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THE IMPORTANCE OF OLIGOSULFIDES IN THE ATTRACTION OF FLY POLLINATORS TO THE BROOD-SITE DECEPTIVE SPECIES *JABOROSA ROTACEA* (SOLANACEAE)

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Premise of research. Brood-site deceptive flowers use dishonest signals—especially floral odors that mimic oviposition substrates—to attract and deceive saprophilous insects to pollinate them. In this work, we recorded the pollinators of the sapromyiophilous species *Jaborosa rotacea* (Solanaceae) endemic to southern South America. Then, we characterized the floral volatiles of this species, and finally, we carried out field experiments to decouple the effects of scent and color as attractants for saprophilous flies.

Methodology. We made direct observations of pollinators in a natural population of *J. rotacea*. We characterized floral volatiles by means of gas chromatography–mass spectrometry. Subsequently, we used a mixture of 2 oligosulfides (dimethyl disulfide and dimethyl trisulfide), which our analyses revealed were the main constituents of the floral scent of *J. rotacea*, as baits to determine the attractiveness of this olfactory signal to flies in a geographical region where *J. rotacea* is not present. Finally, we used the same foul-scented baits in arrays of artificial flowers resembling those of *J. rotacea* to assess the dual importance of olfactory and visual cues in fly attraction.

Pivotal results. Pollination of *J. rotacea* occurs when saprophilous flies belonging to the families Calliphoridae, Muscidae, and Sarcophagidae—with similar body dimensions to the anther-stigma distance in these flowers—acquire and deposit pollen in the flowers in a nototribic mode. Our chemical analyses revealed that *J. rotacea* floral scent is chemically simple and features 2 oligosulfide compounds (dimethyl disulfide and dimethyl trisulfide) commonly found in carrion-mimicking flowers. We found that saprophilous flies belonging to the same families that we recorded as pollinators of *J. rotacea* in its native South American habitat were attracted to foul-scented baits in temperate North America. The flies' visitation frequencies (recorded as approaches and landings on the artificial flowers) depended significantly on the presence of the foul-scented baits.

Conclusions. These results support the hypothesis that oligosulfides are universally effective signals by which deceptive flowers may effect pollen dispersal by attracting flies that use carrion or carnivore feces as brood sites.

Keywords: brood-site deceptive flowers, Diptera, *Jaborosa rotacea*, oligosulfides, scent mimicry, Solanaceae.

Introduction

Several unrelated groups of plants benefit from cross-pollination by biotic agents even though they do not provide any kind of reward, a phenomenon known as pollination by deception (Dafni 1984; Renner 2006). Well-known examples of deceitful plant species include food deceptive, sexually deceptive, and brood-site deceptive flowers (Jersáková et al. 2009; Vereecken 2009; Urru et al. 2011). Flowers pollinated by saprophilous insects—that is, those that use carrion or feces as food and brood sites—have evolved in several unrelated angiosperm families worldwide (Vogel 1954; Wiens 1978; Ackerman 1986; Endress 1994) and include some of the largest and most unusual blossoms (e.g., *Rafflesia*, *Amorphophallus*, *Aristolochia*, and *Stapelia*) known in the natural world (Davis et al. 2008). Sapromyiophilous flowers sensu stricto (i.e., pollinated by saprophilous flies) usually exhibit features characteristic of decaying animal or fecal matter, for example, dull-colored corollas often checkered with dark blotches and showing hairy structures or filiform appendages and, especially, fetid odors (Raguso 2004; Jürgens et al. 2006; van der Niet et al. 2011). Thus, pollinators are deceived by dishonest visual and olfactory floral cues thought to evoke insect sensory responses to decaying substances used by the pollinators as brood sites (Urru et al. 2011).

There is good evidence that the attraction of flies to sapromyiophilous flowers depends greatly on the emission of volatiles that are used by flies as cues to locate food and brood sites. Recent chemical analyses of fetid odors have demonstrated that different sources of putrefaction are typified by very distinct and specific compounds, for example, urine (hexanoic acid, carboxylic acids, and pyrazines), rotting carcasses

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(heptanal and octanal), decaying meat and carnivore dung (oligosulfides), herbivore dung (indole and cresol), and rotting fruit (oxygenated aliphatic compounds, such as acetic acid, acetoin, and 3-methylbutanol; Kite and Hetterscheid 1997; Kite et al. 1998; Stensmyr et al. 2002; Jürgens et al. 2006; Goodrich and Raguso 2009; Johnson and Jürgens 2010; Urru et al. 2011). Insect-trapping studies have established that simple blends of sulfides, indole, cresol, and/or butanoic acid are effective in trapping calliphorid flies across Europe, Africa, and Australia (Hall et al. 2003; Aak et al. 2010). In their study of carrion-mimicking flowers and fungi, Borg-Karlson et al. (1994) mentioned that flies of the genera *Calliphora*, *Lucilia*, and *Sarcophaga* in Scandinavia were attracted to dimethyl disulfide (DMDS). More recently, Stensmyr et al. (2002) obtained high levels of blowfly attraction to odorless second-day inflorescences of *Helicodiceros muscivorus* (Araceae) in Sardinia by augmenting them with dimethyl mono-, di-, and trisulfides, the volatile compounds responsible for the intense foul odor of first-day inflorescences. Similarly, Shuttleworth and Johnson (2010) demonstrated that the addition of these same compounds to the inflorescences of wasp-pollinated South African species of *Eucomis* (subfamily Scilloideae, family Asparagaceae) was sufficient to induce a shift to carrion fly pollination. These results, together with investigations of antennal sensitivity and behavioral bioassays (Cossé and Baker 1996; Stensmyr et al. 2002), support the hypothesis that a few specific scent compounds are sufficient to attract saprophilous flies in a variety of settings and habitats.

The genus *Jaborosa* (Solanaceae) comprises 23 species endemic to southern South America and exhibits astonishing interspecific variation in floral traits, including putative adaptations to sapromyiophilous pollination (Barboza and Hunziker 1998). Variation within the genus ranges from nocturnal white flowers with very long corolla tubes that emit pleasant odors, produce abundant nectar, and are exclusively pollinated by long-tongued hawkmoths (Vesprini and Galetto 2000) to diurnal black flowers with shallow corollas that emit unpleasant odors, produce no nectar, and are pollinated by saprophilous flies (Cocucci 1988, 1999). At least 5 species (*Jaborosa laciniata*, *Jaborosa leucotricha*, *Jaborosa magellanica*, *Jaborosa rotacea*, and *Jaborosa sativa*) exhibit dull-colored flowers that open near the ground surface; emit floral odors reminiscent of feces, garlic, or dead animals; and have a poorly developed or completely absent nectary (Cocucci 1988; 1999). The pollination biology of 1 of these species, *J. rotacea* (Lillo) Hunz. & Barboza, has been studied by Cocucci (1988), who recorded saprophilous flies visiting the flowers. Given these preliminary observations, we hypothesized that *J. rotacea* has a specialized fly pollination system based on brood-site mimicry, which to our knowledge is unique in the Solanaceae. To further characterize the floral biology of *J. rotacea*, we examined 3 aspects of its pollination: (1) we observed pollinators visiting this species in its natural habitat to establish which insect families and species were most represented; (2) we characterized the floral volatiles produced by flowers at different stages of bloom as well as those emitted from distinct flower parts; and (3) we assessed the importance of olfactory and visual cues in the attraction of saprophilous flies to *J. rotacea*, using foul-scented baits and artificial flowers modeled after those of *J. rotacea* at a field site where *J. rotacea* is not present.

Material and Methods

Studied Species

Jaborosa rotacea is a coarse, weedy herbaceous plant distributed in the pre-Puna biogeographical province, from southernmost Bolivia to northwestern Argentina, between 1500 and 3600 m in altitude (Barboza and Hunziker 1998; fig. 1*A*). Each plant can develop hundreds of flowers, which are located near ground and are concealed by relatively large and numerous leaves growing in a loose rosette (fig. 1*A*). Flowers are maroon to black, densely covered by whitish hairs (fig. 1*B*), and emit a strong fetid odor during daytime that can be perceived by humans from a distance of several meters. The floral nectary is vestigial, composed of a group of secretory cells that lack stomata and do not form a disc (Cocucci 1988). However, the adaxial surface of the petals is densely covered by multicellular glandular hairs that produce a sugar solution (Cocucci 1988). Unlike many other plant species with carrion-mimicking flowers (e.g., Aristolochiaceae, Araceae, Hydnoraceae), *J. rotacea* has open and rotate flowers that neither form chambers nor hold visiting flies captive (Cocucci 1988, 1999). Similar rotate flowers are also present in several sapromyiophilous species of Asclepiadoideae that have radiated in the New World, such as *Gonolobus* spp. and *Matelea* spp. or the stapeliads (family Apocynaceae) and *Eucomis* flowers (family Asparagaceae) of the Old World (Ollerton and Liede 1997; Jürgens et al. 2006; Shuttleworth and Johnson 2010).

