Nectar ecology of Datura ferox (Solanaceae): an invasive weed with nocturnal flowers in agro-ecosystems from central Argentina

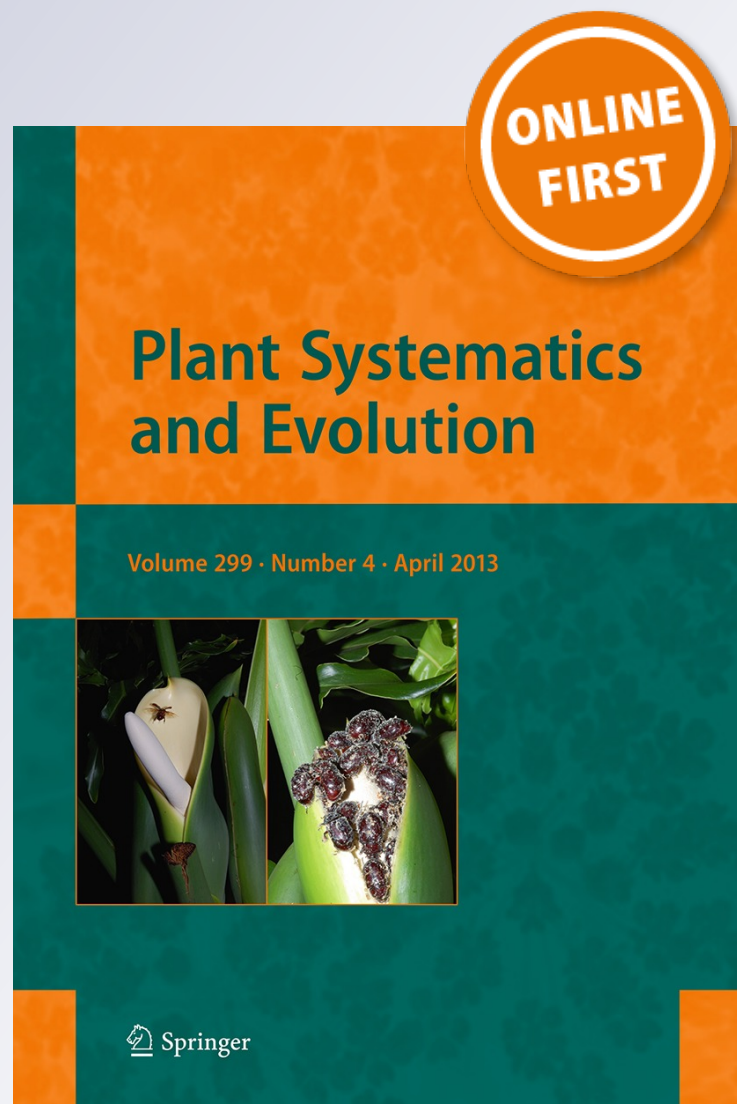## Carolina Torres, Mariana Mimosa & Leonardo Galetto

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Nectar ecology of *Datura ferox* (Solanaceae): an invasive weed with nocturnal flowers in agro-ecosystems from central Argentina

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Abstract Plant–pollinator interactions provide highly important ecological functions, and are influenced by floral nectar characteristics. The night blooming *Datura ferox* is an excellent model to test general hypotheses on the relationship between nectar traits (e.g., nectar secretion patterns, nectar chemical composition), pollinators and reproductive success for invasive, weedy species in highly modified ecosystems as crop fields. We hypothesized an adjustment between nectar composition and secretion dynamics through flower anthesis and the activity and requirements of nocturnal pollinators. Nectar chemical analyses showed low quantities of amino acids and lipids, phenolics, and alkaloids were not detected. *D. ferox* showed sucrose-dominant nectar with comparable amount of hexoses. Sugar proportions did not vary between populations or during flowering season. Most nectar is secreted before flower opening. Nectar resorption was detected at the end of anthesis. Experimentally drained flowers of both populations increased nectar production up to 50 % in the total amount of sugar per flower compared to control flowers. Nectar standing crop was relatively constant during the flowering season, but differences were detected between populations. Nectar traits of *D. ferox* would be favoring cross-pollination and maintaining seed production

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of this weed, since recently open flowers display a higher amount of nectar and they can renew nectar after a pollinator visit or reabsorb it at the end of anthesis. This nectar source may be important for native pollinators considering that human-induced forest fragmentation is related with the impoverishment of native flora from agro-ecosystems.

