Contrasting Pollination Systems of Wild Unction and Devil's Potato Root (Apocynaceae) on San Salvador: Preliminary Observations and Analyses

Authors: Suzanne Koptur, Tatyana Livshultz, Gretchen Ionta, and Ching-Wen Tan

Contrasting Pollination Systems of Wild Unction and Devil's Potato Root (Apocynaceae) on San Salvador: Preliminary Observations and Analyses

Suzanne Koptur,*[1] Tatyana Livshultz,[2] Gretchen Ionta,[3] and Ching-Wen Tan[4]

[1]Department of Biological Sciences, Florida International University, Miami, FL 33199
[2]Department of Botany, Academy of Natural Sciences, and Department of Biodiversity Earth and Environmental Sciences, Drexel University, Philadelphia, PA 19103
[3]Department of Biological and Environmental Sciences, Georgia College and State University, Milledgeville, GA 31061
[4]National Chung Hsing University, Taiwan; present address: Department of Entomology, Pennsylvania State University, University Park, PA 16802

INTRODUCTION

Apocynaceae display highly complex and diverse floral morphologies. Pollen dispersal units include monads (single pollen grains; e.g. *Plumeria*), tetrads (*Apocynum*), and pollen packaged in pollinia, e.g. *Asclepias* and *Cynanchum* (Fishbein et al. 2018). All species produce adhesive from the specialized apex of the gynoecium (the style-head). At anthesis, this adhesive may be amorphous or molded into discrete translators; in either case, it functions to attach the pollen grains to each other and to pollinators, effecting aggregated pollen transport (Fallen 1986, Endress and Bruyns 2000, Livshultz et al. 2018). Species of one large lineage, the APSA clade, which includes ca. 3700 of the ca. 4500 species in the family, share the synapomorphic presence of a gynostegium, the structurally integrated style-head and anthers, which functions to place and remove pollen from visitors (Fishbein et al. 2018). It has been hypothesized that these floral modifications are adaptations that increase "pollen transfer efficiency" and reduce loss of pollen in transit between flowers (Harder and Johnson 2008, Livshultz et al. 2018). Selection on male fitness may favor aggregation and high efficiency at the cost of fewer mating opportunities under conditions of low quantity and/or quality of pollination service (Harder and Johnson 2008, Livshultz et al. 2011). Aggregated pollen also makes it more likely that offspring in the same fruit are full siblings, with implications for pollen competition and pollen to ovule ratios (Harder and Johnson 2008).

Many kinds of floral visitors have been documented for the family, and pollination syndromes in the Apocynaceae are diverse (Ollerton et al. 2018). Insects are the main floral visitors (Endress 1994, Ollerton et al. 2018), including bees (Lopes and Machado 1999, Darrault and Schlindwein 2005, Alvina de Araujo et al. 2011, Nogueira de Moura et al. 2011), beetles (Faria-Vieira and Santos-Fonseca 2011), butterflies (Darrault and Schlindwein 2005, Alvina de Araujo et al. 2011, 2014), moths (Haber 1984, Darrault and Schlindwein 2005, Sugiura and Yamazaki 2005, Barman et al. 2018), flies (Ollerton et al. 2009), and wasps (Wiemer et al. 2011). Recent phylogenetically-informed studies have linked evolution of the gynostegium and pollinia with diversification of both corolla morphology and functional groups of pollinators (Fishbein et al. 2018, Ollerton et al. 2018). These recent syntheses highlight the need for continued studies to test the generality of identified trends.

To obtain information on the natural history of pollinator interactions by species of Apocynaceae, and to learn how their flowers function, we studied two related species that are abundant in

the flora of the Bahamas. Both are lianas belonging to the APSA clade (made up of subfamily Apocynoideae and exemplars of Periplocoideae, Secamonoideae, and Asclepiadoideae; Livshultz et al., 2007) with gynostegia, pollen in monads, and amorphous style-head adhesive, but contrasting corolla morphology. Here we present our preliminary observations on the floral biology and pollination of *Pentalinon luteum* (L.) B.F.Hansen and Wunderlin (Odonadenieae) and *Echites umbellatus* Jacq. (Echiteae).