Study Site

Direct observations of pollinators were performed in a natural population located in Tafí del Valle, Tucumán Province, Argentina (26°52′00″S, 65°40′60″W, 2000 m altitude). This location is in the lower reaches of a semiarid valley with an annual mean rainfall of 420 mm and annual mean temperature of 13.5°C. The region shows severe environmental degradation due to high soil erosion, since natural vegetation has been reduced by deforestation and agricultural development (fig. 1*A*). The lowlands are dominated by the grasses *Cynodon dactylon* and *Nassella neesiana*, but some native shrub and tree species—such as *Acacia caven*, *Acacia aroma*, and *Prosopis alba*—are also present.

Pollinators

Pollinator observations were recorded in 20-min periods from 0800 to 1800 hours on 5 different days, totaling 10 h. Fly behavior was recorded by means of photographs and videos during the flowering seasons of 2008 and 2011. Representative specimens of flies visiting the flowers were captured using a handheld net for later identification. Voucher specimens have been deposited at the Laboratorio de Ecología Evolutiva y Biología Floral (Instituto Multidisciplinario de Biología Vegetal, Córdoba, Argentina).

Presence of Nectar

To assess the presence of nectar, we used capillary tubes of 1 μL ($N = 10$ individuals, 2 flowers per individual). Because no nectar was detected in the flowers, the adaxial surfaces of

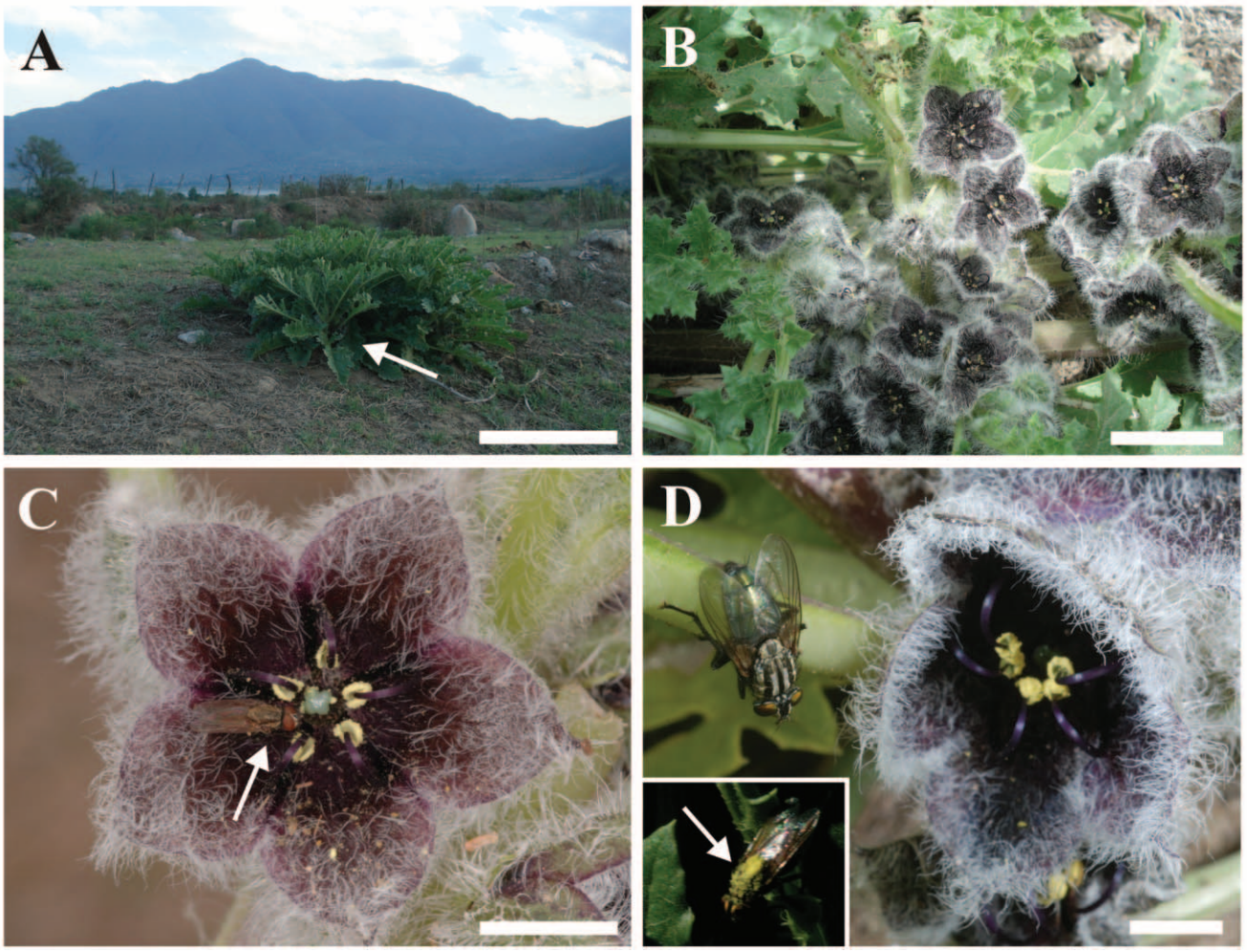


Fig. 1 *Jaborosa rotacea* and its carrion fly pollinators. *A*, Habitat of *J. rotacea* at Tafí del Valle locality in Tucumán, Argentina. *B*, View of *J. rotacea* flowers in situ. Scale bar = 4 cm. *C*, Muscoidea fly (probably belonging to family Anthomyiidae) visiting a flower of *J. rotacea*. Scale bar = 1 cm. *D*, *Sarconesia chlorogaster* (Calliphoridae) near a flower. *Inset*, detail of nototribic pollen deposition. Scale bar = 0.5 cm. Arrows indicate the position of flowers within the plant (*A*), a muscoid fly (*C*), and a blowfly carrying pollen (*D*).

the petals (i.e., bearing the glandular hairs) were pressed onto Glucostix reagent strips to test for the presence of sugar.

Volatile Collection

Floral headspace volatiles were collected from plants growing in a natural population (Tafí del Valle, Tucumán, Argentina) in November–December 2008 and January 2011 as well as from plants cultivated in a greenhouse located at Cornell University (Ithaca, NY) in May–June 2011. Solid phase extraction microtraps (Dötterl et al. 2005; Johnson and Jürgens 2010) were constructed by filling small cut glass capillaries (15 mm long, 0.2 mm internal diameter) with 5 mg Tenax TA (60/80 mesh) between plugs of silanized quartz wool. Living flowers were enclosed within small bags constructed from sealed nylon resin oven bag material (Reynolds) cut to the size needed using an impulse heat sealer. The air was allowed to equilibrate for 1 h, and the headspace volatiles were collected for 5 min using Personal Air Sampler 500 (Spectrex) 9-V battery-operated vacuum pumps. The microextraction traps containing headspace volatiles were stored within amber glass autosampler vials (1.5 mL) at room temperature until they could be analyzed by gas chromatography–mass spectrometry (GC-MS) at Cornell University.