Introduction

Floral nectar is an aqueous solution secreted by flowers as a food source for animal visitors. In addition to the main carbohydrate components, floral nectars also contain varying amounts of different substances such as amino acids, proteins, lipids, alkaloids, phenolics, and minerals (e.g., Percival 1961; Baker and Baker 1983; Galetto and Bernardello 2005; Petanidou 2005; Petanidou et al. 2006; Nicolson and Thornburg 2007). There are some general trends associating nectar sugar composition with the pollinator types; for example, flowers visited mainly by hummingbirds tend to present sucrose-dominant nectars, whereas those visited by bats are hexose predominant (e.g., Baker and Baker 1983; Von Helversen 1993; Chalcoff et al. 2006). Flowers visited mainly by hawkmoths tend to display nectars rich in sucrose (Baker and Baker 1983). However, field observations in central Argentina showed that different sugar ratios—from sucrose dominant species to those with no sucrose—were registered in 17 species visited by this group of pollinators (Galetto and Bernardello 2003, 2004). Adults of hawkmoth species are effective pollinators and tend to visit many plant species for nectar in Chaco forests from central Argentina (e.g.,

Galetto 1997; Galetto et al. 1997; Galetto and Bernardello 2003; Moré et al. 2006a, b).

Flowers with nocturnal anthesis and hawkmoth pollination usually present a characteristic set of traits such as nocturnal flower opening, whitish corolla, strong pleasant scents, and large amount of diluted nectar (Faegri and van der Pijl 1979). The available evidence indicates that hawkmoths can learn to associate specific floral traits with nectar rewards, using visual, olfactory, and gustatory cues to find food sources (Raguso and Willis 2002, 2005; Raguso 2004). Secondary floral attractants (e.g., corolla shape and color, floral odors, CO_2 emissions) play an important role in flower-hawkmoth interactions (Adler and Bronstein 2004; Guerenstein et al. 2004; Thom et al. 2004). In addition, nectar availability (primary attractant) can be recognized by pollinators since hawkmoth visitation activity and the period of nectar secretion are synchronized since flowers produce high levels of CO_2 during nectar production (Guerenstein et al. 2004; Thom et al. 2004; Goyret et al. 2008). Despite the many ways that floral attractants influence interactions between flowers and insect pollinators, we know relatively little about how pollinators respond to the integration of different nectar traits with the pollination process. Although a few native species have been studied, nectar secretion patterns in hawkmoth-pollinated flowers from Chaco forests are consistent and showed a continuous production (Cocucci et al. 1992; Galetto and Bernardello 1993, 2004; Vesprini and Galetto 2000; López and Galetto 2002). In general, nectar secretion is neither inhibited after a removal (i.e., a pollinator visit) nor reabsorbed as the flower ages (Galetto and Bernardello 1993, 2004; Vesprini and Galetto 2000; López and Galetto 2002); only *Nicotiana longiflora* and *Caesalpinia gilliesii* inhibited nectar secretion after removals (Cocucci et al. 1992; Galetto and Bernardello 1993). There are comparatively few data to assess the relationships between nectar composition and secretion dynamics of nocturnal plants with pollination effectiveness and plant reproductive success (Amorim et al. 2013).

The large, scented, nocturnal flowers of *Datura ferox* (Solanaceae) suggest that hawkmoths are likely to be their primary pollinators. Although we have observed hawkmoths visiting the flowers, *D. ferox* were visited and pollinated by sphingids (24 % of the total recorded visits; we recorded 195 visits during a 30-h observation period), coleopterans, and *Apis mellifera* in soybean crop fields from central Argentina (Torres et al. 2013). This invasive weed is self-compatible and autogamous, but populations showed higher fruiting percentages by natural pollination (53–63 %; Torres et al. 2013). *Datura ferox* has combinations of traits (i.e., two flowering peaks, interacts with different pollinators, seed production after autogamy or xenogamy) that provide ecological advantages for establishment and survival processes in agro-ecosystems, hindering the species control (Torres et al. 2013). In many species, pollination effectiveness strongly depends on nectar-seeking pollinators and plants offer a variety of strategies on nectar production to attract them, in addition to particular nectar chemistry (Galetto and Bernardello 2005). Nevertheless, how weedy plants manage nectar production and replenishment to attract fitness-enhancing pollinators in agro-ecosystems is not well understood.