MATERIALS AND METHODS

Study Species

Pentalinon luteum (L.) B.F. Hansen and Wunderlin (syn. *Urechites luteus* (L.) Britton) is a perennial vine (i.e., a slim liana) native to the southeastern U.S. (Florida), islands of the Caribbean (Anguilla; Bahamas; Cayman Islands; Cuba; Dominican Republic; Guadeloupe; Haiti; Jamaica; Netherlands Antilles-Saba; Puerto Rico; St. Kitts and Nevis-St. Kitts; St. Vincent and Grenadines-St. Vincent; Virgin Islands (British); Virgin Islands (U.S.)); and Central America (Honduras) (Liogier 1984, Wunderlin and Hansen 2011, Tropicos 2018). Common names include wild unction (Bahamas), wild allamanda, hammock viper's tail (Florida), *babeiro amarillo* (Puerto Rico), and licebush (Lesser Antilles).

The flowers of *P. luteum* are large (flower face diameter from 5 to 10 cm, varying among individuals), with a sympetalous corolla bearing five petal lobes in a pinwheel shape around a bell with an opening of 1–2 cm, much wider than the short nectar tube subtending the gynostegium (ca. 0.5 cm wide). The anthers are lignified, sagittate, and adhere to the stylehead apex via their adaxial surfaces. Each anther has a terminal appendage, and these appendages are twisted together at anthesis. They remain twisted ("tidy") unless disturbed by a larger probing flower visitor, after which the anther appendages are spread apart from each other ("messy"). Fruits develop from the two-parted ovary into two follicles that dehisce along one side when mature, releasing comose seeds. The two follicles remain attached at their tips during development and at maturity.

Echites umbellatus Jacq. is a perennial vine native to south Florida, West Indies, Mexico, S. America, British Honduras, Colombia, Bahamas, Cuba, Hispaniola, and Grand Cayman (Liogier 1985, Wunderlin and Hansen 2011, Tropicos 2018). Common names include potato vine (Bahamas), rubber vine, devil's potato (Florida), and *vehuco leche* (Puerto Rico).

Echites flowers have white sympetalous corollas with the five corolla lobes in a pinwheel shape (from 2–6 cm wide) around the narrow mouth (2–3 mm) of a very long floral tube (ca. 6–9 cm). The gynostegium is located partway down the tube, with the nectar below, accessible only to visitors with very long, flexible mouthparts. As in *P. luteum*, the gynostegium is formed via adhesion of lignified sagittate anthers to the apex of the stylehead. Fruits develop with the two carpels oriented 180° from each other (i.e., developing along a straight line), the follicles opening along one side when mature, releasing comose seeds.

Study Site

Fieldwork was conducted for three weeks in December 2011 on San Salvador, the easternmost island of the Bahamas, and the location of the Gerace Research Station. For each species we located three sites on San Salvador where plants were blooming prolifically to conduct our studies. Individual flowers were marked and followed, and observations on floral parts, odor, and nectar production were recorded. We conducted pollinator watches and examined the bodies of captured floral visitors to determine if they carried pollen of the species visited.

Field Methods

Floral development was assessed by observing tagged buds over the course of several days to determine the size of flower bud that would open next day. Flower opening time was determined by observation of marked buds from 6 am onward. To establish flower duration individual open flowers were monitored on the day of opening (until early evening), and on subsequent days until senescence.

Floral rewards were quantified by measuring nectar production using microcapillary pipettes and hand-held refractometers, giving us both volume (in microliters) and concentration (% sugar on a wt/wt basis). Bagging flowers of both species on plants in the field prior to anthesis, we measured nectar as described below.

Intact *Pentalinon luteum* flowers (on plants in the field) were sampled twice during the day they were open, and the two volumes summed to make a total for each flower. Sugar concentration was measured (and averaged if there were two or more collections) for each flower.

The long, spiraling, twisted corolla tube of *Echites umbellatus* made it impossible to measure nectar in intact flowers; thus, we cut the corolla tubes beneath the style head and anthers to access the accumulated nectar in the floral tube base. We therefore measured nectar in these flowers only once.

