (approximate 10 µm thick) of perfect flowers, fruits, and seeds (longitudinal and transversal sections) to study their development using a microtome. Xylopodia were analysed in four individuals of each *F. campestris* and *F. oolepis* through permanent slides using a sliding microtome. Serial cuts were stained with astral blue-basic fuchsin and were mounted with Canada balsam (Kraus *et al.* 1998). The images were taken using a light microscope Primo Star-Carl Zeiss and a digital camera Nikon Coolpix 5200. Trichomes were classified according to Ramayya (1962).

Floral visitors

Observations were done for 2-3h in February 2009 and March 2010. Photographs were taken to identify visitors. They were determined to the genus level whenever possible. The total percentage of individuals of each insect order was calculated.

Seed germination

Germination experiment was done using mature full achenes from 4-5 maternal individuals collected in 2010 (4-5 replicates), dried and stored in darkness at room temperature and humidity. Each experiment was made using 30 randomly taken seeds from each maternal individual. Achenes of each maternal individual were leached with water for 24 hours (ISTA 2003), placed in sterilized Petri dishes (each Petri dish was one maternal individual) with moistened filter paper, and kept in a 25°C chamber with alternate conditions of light (12h) and darkness (12h). The experiments were done with seeds of different ages (0.5, 1, 2, 3, 19, and 32 months) to determinate seed longevity and dormancy; for *F. niederleini*, *F. hirta* and *F. tortuosa* 3-months data were not obtained and for *F. leptopoda* data were taken only for 1, 19 and 32 months, considering seed availability. Seeds were considered germinated when their radicle emerged > 2 mm. The numbers of germinated seeds were daily recorded until they stopped to germinate. The average number of days required to start germination for each experiment, i.e., the initial germination time (IGT), mean germination time of each experiment, i.e., when the seeds

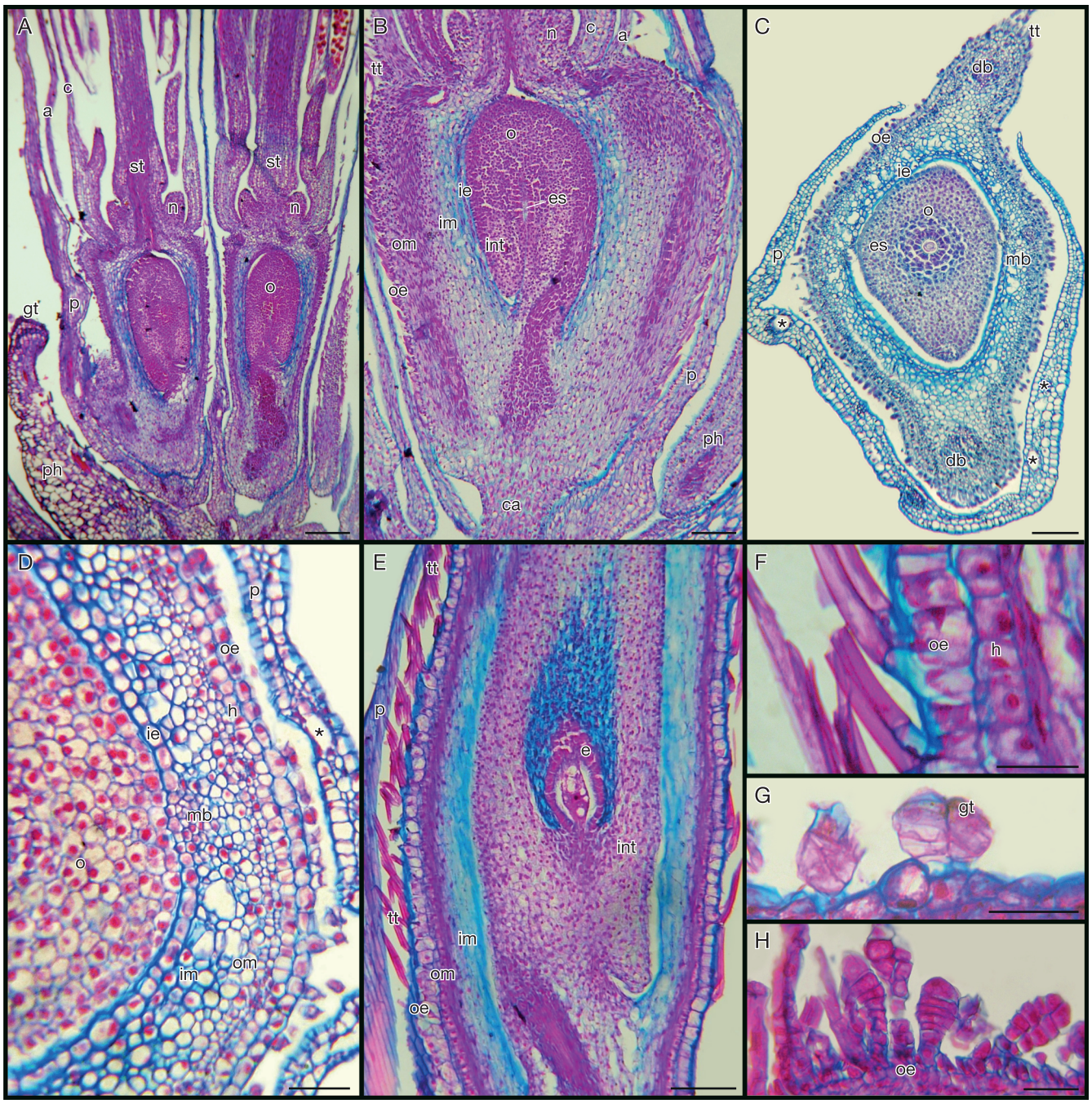

FIG. 3. — Anatomy of tubular flowers in *Flourensia* DC.: **A**-**G**, *F. campestris* Griseb.; **H**, *F. hirta* S.F. Blake; **A**, **B**, **E**, **F**, longitudinal sections; **C**, **D**, **G**, **H**, transversal sections; **A**, capitulum with two tubular flowers; **B**, ovary with one anatropous ovule; **C**, ovary showing two dorsal bundles and paleae; **D**, detail of ovary wall in the carpel fusing region; **E**, mature ovule with embryo sac; **F**, detail of outer epidermis of **E** with twin trichomes and hypodermis; **G**, **H**, glandular trichomes. Abbreviations: **a**, awn; **c**, corolla; **ca**, carpopodium; **db**, dorsal bundle; **e**, endothelium; **gt**, glandular trichome; **h**, hypodermis; **ie**, inner epidermis; **im**, inner mesophyll; **int**, integument; **mb**, marginal bundle; **n**, nectary; **o**, ovule; **oe**, outer epidermis; **om**, outer mesophyll; **p**, paleae; **ph**, phyllary; **st**, style; **tt**, twin trichome. *, secretory duct. Scale bars: A, 200 µm; B-C, E, 100 µm; D, F-H, 50 µm.