In the field, floral scent was collected from 3 individuals (2 replicates). Freshly opened flowers (2–3 per individual) were cut and enclosed in the oven bags. Vegetative ($n = 1$) and air control ($n = 1$) samples were taken. Floral scent was also collected from a single cultivated plant in a greenhouse at Cornell University to identify ontogenic and tissue-specific sources of volatile production. Seeds were germinated from a collection made at the same population where pollinator observations were performed (the voucher specimen is deposited in CORD [AAC 4234]). Poor germination resulted in 3 mature plants, of which 1 bloomed during the duration of this study. In this case, solid phase microextraction (SPME) was used to collect odors from cut and dissected flowers of the same plant. We

q1 used 65-μ divinylbenzene/polydimethylsiloxane SPME fibers (Supelco) previously shown to be ideal for trapping fetid and fermented floral volatiles (Goodrich et al. 2006; Goodrich and Raguso 2009). Cut flowers were enclosed in oven-sterilized 10-mL borosilicate glass beakers covered with cut nylon resin sheets, and the volatiles were allowed to saturate the air (30 min) before they were trapped onto SPME fibers (30 min). All floral samples were standardized to roughly 700 mg (mean + SEM = 0.707 + 0.007 g, $n = 11$) fresh floral material. We prepared 4 different flower samples for SPME analysis, including (1) buds (1–2 d before anthesis); (2) open flowers, before anther dehiscence; (3) open flowers, with anthers dehisced; and (4) open flowers, with anthers dehisced, dissected into male and female sexual organs (stamens plus gynoecium), and the fused corolla plus calyx. Because of the small number of available flowers, male and female organs were not further dissected, so we did not determine whether there were specific volatiles associated with pollen. Volatiles were also collected from vegetative (leaves) and ambient (empty oven bags) samples as controls to identify and account for any nonfloral compounds in our floral scent samples. The full experiment (i.e., all dissections, treatments, and controls) was repeated twice (July 26 and 28, 2010), after which no additional flowers were available.

Gas Chromatographic–Mass Spectrometric Analysis of Floral Scent

Micro extraction traps were eluted through direct thermal desorption in the injection port of a Shimadzu 2010+ gas chromatograph (GC) equipped with a quadrupole, electron impact (70 eV ionization energy) mass spectrometer as a detector. A polar GC column (Stabilwax; inner diameter, 0.25 mm; length, 30 m; film thickness, 0.25 mm [Restek], with cross-linked polyethylene glycol as a stationary phase) was used. After inserting the trap into the injection port liner, the port was purged for 2 min with the split valve open at 30°C, and then the temperature was increased ballistically from 30° to 200°C at 15°C/s in splitless mode (Johnson and Jürgens 2010) to flash desorb and load volatiles onto the GC column. High-purity helium was used as a carrier gas at a fixed flow rate of 1 mL/min and a split ratio of 20 : 1.

SPME fibers were desorbed directly within the injection port of a Shimadzu GC17A gas chromatograph with a Shimadzu QP5000 quadrupole, electron impact (70 eV ionization energy) mass spectrometer as a detector. Analyses were made using splitless injections at 240°C on a polar GC column (inner diameter, 0.25 mm; length, 30 m; film thickness, 0.25 mm [EC-WAX, Alltech]), using high-purity helium as a carrier gas with a flow rate of 1 mL/min and a split ratio of 12 : 1. Oven temperature was held constant at 40°C for 3 min and then ramped up at 10°C/min until reaching 260°C, where it was held for 7 min (Goodrich et al. 2006).

Compounds collected using both methods were tentatively identified using mass spectral libraries (NIST, Wiley, Adams) and confirmed using known standards or Kovats indices (see http://www.pherobase.com/) whenever possible. Peak areas of the total ion chromatograms were integrated using Shimadzu GCMS Solutions software and then were used to calculate crude relative percentages of total emissions per sample for each compound. Because these data were collected using equilibrium-based methods, it is inappropriate to attempt to quantify release rates from flowers (Goodrich et al. 2006).

Field Experiments

Two field experiments were carried out in June/July 2010 at the Liddell Laboratory of Cornell University (Varna, NY). In both cases, we used a 1% solution of 1 : 1 DMDS–dimethyl trisulfide (DMTS) diluted in odorless mineral oil (hereafter DMDS-DMTS solution) as bait. These 2 sulfurous compounds were present in relatively high percentages in the floral scent of *J. rotacea* (see "Results"). Rather than attempting to match the release rates per flower or plant (flower number per plant of *J. rotacea* can vary 10-fold in nature), we envisioned this as a simple test of the potency of these volatiles as potential attractants of saprophilous insects. These volatiles have been described as distinctive components of floral scent in carrion-mimicking deceptive flowers of species belonging to the Apocynaceae, Araceae, Aristolochiaceae, Asparagaceae (subfamily Scilloideae, previously Hyacinthaceae; APG III 2009), and Hydnoraceae (Burger et al. 1988; Kite and Hetterscheid 1997; Stensmyr et al. 2002; Jürgens et al. 2006; Johnson and Jürgens 2010; Shuttleworth and Johnson 2010).

Attraction of fly pollinators to foul-scented traps. To test the attractiveness of *J. rotacea* floral scents to insects, we carried out insect-trapping experiments using 2 different kinds of traps: (1) green cardboard delta traps (Pherocon III D; Trécé, Salinas, CA) folded into a triangular chamber whose inner surface is covered with sticky adhesive; and (2) reused, inverted 2-L polyethylene terephthalate mineral water bottles, modified as described and illustrated by Jofre et al. (2011). Each type of trap was baited with the synthetic DMDS-DMTS scent solution to test whether flies present in the surrounding area were attracted by these volatiles.

Delta traps were hung from small trees and shrubs at ~1.5 m above the ground along a 100-m forest-margin transect. The traps (20 total) were hung in pairs (foul scented and mineral oil control) ~2 m from each other; each pair was separated from its closest neighboring pair of traps by 10 m. Foul-scented traps were baited with 2.5 mL of DMDS-DMTS solution wetted onto a cotton wick placed at the center of the sticky surface ($N = 10$). Control traps were baited with only 2.5 mL of mineral oil ($N = 10$), and all baits were refreshed daily because of evaporation. Traps were removed after 1 wk to score the presence and identity of trapped insects.

Bottle traps were placed 3–5 cm above the ground along 2 parallel transects 32 m long in an old-field meadow. In total, 8 bottle traps (4 per transect) were spaced 8 m from each other along each transect, with the scented traps alternating with the control traps. Beneath each trap, we placed a plastic microcentrifuge tube containing the scent treatment (DMDS-DMTS solution or mineral oil) and bearing a cotton wick that reached the bottom of the tube to facilitate volatile emission. Tubes baited with mineral oil (2.5 mL) were filled first, and tubes with added DMDS-DMTS/mineral oil solution (2.5 mL) were filled in a separate location, using nitrile gloves to avoid contamination. As before, baits were refreshed daily and traps were checked daily for the presence of insects during 2 wk.

All the flies captured in the traps were identified with the aid of specialists (see "Acknowledgments").

Attractiveness of scent and color in artificial flowers. To explore how olfactory and visual cues interact to attract flies, we used arrays of 12 artificial flowers resembling those of *J. rotacea*, independently manipulating flower color (black vs. white) and olfactory cues (DMDS-DMTS solution vs. control mineral oil). We also examined location—with respect to the flowers—of the source of the olfactory signal affected fly attraction by placing odor baits outside of the flower array (fig. 2*A*, 2*B*). Artificial flowers were constructed to simulate the visual (corolla shape, diameter, and spectral reflectance), and olfactory cues presented by *J. rotacea* flowers.