Since plant–pollinator interactions provide highly important ecological functions, and are influenced by the nectar characteristics, the lack of data for invasive weeds on the variations on nectar chemistry, nectar production, and the effects of nectar removals by pollinators on the secretion rhythms is a limitation to better understand the reproductive success of weeds in agro-ecosystems. The theoretical framework and empirical data presented above make night blooming *D. ferox* (but visited by diurnal and nocturnal pollinators) an excellent model to test general hypotheses on the relationship between nectar traits (e.g., nectar secretion patterns, nectar chemical composition), pollinators and reproductive success for invasive, weedy species in highly modified ecosystems as crop fields. We hypothesized an adjustment between nectar composition and secretion dynamics through flower anthesis with the activity and requirements of nocturnal pollinators. Since nectar production has a considerable cost for plants and *D. ferox* is self-compatible, we hypothesized a peak of nectar production at the beginning of anthesis synchronized with nocturnal pollinators and floral capability to reabsorb nectar at the end of anthesis. These two mechanisms can regulate secretion dynamics improving the balance between nectar costs and reproductive success.

In order to evaluate these hypotheses we first identified the constituents of the floral nectar of *D. ferox*. Next, we studied the pattern of nectar secretion during flower anthesis and then we experimentally tested the effect of sequential removals on total nectar production. These data were related to the main pollinators and their effectiveness on fruit set (data from Torres et al. 2013). In summary, here we provide an analysis of a weedy plant's interactions with its floral visitors to elucidate the ecological consequences of nectar traits.

Materials and methods

Study species

Datura ferox L. is an annual herb with large, scented, nocturnal flowers that develops as solitary structures in branch forks. Flowers are large, with a tubular calyx and an infundibuliform, white corolla (Fig. 1a). Nectar is secreted

by an annular nectary that surrounds the ovary; nectar accumulates at the bottom of the corolla tube. Anthesis lasts from late afternoon to the first hours of the next morning. Flowers are visited by sphingids (Fig. 1b), honeybees (Fig. 1c), and Coleoptera (Torres et al. 2013). Flowering occurs from late spring or early summer up to late summer. *Datura ferox* occurs in disturbed and recently opened spaces, such as cultivated fields, crop residues, and road edges. It is a common weed in summer crops (maize, potato, sunflower, sorghum, soybean, and Cucurbitaceae) that may become invasive and form large populations (Torres et al. 2013).

Field studies were conducted in two populations, Morteros and Colonia Isleta, San Justo department, Córdoba province, between January and April 1999. Herbarium specimens are deposited in CORD (Leonardo Galetto y Mariana Mimosa CORD 902, 903). The studied populations of *D. ferox* were located within fields sowed with soybean. Thus, plant densities of this weed at the two locations were lowest compared with those of the crop.

Nectar chemical composition

Nectar samples for chemical analyses were collected in the field from bagged (non-visited flowers, bagged in bud stage) flowers during three intervals of the flowering period, quickly dried and stored on Whatman No 1 chromatography paper in a desiccator with silica at room temperature (Galetto and Bernardello 2005). A total of 36 flowers were sampled, 18 at each population (these flowers were divided in three flower sets of six flowers, each one corresponding to three intervals of the flowering season; for each of the six flower sets, three flowers were sampled at the beginning and three at the end of anthesis; see Table 1 for details). Chemical analyses were performed within the 6 months after the sampling period. Colorimetric assays for

