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

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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
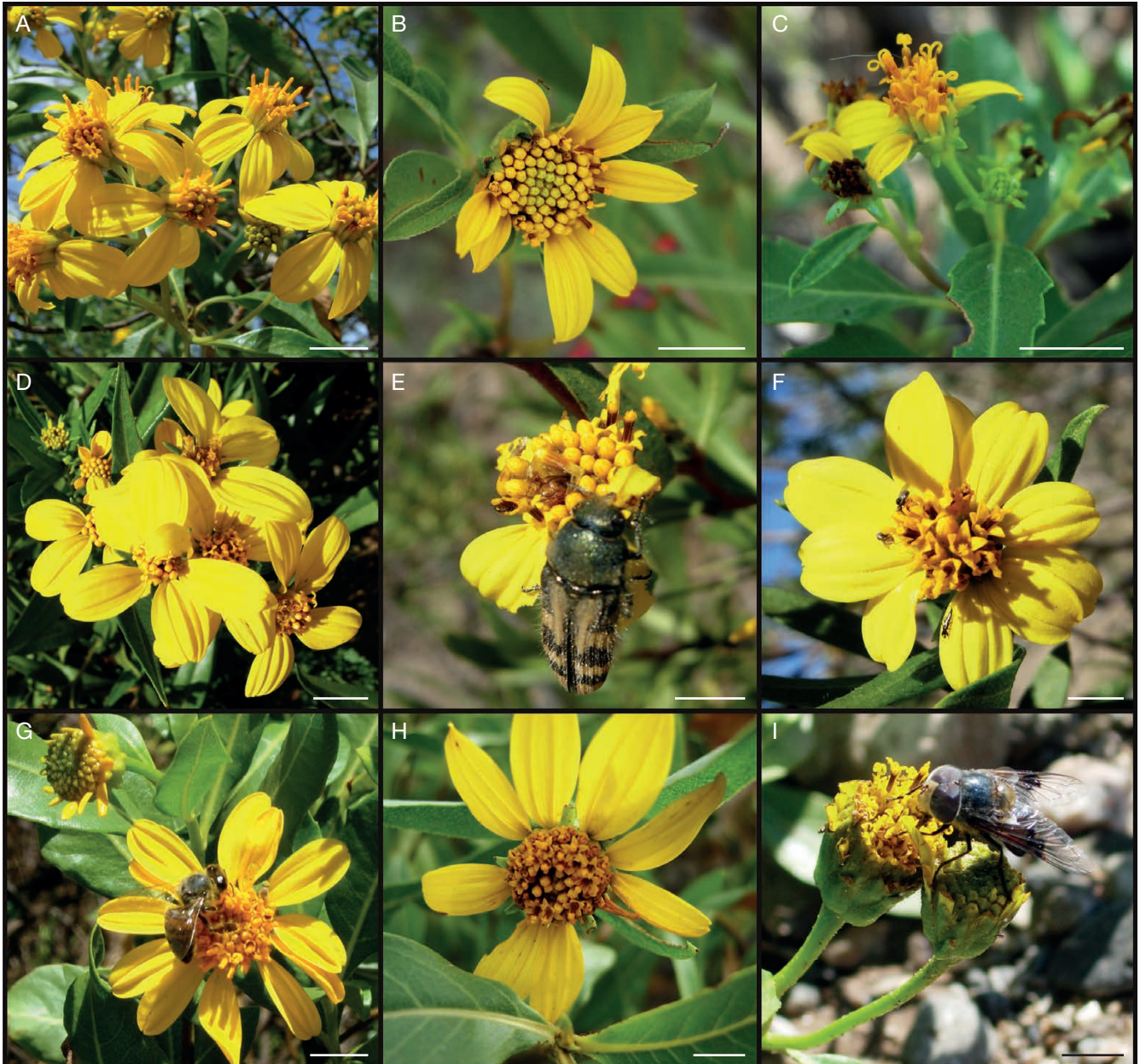
Fɪɢ. 1. — Inflorescences and flower visitors in *Flourensia* DC.: **A**, *F. campestris* Griseb.; **B**, *F. hirta* S.F. Blake; **C**, *F. leptopoda* S.F. Blake; **D-F**, *F. niederleinii* S.F. Blake; **G**, *F. oolepis* S.F. Blake; **H-I**, *F. tortuosa* Griseb.; **E**, Coleoptera; **F**, Thysanoptera; **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
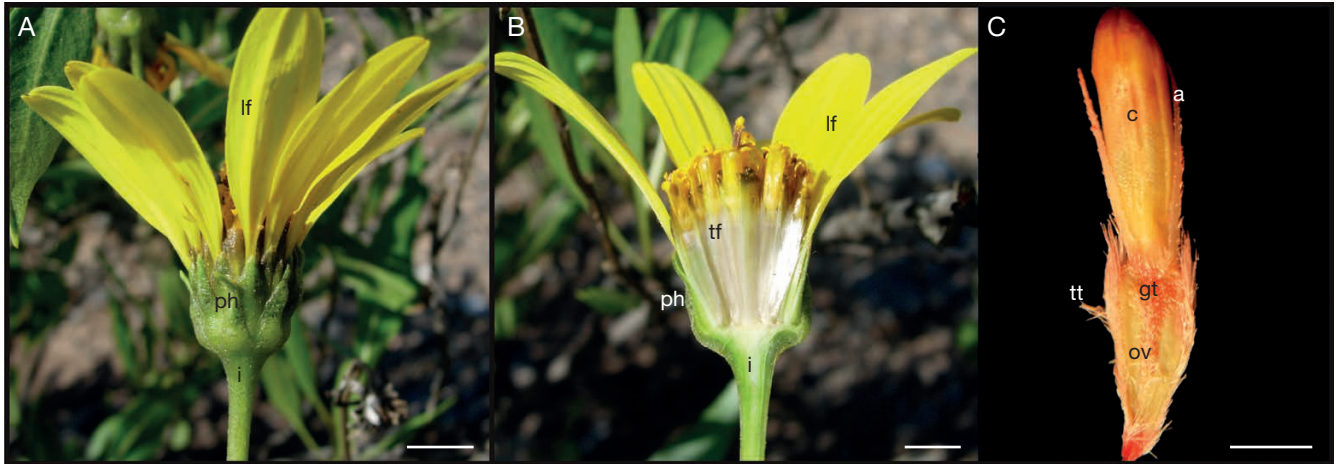
Fɪɢ. 2. — Capitulum and flower in *Flourensia* DC.: **A**, **B**, *F. tortuosa* Griseb.; **C**, *F. campestris* Griseb.; **A**, capitulum lateral view; **B**, capitulum longitudinal section; **C**, tubular flower in preanthesis. Abbreviations: **a**, awn; **c**, corolla; **gt**, glandular trichome zone; **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.

