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Floral scent compounds of Amazonian Annonaceae species pollinated by small beetles and thrips

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This paper is dedicated to Professor Dr. Otto R. Gottlieb on the occasion of his 80th birthday

Abstract

Chemical analysis (GC–MS) yielded a total of 58 volatile compounds in the floral scents of six species of Annonaceae distributed in four genera (*Xylopia*, *Anaxagorea*, *Duguetia*, and *Rollinia*). *Xylopia aromatica* is pollinated principally by Thysanoptera and secondarily by small beetles (Nitidulidae and Staphylinidae), whereas the five other species were pollinated by Nitidulidae and Staphylinidae only. Although the six Annonaceae species attract a similar array of pollinator groups, the major constituents of their floral scents are of different biochemical origin. The fragrances of flowers of *Anaxagorea brevipes* and *Anaxagorea dolichocarpa* were dominated by esters of aliphatic acids (ethyl 2-methylbutanoate, ethyl 3-methylbutanoate), which were not detected in the other species. Monoterpenes (limonene, *p*-cymene, α-pinene) were the main scent compounds of *Duguetia asterotricha*, and naphthalene prevailed in the scent of *Rollinia insignis* flowers. The odors of *X. aromatica* and *Xylopia benthamii* flowers were dominated by high amounts of benzenoids (methylbenzoate, 2-phenylethyl alcohol).

Keywords: *Anaxagorea*; *Duguetia*; *Rollinia*; *Xylopia*; Annonaceae; Beetle pollination; Floral volatiles; Aliphatic esters; Benzenoids; Terpenes

1. Introduction

Floral scent is a major secondary attractant (Faegri and van der Pijl, 1979; Dobson, 1994) and especially important in many beetle-pollinated plants (Proctor et al., 1996; Gottsberger, 1999). The family Annonaceae includes over 2000 species, many adapted to pollination by beetles (Gottsberger, 1989a, b; Schatz, 1990; Nadel, 1990; Nadel and Pena, 1994; Armstrong and Marsh, 1997; Webber, 1996). Beetle pollination evolved from more generalized phytophagy (Gottsberger, 1977; Pellmyr and Thien, 1986; Bernhardt and Thien, 1987) and many contemporary beetle-pollinated flowers exhibit floral fragrances characterized as fruit mimics, with a fruity–spicy odor and ample fleshy tissues or fleshy floral organs that serve both as nutritive resources and brood substrate (Gottsberger, 1977, 1986; Webber, 1981a; Armstrong and Irvine, 1990; Olesen, 1992; Armstrong and Marsh, 1997). A large number of Annonaceae are fragrant due to the presence of essential oils. The constituents of these oils are usually mono- and sesquiterpenes or aromatic compounds (Santos et al., 1998), but esters of aliphatic acids can also be the main compounds (Jirovetz et al., 1998). The objectives of this paper are to provide chemical descriptions of the floral scents in Annonaceae species and to relate scent composition to the pollination biology of these taxa, especially to their beetle pollination.

2. Results and discussion

The genus *Anaxagorea*, considered by several authors to be basal within the Annonaceae (e.g. Doyle and Le Thomas, 1997; Zuilen and Koek-Noorman, 1997), occurs in the Neotropics and in Southeast Asia. The six taxa mentioned in the present study are also from the Neotropics, with a geographical distribution centred mainly in the Amazon region and the Guianas (Fries, 1959; Maas and Westra, 1992). Only *Xylopia aromatica*, which belongs to a genus with pantropical distribution, extends beyond, occurring in the West Indies and from

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Costa Rica in Central America down to southern Brazil and Paraguay (Fries, 1959).