Visual cues. Given the unusually dark and hirsute corolla surfaces of *J. rotacea* flowers (fig. 1*B*, 1*C*), we constructed flower models using black velvet adhered to cardboard with odorless glue stick and placed them at ground level using push pins anchored in the ground (fig. 2*C*). The percentage of light reflectance of natural and artificial flowers was measured from 300 to 700 nm using an Ocean Optics USB4000 miniature fiber optic spectrophotometer with a deuterium-tungsten halogen lamp and a fiber optic probe to provide standardized

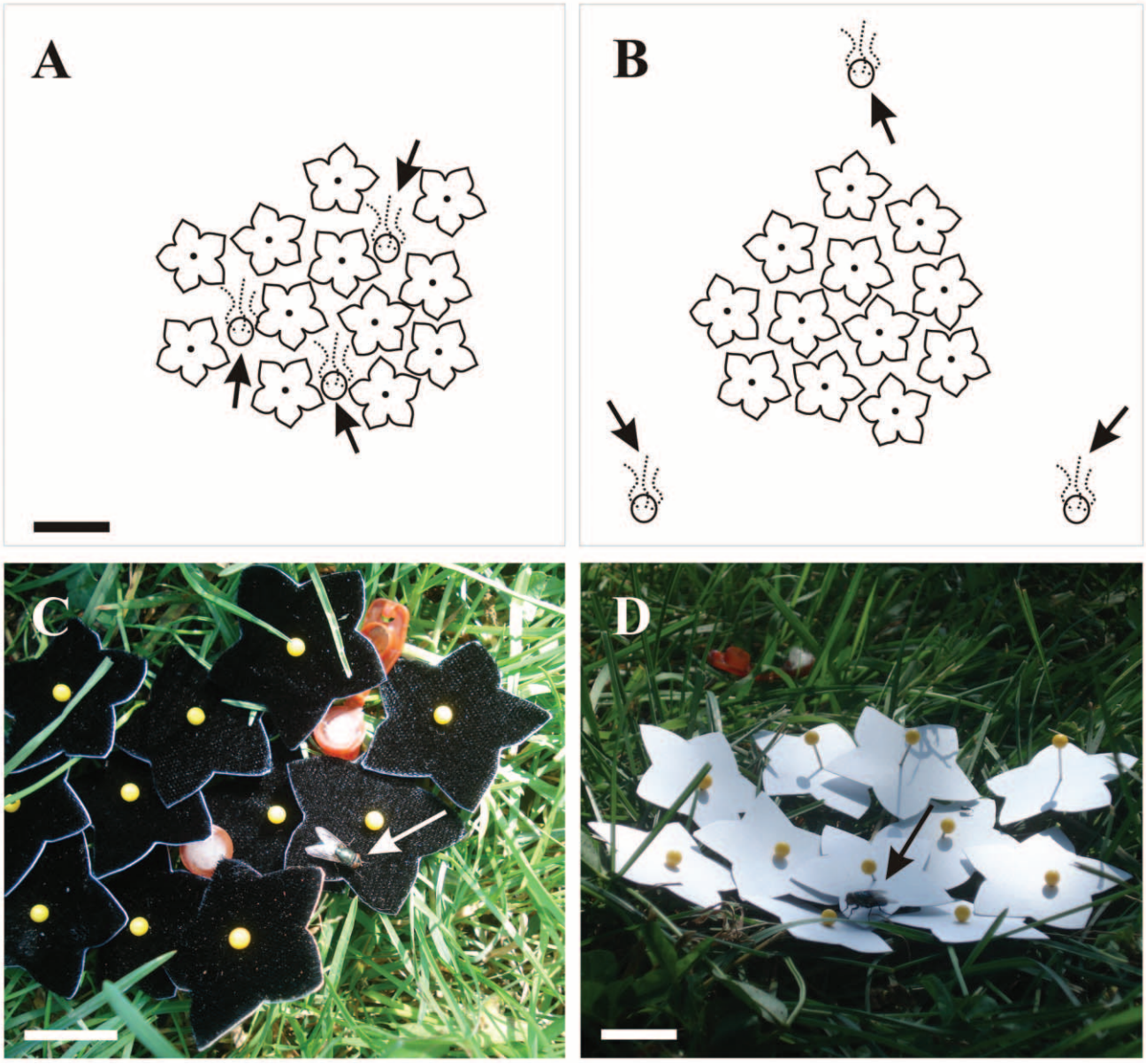


Fig. 2 Arrays of artificial flowers used in the experiment testing scents and visual cues. Scents were presented as baits (arrows) containing mineral oil alone (control) or with added 1% dimethyl disulfide–dimethyl trisulfide, which were placed at ground level either within (*A*) or outside of (*B*) the floral array. Scale bar = 4 cm. *C*, *D*, Experiments were performed using either black velvet (*C*) or white (*D*) flowers. Arrows indicate calliphorid flies that landed on the artificial flowers. Scale bars = 2 cm.

illumination. Measurements were taken from fresh flowers of *J. rotacea* ($N = 10$ individuals, 3 flowers per individual) and from white and black cardboard artificial flowers ($N = 3$). After each measurement, the spectrophotometer was recalibrated using a white diffuse reflectance standard (Ocean PN WS-1). The spectral reflectance of artificial black flowers closely matched that of *J. rotacea* flowers, although they were slightly darker than the natural flowers (fig. 3*A*). We used push pins with yellow plastic heads to imitate the visual display given by the yellow anthers of *J. rotacea* flowers (fig. 2*C*). Since dark flowers were not present in the community where experiments were performed, we also constructed flowers using low ultraviolet (UV)-reflecting white cardboard (figs. 2*D*, 3*A*). To determine the similarity between *J. rotacea* and the artificial flowers, as perceived by carrion fly pollinators, we represented the reflectance spectra of natural and artificial flowers used in the bioassays as loci in the perceptual space of a blowfly color vision model (Troje 1993; Arnold et al. 2009). According to this model, blowflies exhibit a categorical color vision system based on the relative excitations of the 2 pale-type and 2 yellow-type receptors. Thus, color perception depends on the receptor of each pair that is stimulated most strongly, given 4 possible color categories (fly-UV, -blue, -yellow, and -purple). Stimuli with loci in the same color category would be indistinguishable to the fly (Troje 1993; Arnold et al. 2009; Shuttleworth and Johnson 2010). To adjust for relative sensitivity of receptors to the background, we plotted color loci with reference to the leaf of *J. rotacea* (Chittka and Kevan 2005).

Olfactory cues. Foul-scented and control baits were constructed in the same way as described for the bottle trap experiment. The experiment was carried out during 10 consec-

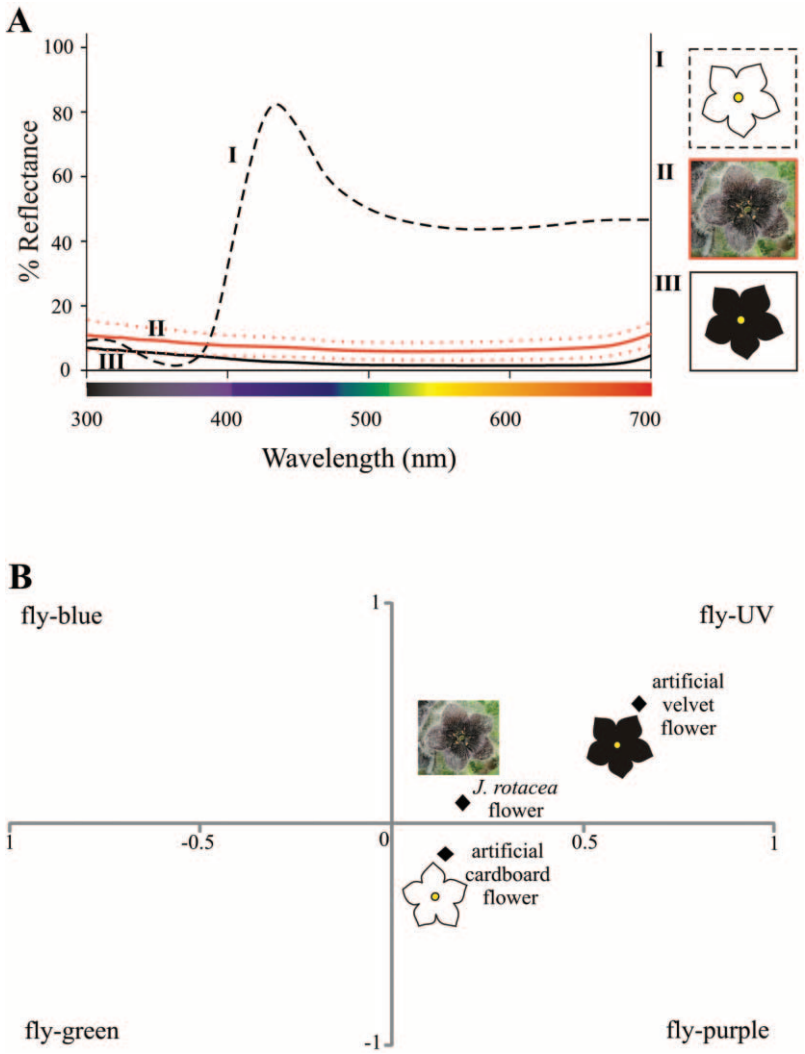


Fig. 3 Visual cues of *Jaborosa rotacea* flowers and artificial flowers used in the bioassays. *A*, Spectral reflectance of natural and artificial flowers. I = white cardboard flower (black dashed line). II = *Jaborosa rotacea* flower (red solid line, mean reflectance spectra; red dotted lines, ±1 SD; $N = 10$ individuals, 2 flowers per individual). III = black velvet flower (black solid line). *B*, Colors of natural and artificial flowers according to how they would be perceived by the blowfly, using the model proposed by Troje (1993) for *Lucilia* sp. (Calliphoridae). Colors in the same quadrant of the graph are not discriminated by the blowfly.