Tᴀʙʟᴇ 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.

Aɴᴀᴛᴏᴍʏ

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).

Fʟᴏʀᴀʟ ᴠɪsɪᴛᴏʀs

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.

Sᴇᴇᴅ ɢᴇʀᴍɪɴᴀᴛɪᴏɴ

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
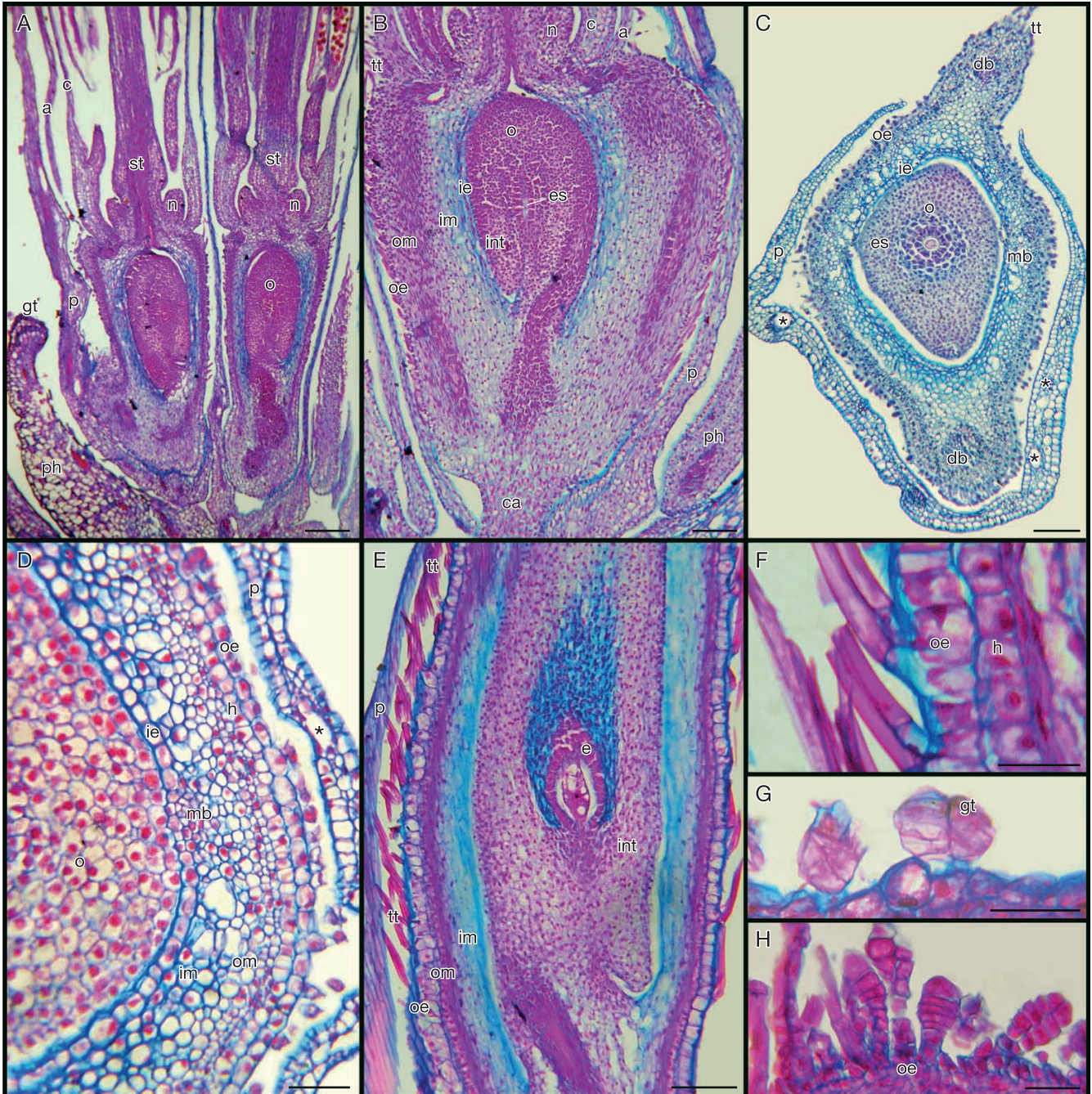
FIG. 3. — Anatomy of tubular flowers in *Flourensia* DC.: **A-G**, *F. campestris* Griseb.; **H**, *F. hirta* S.F. Blake; **A**, **B**, **E**, **F**, longitudinal sections; **C**, **D**, **G**, **H**, transversal sections; **A**, capitulum with two tubular flowers; **B**, ovary