Floral scent production was determined by sniffing flowers on plants in the field at intervals throughout their open phase; all four authors participated. For the longer-lasting flowers of *Echites*, flowers of varying ages were cut from the plants and maintained with their pedicels in tubes with water, allowing us to perform "sniff tests" over several days. This allowed us to monitor changes during the hours we were not in the field, as field station regulations for safety considerations curtailed our nocturnal observations. Data were recorded as scent absent or present, with relative intensity and quality subjectively described.

To observe flower visitors, we conducted 30-minute watches at intervals throughout the daytime hours, from before the *Pentalinon* flowers opened in the early morning until evening, with multiple observers watching a certain number of (different sets of) flowers; from these watches we calculated the number of visits per flower per unit time. We performed morning, afternoon, and evening observations for 30-minute periods on *Echites* flowers. After the observation periods, we waited and caught visitors still active in the area that had been observed visiting flowers (only on *Pentalinon*). Insect species were identified and in the laboratory pollen was removed from their bodies under the dissecting microscope and mounted in fuchsin gel. Pollen samples were examined under a compound microscope and compared to reference slides made from pollen removed from pristine flowers of the two species.

Post-anthesis flowers and buds were collected into 70% ethanol to measure pollen deposition and pollen to ovule ratios. Pollen to ovule ratios were estimated using methods modified from those described in Livshultz et al. (2018). Five buds, each from a different plant, were sampled per species. Because of the much higher pollen content in *P. luteum* and *E. umbellatus* than *Apocynum cannabinum* L., the species studied by Livshultz et al. (2018), the following changes were made. The pollen content of 1 anther was dispersed in drops of Calberla's fluid and the result multiplied by 5, except for 3 *E. umbellatus* flowers where pollen was counted from all 5 anthers. Three to fifteen slides were prepared for each flower. If there was still a lot of pollen visibly clinging to the anther after this procedure, the anther remains were prepared as an additional slide. Each pollen slide was digitally imaged with a dissecting microscope using transmitted light from below and 9.3× magnification. The images were cropped at the boundaries of the coverslip and the pollen counted using an image processing and analysis script by Costa and Yang (2009) for ImageJ (Rasband 1997). The circularity and pixel area of objects counted as pollen grains was adjusted for each slide to minimize both false positives (debris counted as pollen) and negatives (pollen grains not counted) in the observer's judgement. Circularity was set at 0.5–1 for all slides. Objects with area of 90–280 pixels^2 were counted as pollen for *E. umbellatus*; for *P. luteum* dimensions of counted objects varied: 30–130 pixels^2 (2 flowers), 40–150 pixels^2 (2 flowers), and 50–240 pixels^2 (1 flower). Pollen in the "anther remains" slide was counted either with the same image analysis workflow or by eye with the "cell counter" function in ImageJ. Ovule slides were prepared by dissecting out the ovules from both ovaries; they were imaged as the pollen slides and counted using the "cell counter" function in ImageJ.

Vouchers

Vouchers of the studied plant species are deposited in the herbarium of the Academy of Natural Sciences of Drexel University (PH). Vouchers of the insect visitors are retained in the first author's collection at FIU and will be deposited in the Arthropod Collection of the Florida Museum of Natural History.

RESULTS

Both *Pentalinon luteum* and *Echites umbellatus* produce nectar and are visited by insects that collect it, in the process picking up pollen monads via the action of a gynostegium and stylehead adhesive (Livshultz et al. 2011). Both species have salverform corollas with a floral tube and contorted petals.

The large yellow flowers of *Pentalinon luteum* (Figure 1) open early in the morning, and most last for a single day; a few may last more than 24 hours, remaining open for visitation the morning of the second day (three of the more than eighty we observed). Anthesis is terminated by the abscission of the corolla with the gynostegium still attached, leaving behind the ovaries enclosed by the calyx. They have distinctive, long, anther appendages twirled together that emerge from the floral tube into the bell of the corolla (Figure 1, bottom left); it may be that certain kinds of visitors disturb this neatly twisted arrangement. Our observations revealed that after a visit by a bee, the neatly twisted anther appendages are open and askew (Figure 1, bottom left); however, butterfly visits did not disturb the tidy arrangement.

