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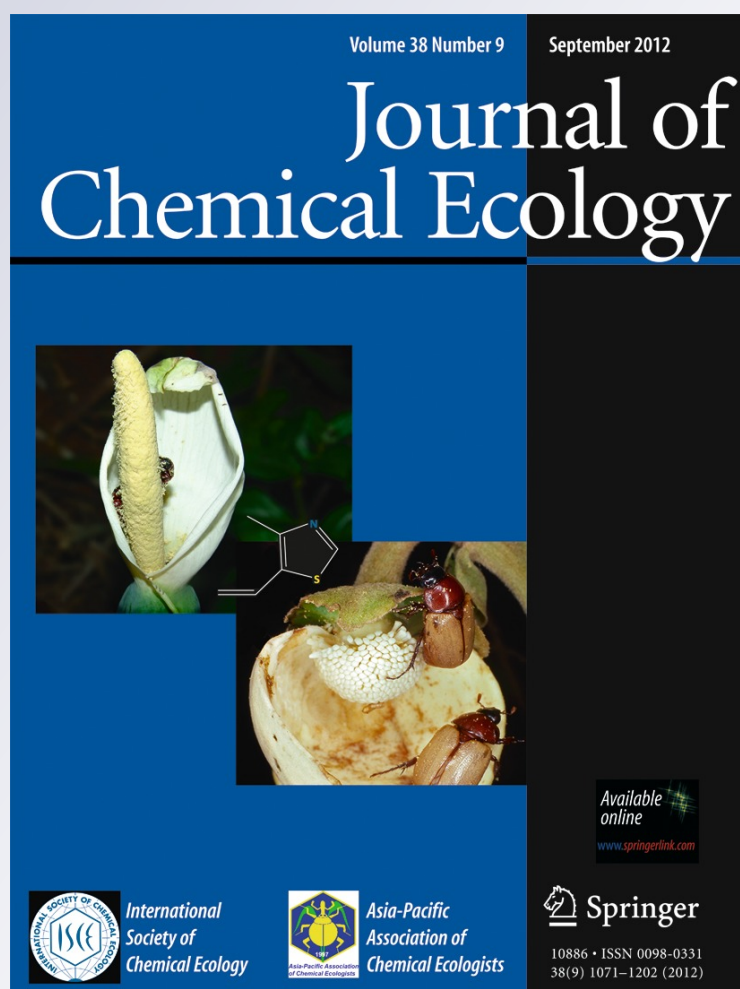
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The Key Role of 4-methyl-5-vinylthiazole in the Attraction of Scarab Beetle Pollinators: a Unique Olfactory Floral Signal Shared by Annonaceae and Araceae

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Abstract Cyclocephaline scarabs are specialised scent-driven pollinators, implicated with the reproductive success of several Neotropical plant taxa. Night-blooming flowers pollinated by these beetles are thermogenic and release intense fragrances synchronized to pollinator activity. However, data on floral scent composition within such mutualistic interactions are scarce, and the identity of behaviorally

active compounds involved is largely unknown. We performed GC-MS analyses of floral scents of four species of *Annona* (magnoliids, Annonaceae) and *Caladium bicolor* (monocots, Araceae), and demonstrated the chemical basis for the attraction of their effective pollinators. 4-Methyl-5-vinylthiazole, a nitrogen and sulphur-containing heterocyclic compound previously unreported in flowers, was found as a prominent constituent in all studied species. Field biotests confirmed that it is highly attractive to both male and female beetles of three species of the genus *Cyclocephala*, pollinators of the studied plant taxa. The origin of 4-methyl-5-vinylthiazole in plants might be associated with the metabolism of thiamine (vitamin B1), and we hypothesize that the presence of this compound in unrelated lineages of angiosperms is either linked to selective expression of a plesiomorphic biosynthetic pathway or to parallel evolution.

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Introduction

For numerous species of extant angiosperms, the emission of floral volatiles plays a role in breeding success. Such species rely on chemically mediated strategies for attracting effective pollinators (Faegri and van der Pijl, 1979; Raguso 2001, 2008). It is generally agreed that the broad spectrum of known flower visitors is matched by an equally vast assortment of floral volatiles of diverse origins, with a myriad of possible fragrant combinations (Dobson, 2006; Knudsen et al., 2006).

This chemical diversity is a portrait of the evolutionary processes of diversification and speciation involved in scent-mediated pollination mutualisms (Dobson, 2006).