with one anatropous ovule; **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.
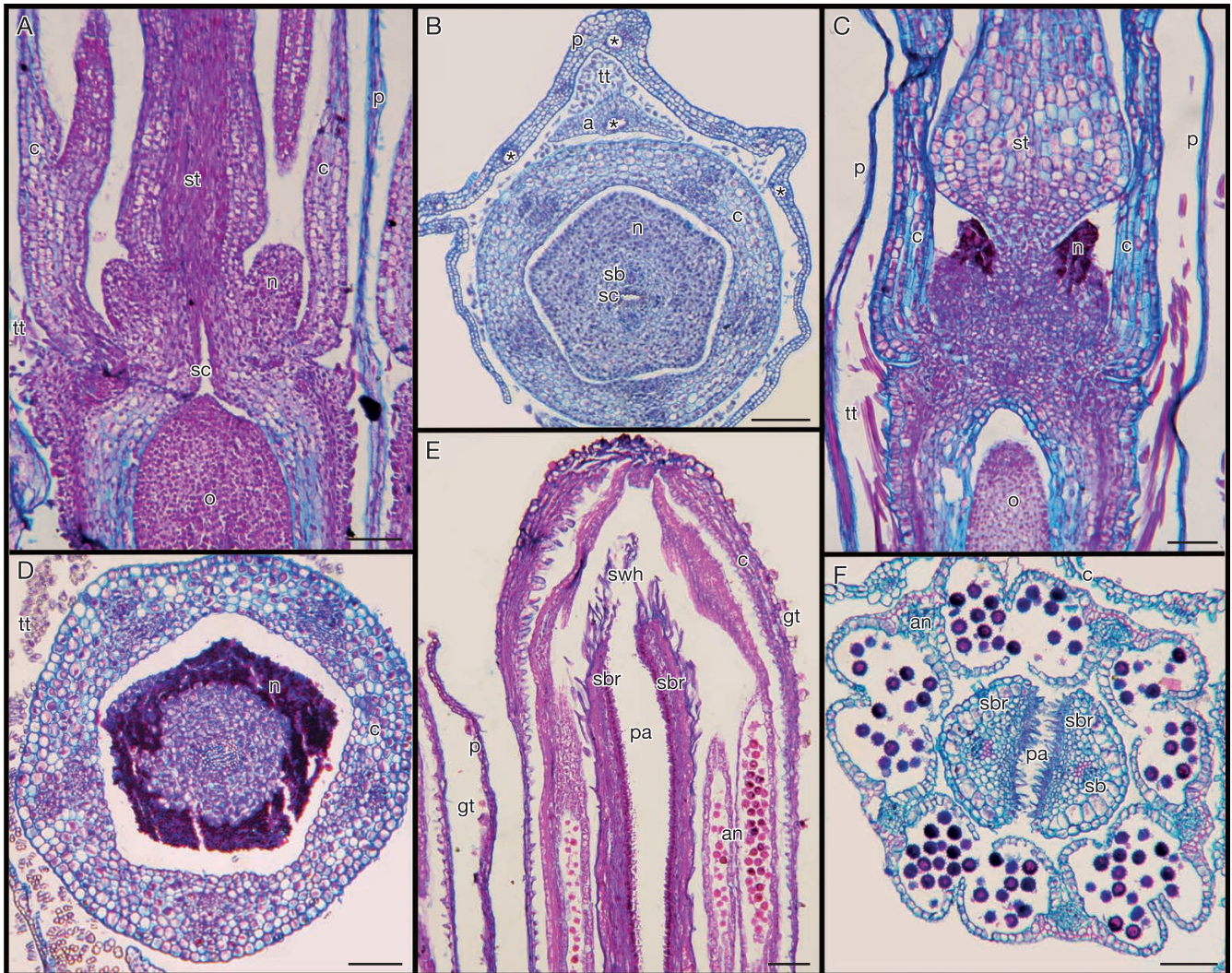
FIG. 4. — Anatomy of nectary, style, stigma and anther in *Flourensia campestris* Griseb.: **A-D**, nectary; **E**, **F**, style, stigma and anther features*;* **A**, **B**, nectary from flower at preanthesis; **C**, **D**, nectary after fertilization; **E**, style branches with sweeping hairs and stigmatic 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).
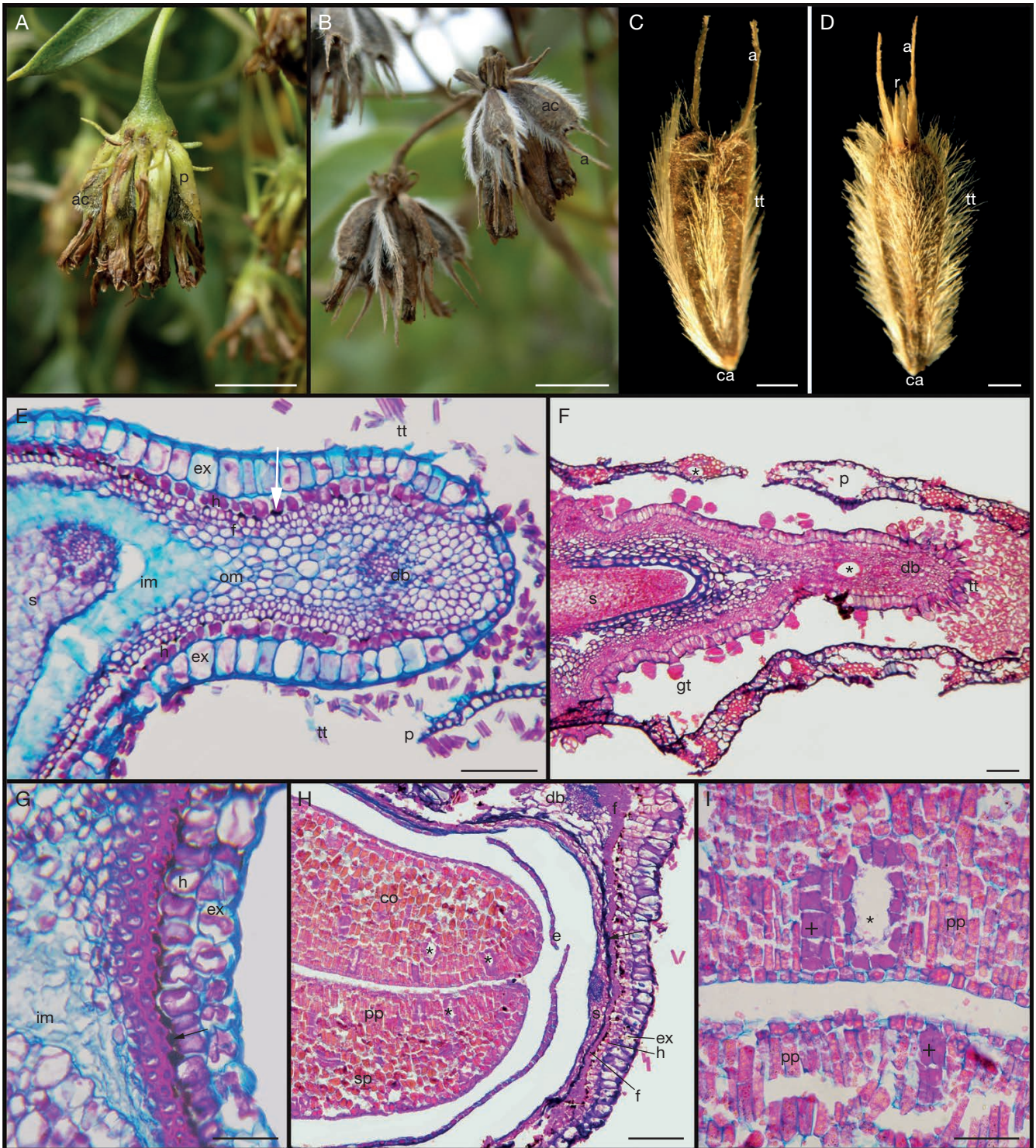