stopped germinating (GT), mean germination percentage for each experiment (%G), and mean daily germination (MDG) determined as germination percentage/germination time, were calculated for each Petri dish and then for each seed age. An additional germination treatment with gibberellic acid (1000ppm) was performed in *F. campestris* and *F. oolepis* seeds of 2-months old.

REGENERATION ABILITY

Four plants of each *F. campestris* and *F. oolepis* were digged out to observe their root system and xylopodia. Part of the community from the studied population of *F. campestris* was accidentally burned in 2010, allowing additional observations on the regeneration of burned individuals.

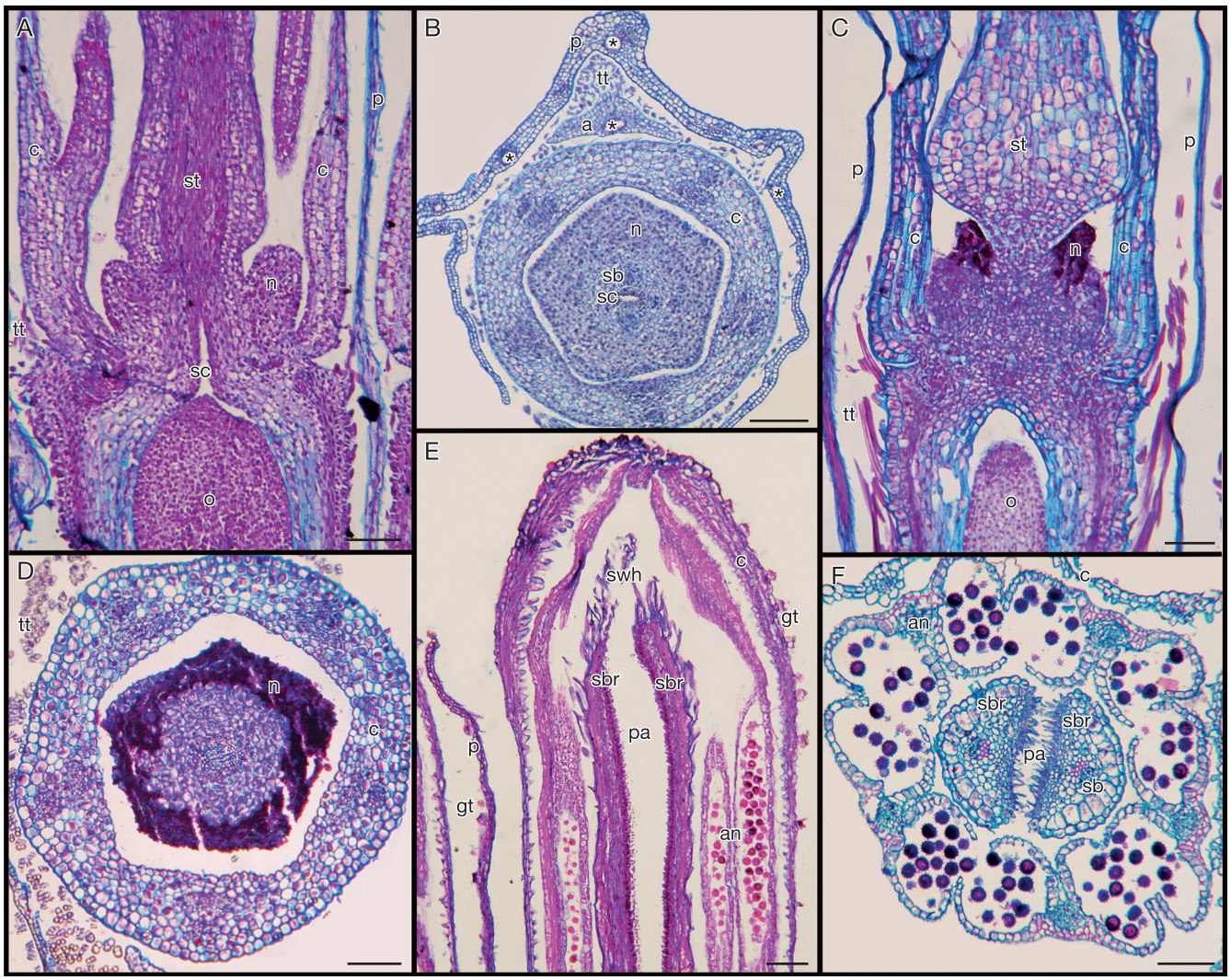

Fig. 4. — Anatomy of nectary, style, stigma and anther in *Flourensia campestris* Griseb.: **A**-**D**, nectary; **E**, **F**, style, stigma and anther features; **A**, **B**, nectary from flower at preanthesis; **C**, **D**, nectary after fertilization; **E**, style branches with sweeping hairs and stigmatic papillae; **F**, mature anthers and style branches; **A**, **C**, **E**, longitudinal sections; **B**, **D**, **F**, transversal sections. Abbreviations: **an**, anther; **a**, awn; **c**, corolla; **gt**, glandular trichome; **n**, nectary; **o**, ovule; **p**, paleae; **pa**, papillae; **st**, style; **sb**, style bundle; **sbr**, style branche; **sc**, style canal; **swh**, sweeping hair; **tt**, twin trichome. *, secretory duct. Scale bars: 100 µm.