Among some highly specialised pollination systems, such as those involving deceptive *Ophrys* orchid flowers and male solitary bees (Schiestl et al., 1999), scent-driven behavioral reactions of pollinators are triggered by common volatile compounds that act synergistically according to specific relative ratios in the overall floral bouquets (Raguso, 2008). In other systems, however, it has been shown that occasionally uncommon single compounds induce taxa-restricted attractive response of pollinators (Dodson et al., 1969; Schiestl et al., 2003). In these cases, described as ‘private communication channels’, the specificity of the olfactory signal is ensured by rare compounds synthesized in unusual pathways, unlikely to be reproduced by chance (Raguso, 2008). As an example, Australian sexually deceptive *Chiloglottis* orchids attract their highly specific pollinators with one or few extremely uncommon volatiles, i.e., chiloglottones. Sympatric species typically emit different single chiloglottones or different blends of compounds that attract different pollinator species (Peakall et al., 2010). Potential ‘private communication channels’ also have been documented among highly specialized plant-pollinator interactions involving male euglossine bees (Apidae, Euglossini) (Whitten et al., 1986) and fig wasps (Agaonidae) (Chen et al., 2009). It is likely that such systems are more common than what is currently documented, but the scarce knowledge of floral scent chemistry is still a barrier in pollination studies (Knudsen, et al., 2006).

Cyclocephaline scarab beetles (Scarabaeidae, Cyclocephalini) are predominant among the highly specialized pollinators in the Neotropics, implicated with the reproductive success of an estimated 900 Neotropical species among the Annonaceae, Araceae, Arecaceae, Cyclanthaceae, Magnoliaceae and Nymphaeaceae (Beach, 1982; Gottsberger, 1986; Schatz, 1990; Ervik and Knudsen, 2003). As the result of convergent adaptive evolution, several lineages within these families exhibit shared features in floral morphology and physiology, configuring what is broadly acknowledged as ‘cyclocephaline scarab pollination syndrome’ (Gottsberger, 1990). The night-active scarabs are attracted to flowers or inflorescences by intense odors given off during episodes of floral thermogenesis (Gottsberger and Silberbauer-Gottsberger, 1991; Seymour et al., 2003). Warm shelter and nutritious flower tissues await the pollinators inside floral chambers, where they feed and mate (Gottsberger, 1990).

Even basic information about floral scent composition within cyclocephaline scarab beetle-pollinated taxa is meager (Dobson, 2006). Nonetheless, a few available studies portray obvious diversity; oxygenated terpenoids, esterified fatty acid derivatives, C5-branched chain compounds, and uncommon methoxylated and hydroxylated benzenoids have all been reported as major constituents in these angiosperms (Azuma et al., 1999; Ervik et al., 1999; Schultz et al., 1999; Ervik and

Knudsen, 2003; Gibernau et al., 2003; Maia et al., 2010). Verified behavioral response, however, only has been described for methyl 2-methylbutyrate, the main component in flowers of *Magnolia ovata* (Magnoliaceae) pollinated by *Cyclocephala literata* (Gottsberger et al., 2012). Compounds mediating other interactions within these highly specialized pollination systems are unknown.

In the present study, gas chromatography—mass spectrometry (GC-MS) was used to investigate the floral scent composition of four species of Annonaceae (magnoliids; *Annona* spp.) and one Araceae (monocots; *Caladium bicolor*), all pollinated by cyclocephaline scarabs of the genus *Cyclocephala*. The behavioral activity of a prominent compound isolated in the analyzed samples was assessed in field bioassays. The main questions we posed were: a) how similar is the floral scent composition in these two phylogenetically distant groups of angiosperms (Annonaceae and Araceae) pollinated by cyclocephaline scarab beetles? b) is the most prominent compound in these floral fragrances sufficient for (specific) pollinator attraction?

Methods and Materials

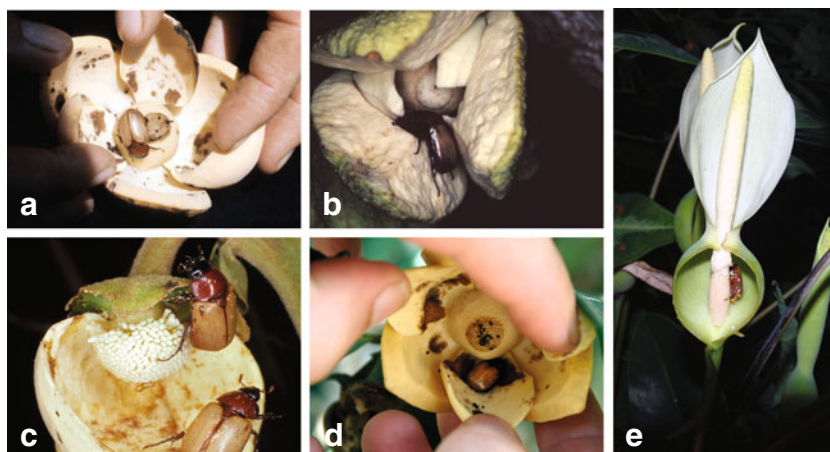
Study Sites Both the collection of floral scent samples and behavioral tests were conducted in two localities in Brazil where native populations of the selected species of *Annona* and *Caladium bicolor* naturally occur (Fig. 1).