Fig. 5. — Achenes in *Flourensia* DC.: **A**, **B**, **E**, **G**, *F. campestris* Griseb.; **C**, *F. niederleinii*; **D**, **H**, **I**, *F. oolepis*; **F**, *F. hirta* S.F. Blake; **A-C**, exomorphology; **E-I**, anatomy and ontogeny of fruit and seed in transversal section; **A**, immature achenes 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).
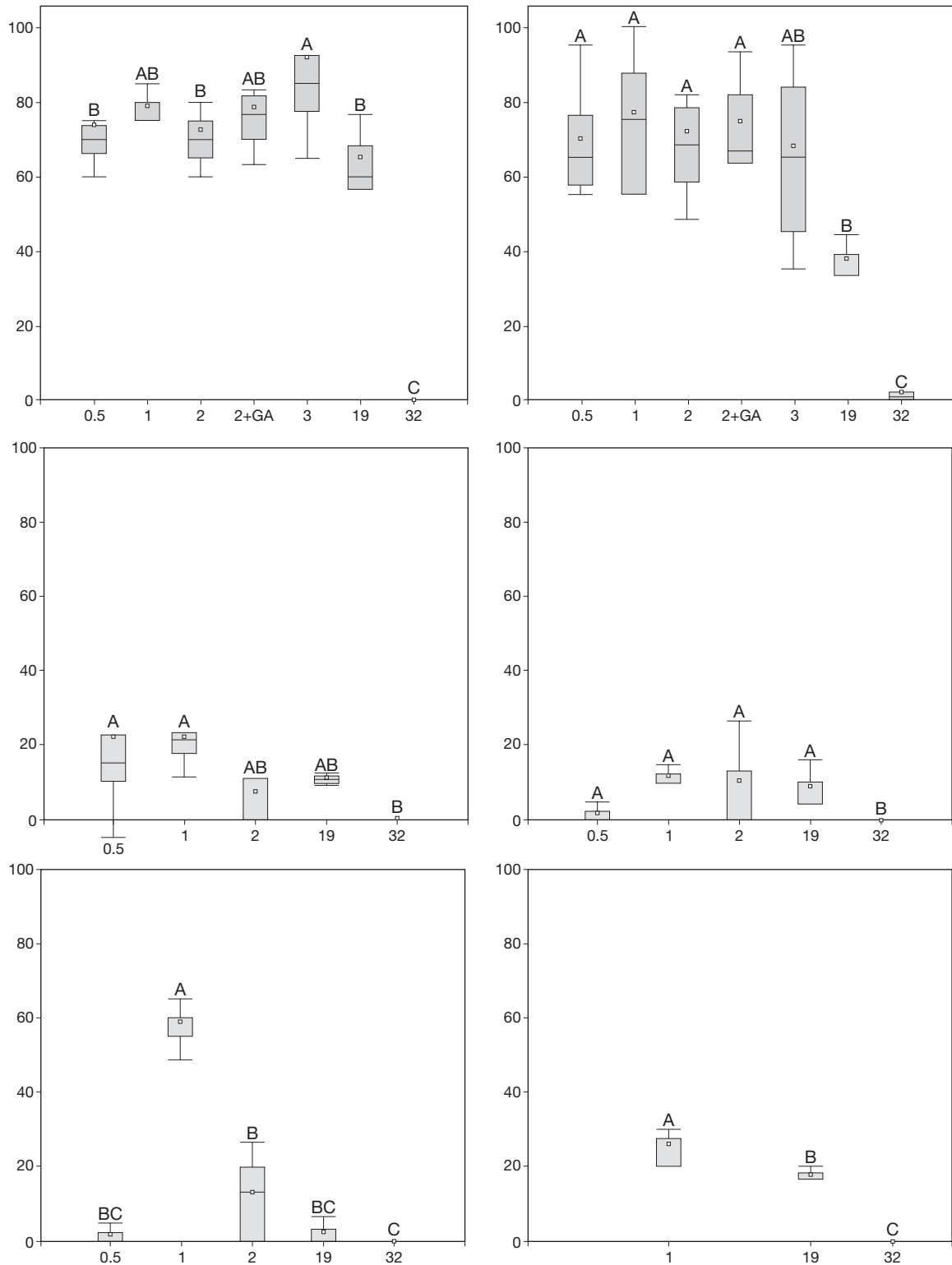
Fig. 6. — Germination in *Flourensia* DC.: germination percentage for experiments made at different seed ages. **+GA**, germination experiment with gibberellic acid. Boxplot: **box**, quartiles 1 and 3; **dot**, mean; **horizontal