Statistical analyses

They were done using InfoStat (Di Rienzo *et al.* 2014). Mean and standard deviation of all variables were calculated for each harvest year (2009 and 2010) and for each species. Ligulate and tubular flowers per capitulum have a normal distribution and homogeneous variances. Full fruits and fruit set data were log2-transformed. One-way Analyses of Variance were used to compare each variable between years. Since no differences between years were found (except for full fruits of *F. hirta*), data were pooled and the variables among all species were compared using Tukey tests.

For germination experiments, mean and standard deviation for all variables were calculated among Petri dishes (maternal individuals) for each seed age. All variables have a normal distribution and homogeneous variances. One-way Analyses of Variance and Tukey tests were used to compare the germination percentage among seed ages for each species. In addition, for all variables and each species, a mean from 0.5 to 19 months seed ages was calculated and Tukey tests were performed to compare each variable among species.

RESULTS

Capitula and flower

The studied species bloomed in the summer rainy season, between November and March. Capitula generally were arranged in dense inflorescences, as in *F. campestris, F. leptopoda*, and *F. niederleinii* (Fig. 1A, C, D), or were solitary or in small lax inflorescences, as in *F. hirta, F. oolepis*, and *F. tortuosa* (Fig. 1D, G, H).

All species had typical yellow radiate capitula (Fig. 1) with bell-shaped hemispherical involucres with 2-3 sets of greenish phyllaries (Fig. 2A). Ray flowers were ligulate, sterile, and arranged in a single outer series (Fig. 2A, B), whereas disc flowers were tubular and perfect (Fig. 2C).

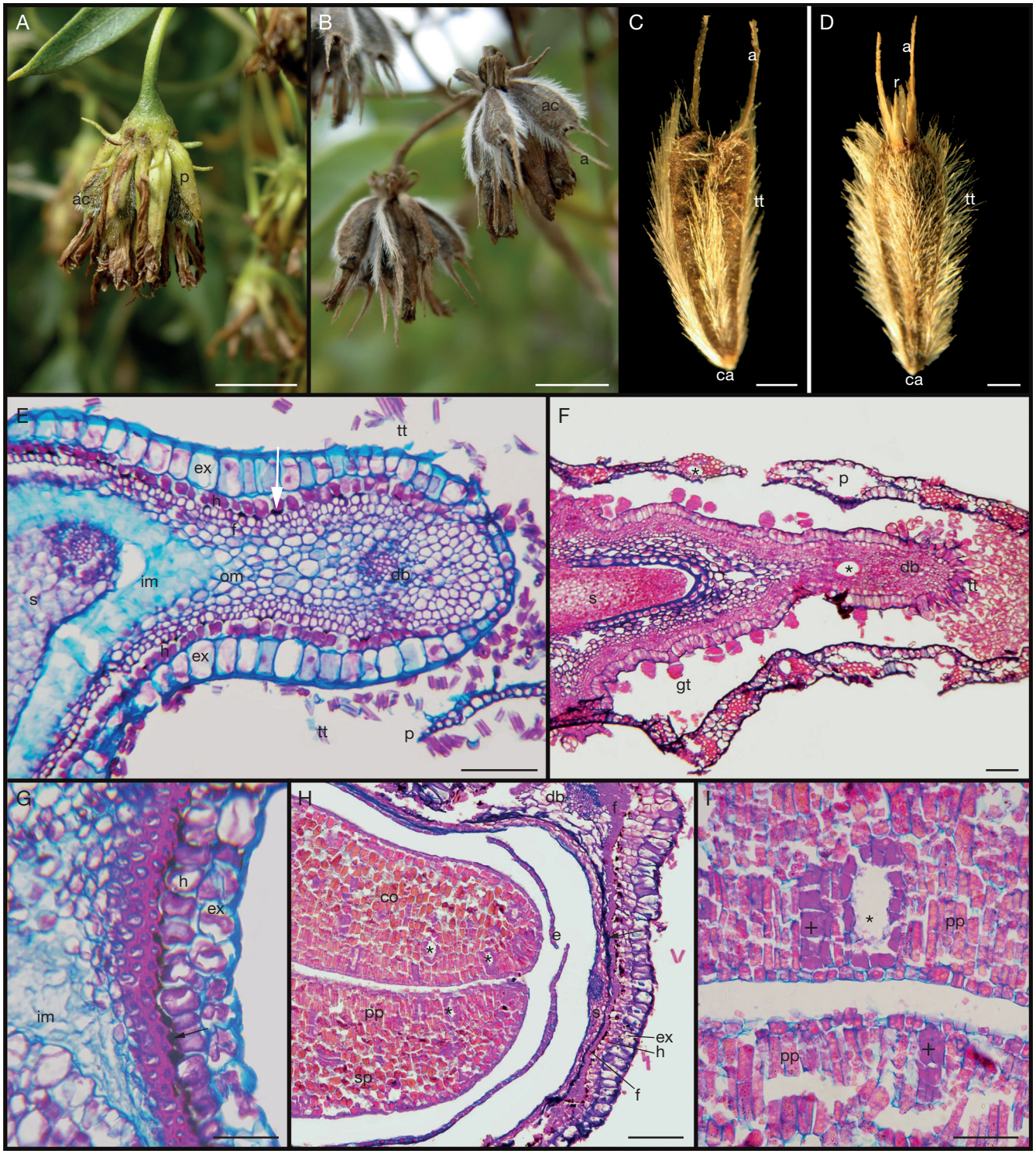

Fig. 5. — Achenes in *Flourensia* DC.: **A**, **B**, **E**, **G**, *F. campestris* Griseb.; **C**, *F. niederleinii*; **D**, **H**, **I**, *F. oolepis*; **F**, *F. hirta* S.F. Blake; **A**-**C**, exomorphology; **E**-**I**, anatomy and ontogeny of fruit and seed in transversal section; **A**, immature achenes with paleae; **B**, mature achenes; **B**, achene with awns and **C**, ring of straws; **E**, young achene showing dorsal bundle; **F**, young achene with glandular trichomes, twin trichomes and ducts; **G**, detail of wall; **H**, mature achene; **I**, detail of cotyledons with ducts. Abbreviations: **a**, awn; **ac**, achene; **ca**, carpopodium; **co**, cotyledon; **db**, dorsal bundle; **e**, endothelium; **ex**, exocarp; **f**, fiber; **gt**, glandular trichome; **h**, hypodermis; **im**, inner mesocarp; **om**, outer mesocarp; **p**, palea; **pp**, palisade parenchyma; **r**, ring of straws; **s**, seed; **sp**, spongy parenchyma; **tt**, twin trichome; **arrow**, phytomelanin layer. Symbols: *, secretory duct; +, developing duct. Scale bars: A, B, 6 mm; C, D, 1 mm; E, F-H, 100 µm. G, I, 50 µm.