The first site corresponds to remnants of *cerrado sensu lato* covering an area of ca. 100 ha, inserted in agricultural land and pastures in the municipality of Botucatu, Central Brazil (22° 52' S, 48°26' W; altitude ca. 850 m). Local vegetation is a mixture of the central Brazilian *cerrado* (open tree and scrub woodland to low-tree and scrub savanna), with scattered patches of mesophytic, more or less evergreen Atlantic forests (Gottsberger and Silberbauer-Gottsberger, 2006). Local rainfall regime is semi-humid, and over 80 % of the average annual precipitation (ca. 1,300 mm) occurs during the warmer months of October through March. Mean monthly temperatures vary between 16 °C in July and 23 °C in February (CEPAGRI, 2012).

At this site, henceforth referred to as *Botucatu*, populations of *Annona crassiflora*, *A. coriacea*, and *A. dioica* grow sympatrically and exhibit staggered flowering peaks between the months of October and December. All three species are essentially pollinated by *Cyclocephala atricapilla*, with erratic visits from few other congenics (Gottsberger, 1989).

The second site corresponds to a private reserve of Atlantic Forest, covering ca. 66 km² comprised of scattered fragments inserted in a sugarcane monoculture matrix, property of Usina São José S/A Sugarcane Company, municipality of Igarassu, north-eastern coast of Brazil (7°49' S, 35°02' W; altitude ca. 110 m). Local rainfall regime is perhumid, and over

Fig. 1 Investigated plant species with *Cyclocephala* pollinators (Scarabaeidae, Dynastinae): **a** *Annona coriacea*, **b** *A. crassiflora*, **c** *A. dioica*, **d** *A. montana* (Annonaceae, magnoliids), **e** *Caladium bicolor* (Araceae, monocots)



80 % of the average annual precipitation (ca. 1,800 mm) occurs between February and August. Mean monthly temperatures vary between 26 °C in March and 23 °C in August (ITEP, 2012).

At this site, henceforth referred to as *Igarassu*, native *Caladium bicolor* populations bloom between March and May (Maia and Schlindwein, 2006). *Annona montana* populations flower year long, but are in full bloom between January and March. *Cyclocephala celata* is the exclusive local pollinator of *Caladium bicolor*. *Cyclocephala vestita* is the only known pollinator locally associated with *A. montana* (H. Teichert, unpublished data).

Volatile Collection and Chemical Analyses Floral scent samples were collected *in situ* using standard dynamic headspace extraction methods (Raguso and Pellmyr, 1998), during the flowering period of the selected species in 2008–2009. During the female phase of anthesis, flowers (*Annona* spp.) or inflorescences (*Caladium bicolor*) were individually enclosed in polyacetate bags. Scented air was drawn from the bags at a constant flow rate of 200 ml.min⁻¹ for 30–120 min through glass cartridges filled with 25 mg adsorbent polymer, a 1:1 weight mix of Tenax and Carbotrap. After collection, the cartridges were eluted with 9: 1 hexane: acetone solvent and analyzed by combined gas chromatography-mass spectrometry (GC-MS) using methods detailed in Kaiser and Tollsten (1995) and Dötterl et al., (2006). To determine the total amount of scent in these samples, we added an internal standard (10 µg 3-chloro-4-methoxy toluene) prior to injection. Smaller cartridges filled with 3 mg of the adsorbent mix were used for sampling unbagged flowers of *Annona coriacea* and *A. crassiflora* (200 ml.min⁻¹ flow, 2 min duration). These samples were thermally desorbed and analyzed by GC-MS, according to methods described by Dötterl et al., (2005). We calculated the total scent emission (absolute amount) of samples analyzed by thermal desorption by injecting known amounts of monoterpenoids, benzenoids, and fatty acid derivatives. The mean response of these compounds (mean peak

area) was used to determine the total amount of scent available in the samples (Dötterl et al., 2006). Simultaneous collections of the surrounding air were performed for both sampling techniques to distinguish between floral/inflorescence compounds and ambient contaminants.