Table 1

Pollinators Species (All Flies, Diptera) Captured on the Flowers of *Jaborosa rotacea* in Its Natural Habitat (Tafí del Valle, Tucumán, Argentina)

Family and genus	Species	Gender	Captured individuals
Anthomyiidae:[a]			
Unidentified			
Calliphoridae:			
Calliphora	*nigribasis*[b]	Female	1
Chrysomya	*albiceps*[b]	Female, male	1
Cochliomyia	*macellaria*[b]	Female, male	4
Lucilia	*eximia*	Female	1
Lucilia	*sericata*[b]	Female	1
Myolucilia	*lyrcea*[b]	Female, male	10
Paralucilia	*fulvicrura*[b]	Female, male	15
Sarconesia	*chlorogaster*[b]	Female	5
Muscidae:			
Unidentified		Female, male	2
Sarcophagidae:			
Oxysarcodexia	*varia*	Male	1
Oxysarcodexia	*paulistanensis*	Female	1
Ravinia	sp.	Female	5

Note. Gender based on captured specimens or identified from photographs.

[a] Flies probably belonging to family Anthomyiidae were photographed pollinating the flowers but were not captured.

[b] Species also recorded pollinating *J. rotacea* flowers by Cocucci (1988).

utive days. We recorded the number of approaches (defined as obvious fly orientation toward the flower array, including landings) and the number of landings by flies on the flower arrays during 15-min observation periods. The number of approaches and landings were recorded regardless of whether they were by the same or different fly specimens. We also examined whether the source of the olfactory signal affected fly attraction, by placing odor baits either within or outside of the flower array (fig. 2). If odor functions as a distance attractant supplanted at close range by visual cues (e.g., when foliage has a rank scent), then flowers themselves do not need to be scented. Artificial flower arrays were observed for a total of 10 h when odor baits were located within arrays and for 17.5 h when baits were located outside of the arrays. Representative fly individuals were photographed when landing at the artificial flowers and identified, at least up to family level, by comparison with the individuals captured in the scented traps.

Statistical Analysis

Data on fly visits to artificial flowers were classified in contingency tables according to 2 factors: scent (foul scented vs. control) and color (black vs. white flowers). The number of approaches and landings was recorded for each combination. Two log-linear models (Agresti 2007) were applied to the data to test whether odor and color affect the probability of approaches (pooling together the number of approaches and landings) or landings. Because no interaction between scent and color was observed—that is, factors were independent—a reduced model was applied to test for differences in fly approaches and landings between scent and color treatments (Quinn and Keough 2002). Calculations were made with R statistical software (R Development Core Team 2011).

Results

Pollinators in Wild Populations

Only saprophilous flies of both sexes were observed both visiting and pollinating the flowers of *Jaborosa rotacea* in natural populations (table 1). Fly visitation was most intense between 0900 and 1500 hours on sunny, warm, and still days. Groups of several flies were frequently observed interacting aggressively (i.e., showing territorial displays) on the vegetation of the plant. Ants were observed patrolling the vegetation, but none of them visited the flowers. Beetles (*Astylus* sp., Melyridae; $N = 4$) were also observed on the plants, but they did not carry pollen of *J. rotacea*. The floral visitors serving as pollinators in natural populations included 10 species of flies belonging to the families Calliphoridae (blowflies), Muscidae (houseflies), and Sarcophagidae (flesh flies) as pollinators (table 1; fig. 1*C*, 1*D*). Other flies, probably belonging to the family Anthomyiidae (fig. 1*C*; root maggot flies), were observed and photographed pollinating the flowers but were not captured. A sarcophagid fly visitor also was observed carrying a pollinarium belonging to an unidentified Asclepiadoideae. In general, flies first landed on the leaves and then flew to and landed on the flowers, which were hidden by the foliage. Once on a flower, carrion flies licked the sugar from glandular petal hairs, which are more densely distributed in the center of the corolla, where anthers and stigma are located (fig. 1*C*). This behavior resulted in pollen being placed mainly on a fly's dorsal thorax and abdomen (i.e., nototribic deposition; fig. 1*C*, 1*D*). The mean distance between anthers and the base of the corolla was 6.76 ± 0.66 mm (mean $\pm$ SE; $N = 10$ plants, 2 flowers per plant), and mean stigma height was 6.77 ± 0.52 mm (mean $\pm$ SE; $N = 10$ plants, 2 flowers per plant). The thorax height

of the flies captured in *J. rotacea* flowers was 4.49 ± 0.28 mm (mean ± SE; $N = 11$ specimens). No nectar was detected within the flowers of *J. rotacea* ($N = 10$ individuals, 3 flowers per plant), although Glucostix reagent strips were faintly stained, indicating the presence of sugar in the glandular hairs.

Floral Scent

In situ dynamic headspace collections of volatiles from living flowers of *J. rotacea* in Argentina identified only 2 consistent odorants, the oligosulfides DMDS and DMTS. However, static headspace collections of volatiles from flowers of a cultivated plant allowed us to detect 14 volatiles, of which 10 could be identified (fig. 4). These included small, highly volatile alcohols (e.g., 2-methyl propanol), 2 oligosulfides, an aromatic ketone (acetophenone), as well as α-pinene and 2 green leaf volatiles (hexanal and heptanal) that were also detected in the vegetative control (fig. 4). The most abundant volatile detected in SPME-GC-MS analyses was acetophenone, which varied temporally (as flowers matured) and spatially (across different floral organs; fig. 4). Buds and predehiscent open flowers all produced low amounts of α-pinene, hexanal, heptanal, and DMDS (fig. 4), suggesting that they are emitted by the dense trichomes covering these tissues. Four-fold larger amounts (peak areas not shown) of the oligosulfides were emitted from the fused petals (dissected corolla) of dehiscent flowers, whereas the yeast- or urine-scented, short-chain oxygenated compounds (acetoin, 2-methyl butanol, 2-heptanone) and sweet floral-scented acetophenone were localized to the sexual organs of these flowers when pollen was present.

Field Experiments

Attraction of fly pollinators to foul-scented traps. In general, the same kinds of insect were captured on the scented delta traps and bottle traps; therefore, the collection data were pooled (table 2). Insects of 5 orders were captured in the traps: Coleoptera, Diptera, Hemiptera, Homoptera, and Hymenoptera. Diptera were the most abundant in both the foul-scented (78.52%) and the control (54.24%) traps. We captured a total of 135 insects in the foul-scented traps, 29 of which belonged to the same families of saprophilous flies (Anthomyiidae, Calliphoridae, Muscidae, and Sarcophagidae) recorded pollinating the flowers in their natural habitat (table 2). These included the widespread calliphorids *Lucilia silvarum* and *Pollenia vagabunda*. In contrast, only 59 insects were collected in the unscented control traps, and of these, only 4 individuals were saprophilous flies (table 2).

Attractiveness of scent and color in artificial flowers. Saprophilous fly species—primarily muscids, calliphorids, and sarcophagids—were observed approaching and landing on the arrays of black and white artificial flowers when foul-scented baits were present either within or outside of the flower array (fig. 2). None of the dryomyzids or sciomyzids that were so abundant in our traps were observed at the artificial flowers. A total of 261 fly landings occurred among scented artificial flowers, whereas only 3 occurred on the unscented controls. After landing, flies exhibited distinct behaviors: they remained immobile for several seconds and then either flew away or walked among flowers. Very few (4) landings occurred on the scented odor sources themselves, as opposed to the 205 landings observed on the flowers. Groups of several flies (up to 10 flies) were frequently observed displaying territorial contests (i.e., as if they were fighting for territories on a oviposition substrate) at the flower arrays or in the vicinity of the foul-scented baits, as was observed in natural populations. On 1 occasion, a single *Apis mellifera* bee was attracted to a white artificial flower equipped with foul-scented bait. It is noteworthy that besides the saprophilous flies, only 1 honey bee visited our floral arrays, given that several experimental colonies housing thousands of actively foraging bees were located within 20 m of the floral arrays.