The total number of tubular and ligulate flowers per capitulum did not vary significantly between the different collection years for each species (p: 0.1 to 0.96, F: 2.60E-03 to 0.31, df: 9),but the number of both flower types were different among species, with *F. oolepis* having the highest number and *F. leptopoda* the lowest (Table 2).

TABLE 2. — Flower number and fruits per capitulum in *Flourensia* DC. species. Data are mean values (standard deviation) from 2009 and 2010 data. ANOVAs F: 94.5, 133.9, 248.7 and 122.5 respectively; **df**, 59, $p < 0.05$; **Tukey tests df**, 54, $p < 0.05$. Different letters indicate significant differences with Tukey tests.

Species	Ligulate flowers	Tubular flowers	Full fruits	Fruit set
Flourensia campestris Griseb.	5.3 (0.4) c	17.9 (2.04) ab	15.5 (1.6) c	0.86 (0.03) d
F. hirta S.F. Blake	5.6 (0.6) c	20.6 (1.59) ab	7.4 (1.7) a	0.4 (0.06) a
F. leptopoda S.F. Blake	3.9 (0.2) c	13.25 (0.7) a	11.1 (0.7) b	0.8 (0.03) d
F. niederleinii S.F. Blake	5.4 (0.4) c	22.6 (2.91) b	14.6 (2.7) c	0.6 (0,07) c
F. oolepis S.F. Blake	11 (0.7) a	68.3 (5.7) c	61.8 (6.1) e	0.9 (0.07) d
F. tortuosa Griseb.	8.9 (0.1) b	63 (14.9) c	33.1 (6.8) d	0.5 (0.06) b

TABLE 3. — Insect floral visitors in *Flourensia* DC. Total number of visits and percentage by order.

Order	Family	Genus	Total	Visits by order (%)
Coleoptera	Cerambicidae	*Basiptera* sp.	1	9.7
	Buprestidae	*Dactilozoides* sp.	1	
	Chrysomelidae	*Cacoscelis* sp.	1	
Diptera	Bibionidae	Unidentified	1	16.1
	Muscidae	Unidentified	1	
	Syrphidae	*Erystalis* sp.	2	
		Unidentified	1	
Hymenoptera	Apidae	*Apis mellifera*	9	45.2
	Formicidae	*Solenopsis* sp.	4	
	Sphecidae	*Larra* sp.	1	
Orthoptera	Acrididae	*Trimerotropis* sp.	1	3.2
Thysanoptera	Thripidae	*Frankliniella* sp.	7	22.6

Ray flowers had not anthers and generally had an unfunctional pistil, being infertile. In cross section, their corollas were anatomically similar to a leaf. Disc flowers were typically epigynous, had pappus with two awns, and were surrounded by paleae (Fig. 2C, 3A). They were inserted to the receptacle by a small piece of parenchyma that later will become the carpopodium (Fig. 3B). The two-carpelled ovary had an elliptical shape with two ribs in cross section (Fig. 3C). Both carpels had two developed collateral dorsal bundles (Fig. 3C) and two smaller marginal bundles with an uniseriate inner epidermis (Fig. 3D). In the ovary wall, the mesophyll had two zones: a loose inner one with large intercellular spaces and a compact outer one with small stained cells with dense contents (Fig. 3C-E). The only ovule of each ovary was anatropous with basal placentation and had an integument (Fig. 3B).

In anthesis, the outer epidermal cells were larger, had thickened cuticles (Fig. 3E, F), and showed two trichome types: non-glandular twin (with four cells: two basal short and two apical elongated lignified; Fig. 3F) and glandular biseriate vesicular (with a 2-celled foot and a multicellular biseriate head; Fig. 3G). Both types were found in all species, being more numerous in *F. hirta* (Fig. 3H). Immediately below, there was a hypodermis with larger cells and dense contents (Fig. 3F). The mature embryo sac (Fig. 3E) was surrounded by an endothelium and the integument cells were disrupted.

An ovarian annular nectary, pentagonal in cross-section, was located on top of the inferior ovary, surrounding the style base (Figs 3A; 4A-D). Before anthesis, the secretory parenchyma had clear cells (Fig. 4A, B), but during anthesis, they showed more dense contents. After fertilization, the nectary collapses (Fig. 4C, D).

The style has two bundles and a stylar channel (Fig. 4B). Apically, there were two stigmatic branches, each with a bundle (Fig. 4E, F), that had an outer epidermis with sweeping hairs at the apex (Fig. 4E), and an inner epidermis with many papillae (Fig. 4E, F). The five stamens formed a tube around the style (Fig. 4F).

Secretory ducts were observed in paleae (Figs 3C; 4B), pappus' awns (Fig. 4B, C), some floral dorsal bundles and immature achenes (Fig. 5F). Glandular trichomes were found in phyllaries (Fig. 3A), paleae (Figs 3A; 4E), awns (Fig. 4B), and corollas (Fig. 4E). In addition, in paleae and phyllaries of *F. hirta* and *F. tortuosa* eglandular uniseriate multicellular trichomes were found.

Flower visitors

Capitula were visited by a variety of insects, including a total of five orders and 11 families (Table 3). Hymenoptera was the most abundant group (Fig. 1G), followed by Thysanoptera (Fig. 1F) and Diptera (Fig. 1I). Coleoptera (Fig. 1E) and Orthoptera were infrequent. *Apis mellifera*, the honeybee, was the most abundant species observed (Fig. 1G), followed by the thrip *Frankliniella* sp. (Fig. 1F). Visitors generally look for pollen and/or nectar in several capitula of a plant and, consecutively, several plants in a population.