Behavioral Activity of 4-Methyl-5-vinylthiazole At Botucatu, biotests were conducted during November 2009. Undiluted 4-methyl-5-vinylthiazole (≥ 97 % purity, Sigma-Aldrich; 80 µl) was applied to pieces of white filter paper, cut to the approximate size of the perianth of *Annona* flowers. To determine the total amount of scent released by decoy flowers ($N=5$), we applied the same sampling method used for inflorescences of *Caladium bicolor* and flowers of *Annona dioica* and *A. montana* (see above). The total amount of 4-methyl-5-vinylthiazole emitted by the decoy flowers was calculated at 100 µg h⁻¹, similar to the values obtained from a single bagged flower of *Annona* under the same environmental conditions (Table 1). The decoys were fixed to branches of *Annona* trees or laid on the ground. On four different occasions, we installed sets of 2, 2, 4 and 5 decoys distant ca. 2 m from each other. At regular 10 min intervals, we collected the insects that settled over the decoys between 17:30–21:00 h, when cyclocephaline scarab beetles are most active. Baitless paper flowers were used as paired controls. Biotests at *Igarassu* took place between January and April 2010. We cut out pieces of dental cotton roll (2 cm sections) and pushed them into 4 ml clear glass vials (Supelco, Bellefonte, PA, USA), so that the tip of the cotton rolls leveled with the rim opening of the vials. Each dispenser was impregnated with 100 µl undiluted 4-methyl-5-vinylthiazole and applied to ‘Japanese beetle’ traps built based on a model by Trécé, USA (Bengtsson et al., 2009). Traps were hung chest-high from tree branches along borders of rainforest patches, 25 m apart from one another. Loaded dispensers ($N=5$) were bagged individually and sampled through dynamic headspace extraction, applying the same method used for the filter

Table 1 Relative amounts (mean±SD) of 4-methyl-5-vinylthiazole and other tentatively identified floral scent compounds (pooled according to compound class) in four species of *Annona* (Annonaceae, magnoliids) and *Caladium bicolor* (Araceae, monocots), all of which exclusively pollinated by cyclocephaline scarab beetles (Scarabaeidae, Cyclocephalini). Floral scent samples were obtained during the female phase of anthesis

Compound	<i>mw</i>	<i>A. cor.</i>	<i>A. cra.</i>	<i>A. dio.</i>	<i>A. mon.</i>	<i>C. bic.</i>
Total number of compounds		6	4	8	3	17
Number of analyzed samples		2	6	3	4	4
Average (min-max) total amount of scent per flower/inflorescence (µg/h) ^a		95 (61-130)	45 (tr-148)	9 (2-16)	90 ^b (6-194)	2,178 (113-6,206)
Aliphatics						
3-Pentanyl acetate	130	–	–	–	–	0.81
Benzenoids and phenylpropanoids						
<i>p</i> -Methoxystyrene	134	–	–	0.71	–	–
Anisaldehyde	136	–	–	0.07	–	–
Veratrole	138	–	–	–	–	0.30
Methyl salicylate	152	–	–	–	–	15.56
Eugenol	164	–	–	0.22	–	–
Methyl 2-methoxybenzoate	166	–	–	–	–	0.43
1,2,4-Trimethoxybenzene	168	–	–	–	–	0.01
1,3,5-Trimethoxybenzene	168	–	–	–	–	31.49
1,2,3,5-Tetramethoxybenzene	198	–	–	–	–	0.01
Nitr. and/or sulp. cont. compounds						
Indole	117	–	–	0.05	–	0.01
4-Methyl-5-vinylthiazole	125	97.49	97.95	98.36	99.63	36.25
4-Methyl-5-formylthiazole	127	0.22	1.58	0.24	0.05	0.03
Terpenoids						
α -Pinene	136	–	0.43	–	–	–
β -Pinene	136	–	0.04	–	–	–
β -Myrcene	136	–	–	–	–	0.01
(<i>E</i>)- β -Ocimene	136	–	–	–	0.31	0.01
Linalool	154	–	–	–	–	8.73
Dihydro- β -Ionone	194	2.26	–	–	–	0.07
β -Caryophyllene	204	–	–	0.32	–	–
α -Humulene	204	–	–	0.02	–	–
Unid. <i>m/z</i> : 119,91,121,43,105,134		0.01	–	–	–	–
Unid. <i>m/z</i> : 121,107,91,93,136,192		0.01	–	–	–	–
Miscellaneous cyclic compounds						
(<i>E</i>)-Jasmone	150	–	–	–	–	0.05
(<i>Z</i>)-Jasmone	150	–	–	–	–	6.22
Methyl jasmonate	210	0.01	–	–	–	0.02
Fatty acid derivatives						
Benzenoids and phenylpropanoids		–	–	1.00	–	47.79
Nitr. and/or sulp. cont. compounds		97.71	99.53	98.65	99.68	36.29
Terpenoids		2.28	0.47	0.34	0.31	8.82
Miscellaneous cyclic compounds		0.01	–	–	–	6.29