bar**, median; **whiskers**, maximum and minimum observation. Different 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).
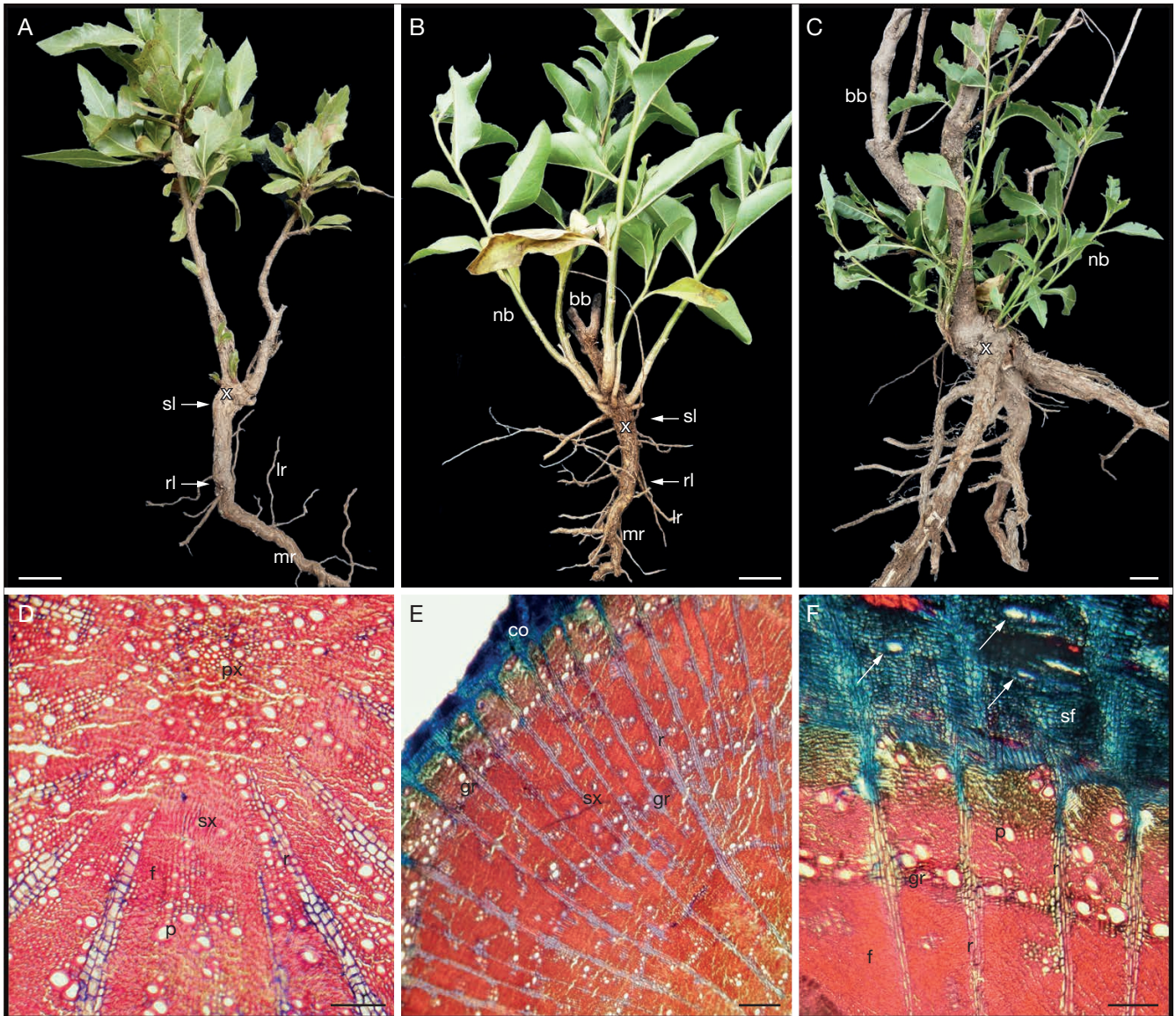
Fig. 7. — Xylopodia in *Flourensia* DC.: **A**, **D-F**, *F. oolepis*; **B**, **C**, *F. campestris* Griseb.; **A**, young plant with developing xylopodium; **B**, young burned plant with new branches; **C**, mature burned plant with new branches from xylopodia; **D**, cross section with primary xylem 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 dispersal, 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).