Fruits

At the beginning of their development, they were protected by paleae and the wilted parts of the perianth (Fig. 5A), which later fell (Fig. 5B). Achenes were obovate to obconic, slightly compressed, dark brown, hairy (Fig. 5C, D), and showed a two-awned persistent pappus (Fig. 5C); only *F. oolepis* additionally had a ring of straws, shorter than the awns (Fig. 5D).

The exocarp of young fruits had large rectangular cells with thick cuticles (Fig. 5E) and abundant twin and glandular trichomes, especially on the ribs (Fig. 5E, F). Between hypodermis and mesocarp, a deposition of phytomelanin secreted by hypodermical cells was observed (Fig. 5E, arrow). The outer mesocarp consisted of 2-3 cell layers, that later lignified (Fig. 5G), whereas the inner mesocarp disorganized (Fig. 5E, G). Dorsal bundles can have secreting ducts (Fig. 5F). Awns of pappus and paleae presented fibers associated with bundles and ducts (Fig. 5F).

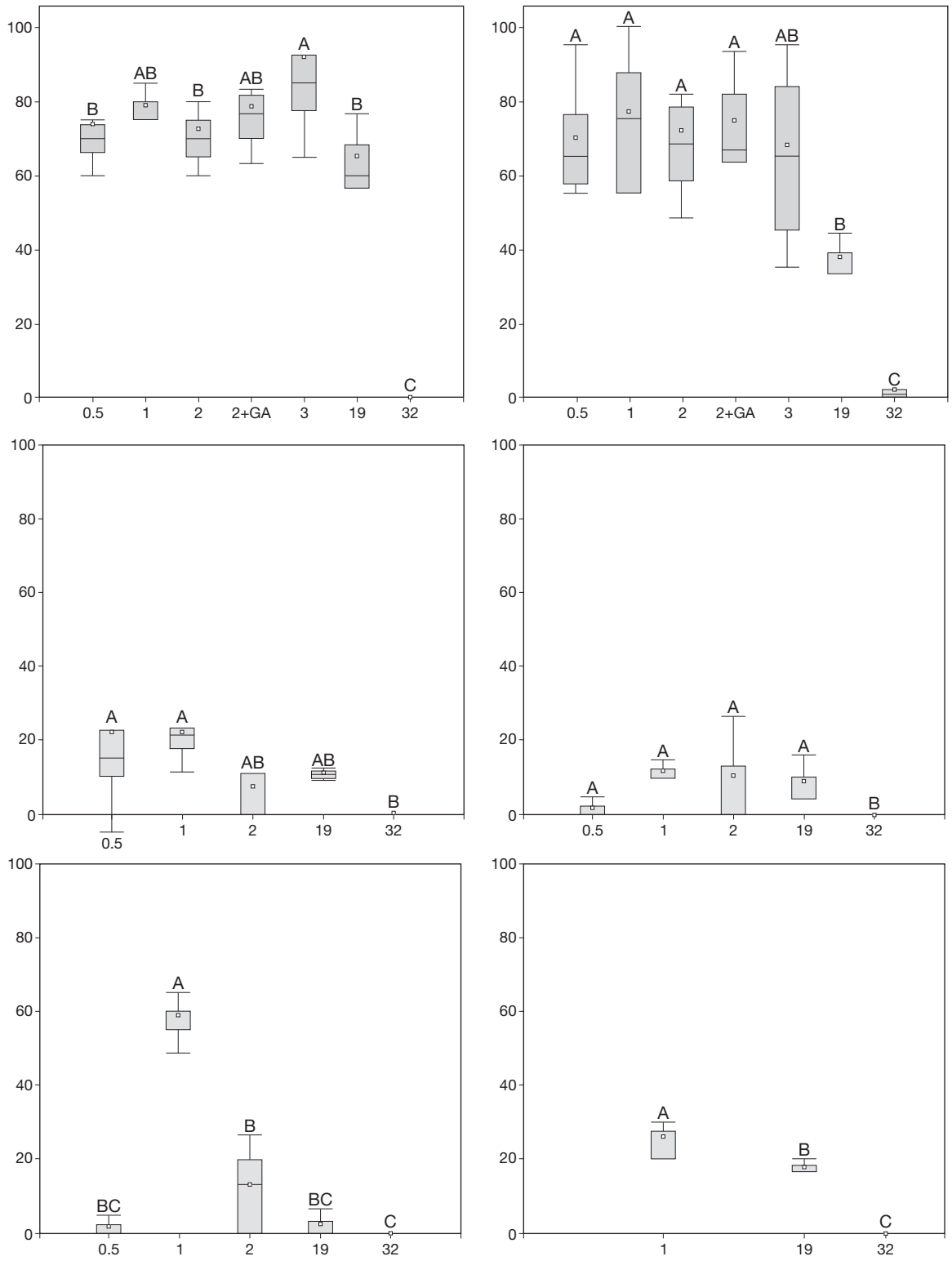

Fig. 6. — Germination in *Flourensia* DC.: germination percentage for experiments made at different seed ages. **+GA**, germination experiment with gibberellic acid. Boxplot: **box**, quartiles 1 and 3; **dot**, mean; **horizontal bar**, median; **whiskers**, maximum and minimum observation. Different letters for each species indicate significant differences with Tukey test.

Seeds had no endosperm. The episperm was composed of 2-3 parenchyma layers and an endothelium (Fig. 5H). The embryo showed 2-4 layers of palisade and 6-7 of spongy parenchyma (Fig. 5H, I), both with many schizogenous ducts (Fig. 5I).

Fruit set

The percentage of full fruits varied significantly among species (Table 2): *F. oolepis, F. campestris* and *F. leptopoda* had the highest fruit set, while *F. hirta* the lowest (Table 2). On the

Table 4. — Germination experiments in *Flourensia* DC. species. **IGT**, initial germination time; **GT**, germination time; **%G**, total germination percentage; **MDG**, mean daily germination (as germination percentage/germination time). **+ GA**, experiments with addition of gibberellic acid. Data are total mean from 0.5- to 19-month old seed experiments (standard deviation). ANOVAs F, IGT 9.64, %G 21.66, MDG 17.11, df: 25, p< 0.05; **Tukey tests**, df: 20, p< 0.05; ANOVAs F, GT 0.49, df: 25, p: 0.78. Different letters indicate significant differences with Tukey tests.