The flowers apparently produce nectar in the morning, and little or none in the afternoon. The total amount of nectar produced by individual flowers was 11.7 ± 7.4 microliters per flower, with a range from 1–32 microliters (n = 38). The sugar concentrations measured in this species ranged from 25–41% sugar on a wt/wt basis, with an average of 34.2% ± 4.8% (n = 38).

Pentalinon luteum flowers receive insect visits in the morning hours, primarily by butterflies, and occasionally, bees. Though we observed visitors to other species (such as *Pithecellobium keyense* Britt. ex Britt. and Rose and other wildflowers) nearby in the afternoon, we saw no visits to *Pentalinon* flowers later in the day. We observed and caught specimens of four species of large butterflies, and a small sweat bee visiting the flowers (Table 1); we observed other larger bees visiting flowers higher up, but were not able to catch them. Many of the visitors we caught, but not all, had pollen on their mouthparts (Table 1; Figure 2).

The long white flowers of *Echites umbellatus* open from mid-morning to late afternoon and can last from seven to at least ten days, potentially longer (Figure 3). Our observations (day and night) revealed no visitors to *E. umbellatus*, but we expect that they are visited by hawkmoths, as the flowers develop a sweet smell in the late evening that gets stronger through the night. We also found scales from lepidopteran wings inside one flower.

The *Echites umbellatus* flowers destructively sampled yielded nectar with an average volume of 5.4 ± 10.1 microliters, with a range from zero–40.4 microliters, n = 15). Sugar concentration was on average 26% ± 4.7%, and ranged from 20–31%, (n = 15).

Echites flowers of different ages used in "sniff tests" over the course of several days at varying times (day and night) were markedly less fragrant than many other moth-pollinated plants (personal observations). We detected no noticeable scent during daylight hours, nor in the early evening. The scent grew faintly sweet from several hours after dark until early morning hours, with individual flowers fluctuating in their scent intensity through the night. It must be noted that scent perception by humans is not a meaningful representation of scent detection by flower visiting insects, but only a first approximation of the scent at different times of day.

Pentalinon luteum flowers had an average of 9,900 ± 2900 pollen monads (range 7,489–14,449) and 208 ± 16 ovules (range 188–226). Pollen to ovule ratio was 48 ± 15 (range 36–73). *Echites umbellatus* flowers had 24,656 ± 5893 pollen monad (range 19,060–31,144) and 586 ± 156 ovules (range 417–783). Pollen to ovule ratio was 42 ± 8 (range 35–55).

Some dissected flowers of both species revealed pollen deposited on the receptive stigmatic area

Figure 1. Flowers of *Pentalinon luteum* on San Salvador, Bahamas. Top row: side view and plant habit. Bottom: flower faces, with stamen appendages tidy (left) and messy (right).

of their style heads, evidence of pollination during the time they were open. At several sites we saw many fruit produced on *Pentalinon*, evidence of a substantial number of successful pollinations. We rarely observed *Echites* with multiple fruits.

DISCUSSION

A bright colorful flower in a sea of green stands out to an observer; multiple flowers on an individual plant enhance the display. Both species studied have relatively inconspicuous vegetative parts; they

Table 1. Visitors to *Pentalinon luteum* captured and closely observed. Each line represents a single individual caught and examined. Voucher specimens in Koptur lab collection, for deposit at FMNH.