REFERENCES

ARIZA ESPINAR L. 2000. — *Familia Asteraceae. Tribu Heliantheae. Pródromo de la flora fanerogámica de Argentina Central 2.* Museo Botánico, FCEFyN, Universidad Nacional de Córdoba, Córdoba, 111 p.

BATALHA VELTEN S. & SOUZA GARCIA Q. 2005. — Efeitos da luz e da temperatura na germinação de sementes de *Eremanthus* (Asteraceae), ocorrentes na Serra Do Cipó, Mg, Brasil. *Acta Botanica Brasilica* 19: 753-761. https://doi.org/10.1590/S0102-33062005000400010

BERNARDELLO G. 2007. — A systematic survey of floral nectaries, *in* NICOLSON S., NEPI M. & PACINI W. (eds), *Nectaries and Nectar.* Springer-Verlag, Dordrecht: 19-128.

BIESMEIJER J., ROBERTS S., REEMER M., OHLEMULLER R., EDWARDS M., PEETERS T., SCHAFFERS A., POTTS S., KLEUKERS R., THOMAS C., SETTELE J. & KUNIN W. 2006. — Parallel declines in pollinators and Insect-pollinated plants in Britain and the Netherlands. *Science* 313: 351-354. https://doi.org/10.1126/science.1127863

BLAKE S. F. 1921. — Revision of the genus *Flourensia. Contributions from the United States National Herbarium* 20: 393-409.

BYERS D. 1995. — Pollen quantity and quality as explanations for low seed set in small populations exemplified by *Eupatorium* (Asteraceae). *American Journal of Botany* 82: 1000-1006. https://doi.org/10.2307/2446229

CHARLES H. & DUKES J. 2007. — Impacts of invasive species on ecosystem services, *in* NENTWIG W. (ed), *Biological Invasions.* Springer, Berlin: 217-237.

CICCARELLI D., GARBARI F. & PAGNI A. 2007. — Glandular hairs of the ovary: a helpful character for Asteroideae (Asteraceae) taxonomy? *Annales Botanici Fennici* 44: 1-7. http://www.jstor.org/stable/23727679

CURY G. & APPEZZATO DA GLÓRIA B. 2009. — Internal secretory spaces in thickened underground systems of Asteraceae species. *Australian Journal of Botany* 57: 229-239. https://doi.org/10.1071/BT08139

DELBÓN N., COSA M. & DOTTORI N. 2007a. — Anatomía de órganos vegetativos en *Flourensia campestris* y *F. oolepis* (Asteraceae), con especial referencia a las estructuras secretoras. *Arnaldoa* 14: 61-70.

DELBÓN N., COSA M., DOTTORI N. & STIEFKENS L. 2007b. — Estudio de la epidermis foliar en *Flourensia campestris* y *F. oolepis* (Asteraceae). *Boletín de la Sociedad Argentina de Botánica* 42: 45-50.

DELBÓN N., COSA M. & BERNARDELLO G. 2012. — Exomorfología y anatomía de órganos vegetativos aéreos en especies de *Flourensia* DC. (Asteraceae) con importancia fotoquímica. *Acta Botanica Brasilica* 26: 2-10. https://doi.org/10.1590/S0102-33062012000100002

DELBÓN N. & EYNARD C. 2006. — Cultivo de *Flourensia oolepis* (Asteraceae). Libro de resúmenes de la II Jornadas Nacionales de Flora Nativa y III Encuentro de Cactáceas, Córdoba, 1-3.

DI RIENZO J., CASANOVES F., BALZARINI M., GONZALEZ L., TABLADA M. & ROBLEDO C. 2014. — InfoStat versión 2014. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Córdoba. http://www.infostat.com.ar (last consultation on 28 April 2017).

DILLON M. 1984. — A systematic study of *Flourensia* (Asteraceae, Helantheae). *Fieldiana Botany* 16: 1-67. https://doi.org/10.5962/bhl.title.2565

FAHN A. 2002. — Functions and location of secretory tissues in plants and their possible evolutionary trends. *Israel Journal of Plant Sciences* 50: 59-64.

FERRER M. & GOOD-AVILA S. 2007. — Macrophylogenetic analyses of the gain and loss of self-incompatibility in the Asteraceae. *New Phytologist* 173: 401-414. https://doi.org/10.1111/j.1469-8137.2006.01905.x

FERRER M., GOOD-AVILA S., MONTAÑA C., DOMINGUEZ C. & EGUIARTE L. 2009. — Effect of variation in self-incompatibility on pollen limitation and inbreeding depression in *Flourensia cernua* (Asteraceae) scrubs of contrasting density. *Annals of Botany* 103: 1077-89. https://doi.org/10.1093/aob/mcp033

FREIRE S. & KATINAS L. 1995. — Morphology and ontogeny of the cypsela hairs of Nassauviinae (Asteraceae, Mutisieae), *in* HIND D., JEFFREY C. & POPE G. (eds), *Advances in Compositae Systematics.* Royal Botanical Gardens, Kew: 107-143.

FRÍAS D. 1985. — Cuatro nuevas especies Chilenas del género *Trupanea* Srank (Diptera Tephritidae). *Revista Brasileira de Zoologia* 2: 363-381. https://doi.org/10.1590/S0101-81751984000200008

FUENTES M. & SCHUPP E. 1998. — Deceptive fruits reduce seed predation by birds in *Juniperus osteosperma. Evolutionary Ecology* 12: 823-827. https://doi.org/10.1023/A:1006594532392

FUNK V. A., SUSANNA A., STUESSY T. & ROBINSON H. 2009. — Classification of Compositae, *in* FUNK V. A., SUSANNA A., STUESSY T. & BAYER R. (eds), *Systematics, Evolution and Biogeography of Compositae.* Sheridan Books, Inc., Ann Arbor: 171-192.

GALETTO L. & BERNARDELLO G. 2003. — Nectar sugar composition in angiosperms from Chaco and Patagonia (Argentina): an animal visitor's matter? *Plant Systematics and Evolution* 238: 69-86. https://doi.org/10.1007/s00606-002-0269-y

GALÍNDEZ G., BIGANZOLI F., ORTEGA BAES P. & SCOPEL A. 2009a. — Fire responses of three co-occurring Asteraceae shrubs in a temperate savanna in South America. *Plant Ecology* 202: 149-158. https://doi.org/10.1007/s11258-008-9537-4

GALÍNDEZ G., ORTEGA BAES P., DAWA M., SCOPEL A. & PRITCHARD H. 2009b. — Seed mass and germination in Asteraceae species of Argentina. *Seed Science and Technology* 39: 786-790.