Species	IGT (days)	GT (days)	%G	MDG
F. campestris Griseb.	5.8 (2.1) ab	12.3 (4.6) a	66.6(14.5) a	6.2 (2) a
F. campestris + GA	4.2 (0.5) ab	13 (3.8) a	74.7 (14.2) a	5.7 (1.2) a
F. hirta S.F. Blake	14.8 (4.8) c	9 (6.7) a	8.5 (4.5) b	0.8 (0.5) b
F. leptopoda S.F. Blake	9.2 (5.8) ab	10 (4.5) a	22 (5.7) b	2.3 (0.5) b
F. niederleinii S.F. Blake	6.6 (2.4) a	7.8 (2.4) a	15.6 (7.6) b	1.7 (0.5) b
F. oolepis S.F. Blake	4.5 (2.7) ab	10.7 (2.4) a	77 (8.9) a	8.1 (2.2) a
F. oolepis + GA	2 (0.2) a	7.4 (2.1) a	78.7 (8.7) a	10.6 (2.1) a
F. tortuosa Griseb.	5.2 (2.9) ab	8.1 (4.5) a	19.2 (27) b	1.4 (1.3) b

other hand, there were no differences between both collecting years (F, 0.01 to 9.7; p, 0.07 to 0.9; df, 9), except in *F. hirta* (F, 9.6; p, 0.01; df, 9).

Spontaneous autogamy experiments in *F. campestris* and *F. oolepis* showed a much lower fruit set (0.06±0.02 and 0.13±0.03, respectively) than open pollinated capitula (F, 677.5 and 1137.6, respectively; p, 0.0001; df, 14), suggesting that both species may be self-incompatible.

Seed germination

All species did not show dormancy, except *F. tortuosa* in which seeds started to germinate after one month. In all cases, the germination percentage decreased after 19 months, being almost completely lost after 32 months (Fig. 6). IGT varied among the species from four to nine days, being significantly different only in *F. hirta* with almost 15 days (Table 4). The GT did not show significant differences among the species (Table 4), being the total mean 10.7 ± 2.1 days.

Flourensia oolepis and *F. campestris* had the highest germination percentages, more than 60% in all experiments (Fig. 6). At the same time, both species had higher %G and MDG (Table 4). The addition of gibberellic acid in two-months old seeds in both species did not influence their germination (Fig. 6; Table 4), with the exception of the IGT in *F. oolepis* that decreased from 4.5 to 2 (Table 4).

The remaining species had comparatively lower germination percentages, mostly below 30% in all experiments (Fig. 6) and had lower %G and MDG (Table 4), except for *F. tortuosa* one month-old seeds with 56%.

Regeneration ability

All species showed xylopodia, i.e. underground irregular thickenings of the main roots with a rough vegetative bark and numerous buds (Fig. 7A-C). Anatomical studies in *F. campestris* and *F. oolepis*, showed that xylopodia were root and stem modifications (Fig. 7D-F). At the root level, the primary xylem was located in the central area, having a variable number of metaxylem poles (Fig. 7D), whereas at the stem level there was a parenchymatic pith. In both levels, a normal secondary xylem with many growth rings and round porosity was detected (Fig. 7E); vessels were comparatively scarce, but fibers and rays were abundant (Fig. 7E, F). In the secondary phloem, fiber groups and secretory ducts were present (Fig. 7F).

Completely burned individuals of *F. campestris* actively resprouted from buds at their bases (Fig. 7B, C). The origin of these new branches was from numerous underground buds of xylopodia that also produced adventitious roots. However, no new plants developed from them.

DISCUSSION

Flowers of capitula may have different forms, sex and arrangements, displaying a great diversity in Asteraceae (Jeffrey 2009; Funk *et al.* 2009). *Flourensia* species studied had attractive yellow radiated capitula whit perfect tubular flowers and infertile ligulate flowers, as in most species of the genus (Dillon 1984; Ariza Espinar 2000; Urzúa *et al.* 2007). The numbers of both ligulate and tubular flowers were fundamental to differentiate the species.

Anatomically, *Flourensia* tubular flowers had two interesting traits: trichomes and secretory ducts. Trichomes are taxonomically important in the family and have been used in phylogeny (Ciccarelli *et al.* 2007; Marzinek & Trombert Oliveira 2010). They were abundant in reproductive organs, as reported for aerial vegetative organs of these species (Delbón *et al.* 2007a, b, 2012; Silva *et al.* 2015). Glandular trichomes in ovary and ripe fruit are here reported for the first time in *Flourensia*, although there were registered for other floral whorls in *Encelia* Adans. and *Flourensia campestris* (Sanders & Clark 1987; Silva *et al.* 2015). On the other hand, twin trichomes were never reported *Flourensia* in any organ, although they have been reported in *Encelia* (Sanders & Clark 1987). Its function would be related to water absorption and retention (Freire & Katinas 1995; Sancho & Katinas 2002).

Secretory ducts were previously found in *Flourensia* aerial vegetative organs and flowers in all species studied (Delbón *et al.* 2007a, 2012; Silva *et al.* 2015), but were here described for fruit, embryo, and xylopodium. The secretion of glandular trichomes and ducts generally contain terpenoids (Urzúa *et al.* 2007; López *et al.* 2014; Silva *et al.* 2015), chemicals that would protect the plants against herbivores or pathogens (Fahn 2002; Jaime *et al.* 2013), as might occur in *Flourensia*. In the reproductive organs of *F. thurifera*, the volatile terpenoids secreted would be a stimulus for insects visiting the capitula as they turn them very fragrant (Urzúa *et al.* 2007).