Order: family Specimen	Common Name	History	Pollen?
Hymentoptera: Apidae			
Dialictus parvus	tiny sweat bee	in bell of corolla	pollen on body, mounted on slide
Lepidoptera: Heliconiidae			
Agraulis vanillae insularis	gulf fritillary	visited flower, very dilapidated specimen	no pollen
Dryas iulia carteri	Julia or flambeau	visited flowers	lots of pollen, in row along proboscis
Lepidoptera: Pieridae			
Ascia monuste eubotea	great southern white	visited flower	no pollen
Phoebis agarithe antillia (male)	large orange sulfur	visited flowers	few pollen grains
Phoebis agarithe antillia (male)	large orange sulfur	visited flowers	no pollen
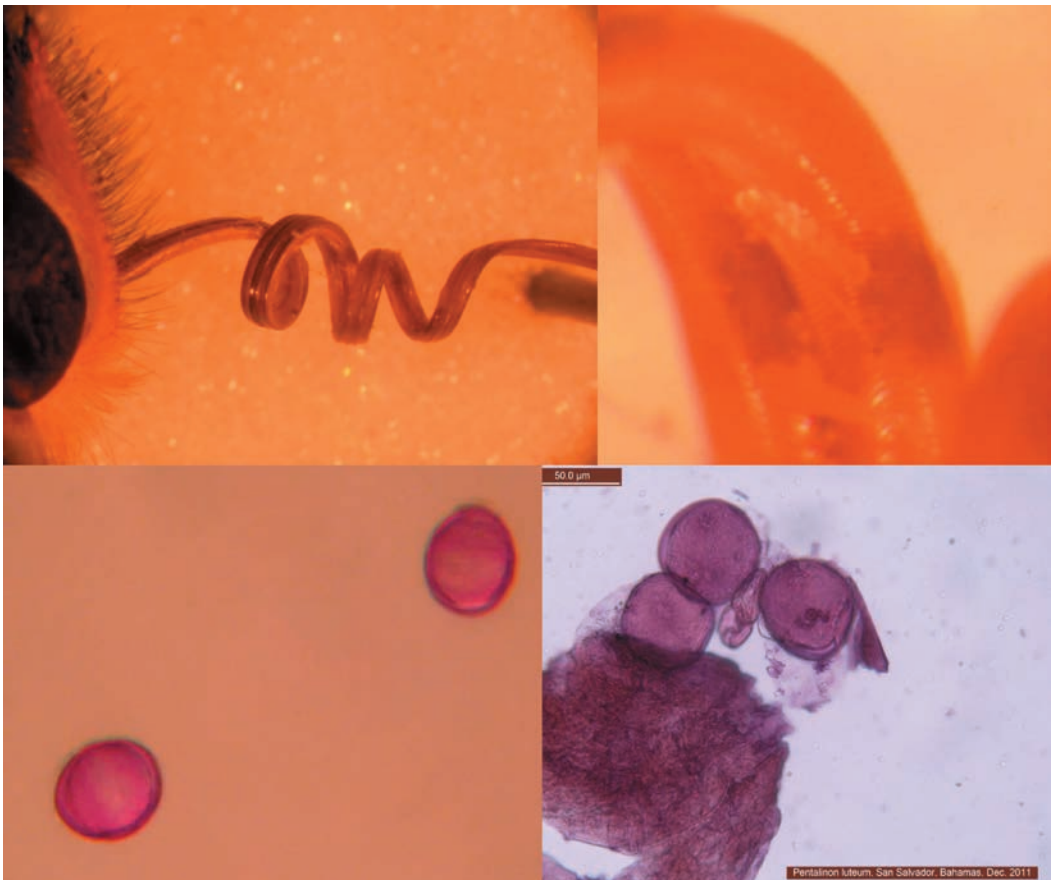
Figure 2. Evidence of pollen transfer by *Pentalinon luteum* visitor *Dryas iulia carteri*. Upper left: visitor proboscis with pollen on its surfaces; upper right: closeup of coiled proboscis with pollen grains; lower left: reference slide of *Pentalinon luteum* pollen; lower right: pollen scraped from proboscis, mounted in fuchsin gel. Its dimensions and shape confirm it is *Pentalinon* pollen.

Figure 3. Flowers of *Echites umbellatus* on San Salvador, Bahamas. Top: habit shot, in full sun. Bottom: older flowers, left, and fresh flowers, right. Individual flowers may last ten days or more.