Loci for *J. rotacea* and black artificial flowers fall within the same quadrant (fly-UV) in the blowfly model, and in the other side, white artificial flowers fall in a different quadrant (fly-purple; fig. 3*B*). Thus, flies would be unable to distinguish *J. rotacea* flowers from the black artificial ones, but white artificial flowers would be perceived as different.

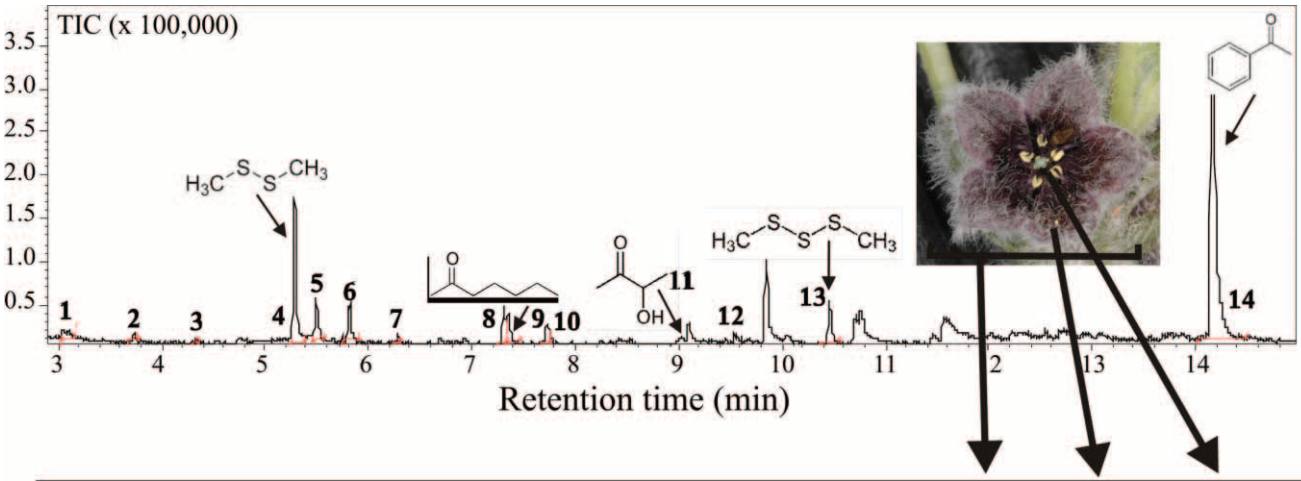
The presence of foul-scented baits resulted in significantly more approaches and landings at the artificial flowers when the baits were located both within (scent odds = 14.91, $P < 2E{-}16$) or outside of (scent odds = 21.67, $P = 1.39E{-}06$) the flower arrays. Furthermore, the presence of white artificial flowers resulted in significantly more landings (color odds = 2.09, $P = 0.008$), but no significant effect was observed for approaches (color odds = 1.01; fig. 5). When foul-scented odor baits were placed outside of the flower array, the presence of odor and white artificial flowers resulted in significantly more approaches (scent odds = 50.67, $P < 2E{-}16$ [$P < 0.001$]; color odds = 1.31, $P < 2E{-}16$ [$P < 0.001$]) and landings (scent odds = 69.5, $P < 2E{-}16$ [$P < 0.001$]; color odds = 6.05, $P < 2E{-}16$ [$P < 0.001$]; fig. 5*B*).

Discussion

We have shown that *Jaborosa rotacea* flowers exhibit olfactory cues—that is, floral scent including 2 oligosulfides, DMDS and DMTS—resembling the substrates used by saprophilous flies as brood sites. The pollinator assemblage of *J. rotacea* in a natural population was constituted exclusively by saprophilous flies belonging to the Anthomyiidae, Calliphoridae, Muscidae, and Sarcophagidae. Our trapping experiments, performed outside the native range of *J. rotacea* in temperate North America, showed that DMDS and DMTS are sufficient to trap flies belonging to the same families that pollinate *J. rotacea* in its native habitat. Moreover, bioassays performed using artificial flowers show that fly attraction is greater when foul-scented baits are present and visual cues (i.e., white flowers) contribute to attraction but in a direction contra the *J. rotacea* floral phenotype. These results place *J. rotacea* among a growing list of flowering plants, mosses, and fungi worldwide that utilize sulfur volatiles to attract saprophilous flies as pollen or spore vectors (Marino et al. 2009; Johnson and Jürgens 2010; Urru et al. 2011). We discuss ecological and evolutionary aspects of carrion mimicry as it relates to this unusual genus of nightshade plants.

Pollinator Behavior and Effectiveness

Pollination of *J. rotacea* occurs when blowflies, flesh flies, and houseflies of similar dimensions to the flowers' anther-



Peak #	Compound	RT	ID	buds	indehiscent flower	dehiscent flower	corolla	sex organs
1	unknown m/z **43**	3.05	NA ?			1.27	1.36	0.48
2	unknown m/z **43**, 57, 86	3.72	35 ?			0.77		2.67
3	α-pinene	4.38	988 STD	10.29	5.86	0.41		0.94
4	dimethyl disulfide	5.28	1051 STD	89.71	83.31	15.11	81.68	0.42
5	hexanal	5.51	1067 KI	<0.10	<0.10	2.46		0.53
6	2-methyl propanol	5.82	1088 KI		8.42	5.20	3.06	1.76
7	2-propanol	6.28	1116 ?			0.66		0.67
8	2-heptanone	7.31	1173 KI			3.73		11.36
9	2-heptanal	7.33	1174 KI	<0.10	<0.10	1.96		<0.10
10	2-methyl butanol	7.72	1196 KI			1.88	2.14	4.37
11	acetoin	8.97	1271 STD				0.57	3.08
12	unknown m/z **45**, 55, 73	9.56	1308 ?			0.41		1.78
13	dimethyl trisulfide	10.45	1371 STD			2.41	5.49	
14	acetophenone	14.15	1644 STD			63.53	5.7	71.94
				11.42	**11.25**	**100**	**47.17**	**64.43**

Fig. 4 Olfactory signal of *Jaborosa rotacea* flowers. Total ion gas chromatogram (*top*) of headspace volatiles collected by solid phase microextraction from intact, mature flowers of *J. rotacea*. Numbered peaks are the identified floral compounds listed in the table (*bottom*) in order of increasing retention time (min). *Bottom*, table of the relative percentage (out of the total trapped scent) of each volatile detected in the floral scent. Compounds in green were also found in foliage control samples. Columns (left to right) present data from intact buds, open indehiscent flowers, open dehiscent flowers, dissected corollas, and dissected sex organs (stamens and gynoecium). Numbers in each column are relative percentages (out of 100%) of total peak areas, whereas bold numbers below each column express the sum of gas chromatography (GC) peak areas for each treatment as a percentage of total GC peak area from uncut, dehiscent flowers.

stigma distance establish physical contact with these sexual organs. The center of the corolla is densely covered by glandular hairs that may function to position the flies in the correct position to acquire and deposit pollen in a nototribic mode. Because flowers of this species do not provide suitable oviposition sites, female insect pollinators are duped by deceitful signals when they are searching for a brood site. The absence of a well-developed floral nectary suggests that there are almost no compensatory energetic benefits to floral visitation. The presence of rewarding glandular hairs could promote subsequent fly visits to *J. rotacea* flowers necessary to effect pollination. Although we have not formally studied the breeding system of *J. rotacea*, the absence of fruit production in plants grown in greenhouses—combined with high fruit production (M. Moré and A. A. Cocucci, personal observation) and nearly exclusive visitation by saprophilous flies in the plants' natural habitat—suggests that these flies are necessary and effective pollinators of *J. rotacea*.