Fig. 1 **a** A solitary open flower of *Datura ferox*. **b** *Xylophanes tersa* visiting a flower of *D. ferox*. **c** *Apis mellifera* visiting a flower of *D. ferox*
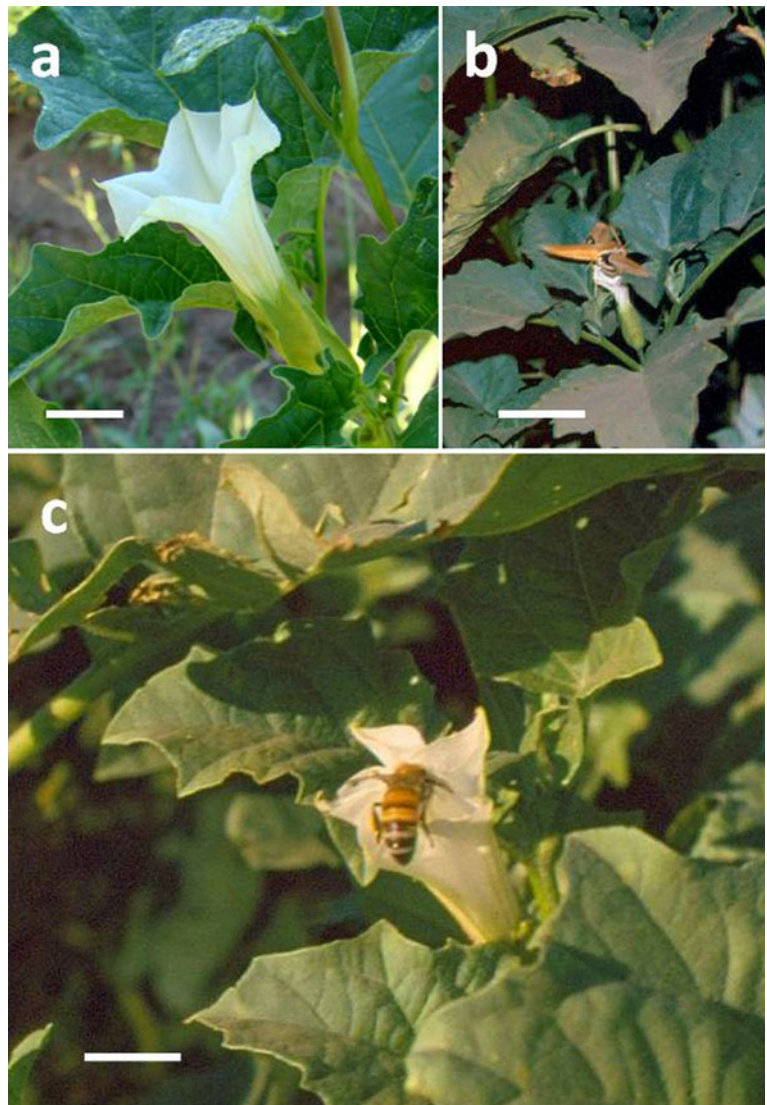
Table 1 Nectar sugar composition in two populations of *Datura ferox* during flower anthesis and during flowering season

Population	Sucrose	Fructose	Glucose
Morteros (n = 17)	53.3 ± 4.4	23.4 ± 2.2	23.3 ± 2.5
Colonia Isleta (n = 18)	62.2 ± 13.1	18.8 ± 6.3	19.0 ± 6.8
	$H = 1.5$; $p = 0.23$	$H = 3.1$; $p = 0.08$	$H = 1.4$; $p = 0.24$
Flower anthesis (sampling hour)			
19 h (n = 18)	62.3 ± 12.4	19.0 ± 6.2	18.7 ± 6.3
09 h (n = 17)	52.7 ± 4.4	23.4 ± 2.3	23.9 ± 2.5
	$H = 5.3$; $p = 0.02$	$H = 3.3$; $p = 0.06$	$H = 6.5$; $p = 0.01$
Flowering season (date in 1999)			
01/30 (n = 12)	60.5 ± 12.5	19.7 ± 6.1	19.8 ± 6.5
02/09 (n = 12	54.8 ± 2.2	22.8 ± 1.1	22.4 ± 1.9
02/19 (n = 11)	58.2 ± 13.2	20.7 ± 6.5	21.1 ± 6.6
	$H = 0.6$; $p = 0.74$	$H = 0.4$; $p = 0.82$	$H = 1.1$; $p = 0.58$
Overall (n = 35)	57.9 ± 10.7	21.0 ± 5.3	21.1 ± 5.5

Values represent percentages (means ± standard deviation)

n number of individuals sampled (one flower per individual; one of the bagged flowers at Morteros population was lost due to herbivory)

the detection of alkaloids, lipids, phenolics, and reducing acids were performed according to the suggestions of Baker and Baker (1975). Amino acids were quantified through the histidine scale (Galetto and Bernardello 2005). For sugar analyses, nectar was re-dissolved and sugar separation was accomplished by gas–liquid chromatography (GLC) to determine the types of sugar in nectar and their respective proportions (Galetto and Bernardello 2005). Nectar was lyophilized and silylated according to Sweeley et al. (1963). The derivatives were then injected into a Konik KNK 3000-HRGS gas chromatograph equipped with a Spectra-Physics SP 4290 data integrator, a flame ionization detector, and a SE 30 capillary column (30 m long, 0.25 mm diameter, and 0.25 μm thickness of the inner pellicle). Nitrogen was the carrier gas (2 ml/min), and the following temperature program was followed: 200 °C/1 min, 1 °C/min until 208 °C, 10 °C/min until 280 °C for 2 min. Carbohydrate standards (Sigma Chem.) were prepared using the same method. The sucrose/hexose ratio was calculated following Baker and Baker (1983). Finally, the hexose ratio was calculated as glucose/fructose.