In flowers of both species, the gynostegium restricts access to the nectar. The mouthparts of pollinators must fit into the gap between the hardened margins of two adjacent anthers to access the nectar. Examination of floral morphology indicates that upon retraction, the pollinator's mouthparts must be trapped between the pair of guiderails formed by the sagittate bases of two adjacent anthers, and guided first to the receptive stigma below a "collar" at the base of the stylehead, where any pollen they picked up previously might be deposited. The mouthparts then receive adhesive produced by the central portion of the stylehead, and finally pick up the pollen for export that is held in a chamber, its base formed by the apex of the stylehead and the sides by the five tightly packed anthers. The adhesive on the mouthparts causes the pollen to stick, until it can be deposited in another flower. The floral tube of *Pentalinon luteum*, located at the base of the bell of the corolla, is short compared to overall flower length (Figure 4), and the nectar is accessible to a wide variety of visitors that can insert a proboscis into the rather large gaps below the anther margins. The long narrow upper floral tubes in flowers of *Echites umbellatus* restrict access to the gynostegium. They are twisted with five hair-line channels spiraling along their lengths (Figure 5). These may serve to guide the flexible, long tongue of a hawkmoth to one of the 5 small gaps between adjacent anther margins at the base of the gynostegium as it seeks the nectar at the base of the tube, such that retraction of the proboscis may result in deposition of pollen from a previously-visited flower on the stigmatic surface, while also picking up pollen from the flower from which they are taking nectar.

Both species produce a large nectar reward, with concentrations corresponding to those preferred by Hymenoptera and Lepidoptera (Willmer 2011). Though we observed a variety of visitors to flowers of *Pentalinon*, some may be more effective pollinators than others. In a study of *Angadenia berteroi* (A. DC.) Miers in south Florida, Barrios et al. (2016) found that only visits from large bees produced fruit with a single visit; measurements and experiments revealed that their mouthparts were wider, and picked up more pollen than did butterflies, skippers, and smaller bees.

Echites umbellatus nectar was more dilute than that of *Pentalinon luteum*, which is appropriate for a longer floral tube, as a higher sugar concentration makes nectar more viscous and harder to collect by a long-tongued visitor (Baker 1975). Though we saw no visitors during any of our watches, it seems likely that hawkmoths are the appropriate visitors. Hawkmoths do occur on San Salvador, and we observed two species (*Erinnyis ello* L., and *Xylophanes tersa* L.) in the preserved insect collection at the Gerace Research Centre; one more, *Pseudosphinx tetrio* L., is listed for San Salvador (Elliott et al. 2009).

Echites umbellatus flowers produce more than twice as many pollen grains and ovules as those of *Pentalinon luteum*. This suggests that *E. umbellatus* stigmas must receive more than twice as much pollen to initiate fruit development, if the threshold ratio of pollen receipt is the same between the two species. Perhaps this can explain much greater floral longevity of *E. umbellatus*, if it must receive visits from more pollinators to pass this threshold, and our qualitative impression of more abundant fruiting by *P. luteum*. This hypothesis would predict similar amounts of pollen deposition per pollinator visit between the two species and higher levels of multiple paternity in *Echites umbellatus*. Alternatively, low fruit set in *E. umbellatus* may occur because their long tubular flowers restrict pollinators to a smaller set of visitors, and those visitors were not frequent at the time of our study.

The pollen to ovule ratios of the two species are very similar (42 versus 48). These fall within the range of ratios reported for Apocynaceae species with monads (range = 31–204, N = 9 species) but much higher than that for species with pollinia (range = 2.7–21.9, N = 46 species) (Livshultz et al. 2018) and are consistent with the very low pollen to ovule ratios in Apocynaceae compared to other xenogamous or facultatively xenogamous flowers. Low pollen to ovule ratios have been correlated with aggregated pollen transport and high pollen transfer efficiency (Livshultz et al., 2018). The consistently low values reported across Apocynaceae suggest that all the diverse floral morphologies in the family share these functional similarities, perhaps due to the shared presence of stylehead adhesive.

Figure 4. Flower details of *Pentalinon luteum*. Upper left: Intact corolla, side view. Upper right: corolla cut to expose anthers, stamen filaments are adnate with lower part of corolla tube, and introrsely dehiscent around style head. Lower left: one anther pulled out to reveal style head and sticky glue; middle left: anther with its appendage, note glue apparent on lower side of background surface. Lower right: style and style head with sterile apical cap, mid-region that produces glue, and base with receptive stigmatic surface. Dissection and photo by Tanya Livshultz.