In the native range of *J. rotacea*, the same blowfly and flesh fly species that were documented pollinating the flowers are also commonly used locally as indicators in forensic studies and are routinely captured on baits of dog feces, rotten cow liver, and pig carcasses (Battán-Horestein et al. 2010; Mulieri et al. 2010). Five calliphorid species (*Chrysomya albiceps*, *Cochliomyia macellaria*, *Lucilia eximia*, *Lucilia sericata*, and *Sarconesia chlorogaster*) and the 2 sarcophagid species (*Oxysarcodexia* spp.) captured in the native habitat are particularly abundant in rural areas of Argentina throughout the flowering season of *J. rotacea* (Battán-Horestein et al. 2010; Mulieri et al. 2011). In addition, *Oxysarcodexia* species have

Table 2

Total No. Insect Specimens in Different Orders and Families That Were Captured on Scented (1% Dimethyl Disulfide–Dimethyl Trisulfide) and Control (Mineral Oil) Baits in Delta and Bottle Traps in the Field (New York)

	Foul-scented baits ($N = 135$)		Control baits ($N = 59$)	
Order and family	No.	Percent	No.	Percent
Coleoptera:				
Cantharidae	4	2.96		
Cleridae	1	.74		
Cryptopagidae	1	.74		
Dermestidae			1	1.69
Lampryiidae	2	1.48	1	1.69
Mordellidae			1	1.69
Mycetophagidae			2	3.39
Ptilodactylidae	1	.74		
Throscidae			2	3.39
Unidentified			1	1.69
Total	9	6.67	8	13.56
Diptera:				
Agromyzidae	2	1.48		
Anthomyiidae[a]	5	3.70	1	1.69
Calliphoridae[ab]	5	3.70	1	1.69
Cecidomyiidae	1	.74		
Chaoboridae	1	.74		
Chironomidae	4	2.96		
Chloropidae	6	4.44	4	6.78
Clusiidae			1	1.69
Dolichopodidae	6	4.44	5	8.47
Dryomyzidae				
Empididae	1	.74		
Heleomyzidae	7	5.19		
Lauxaniidae	5	3.70		
Lonchopteridae			1	1.69
Mycetophilidae			1	1.69
Muscidae[ab]	16	11.85		
Opomyzidae	8	5.93	3	5.08
Otitidae	1	.74		
Phoridae	1	.74	1	1.69
Piophilidae	1	.74	1	1.69
Platystomatidae	1	.74		
Psychodidae			1	1.69
Unidentified			1	1.69
Sarcophagidae[ab]	3	2.22	1	1.69
Scathophagidae			1	1.69
Sciaridae	2	1.48		
Sciomyzidae	10	7.41	1	1.69
Simulidae	1	.74		
Stratiomyidae	3	2.22	1	1.69
Syrphidae			1	1.69
Tabanidae			1	1.69
Tipulidae	1	.74		
Unidentified	3	2.22	5	8.47
Total	106	78.52	32	54.24
Hemiptera:				
Miridae	1	.74	2	3.39
Homoptera:				
Cercopidae			1	1.69
Cicadellidae	11	8.15	7	11.86
Derbidae	1	.74		
Total	12	8.89	8	13.56
Hymenoptera:				
Braconidae	1	.74	2	3.39
Cynipidae	1	.74		
Formicidae	1	.74		
Ichneumonidae	2	1.48	6	10.17
Platygasteridae	1	.74		
Tenthridinidae	1	.74		
Unidentified			1	1.69
Total	7	5.19	9	15.25

Note. Percent indicates percentage representation for each family and order.

[a] Families recorded pollinating.

[b] Families that were also recorded visiting scented artificial flowers.

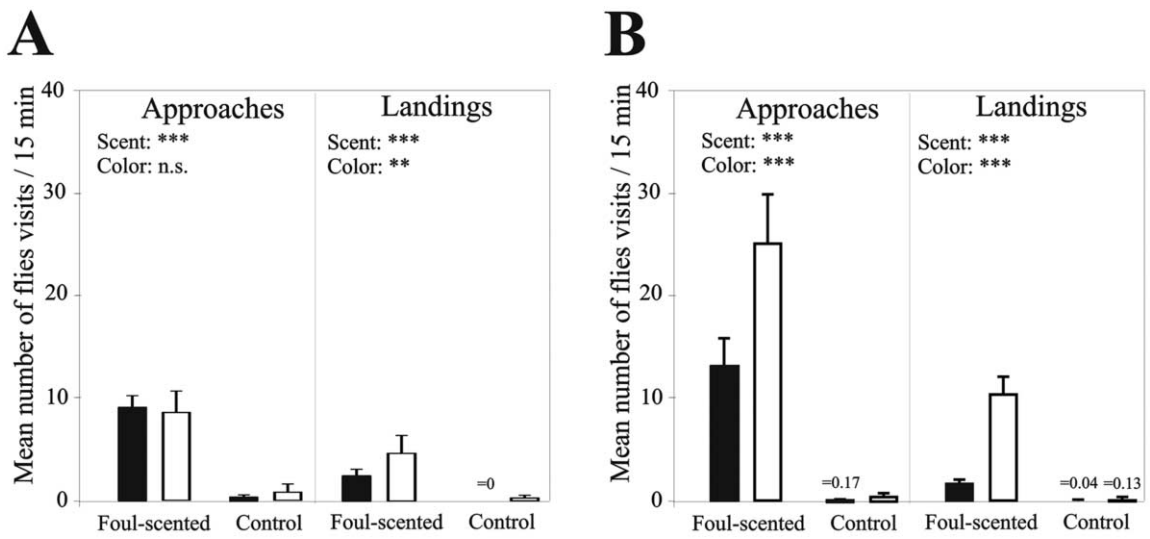


Fig. 5 Visitation of flies to artificial flowers expressed as mean number (±SE) of flies that approached and landed on artificial flowers with and without scents. Filled bars represent black flowers arrays, and open bars represent white flowers arrays, conducted with odor baits located either within (*A*) or outside of (*B*) each floral array. Scent and, to a lesser degree, color are significant predictors of fly visitation. Three asterisks, $P < 0.001$; two asterisks, $P < 0.01$; n.s., not significant.

been observed visiting nectar-rewarding flowers in species of Apiaceae, Euphorbiaceae, and Rhamnaceae in Argentina (Mulieri et al. 2010). We observed eggs and small larvae laid by female flies on *J. rotacea* plants, suggesting that a close interplay between olfactory, visual, and mechanosensory floral cues triggers the oviposition behavior of duped female pollinators. Fly oviposition within flowers has been observed in other sapromyiophilous systems, including stapeliads (Meve and Liede 1994), *Rhizanthes* (Rafflesiaceae) in Thailand (Bänziger 1996), *Helicodiceros muscivorus* (Araceae) in Sardinia (Angioy et al. 2004), and *Satyrium pumilum* (Orchidaceae) in South Africa (van der Niet et al. 2011), which represents to the flies a fitness cost (in addition to opportunity costs) accruing to deceived pollinators.

Chemistry of floral scent. The results of our floral scent analyses show that *J. rotacea* has a chemically simple floral scent that includes 2 oligosulfide compounds (DMDS and DMTS), which match the global pattern predicted for carrion and dung mimicry (Borg-Karlson et al. 1994; Stensmyr et al. 2002; Jürgens et al. 2006). These 2 oligosulfides and 2-heptanone, emitted by mature *J. rotacea* flowers (fig. 4), are commonly emitted from putrefying meat and carnivore/omnivore feces (Urru et al. 2011 and references therein). Low amounts of DMDS were also found in buds and open flowers with immature anthers (fig. 4), presumably emitted from trichome hairs located on the outer floral surfaces. However, flowers of *J. rotacea* emit other volatiles that we did not include in our behavioral assays. These compounds include acetoin and 2-methyl butanol, common to fermenting fruits and yeast (Goodrich et al. 2006), as well as acetophenone, an aromatic compound shown to repel bumblebees in *Antirrhinum majus* flowers (Suchet et al. 2010). These compounds and 2-heptanone were present only in open flowers with pollen and were persistent in dissected flowers lacking perianth tissues (corolla and calyx), suggesting their potential to be emitted specifically by pollen or by mature, receptive stigmatic tissue. Unfortunately, we did not have enough flowers to verify the source tissue for these volatiles. However, given the known defensive functions of volatile ketones in pollen (Dobson and Bergström 2000), 2-heptanone and acetophenone may play roles other than pollinator attraction or manipulation in *J. rotacea*. q7

Because oligosulfides are pervasive attractants for insects that oviposit in carrion (Urru et al. 2011), brood-site deceptive flowers are primarily pollinated by gravid female insects that sometimes even deposit eggs or live larvae on flowers (Bänziger 1996; Stensmyr et al. 2002; van der Niet et al. 2011). However, males seeking mating opportunities might also be attracted and eventually act as pollinators, potentially being rewarded with sexually mature females (Urru et al. 2011). Our observations of territorial behavior on wild plants in Argentina and artificial flower arrays in the United States and our records of males visiting the flowers of *J. rotacea* in its native habitat (table 1) suggest that male carrion and dung flies are also attracted by fetid odors, since they are known to aggregate at brood sites and compete for females (e.g., scathophagids; Simmons and Parker 1992). Thus, future experiments should explore the relative roles of females and males as pollinators of *J. rotacea*.