GIORGIS M., CINGOLANI A. & CABIDO M. 2013. — El efecto del fuego y las características topográficas sobre la vegetación y las propiedades del suelo en la zona de transición entre bosques y pastizales de las Sierras de Córdoba, Argentina. *Boletín de la Sociedad Argentina de Botánica* 48: 493-513. http://ref.scielo.org/z4twgh

GIORGIS M., CINGOLANI A., CHIARINI F., CHIAPELLA J., BARBOZA G., ARIZA ESPINAR L., MORERO R., GURVICH D., TECCO P., SUBILS R. & CABIDO M. 2011. — Composición florística del Bosque Chaqueño Serrano de la Provincia de Córdoba, Argentina. *Kurtziana* 36: 9-43. http://ref.scielo.org/fdqxnb

GURVICH D., ENRICO L. & CINGOLANI A. 2005. — Linking plant functional traits with post fire sprouting vigour in woody species in Central Argentina. *Austral Ecology* 30: 789-796. https://doi.org/10.1111/j.1442-9993.2005.01522.x

HOWELL G., SLATER A. & KNOX R. 1993. — Secondary pollen presentation in Angiosperms and its biological significance. *Australian Journal of Botany* 41: 417-438. https://doi.org/10.1071/BT9930417

HOYOS L., GAVIER PIZARRO G., KUEMMERLE T., BUCHER E., RADELOFF V. & TECCO P. 2010. — Invasion of glossy privet (*Ligustrum lucidum*) and native forest loss in the Sierras Chicas of Córdoba, Argentina. *Biological Invasions* 12: 3261-3275. https://doi.org/10.1007/s10530-010-9720-0

ISTA 2003. — *International Rules for Seed Testing.* International Seed Testing Association, Bassersdorf.

JAIME R., REY P., ALCÁNTARA J. & BASTIDA J. 2013. — Glandular

trichomes as an inflorescence defense mechanism against insect herbivores in Iberian Columbines. *Oecologia* 172: 1051-1060. https://doi.org/10.1007/s00442-012-2553-z

JANA B. & MUKHERJEE S. 2012. — Comparative morphological and anatomical studies of cypselas or some members of the tribe Millirieae. *International Journal of Pharmaceutical Research and Bioscience* 1: 317-330.

JEFFREY C. 2009. — Evolution of Compositae flowers, *in* FUNK V. A., SUSANNA A., STUESSY T. & BAYER R. (eds), *Systematics, Evolution and Biogeography of Compositae.* Sheridan Books Inc., Ann Arbor: 131-138.

JORAY M., DEL ROLLÁN M., RUIZ G., PALACIOS S. & CARPINELLA M. 2011. — Antibacterial activity of extracts from plants of central Argentina. Isolation of an active principle from *Achyrocline satureioides*. *Planta Medica* 77: 95-100. https://doi.org/10.1055/s-0030-1250133

KATINAS L., GUTIERREZ D., GROSSI M. & CRISCI J. 2007. — Panorama de la familia Asteraceae (= Compositae) en la República Argentina. *Boletín de la Sociedad Argentina de Botánica* 42 (1-2): 113-129. http://ref.scielo.org/3kd4hn

KRAUS J., DE SOUSA H., REZENDE M., CASTRO N., VECCHI C. & LUQUE R. 1998. — Astra blue and basic fuchsin double staining of plant materials. *Biotechnic & Histochemistry* 73 (5): 235-243. https://doi.org/10.3109/10520299809141117

LANE M. 1996. — Pollination biology of Compositae, *in* CALIGARI P. & HIND D. (eds), *Compositae: Biology & Utilization.* Royal Botanical Garden, Kew: 61-80.

LÓPEZ D., PIAZZA L., SILVA M., LÓPEZ RIVILLI M., CANTERO J., TOURN G. & SCOPEL A. 2014. — Distribution of (–)-hamanasic acid A in South American species of *Flourensia* and phytotoxic effects of leaf aqueous extracts. *Natural Product Communications* 9: 341-345.

MALCOLM J., LIU C., NEILSON R., HANSEN L. & HANNAH L. 2006. — Global warming and extinctions of endemic species from biodiversity hotspots. *Biological Conservation* 20: 538-548. https://doi.org/10.1111/j.1523-1739.2006.00364.x

MARZINEK J. & TROMBERT OLIVEIRA D. 2010. — Structure and ontogeny of the pericarp of six Eupatorieae (Asteraceae) with ecological and taxonomic considerations. *Anais da Academia Brasileira de Ciencias* 82: 279-291. https://doi.org/10.1590/S0001-37652010000200004

MAUCHAMP A., MONTAÑA C., LEPART J. & RAMBLA S. 1993. — Ecotone dependent recruitment of a desert shrub, *Flourensia cernua*, in vegetation stripes. *Oikos* 68: 107-116. https://doi.org/10.2307/3545315

MONTAÑA C., LOPEZ PORTILLO L. & MAUCHAMP A. 1990. — The response of two woody species to the conditions created by a shifting ecotone in an arid ecosystem. *Journal of Ecology* 79: 789-798. https://doi.org/10.2307/2260899

MYERS N., MITTERMEIER R., MITTERMEIER C., DA FONSECA G. & KENT J. 2000. — Biodiversity hotspots for conservation priorities. *Nature* 403: 853-858. https://doi.org/10.1038/35002501

PANDEY A. & DHAKAL M. 2001. — Phytomelanin in Compositae. *Current Science* 80: 933-940.

PANERO J. 2005. — New combinations and infrafamilial taxa in the Asteraceae. *Phytologia* 87 (1): 1-14.

PIMM S., RUSSELL G., GITTLEMAN J. & BROOKS T. 1995. — The future of biodiversity. *Science* 269: 347-349. https://doi.org/10.1126/science.269.5222.347

PRIMACK R., ROZZI R., FEINSINGER P., DIRZO R. & MASSARDO F. 2001. — *Fundamentos de conservación biológica.* Fondo de Cultura Económica, México, D.F., 797 p.