Figure 5. Floral details of *Echites umbellatus*. Longitudinal section of floral tube, revealing structures near the base. From left, ovary (green) with style (white) terminating in style head, surrounded by cone of anthers. Pollen is deposited on sterile style head, shown here in the pollen chamber. Middle: fishing line of appropriate diameter (approximating width of hawkmoth tongue) was inserted to base of corolla tube, contacting glue, and extracted, covered with pollen. Bottom: cross-section of floral tube, showing hairs that guide the tongue down the spiraling paths through the anther guide rails to the nectar at the base of the tube and past the pollen chamber and style head. Dissections and photos by Gretchen Ionta.

Clearly more work is needed, and fortunately, these species are found in other locations in the Caribbean. We are continuing our studies of these species in south Florida, hoping to elucidate how the pollination mechanisms relate to pollen transfer efficiency and plant reproductive success. Our findings here provide much-needed information on pollinator/plant relationships and floral evolution in earlier diverging clades of the family Apocynaceae.

ACKNOWLEDGMENTS

LITERATURE CITED

Alvina de Araújo, L.D., Z.G.M. Quirino, and I.C. Machado. 2011. Fenologia reprodutiva, biologia floral e polinização de *Allamanda blanchetii*, uma Apocynaceae endêmica da Caatinga. Revista Brasil. Bot. 34:211–222.

Alvina de Araújo, L.D., Z.G.M. Quirino, and I.C. Machado. 2014. High specialisation in the pollination system of *Mandevilla tenuifolia* (J.C. Mikan) Woodson (Apocynaceae) drives the effectiveness of butterflies as pollinators. Pl. Biol. 16:947–955.

Baker, H.G. 1975. Sugar Concentrations in Nectars from Hummingbird Flowers. Biotropica 7(1): 37–41.

Barman, C., V.K. Singh, S. Das, and R. Tandon. 2018. Floral contrivances and specialized pollination mechanism confer strong influence to elicit mixed-mating in *Wrightia tomentosa* (Apocynaceae). Pl. Biol. 20:546–554.

Barrios, B., S.R. Pena, A. Salas, and S. Koptur. 2016. Butterflies visit more frequently, but bees are better pollinators: the importance of mouthpart dimensions in effective pollen removal and deposition. AoB PLANTS 8:plw001; doi:10.1093/aobpla/plw001

Cortés-Flores, J., K.B. Hernández-Esquivel, A. González-Rodríguez, and G. Ibarra-Manríquez. 2017. Flowering phenology, growth forms, and pollination syndromes in tropical dry forest species: Influence of phylogeny and abiotic factors. Amer. J. Bot. 104:39–49.

Costa, C.M., and S. Yang, S. 2009. Counting pollen grains using readily available, free image processing and analysis software. Ann. Bot. 104:1005-1010.

Darrault, R.O., and C. Schlindwein. 2005. Limited fruit production in *Hancornia speciosa* (Apocynaceae) and pollination by nocturnal and diurnal insects. Biotropica 37:381–388.

Elliott, N.B., D.L. Smith, and S.G.F. Smith. 2009. Field Guide to the Insects of San Salvador. Gerace Research Centre, San Salvador, Bahamas.

Endress, M.E., and P.V. Bruyns. 2000. A revised classification of the Apocynaceae *s.l.* Bot. Rev. 66:1–56.

Endress, P.K. 1994. Diversity and Evolutionary Biology of Tropical Flowers. Cambridge University Press, Cambridge, UK.

Fallen, M.E. 1986. Floral structure in the Apocynaceae: morphology, functional, and evolutionary aspects. Bot. Jahrb. Syst. 106:245–286.

Faria Vieira, M., and R. Santos Fonseca. 2011. A casual cantharophily: the meeting between *Astylus variegatus* (Coleoptera: Myleridae) and *Oxypetalum banksia* (Apocynaceae: Asclepiadoideae). J. Pollinat. Ecol. 5:86–89.

Fishbein, M., T. Livshultz, S.C.K. Straub, A.O. Simões, J. Boutte, A. McDonnell, and A. Foote. 2018. Evolution on the backbone: Apocynaceae phylogenomics and new perspectives on growth forms, flowers, and fruits. Amer. J. of Bot. 105:1–19.