A. cor. *Annona coriacea*, *A. cra.* *A. crassiflora*, *A. dio.* *A. dioica*, *A. mon.* *A. montana*, *C. bic.* *Caladium bicolor*

^a Scent amount trapped cannot be compared among species as different methods were used for scent collection (e.g. ,bagged vs. unbagged, see [Methods and Materials](#))

^b total amount was determined for three of the four samples

paper decoy flowers (see above). The amount of 4-methyl-5-vinylthiazole emitted by each dispenser was calculated between 700–950 µg h⁻¹, similar to the value

obtained from a single bagged *Caladium bicolor* inflorescence under the same environmental conditions (Table 1). The traps (91 in total) were installed between 17:30–18:30 h

and retrieved 3–4 h later. Traps with unloaded dispensers were used as paired controls.

Results and Discussion

GC-MS analyses of floral scent samples of the studied species revealed a total of 26 compounds, divided into several chemical classes (see Knudsen et al., (2006) for details): aliphatics (1), benzenoids and phenylpropanoids (9), nitrogen- and/or sulphur-containing compounds (3), terpenoids (10), and miscellaneous cyclic compounds (3) (Table 1). The bouquets of the *Annona* species were each comprised of eight or less compounds, and the bouquet of *Caladium bicolor* of seventeen.

Fragrances of the four species of *Annona* consisted almost entirely of a single compound, 4-methyl-5-vinylthiazole (Fig. 2), which alone accounted for over 97 % of relative flower scent discharge. This sulphur- and nitrogen-containing heterocyclic, reported here for the first time as a floral volatile, also was identified as a major constituent (36.25 %) in samples of the aroid *C. bicolor* (Fig. 3; Table 1).

Derived from the metabolism of amino acids (Schwab et al., 2008), sulphur-containing floral volatiles are found in less than 40 % of the extant angiosperm orders (Knudsen et al., 2006). Among them, thiazoles are largely unknown in flower scents. Recently, two thiazole compounds (2-methylthiazole and 2-methyl-4,5-dihydrothiazole) were reported as minor constituents (≤ 2.5 % relative discharge) in the floral scent of wasp-pollinated *Eucomis* spp. (Hyacinthaceae) (Shuttleworth and Johnson, 2010). Two other compounds, benzothiazole and benzothiazolone, isolated in less than 10 plant species as minor constituents, are actually recognized as ubiquitous environmental contaminants, and their natural occurrence in flowers is subject to controversy (Azizian et al., 2003; Valdes

and Zaror, 2006). Unlike sulphides present in the fetid, pungent aroma of bat- or carrion insect-pollinated species (Dobson, 2006), thiazole fragrances commonly emanate notes of tropical fruits, nuts, and green leaves (Maga, 1975; Goeke, 2002). All samples we analyzed emitted a characteristic roasted nut reminiscent aroma, as does pure 4-methyl-5-vinylthiazole.

Most plant volatiles originate from primary metabolism, arising as secondary metabolites of countless feasible alterations of pre-existing biosynthetic pathways (Pichersky et al., 2006; Schwab et al., 2008). We can only speculate about the occurrence of 4-methyl-5-vinylthiazole in flowers. However, while previously unknown as a floral scent volatile, it has been identified as a trace constituent in fruits of *Annona muricata* (Wong and Khoo, 1993; Pino et al., 2001). It also is found in passion fruits (*Passiflora edulis* f. *flavicarpa*, Passifloraceae) (Engel and Tressl, 1991), cupuaçu (*Theobroma grandiflorum*, Malvaceae) (Fischer et al., 1995), and garlic bulbs (*Allium sativum*, Alliaceae) (Yu et al., 1989), a clear indication that several plant lineages bear the necessary biochemical apparatus for its synthesis. Although yet untested, it is plausible that the origin of 4-methyl-5-vinylthiazole in angiosperms is related to the thiazole pathway involved in the multi-enzyme biosynthesis of thiamine (vitamin B1). This compound is an essential co-factor to all living organisms, synthesized *de novo* in all major groups of higher plants (Jurgenson et al., 2009). Through dephosphorylation and dehydration reactions, yet to be elucidated, the precursor of the thiazolic moiety of thiamine, 4-methyl-5- β -hydroxyethylthiazole phosphate, could be converted to 4-methyl-5-vinylthiazole.