RAMAYYA N. 1962. — Studies on the trichomes of some Compositae I. General structure. *Bulletin of the Botanical Survey of India* 14: 177-188.

RICHARDS A. 1997. — *Plant Breeding Systems.* Chapman and Hall, London, xii + 529 p. https://doi.org/10.1007/978-1-4899-3043-9

RICHERSON J. & BOLDT P. 1995. — Phytophagus insect fauna in *Flourensia cernua* (Asteraceae, Heliantheae) in Trans-Pecos Texas and Arizona. *Environmental Entomology* 24: 588-594. https://doi.org/10.1093/ee/24.3.588

SANCHO G. & KATINAS L. 2002. — Are the trichomes in corollas of Mutisieae (Asteraceae) really twin hairs? *Botanical Journal of the Linnean Society* 140: 427-433. https://doi.org/10.1046/j.1095-8339.2002.00113.x

SANDERS D. & CLARK C. 1987. — Comparative morphology of the capitulum of *Enceliopsis* (Asteraceae: Heliantheae). *American Journal of Botany* 74: 1072-1086. https://doi.org/10.2307/2443948

SILVA M., PIAZZA L., LÓPEZ, D., LÓPEZ RIVILLI M., TURCO M., CANTERO J., TOURN G. & SCOPEL A. 2012. — Phytotoxic activity in *Flourensia campestris* and isolation of (-)-Hamanasic Acid A as its active. *Phytochemistry* 77: 140-148. https://doi.org/10.1016/j.phytochem.2011.09.020

SILVA M., TOURN G., LÓPEZ D., GALATI B., PIAZZA L., ZARLAVSKY G., CANTERI J. & SCOPEL A. 2015. — Secretory structures in *Flourensia campestris* and *F. oolepis*: ultrastructure, distribution, and (-)-Hamanasic Acid A secretion. *American Journal of Plant Sciences* 6: 925-942. https://doi.org/10.4236/ajps.2015.67100

STUESSY T., TAKAYAMA K., LÓPEZ SEPÚLVEDA P. & CRAWFORD D. 2014. — Interpretation of patterns of genetic variation in endemic plant species of oceanic islands. *Botanical Journal of the Linnean Society* 174: 276-288. https://doi.org/10.1111/boj.12088

TORRES C. & GALETTO L. 2002. — Are nectar sugar composition and corolla tube length related to the diversity of insects that visit Asteraceae flowers? *Plant Biology* 4: 360-366. https://doi.org/10.1055/s-2002-32326

TORRES C. & GALETTO L. 2008. — Importancia de los polinizadores en la reproducción de Asteraceae de Argentina Central. *Acta Botanica Venezuelica* 31: 473-494.

TORRES DÍAZ C., CAVIERES L., MUÑOZ RAMÍREZ C. & ARROYO M. 2007. — Consecuencias de las variaciones microclimáticas sobre la visita de insectos polinizadores en dos especies de *Chaetanthera* (Asteraceae) en los Andes de Chile Central. *Revista Chilena de Historia Natural* 80: 455-468. https://doi.org/10.4067/S0716-078X2007000400007

URZÚA A., SANTANDER R. & ECHEVERRÍA J. 2007. — Analysis of surface and volatile compounds of flower heads of *Flourensia thurifera* (Mol) D.C. *Journal of the Chilean Chemical Society* 52: 1244-1245. https://doi.org/10.4067/S0717-97072007000300011

VALENCIA DÍAZ S. & MONTAÑA C. 2003. — Effects of seed age, germination substrate, gibberelic acid, light and temperature on seed germination in *Flourensia cernua* (Asteraceae), a Chihuahuan Desert shrub. *Southwestern Naturalist* 48: 1-13. https://doi.org/10.1894/0038-4909(2003)048<0001:EOSAGS>2.0.CO;2

VALENCIA DÍAZ S. & MONTAÑA C. 2005. — Temporal variability in the maternal environment and its effect on seed size and seed quality in *Flourensia cernua* DC. (Asteraceae). *Journal of Arid Environments* 63: 686-695. https://doi.org/10.1016/j.jaridenv.2005.03.024

VERDÚ M. & GARCÍA FAYOS P. 2001. — The effect of deceptive fruits on predispersal seed predation by birds in *Pistacia lentiscus*. *Plant Ecology* 156: 245-248. https://doi.org/10.1023/A:1012653002598

VILHALVA D. & APPEZZATO DA GLÓRIA B. 2006. — Morfoanatomia do sistema subterrâneo de *Calea verticillata* (Klatt) Pruski e *Isostigma megapotamicum* (Spreng.) Sherff – Asteraceae. *Brazilian Journal of Botany* 29: 39-47. https://doi.org/10.1590/S0100-84042006000100005

VILLAMIL C., DE VILLALOBOS A. & SCOFFIELD R. 2010. — Plantas endémicas de Argentina. http://www.lista-planear.org (last consultation on 27 April 2017).

W<small>IST</small> T. & D<small>AVIS</small> A. 2006. — Floral nectar production and nectary anatomy and ultrastructure of *Echinacea purpurea* (Asteraceae). *Annals of Botany* 97: 177-193. https://doi.org/10.1093/aob/mcj027

Z<small>AK</small> M., C<small>ABIDO</small> M., C<small>ÁCERES</small> D. & D<small>ÍAZ</small> S. 2008. — What drives accelerated land cover change in Central Argentina? Synergistic consequences of climatic, socioeconomic, and technological factors. *Journal of Environmental Management* 42: 181-189. https://doi.org/10.1007/s00267-008-9101-y

Z<small>ULOAGA</small> F., M<small>ORRONE</small> O. & R<small>ODRIGUEZ</small> D. 1999. — Análisis de la biodiversidad en plantas vasculares de la Argentina. *Kurtziana* 27: 17-167.