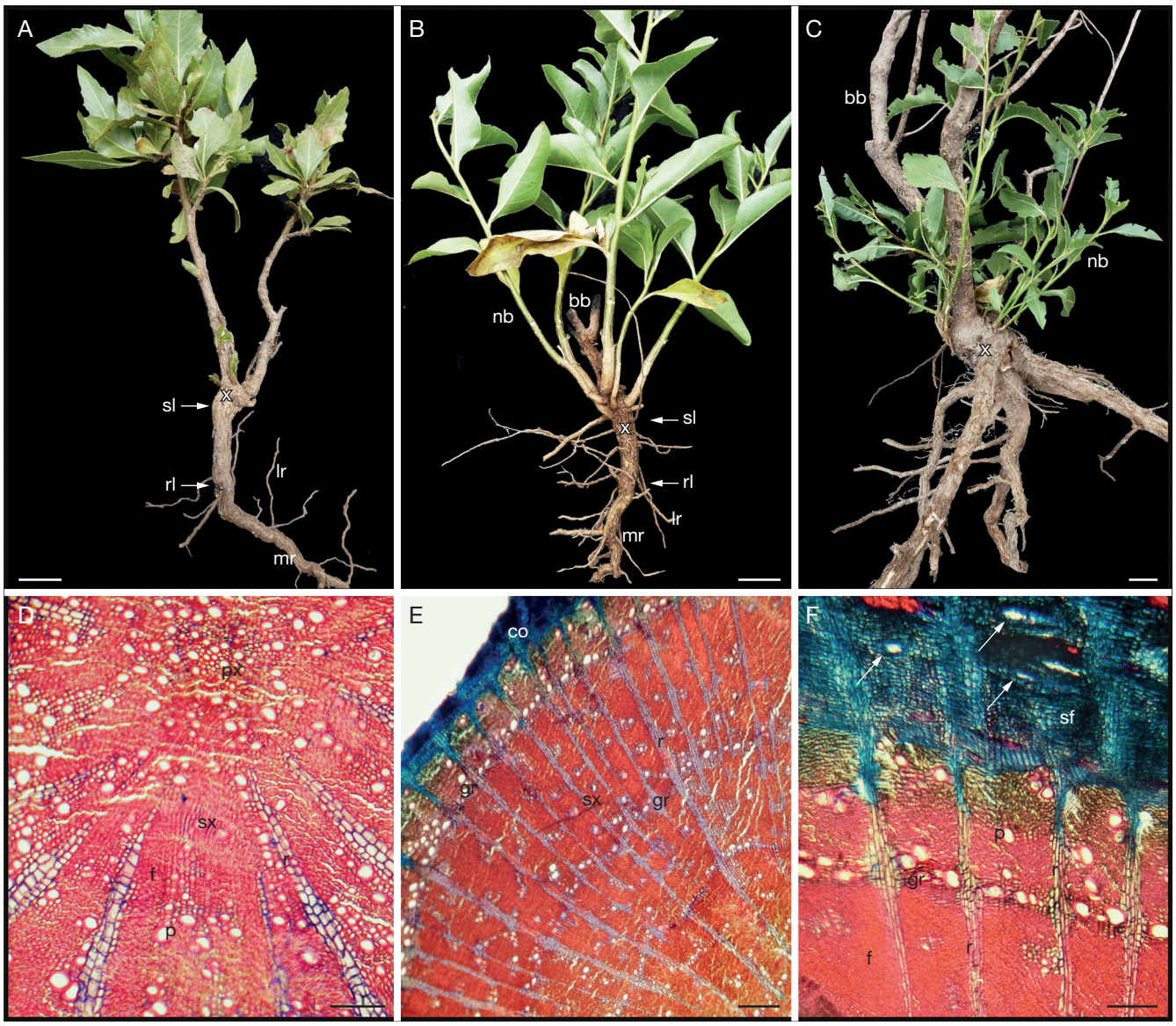

FIG. 7. — Xylopodia in *Flourensia* DC.: **A**, **D**-**F**, *F. oolepis*; **B**, **C**, *F. campestris* Griseb.; **A**, young plant with developing xylopodium; **B**, young burned plant with new branches; **C**, mature burned plant with new branches from xylopodia; **D**, cross section with primary xylem at root level indicated in **A** with **rl**; **E**, **F**, cross section at stem level indicated in **A** with **sl**; **E**, general view of wood with growth rings; **F**, detail of cortex with secretory ducts. Abbreviations: **bb**, burned branch; **co**, cortex; **f**, fiber; **gr**, growth ring; **lr**, lateral root; **mr**, main root; **nb**, new branch; **p**, pore; **px**, primary xylem; **r**, ray; **rl**, root level; **sl**, stem level; **sf**, secondary phloem; **sx**, secondary xylem; **x**, xylopodium. **Arrow**, secretory duct. Scale bars: A-C, 2 cm; D-F, 200 µm.

An ovarian nectary is common in Asteraceae secreting nectar through stomata (Wist & Davis 2006; Bernardello 2007). Nectar analyses of Argentinean Asteraceae indicated they generally had higher proportion of hexoses than sucrose, including *F. campestris* (Torres & Galetto 2002; Galetto & Bernardello 2003).

Most Asteraceae are pollinated by generalist species (e.g., Lane 1996; Torres Díaz *et al.* 2007). Accordingly, *Flourensia* species studied were visited by a variety of insects that according to their behavior can be considered as pollinators, as also informed for *F. thurifera* and *F. campestris* (Torres & Galetto 2002, 2008; Urzúa *et al.* 2007). Nevertheless, there is a Mexican species, *F. cernua* that has small capitula with no ray flowers and is wind-pollinated (Valencia Díaz & Montaña 2005); other North American species with the same type of capitula might also be wind-pollinated (Dillon 1984).

Asteraceae have a great diversity of reproductive systems. About 65% of the studied species have sporophytic self-incompatibility, a prezygotic system that ensures high genetic variability in the offspring (Ferrer & Good-Avila 2007; Jeffrey 2009). At the same time, there are partially self-compatible or self-compatible species (Torres Díaz *et al.* 2007; Torres & Galetto 2008; Ferrer *et al.* 2009). Few data are available on the reproductive system of the genus: *Flourensia campestris* was reported as self-incompatible (Torres & Galetto 2008) and *F. cernua* as partially self-incompatible (Ferrer *et al.* 2009). According to our data, *F. campestris* and *F. oolepis* could also be self-incompatible, as probably would be the remaining

species. Additional floral traits favor cross pollination in *Flourensia* species studied, as it is frequent in Asteraceae, i.e., protandry and secondary pollen presentation (Howell *et al.* 1993; Jeffrey 2009).