The fragrant decoys and traps baited with 4-methyl-5-vinylthiazole installed at both study sites lured exclusively male and female scarab beetles belonging to three species of the genus *Cyclocephala* (Table 2). We did not observe insects approaching unbaited decoy flowers at *Botucatu*, nor

Fig. 2 Quadrupole mass spectra of 4-methyl-5-vinylthiazole (m/z 125.0)

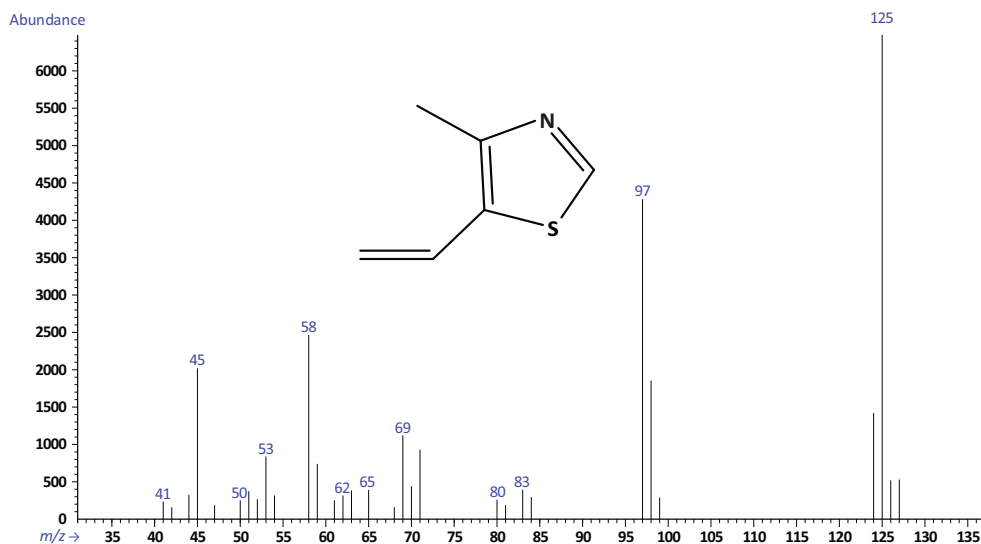
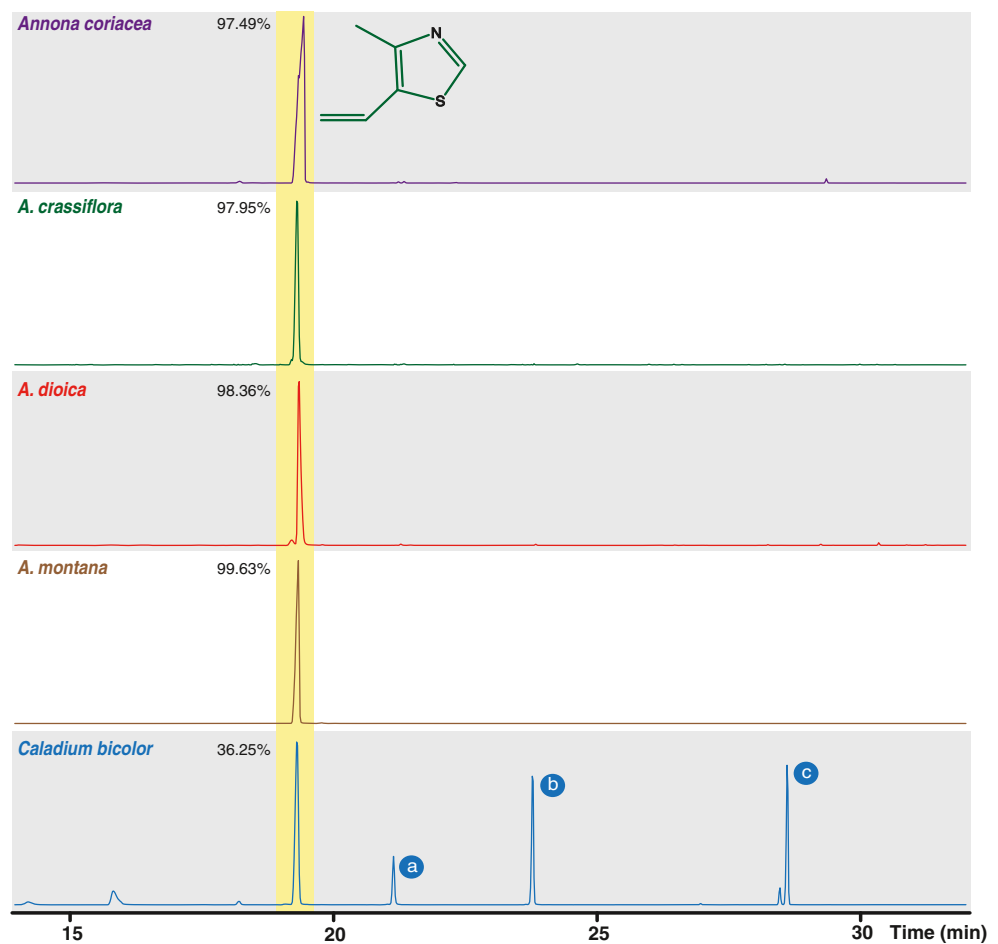