Nectar secretion dynamics and removal effects

In each of the two populations nectar secretion dynamics and floral response to repeated nectar removals were performed according to Torres and Galetto (1998) and Galetto and Bernardello (2005). Nectar volume was measured using graduated micro-capillaries (10 μL), sugar concentration (percentage sucrose, mass/mass) was measured with a pocket refractometer (0–32 %), and nectar solutes were calculated from these data according to Galetto and Bernardello (2005). The nectar secretion pattern was determined using six plants in which three flowers were isolated in nylon fine mesh bags (mesh size of 0.5 mm/500 μm per aperture) during the bud stage. Thus, each flower set consisted of flowers from different individuals to cover inter-plant variations. Nectar dynamics were studied during flower anthesis, measuring variables (volume, concentration, and milligrams of solutes) at three different intervals (18:30, 20:30, 08:00; two of them at the beginning of anthesis and the last one early in the next morning). The nectar was drained and measured once in each flower set, allowing the nectar to accumulate until the next measurement in an untouched new flower set. The different phases of the nectar dynamics linked to the accumulated nectar in the different flower sets (i.e., active nectar secretion, cessation and resorption) were inferred from the plotted values of these data. The net nectar production rate per hour (NNPR) was calculated as the ratio of the amount of sugar increased (in mg) after an interval by the number of hours of the interval. The net nectar resorption rate (NNRR) was similarly calculated as the ratio of the amount of nectar decreased (in mg) after an interval by the number of hours of the last interval (Torres and Galetto 1998; Galetto and Bernardello 2005).

Floral response to successive nectar removals was evaluated in an experimental design simulating the activity of flower visitors (i.e., multiple visits to the same flowers) over the course of anthesis. We compared total accumulated nectar between 'visited' (i.e., nectar experimentally drained) and unvisited flowers (control) in three sessions at the described 3-intervals during the flower lifespan in three flower sets. A new untouched flower set was included in each subsequent removal session. Thus, in the first set of

flowers (set 3), the nectar was drained three times, the second (set 2) two times, and flowers of set 1 was measured at the end of the experiment (i.e., this was the control in which nectar was drained only once allowing its accumulation until the end of anthesis).

Nectar standing crop

The availability of nectar per flower to pollinators (nectar standing crop) was estimated sampling open flowers (10–15 flowers for each sample) in each of the two studied populations, at two different time-points of anthesis (at the beginning, 19 h, and at the end of anthesis, 9 h of the next day) and throughout the flowering season (sampling between 15 days from January to the end of February). The variables used to characterize nectar standing crop were volume, concentration, and milligrams of solutes.

Statistical analyses

Distributions were evaluated considering parametric assumptions through run tests, Levene tests, and Kolmogorov–Smirnov's goodness of fit tests. To analyze nectar sugar composition between populations, at two different time-points of anthesis (at the beginning, 19 h, and at the end of anthesis, 9 h of the next day) and throughout the flowering season (three sampling periods from January to the end of February), non-parametric analyses were performed because data did not match the parametric assumptions. To analyze the variations of nectar availability to pollinators between populations, at two different time-points of anthesis (at the beginning, 19 h, and at the end of anthesis, 9 h of the next day) and throughout the flowering season (three sampling periods from January to the end of February), a three-way ANOVA ("population", "hours of the day", and "flowering period") was performed. To analyze the effect of nectar removals between populations a two-way ANOVA ("population", "number of removals") was performed. Data were transformed to comply with the assumptions of these analyses. For those variables that showed significant differences among treatments, the a posteriori Bonferroni test was used. Statistical analyses were performed using SPSS (1999).

Results

Nectar chemical composition

Overall nectar sugar composition in *D. ferox* showed sucrose predominance with a comparable amount of hexoses (Table 1). Sugar proportions did not vary between populations or during flowering season (Table 1). On the other hand, newly opened flowers tended to present higher percentages of sucrose in nectar compared to samples obtained at the end of anthesis (Table 1). Nectar chemical analyses showed small amount of amino acids (1–2 μg/ml) but lipids, phenolics, and alkaloids were not detected.

Nectar secretion dynamics

Nectar secretion started before flower opening and newly opened flowers contained most of the naturally secreted nectar (first measurements of each treatment; Table 2). Each flower produced around 8–12 μl of nectar, with a concentration of 20–30 %. These values correspond to ca. 2.5 mg of solutes per flower (Table 2). Activity of nectar secretion in bagged flowers was registered during the initial hours after flower opening with a NNPR of 0.31 and 0.35 mg h^{-1} for Morteros and Colonia Isleta populations, respectively. These values were obtained comparing the increase in the milligrams of solutes per flower between 18:30 and 20:30, with a total of 0.63 and 0.69 mg in a period of 2-h for Morteros and Colonia Isleta populations, respectively. A reduction in the nectar solutes of the bagged flowers was detected at the first hours of the next morning (Table 2). This period was interpreted as one of active nectar resorption, in which nectar recovery by the flower started a few hours before flower senescence. Nectar resorption in terms of sugar solutes was detected in both populations with a NNRR of 0.10 and 0.03 mg h^{-1} for these populations, respectively. These values were obtained comparing the decrease in the milligrams of solutes per flower between 20:30 and 08:00, with a total of − 1.22 and −0.39 mg in a period of 12 h for Morteros and Colonia Isleta populations, respectively.