In the achene pericarp, there is a layer of phytomelanin which is considered both a defense against insect attack and a barrier against light (Pandey & Dhakal 2001; Jeffrey 2009). It is a synapomorphy used to delimit the *Phytomelanin Cypsela* Clade that comprises more than 5000 species (e.g. Heliantheae, Helenieae and Eupatorieae; Jeffrey 2009). In the seeds, it is noteworthy the presence and persistence of an endothelium which accumulates nutrients, breaks them down, and transfers them to the embryo, possibly acting as a restrictive barrier (Fahn 2002). The schizogenous secretory ducts observed in the cotyledons were observed for the first time in the genus and would provide protection against herbivores to both seeds and seedlings. This trait is rare in the family and was previously found in some Senecioneae (Jeffrey 2009) and Millerieae (Jana & Mukherjee 2012); probably, it might be present in all *Flourensia* species.

Achenes of the studied species have no adaptations for dispersal over long distances, even though it is common in composites by the presence of pappus (Funk *et al.* 2009). They tend to fall close and remain near to the maternal plant or may be dispersed at short distances by ants and other small animals, as detected in *F. cernua*, *F. thurifera* and several species of the closely related genus *Encelia* (Montaña *et al.* 1990; Mauchamp *et al.* 1993). This limitation in seed dispersal would explain their distribution: generally in dense clumps forming large almost monospecific communities covering slopes of hills (Montaña *et al.* 1990; Mauchamp *et al.* 1993; Urzúa *et al.* 2007).

A significant number of infertile achenes, in many cases by parasitism, was identified in the species studied. Previously, parasitism was pointed out in *F. thurifera* by Diptera females that oviposit on the flowers and their larvae develop in the ovaries (Frías 1985) and in *F. cernua* by Diptera and Coleoptera larvae (Richerson & Boldt 1995; Valencia Díaz & Montaña 2003). Achenes with abortive embryos have also been informed in *F. cernua* (Valencia Díaz & Montaña 2003, 2005) and other Asteraceae (e.g. Byers 1995; Batalha Velten & Souza Garcia 2005), as here found. This fact could be due to several causes: mechanisms of self-incompatibility, low genetic variability, low quality and/or quantity of the transferred pollen (e.g. Byers 1995; Valencia Díaz & Montaña 2003). Incomplete fruit set may reflect deposition of insufficient quantity of pollen or incompatible pollen; pollen limitation and unavailability of compatible mates may interact together to decrease fruit set (Byers 1995). In other plant families, fruits with empty seeds would be an adaptation to decrease parasitism and predation by insects or birds (e.g. Fuentes & Schupp 1998; Verdú & García Fayos 2001).

As the seeds of the studied species did not show dormancy, except *F. tortuosa*, and were dispersed in the summer rainy season, they could immediately germinate in optimum temperature and humidity. On the other hand, *F. cernua* is the only species previously reported to have a dormancy mechanism (Valencia Díaz & Montaña 2003); thus, it would be a variable trait in the genus. As a whole, seeds did not have long viability, as they have to germinate in less than 32 months. In addition, four species showed low germination (< 30%), similar to that reported for *F. cernua*, a species in which the low reproductive success would be a consequence of inbreeding depression (Valencia Díaz & Montaña 2003, 2005); the same situation could be applied here, although additional research on the subject is needed. By contrast, *F. campestris* and *F. oolepis* showed high germination rates in all tests (>60%), consistent with results previously reported (Galíndez *et al.* 2009b).

The xylopodia anatomy was here reported for the first time in the genus, including the presence of secretory ducts. They proved to be essential in the regrowth of burned plants. This regenerative function is considered an adaptation to the xeric environments where *Flourensia* species grow in Central Argentina, in which fires and drought periods occur. Xylopodia with similar function in other Asteraceae of arid areas were reported (Vilhalva & Appezzato da Glória 2006; Cury & Appezzato da Glória 2009; Galíndez *et al.* 2009a).

CONCLUSION

The six species studied are endemics with restricted distribution. They are rare and in the past decades have suffered considerable habitat reduction. No data are available on their genetic diversity to know if genetic flux exists among the extant populations that have become fragmented and reduced in size. Their distribution could be explained taking into account the features of their reproductive biology: self-incompatibility, low production of fertile achenes, limited fruit dispersion, low germination rates, and comparatively short seed viability. *Flourensia hirta*, *F. leptopoda*, *F. niederleinii* and *F. tortuosa* would be the most vulnerable, because they are rarer and showed less reproductive success. *Flourensia tortuosa* is in category 3 because it comparatively has greater range, but considering our findings, it should be in a higher risk category. By contrast, *F. campestris* and *F. oolepis* are in less danger since they had greater germination, reduced number of unviable fruits showing greater abundance in the field.

Another issue is that seed germination and cultivation of these plants from seed is easy. *Flourensia oolepis* may be an interesting ornamental, as analyzed by Delbón & Eynard (2006), and also *F. campestris* could be cultivated. Both, and the remaining species, would be suitable for restoration of degraded and burned areas, as their xylopodia would have regenerative capacity. This feature is vital in the areas where they inhabit in which the fire frequency is high in winter, especially in Cordoba hills (Gurvich *et al.* 2005; Giorgis *et al.* 2011, 2013). Although these *Flourensia* are not in immediate danger of extinction, strategies should be implemented to protect and conserve them: to preserve its habitat together with attempts to increase their population sizes and maintain their pollinators, as it is well known that there are parallel declines in pollinators and insect-pollinated plants (Biesmeijer *et al.* 2006).

Acknowledgements
We thank Drs Luis Ariza Espinar for his assistance in species' identification and Lorena Ashworth and Ana Calviño for help with literature and statistical methods. Drs Thierry Deroin and Michael Dillon reviews were useful in improving the manuscript.

This research was supported by SECyT-UNC and CONICET. ND has a doctoral fellowship from CONICET and GB is research member from CONICET.

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Submitted on 28 September 2016;
accepted on 26 January 2017;
published on 30 June 2017.