Fig. 3 Chromatograms (total ion counts) of dynamic headspace samples from flowers of four *Annona* species (Annonaceae, magnoliids) and from an inflorescence of *Caladium bicolor* (Araceae, monocots). The peak of 4-methyl-5-vinylthiazole is highlighted and relative percentage of the compound in the overall scent blends is shown for each studied species. Other prominent compounds are: (a) linalool, (b) methyl salicylate, and (c) 1,3,5-trimethoxybenzene



did we recover insects from any of the paired negative control traps at *Igarassu*. Attraction was selective at both sites, as only these three species were recovered out of a wider array of sympatric congeneric species (see Gottsberger (1989) and Maia et al., (2010) for details).

We have gathered evidence of a biochemical convergence between unrelated lineages of angiosperms (Annonaceae and

Araceae) towards a specific set of pollinators, either linked to selective expression of a plesiomorphic biosynthetic pathway or to parallel evolution leading to independent floral scent compound production. Convergent evolution in plant metabolism is thoroughly exemplified in plant-pollinator interactions (Pichersky and Lewinsohn, 2011), and sometimes the production of certain floral volatiles can even be assumed as predictors of different pollination syndromes (Dobson, 2006). For example, plants emitting oligosulfides are typically pollinated by carrion/dung flies (Jürgens et al., 2006; Johnson and Jürgens, 2010) or bats (Knudsen and Tollsten, 1995; Von Helversen et al., 2000). Nocturnal plants emitting floral scents rich in lilac aldehydes are associated with noctuid moths (Plepyš et al., 2002; Dötterl et al., 2006; Chess et al., 2008).

What is particularly remarkable in our observations is that the attraction of the observed species of *Cyclocephala* towards their flower hosts is highly specific (as aforementioned, other congenics were not attracted) and linked to the presence of a single compound. This serves as one example of a rarely documented scent-driven ‘private communication channel’ (Raguso, 2008). A similar scenario was found within reward-based pollination systems involving fragrance seeking male euglossine bees (Apidae, Euglossini) (Whitten et al.,

Table 2 Attraction of pollinator scarab beetles (Scarabaeidae, Dynastinae) of the genus *Cyclocephala* to synthetic 4-methyl-5-vinylthiazole. Field experiments conducted in two localities in Brazil (Botucatu, 22° 52' S, 48°26' W; and Igarassu, 7°49' S, 35°02' W). Installed baited traps were paired to negative controls (unbaited). No insects were recovered from negative controls at either site

	Botucatu	Igarassu
Type of trap	paper flower decoy	'Japanese beetle trap'
# installed traps (baited)	13	91
% traps with captures	46.1	40.7
Total # of captures	26	59
Average captures per trap	2.0	0.65
Species collected (male: female sex ratio)	<i>C. atricapilla</i> (1.4: 1)	<i>C. celata</i> (1: 1); <i>C. vestita</i> (7.2: 1)