Bioassays Using Foul-Scented Baits and Artificial Flowers

Our field bioassays using traps scented with DMDS and DMTS have shown that olfactory cues alone are sufficient to attract saprophilous flies in 2 spatial contexts (ground level and low-lying foliage) thousands of miles beyond the natural range of *J. rotacea*. The fly species attracted to these odor cues in the nonnative habitat (low-elevation, temperate deciduous forests and meadows of northeastern United States) belong to the same families as the flies that pollinate *J. rotacea* in its

native habitat (high-elevation, open xeric habitats in southern South America). Although the attracted fly species are not exactly the same, our findings support the idea that oligosulfide volatiles function as a universal signal to attract flies that use carrion or carnivore feces as brood sites. Accordingly, these sulfide compounds are present in different groups of living organisms (e.g., plants, dung mosses, and unrelated groups of fungi) that use saprophilous flies as dispersal agents of pollen or spores (Marino et al. 2009; Vereecken and McNeil 2010; Urru et al. 2011). By producing volatile oligosulfide compounds, diverse organisms in different geographical regions may reliably attract the same potential dispersal agents (Borg-Karlson et al. 1994; Stensmyr et al. 2002; Bänziger and Pape 2004; Ollerton and Raguso 2006; Johnson and Jürgens 2010; van der Niet et al. 2011). Nevertheless, there are ecological contexts in which saprophilous flies may not be the primary recipients of oligosulfide volatile signals.

The partially buried carrion-mimicking flowers of the South African parasitic plant *Hydnora africana* (Hydnoraceae) emit oligosulfides (Burger et al. 1988), and whereas they are primarily visited by blowflies in South Africa, across the border in Namibia they are pollinated by *Dermestes* beetles (Dermestidae) that feed on dead animals (Bolin et al. 2009). In the tropical rainforests of Central America, oligosulfides (or similar S-volatiles) are the primary attractants in a guild of night-blooming flowers that are pollinated by glossophagine bats (von Helversen et al. 2000), while daytime blooming *Aristolochia* species appear to use the same volatile compounds to attract saprophilous flies as pollinators (Blanco 2002). Finally, beyond attracting specific guilds of pollinators, flowers that look and smell like carrion may represent a strategy by which sapromyiophilous plants reduce the risk of mammalian herbivory (Strauss et al. 1996; Lev-Yadun et al. 2009), a hypothesis that merits further experimental exploration.

Bioassays performed outside the natural range of a pollinator can provide insight into the universality of a sensory signal. For example, Skubatz et al. (1996) showed that the voodoo lily (*Sauromatum guttatum* [=*S. venosum*; Araceae]), a tropical Himalayan plant, can attract a broad spectrum of saprophilous insects with its fetid odors in cool-temperate United States. Similarly, Ollerton et al. (2009) observed that some species of *Ceropegia* are pollinated by dipterans of the same genus in their native habitat and in cultivation. Heiduk et al. (2010) demonstrated that Chinese *Ceropegia dolichophylla* (Apocynaceae) can attract relatives of their native pollinators using their unusual odors in Germany, where the pollinators (kleptoparasitic milichiid flies) also are native, and are commonly found in greenhouses. Such patterns are to be expected when plants exploit widespread preexisting sensory biases in insects and other animals to utilize them as pollinators or spore-dispersal agents (Schaefer and Ruxton 2009).

Olfactory versus Visual Floral Cues and the Evolution of Fly Pollination in Jaborosa

Most studies indicate that olfactory and visual stimuli combine to attract pollinators to flowers across the spectrum of specialized to generalized cases (Dobson 2006; Raguso 2008). However, the darkly pigmented, often concealed flowers of *J. rotacea* (fig. 1) are not visually conspicuous, which led us to question the role of visual cues in fly attraction in this system. Our findings from the scented trapping experiment clearly demonstrate that volatile sulfides alone are sufficient to attract the kinds of flies that pollinate sapromyiophilous flowers (table 2). Not surprisingly, the presence of these volatiles significantly increased fly approaches and landings in all treatments of the artificial flower array experiment (fig. 5). Surprisingly, we found that foul-scented baits did not need to be located within the artificial flower array to effectively attract flies. In bioassays testing fly attraction, flies often land and walk to an odor source or a visually conspicuous target (Troilo and Cameron 1981), as we observed in fly visits to *J. rotacea* in its native habitat. Similar results have been described in *Eucomis*, where flies were attracted to and visited the flowers even though the odor source was placed at the base of the inflorescence (Shuttleworth and Johnson 2010), and *Helicodiceros* (Araceae), in which sulfides attract flies to the inflorescence but heat and other features direct them into the floral chamber (Angioy et al. 2004). At the very least, these results support the idea that the evolution of sapromyiophily does not require flowers themselves to be scentless, as long as bracts, leaves, or other plant organs produce the appropriate odors when flowers are receptive.

Another interesting finding was that when scented lures were placed outside of the floral array, the white artificial flowers were more attractive than black ones, which indicates that visual cues also mediate flower choice by these flies. White flowers generally are more visually conspicuous to flies (Arnold et al. 2009), and it is possible that the flies in our bioassays already had visited rewarding flowers of similar reflectance in the study area, where black flowers are not present. If black flowers are less visually attractive to saprophilous flies, why aren't the flowers of *J. rotacea* white? It is possible that dark-colored sapromyiophilous flowers are less visually conspicuous because they are filtering out other visitors, such as bees, who might be less flower constant. It is also possible that white coloration was lost during evolutionary transitions from moth-pollinated ancestors in *Jaborosa* as a prezygotic floral isolation mechanism. Molecular phylogenetic reconstruction of the genus suggests that brood-site mimicry has evolved from a white-flowered ancestral species that offered nectar as reward and (inferred from extant species) was pollinated by moths (M. Moré et al., unpublished data). The evolutionary shift in *Jaborosa* species to pollination by carrion flies could have been initiated by the emission of volatile sulfides that attract flies (as outlined by Shuttleworth and Johnson 2010) and then followed by changes in flower color and morphology to optimize reproductive success through pollinator behavior and pollen deposition and, potentially, by narrowing the visitor spectrum. Chemical studies of floral scent in other sapromyiophilous species of *Jaborosa* (*Jaborosa laciniata*, *Jaborosa leucotricha*, *Jaborosa magellanica*, and *Jaborosa sativa*) are currently being carried out to determine whether they have the same floral scent compounds as *J. rotacea* or whether they rely on different volatile compounds to attract saprophilous flies. Carrion mimicry has produced some of the world's largest and most unusual flowers (Davis et al. 2008), yet relatively few plant families have evolved carrion mimicry, and we have few insights on how such derived flowers evolve (Shuttleworth and Johnson 2010). Our studies of the genus *Jaborosa* have the potential

to reveal the critical steps in floral modification leading to carrion mimicry, through combining phylogenetic reconstruction with the functional analyses presented in this article and elsewhere for related plants (Kaczorowski et al. 2012).

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QUERIES TO THE AUTHOR

q1. Should 65-μ be 65-μm? Also, is DVB/PDMS spelled out correctly?

q2. Is there a word missing in "signal affected fly"?

q3. Is there a word missing in "distance attractant supplanted"?

q4. Quinn and Keough 2002 is not listed in the literature cited. Please provide reference information.

q5. In fig. 4, is GC spelled out correctly?

q6. Per IJPS style, asterisk footnotes are not generally used in the text, so I have deleted the asterisks and added $P < 0.001$ (per fig. 5). Are changes okay?

q7. Dobson and Bergström 2000 is not listed in the literature cited. Please provide reference information.

q8. Dobson 2006 and Raguso 2008 are not listed in the literature cited. Please provide reference information.

q9. Please provide all author names for the unpublished data.