Gentry, A.H. 1991a. Breeding and dispersal systems of lianas. p. 393–423. *In*: Putz, F.E. and H.A. Mooney (eds.) The Biology of Vines. Cambridge University Press, Cambridge, UK.

Gentry, A.H. 1991b. The distribution and evolution of climbing plants. p. 3–49. *In*: Putz, F.E. and H.A. Mooney (eds.) The Biology of Vines. Cambridge University Press, Cambridge, UK.

Haber, W.A. 1984. Pollination by deceit in a mass-flowering tropical tree *Plumeria rubra* L. (Apocynaceae). Biotropica 16:269–275.

Harder, L.D. and S.D. Johnson. 2008. Function and evolution of aggregated pollen in angiosperms. Int. J. Plant Sci. 169:59–78.

Liogier, H.A. 1985. Descriptive flora of Puerto Rico and adjacent islands. Editorial de la Universidad de Puerto Rico; 1a edition.

Livshultz, T., D.J. Middleton, M.E. Endress, and J.K. Williams. 2007. Phylogeny of Apocynoideae and the APSA Clade (Apocynaceae s.l.). Ann. Missouri Bot. Gard. 94:324–59.

Livshultz, T., J.V. Mead, D.J. Goyder, and M. Brannin. 2011. Climate niches of Milkweeds with plesiomorphic traits (Secamonoideae; Apocynaceae) and the Milkweed sister group link ancient African climates and floral evolution. Amer. J. Bot. 98:1966–1977.

Livshultz, T., S. Hochleitner, and E. Lakata. 2018. Pollen transfer efficiency of *Apocynum cannabinum* (Apocynaceae): a comparative perspective. J. Pollinat. Ecol. 22:35–48.

Lopes, A.V. and I.C. Machado 1999. Pollination and reproductive biology of *Rauvolfia grandiflora* (Apocynaceae): secondary pollen presentation, herkogamy and self-incompatibility. Pl. Biol. 1:547–553.

Nogueira de Moura, T., A.C. Webber, and L.N. Melo Torres. 2011. Floral biology and a pollinator effectiveness test of the diurnal floral visitors of *Tabernaemontana undulata* Vahl. (Apocynaceae) in the understory of Amazon Rainforest, Brazil. Acta Bot. Brasil. 25:380–386.

Ollerton, J., S. Masinde, U. Meve, M. Picker, and A. Whittington. 2009. Fly pollination in *Ceropegia* (Apocynaceae: Asclepiadoideae): biogeographic and phylogenetic perspectives. Ann. Bot. 103: 1501–1514.

Ollerton, J. et al. (75 authors). 2018. The diversity and evolution of pollination systems in large plant clades: Apocynaceae as a case study. Ann. Bot. 123:311–325.

Rasband, W.S. 1997-2019. ImageJ, U. S. National Institutes of Health, Bethesda, Maryland (**https://imagej.nih.gov/ij/**, 13 March 2019).

Sugiura, S. and K. Yamazaki. 2005. Moth pollination of *Metaplexis japonica* (Apocynaceae): pollinaria transfer on the tip of the proboscis. J. Pl. Res. 118:257–262.

Tropicos. 2018. Missouri Botanical Garden (**http://www.tropicos.org**, 9 Nov 2018).

Wiemer, A.P., A.N. Sérsic, S. Marino, A.O. Simões, and A.A. Cocucci. 2012. Functional morphology and wasp pollination of two South American asclepiads (Asclepiadoideae–Apocynaceae). Ann. Bot. 109:77–93.

Willmer, P. 2011. Pollination and Floral Ecology. Princeton University Press, Princeton, New Jersey.

Wolff, D., U. Meve, and S. Liede-Schumann. 2008. Pollination ecology of Ecuadorian Asclepiadoideae (Apocynaceae): how generalized are morphologically specialized flowers? Basic Appl. Ecol. 9:24–34.

Wunderlin, R.P. and B.F. Hansen. 2011. Guide to the vascular plants of Florida. 3rd edition. University Presses of Florida, Gainesville, Florida.