1986). The monoterpene *trans*-carvone oxide, common to floral scents of several species of orchids (Kaiser, 1993) and unrelated Euphorbiaceae of the genus *Dalechampia* (Armbruster et al., 1989), *Unonopsis stipitata* (Annonaceae) (Teichert et al., 2009), and *Anthurium* spp. (Araceae) (Schwerdtfeger et al., 2002) induced attractive response of pollinator bees of the genera *Eulaema* and *Euglossa*. The occurrence of *trans*-carvone oxide in angiosperms otherwise is extremely rare (Knudsen et al., 2006), and until now this compound has been isolated solely in plant taxa pollinated by fragrance seeking male euglossine bees. Such a level of chemosensory pollinator selectivity also is exemplified in studies of the diverse, pantropical *Annona*. While 4-methyl-5-vinylthiazole is a dominant element in the scent of the species targeted in our study, it is entirely absent from the bouquet of *Annona glabra*. This species is pollinated instead by small beetles (Chrysomelidae), and its flowers give off a different odor: a sharp, acetone-like fragrance rich in 1,8-cineole, 3-pentanyl acetate and 3-pentanol (Goodrich and Raguso, 2009).

Both *trans*-carvone oxide and 4-methyl-5-vinylthiazole function as main attractive signals for selective subsets of insect visitors, and systems involving these two groups of pollinators show analogous luring strategies (e.g., intense fragrance discharge, high relative amounts in overall blend). Floral scent trails for cyclocephaline scarab beetles, male and female alike, indicate specific micro-niches where they consistently find shelter, reliable food sources, and most importantly, optimal mating opportunities (Gottsberger, 1990). Some authors even speculate that sexual aggregation in anthophilous cyclocephaline scarabs rely on allelochemicals present in floral scents of their preferred host plants (Schatz, 1990; Dieringer et al., 1999; Gibernau et al., 1999). For male euglossine bees, fragrances themselves are coveted rewards used in courtship behavior (Eltz et al., 2005).

It may be that other angiosperms pollinated by cyclocephaline scarabs have similarly adopted 'private communication channel' strategies involving peculiar bioactive compounds, strategically ensuring efficient specific attraction of pollinators. The floral scent of *Cyclanthus bipartitus* (Cyclanthaceae), whose thermogenic inflorescences are visited by two species of *Cyclocephala*, is dominated by an exclusive homoterpene, (*E*)-cyclanthone (> 85 % of the floral blend) (Schultz et al., 1999). Unusual methoxylated esters found in the scent of *Nymphaea lotus* L. (Nymphaeaceae) could well be involved in the attraction of *Ruteloryctes morio*, the effective pollinator of indigenous populations of this water lily in West Africa (Ervik and Knudsen, 2003). The large night-blooming flowers of Central American *Magnolia tamulipana* and *M. schiedeana*, attractive to *Cyclocephala caelestis* and *C. jalapensis*, respectively (Dieringer and Espinosa, 1994; Dieringer et al., 1999), give off a strong scent dominated by an exclusive methoxylated monoterpene, geranyl methyl

ether (Azuma et al., 1999). Methyl 2-methylbutyrate, highly attractive to the pollinator of *Magnolia ovata* (Gottsberger et al., 2012), is more broadly distributed in floral scents, although usually as a minor constituent (Knudsen et al., 2006).

It has been suggested that certain flowering plants exploit pre-existing sensory preferences of specific pollinators (Schiestl, 2010). Floral fragrances might accurately mimic sex pheromones (Schiestl et al., 1999), the scent of preferred oviposition substrates (Stensmyr et al., 2002), and even alarm pheromones of honeybees, an optimal signal for hunting wasps (Brodmann et al., 2009). Both adults and larvae of *Cyclocephala* and other scarab beetles feed on non-floral plant materials (e.g., roots, fruits), to which these insects also might be attracted by olfactory cues (Gottsberger, 1990; Bernhardt, 2000). Attractive compounds present in floral scents of plants pollinated by cyclocephaline scarabs could have been selected through feeding-oriented neuronal preferences of the insects (Schiestl, 2010). Although we do not have evidence that 4-methyl-5-vinylthiazole is part of the innate communication system of *Cyclocephala* beetles, our findings show that it plays a key role in long-range signalling for appropriate feeding and/or mating sites.

Overall, our study led to the identification of 4-methyl-5-vinylthiazole as a floral signal utilized by several *Annona* and an unrelated aroid (*Caladium bicolor*) that convergently attracts their *Cyclocephala* beetle pollinators. Further investigations directed at the biosynthesis of 4-methyl-5-vinylthiazole and its prominence in floral fragrances will show how deep the entanglement of this novel floral scent compound goes into the basis of other lineages of cyclocephaline scarab-pollinated angiosperms.

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