Nectar removal effects on total nectar production

Experimentally drained flowers of both populations increased nectar production up to 50 % in the total amount of sugar per flower compared to control flowers (Table 2). Two-way ANOVA showed significant differences between flowers of the control set (#1) and drained flowers (sets # 2 and 3; Table 2; $F_{[2,33]=}$ 14.9; $P < 0.00001$) but not between populations (for milligrams of solutes, volume, and concentration: $F_{[1,18]} = 2.5$, $P = 0.12$; $F_{[1,18]=}1.17$, $P = 0.29$; $F_{[1,18]=}2.6$; $P = 0.12$).

Nectar standing crop

Nectar standing crop was relatively constant during flower lifespan as well as the flowering season, although a higher amount can be found at the end of the flowering season. Significant differences were detected between populations

Table 2 Nectar secretion dynamics and removal effects in two populations of *Datura ferox*

Hour	18:30		20:30		08:00		Total nectar production	
Population	1	2	1	2	1	2	1	2
Set 3								
µl	8.23 ± 2.47	5.83 ± 0.75	5.83 ± 1.33	2.67 ± 1.37	2.83 ± 2.13	3.83 ± 2.4	16.90 ± 3.62	12.33 ± 3.56
%	20.9 ± 5.14	29.9 ± 4.84	22.6 ± 2.2	19.5 ± 3.1	19.4 ± 6.4	15.2 ± 7.5	21.2 ± 3.8	23.2 ± 3.2
mg	1.86 ± 1.20	1.94 ± 0.27	1.45 ± 0.37	0.56 ± 0.31	0.53 ± 0.35	0.60 ± 0.35	3.84 ± 0.70[a]	3.06 ± 0.54[a]
Set 2								
µl			8.17 ± 2.32	8.17 ± 2.93	6.83 ± 2.64	4.67 ± 1.51	15.00 ± 2.83	12.83 ± 3.76
%			26.8 ± 5.9	27.4 ± 4.7	12.5 ± 6.8	16.4 ± 6.4	19.7 ± 6.0	21.9 ± 2.6
mg			2.49 ± 1.05	2.63 ± 1.32	0.77 ± 0.22	0.76 ± 0.26	3.26 ± 1.14[a]	3.39 ± 1.34[a]
Set 1								
µl					12.33 ± 3.88	6.33 ± 1.86	12.33 ± 3.88	6.33 ± 1.86
%					10.1 ± 4.4	31.2 ± 1.1	10.1 ± 4.4	31.2 ± 1.1
mg					1.27 ± 0.62	2.24 ± 0.66	1.27 ± 0.62[b]	2.24 ± 0.66[b]

Nectar volume, concentration, and milligrams of solutes data are presented at three different intervals (18:30, 20:30, 08:00 h) during flower anthesis. Floral response to successive nectar removals was evaluated simulating the activity of flower visitors (i.e., multiple visits to the same flowers) over the course of anthesis (total nectar production). Thus, in set 3, the nectar was drained three times, in set 2 two times, and in set 1 nectar was drained only once allowing its accumulation until the end of anthesis (see "Methods and Materials" section). Values represent means ± SD. Different letters indicate significant differences (Bonferroni a posteriori results). A total of 18 flowers (six for each set) were used for the experiment

1 Morteros, *2* Colonia Isleta

Table 3 Availability of nectar per flower to pollinators (nectar standing crop) in two natural populations of *Datura ferox* throughout the flowering season and at two different time intervals of anthesis (19 h, at the beginning; 9 h, at the end of anthesis)

Variables	Volume (µl)	Concentration (% weight/weight)	Sugar per flower (mg)
Flowering season (date in 1999)			
01/30	5.89 ± 2.65	22.60 ± 9.37	1.37 ± 0.71[a]
02/09	6.41 ± 2.71	22.76 ± 8.62	1.57 ± 0.68[a]
02/19	7.71 ± 4.60	22.42 ± 8.15	2.18 ± 1.41[b]
Flower anthesis			
19 h	7.56 ± 3.40[a]	25.73 ± 7.60[a]	2.10 ± 1.04[a]
9 h	5.63 ± 3.31[b]	17.76 ± 7.97[b]	1.07 ± 0.57[b]
Population			
Morteros	6.97 ± 3.43	20.33 ± 9.41[a]	1.50 ± 0.73[a]
Colonia Isleta	6.40 ± 3.53	24.52 ± 7.49[b]	1.86 ± 1.19[b]

Ten to 15 individuals were sampled for each data set. Values represent means ± SD. Different letters indicate significant differences (Bonferroni a posteriori results)

and when comparing nectar standing crop at different intervals during the flowering period (Tables 3, 4).

Discussion

Many species visited by hawkmoths tend to display nectars with high proportions of sucrose (Baker and Baker 1983), including some species from Chaco with the largest corollas and visited by this group of pollinators such as Onagraceae, Orchidaceae, Convolvulaceae, and Apocynaceae (Galetto and Bernardello 2003, 2004). *Datura ferox* and *D. wrightii*

(this work and Riffell et al. 2008, respectively) fit with this general trend of nectar with sucrose predominance, whereas other native hawkmoth-pollinated Solanaceae and Fabaceae presented nectars with predominance of hexoses (Galetto and Bernardello 2003). Experimental tests showed that hawkmoths have a preference for sucrose-dominant than for hexose-dominant nectars; moreover, hawkmoths learned to associate colors with the preferred sugar (Kelber 2003) suggesting the importance of sugar composition in their preferences when visiting flowers of the community. Riffell et al. (2008) have demonstrated that hawkmoths present innate preference for certain combination of floral traits but

Table 4 ANOVA results showing nectar standing crop (volume, concentration, and milligrams of solutes) variability between populations and during both flower anthesis and flowering period (details in Table 3 and "Materials and methods")

Source of variation	SS	df	F	P
Volume				
Mean effects	186.5	4	6.411	0.0001
Population	27.6	1	3.800	0.053
Hour	130.9	1	17.997	0.0001
Date	17.1	2	1.177	0.311
Concentration				
Mean effects	5532572023	4	18.542	0.001
Population	1206588988	1	16.175	0.0001
Hour	4786742722	1	64.169	0.0001
Date	40566312	2	0.272	0.762
Sugar (mg)				
Mean effects	8.72	4	12.58	0.0001
Population	0.065	1	5.417	0.022
Hour	0.944	1	78.765	0.0001
Date	0.98	2	4.100	0.019

they can visit other species of the community through their learning capabilities and sensory flexibility. Agro-ecosystems present high spatiotemporal variability in floral resources that may have ecological consequences for both pollinators and plants on which they depend.

Flowers can secrete secondary metabolites in nectar (i.e., phenolics, alkaloids, amino acids) in addition to sugars, which can be studied in the context of pollinator behavior. Some nectars of Solanaceae have been examined considering repellent and attractant compounds showing that those experimental plants lacking alkaloids presented more nectar removed per night than wild-type plants (e.g., Kessler and Balwin 2006). We could not detect alkaloids or phenolics (repellent compounds) in the nectar samples of *D. ferox*, situation that could be favoring pollinator visits in unpredictable habitats as agro-ecosystems.

Different patterns of nectar production can be pointed out within the genus *Datura*. Most nectar is secreted in the bud stage in *D. ferox* but a small nectar amount can be found at the beginning of anthesis in *D. meteloides* and *D. stramonium*. In the last two species, most nectar is secreted after flower opening (Grant and Grant 1983; Nuñez-Farfán et al. 1996). Moreover, *D. ferox* presented lower nectar amounts than other hawkmoth-pollinated *Datura* (*D. meteloides*, *D. ceratocaula*, and *D. stramonium*; Cruden et al. 1983) but comparable to *D. wrightii* (Riffell et al. 2008). It is reasonable to assume that *D. ferox* flowers tend to increase the number of pollination events at the beginning of anthesis during the initial night hours, because most nectar is secreted before the flower opens and flower traits are mainly related to attract hawkmoths. Moreover, a

higher amount and also a renewed reward after experimental drained nectar would enhance additional visits to the flower. These results are in accordance with our hypothesis indicating a better adjustment between nectar composition and secretion dynamics through flower anthesis with the activity and requirements of nocturnal pollinators rather than diurnal pollinators. It is interesting to relate recent studies that have demonstrated a relationship between the active period of nectar secretion with the production of high levels of CO_2 emission by newly opened *Datura wrightii* flowers (Guerenstein et al. 2004). This may be important for hawkmoths as a sign to detect nectar availability and determine their foraging behavior indicating food-source profitability (Guerenstein et al. 2004; Thom et al. 2004; Goyret et al. 2008). .

Datura ferox, an exotic weed interacting with native and exotic pollinators, is the only species studied so far in the Chaco region that showed an increase in total production after removals. Some tree *Inga* species visited by diurnal and nocturnal pollinators display flowers, in which removals increased the total amount of nectar secreted (Koptur 1984; Amorim et al. 2013), mainly when it is removed by nocturnal flower visitors (Amorim et al. 2013). Nevertheless, native species from Chaco mainly pollinated by hawkmoths showed, in general, a continuous production that is not affected by removals and the absence of nectar resorption as the flower ages (Cocucci et al. 1992; Galetto and Bernardello 1993, 2004; Vesprini and Galetto 2000; López and Galetto 2002). Interestingly, recent experiments showed a flexible response of hawkmoths to floral CO_2 emission suggesting the possibility that these animals may exploit not only plants such as *Datura*, but also plants in which CO_2 does not indicate floral profitability (Guerestein et al. 2004; Thom et al. 2004). Some of the nectar traits of *D. ferox* would be related with the reproductive success of this weed, since recently open flowers display a higher amount of nectar and they can renew nectar after a pollinator visit or reabsorb it at the end of anthesis. In particular, nectar resorption may serve as an important strategy to save and reallocate resources for the production of more fruits and seeds (Pyke 1991; Nepi and Stpiczynska 2008).

When the nectar evidence is considered altogether, it is interesting to highlight the general pattern for nectar standing crop data of the two populations studied of *D. ferox*, which showed that some nectar was found in flowers throughout both the anthesis and the flowering period. The differences in nectar standing crop between populations may indicate variations in nectar production by flowers of different size and/or dissimilarities in the frequency of pollination visits. Furthermore, nectar standing crop data are indicative of pollinator activity, removing some of the produced nectar. Nevertheless, flowers of both populations presented remaining nectar standing crops through both

flower anthesis and flowering season that could be interpreted as low frequencies of visits by pollinators or as some deterrent properties of nectar that deserve new studies. For instance, the variability of nectar traits and responses to simulated pollinator activity in *D. ferox* can be also related to complex relationships of other *Datura* species and their pollinators (adult hawkmoths) and herbivores (larvae of the same species); for example, greater nectar rewards in *Datura stramonium* increased oviposition of herbivorous larvae by pollinating hawkmoths (Adler and Bronstein 2004). Therefore, attracting pollinators without also attracting herbivores may be a challenge for flowering plants that require animal pollinators to reproduce themselves (Alarcón et al. 2008; Bronstein et al. 2009).

Within an evolutionary framework, flowers of *D. ferox* seem to be mainly related with nocturnal insect pollination, offering high amounts of diluted nectar, a weak smell, and a white corolla as advertisements. They can be considered specialized since nectar is concealed deep in the corolla tube. In contrast, this species is self-compatible and fruit set would be mediated by diurnal and nocturnal pollinators (Torres et al. 2013). Cross-pollinations would be significant for *D. ferox* since populations showed higher seed sets by natural than autonomous or experimental self-pollinations (Torres et al. 2013), suggesting the importance of nectar traits in the interaction between flowers and pollinators. Hawkmoths would be important for cross-pollinations because their capability for rapid, long-distance flights (Amorim et al. 2013) in comparison with diurnal pollinators (coleopterans and honeybees) of *D. ferox* (Torres et al. 2013), and also because populations of this species are usually located within crops or in recently disturbed habitats (Ballaré et al. 1987) that make difficult pollinator movements between native habitats.

Weeds are a management concern in most agricultural systems. The development of weed management systems that minimize the negative impacts caused by the excessive use of pesticides (herbicides) would be privileged. Most of the weeds in arable crops show tolerance to the control strategies such as tillage, mowing, crop rotations, fallow, and herbicides. The efficacy of these techniques requires a detailed knowledge of the weed species' biology (Recasens et al. 2005). If pollination is unpredictable as in many agro-ecosystems, *D. ferox* can produce selfed seeds in the absence of pollen vectors (Torres et al. 2013). Nectar traits seem to be favoring cross-pollination and maintaining seed production of this weed. On the other hand, this nectar source may be important for native pollinators considering human-induced forest fragmentation diminish populations of native plants species from agro-ecosystems. In consequence, the study of nectar ecology is necessary to better understand animal-plant interactions impacts on both weed control and conservation strategies.

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