Characteristics of the floral biology in all six species are summarized in Table 1. The floral scent compounds present in the six studied taxa listed in Table 2 are ordered in classes, which to some degree reflect their biosynthetic origin (see Knudsen et al., 1993). Aliphatic esters and other fatty acid derivatives are products of the malonic acid pathway (Croteau and Karp, 1991). The benzenoids are derived from shikimic acid via phenylanaline, cinnamic acid, and further decarboxylation and ring oxidation (Croteau and Karp, 1991). The monoterpenes and sesquiterpenes are derived from the mevalonic acid pathway via farnesyl pyrophosphate (Croteau and Karp, 1991).

Each of the six species of Annonaceae produced large amounts of only a few scent chemicals (Table 2). Although the species clearly differ in scent composition and amounts of the main compounds, similarities in the biochemical origin of scent compounds were found between species belonging to the same genus. In the two *Anaxagorea* species aliphatic esters were the main compounds, whereas both *Xylopia* species were characterized by high relative amounts of benzenoids; aliphatic esters were not found. Benzenoids are often present in head-space samples of floral scent in many plant families (Knudsen et al., 1993). However, the main compounds of these *Xylopia* species (methylbenzoate, 2-phenylethyl alcohol) have been reported as main components in the floral scent of some Magnoliaceae (Azuma et al., 1997, 1999). Naphthalene, which falls in a miscellaneous category, dominated the scent in *Rollinia insignis*, and was also a main compound in *X. aromatica*. Although naphthalene is known as an insect-repellant of anthropogenic origin (moth-balls), it was sometimes found in flower scents, for example by Zeng et al. (1990) in the floral scent of *Chimonanthus praecox* L. and by Borg-Karlson et al. (1985) in the floral scent of *Ophrys* species. Isoprenoids, especially monoterpenes were the main compounds in *Duguetia asterotricha*. All species have terpenoids and generally small amounts of fatty acid derived alkanes, ketones, and alcohols. However there is a clear pattern in the occurrence of fatty acid derived esters and benzenoid compounds, which are in general prominent compounds of the fragrances, where either one is present to the exclusion of the other in all but one species (*D. asterotricha*). All species studied have protogynous flowers, which is a common characteristic of Annonaceae flowers. The white, cream or yellow coloured flowers emit a strong, mostly fruity scent during their two days lasting anthesis. All species are diurnal, except *Xylopia benthamii* R. E. Fries which is nocturnal. During the first day of anthesis the flowers are in the female stage, which means the stigmas are receptive. On the second day flowers initiate the male stage, in which pollen is liberated. All species, except *X. aromatica* (Lam.) Mart., are mainly pollinated by Nitidulidae and Staphylinidae species which enter the pollination chamber. The beetles are released when flowering is abruptly terminated by the dropping of petals and stamens or flower desintegration after the male flowering stage. *X. aromatica* is mainly pollinated by thrips (Thysanoptera) and some nitidulid and staphylinid beetles (Webber, 1996; Gottsberger, 1999).

On the first day of anthesis the flowers of *Anaxagorea brevipes* Benth. opened at 6 am and emitted a weak fruity odor, which became stronger and at the beginning of the afternoon could be described as a mixture of several fruit odors. In the late afternoon, the odor weakened and a banana-like odor prevailed, and by 8 pm the odor had completely disappeared. In the morning of the second day, the flowers emit a fruit-like odor again. The odor emissions in the morning of the first and the second day were accompanied by temperature elevations in the flowers (thermogenesis), which reached a maximum 1.5°C above air temperature during the female phase and 0.9°C during the male phase. In *A. brevipes* the main scent compounds were aliphatic esters, with a dominance of ethyl 2-methylbutanoate (52.5%). Floral visitors were principally Nitidulidae (see Table 1), which made up 90% of the visits, and more occasionally Staphylinidae (6%), Curculionidae (4%), and Chrysomelidae (<1%). The beetles apparently were attracted to the flowers by the scent emissions and approached them principally during the afternoon of the first day and the morning of the second day of anthesis. Only the Nitidulidae and Staphylinidae species entered the pollination chamber (interior of the flower) and are pollinators; the others fed on the petals (Webber, 1996).

The anthesis and floral ecology of *Anaxagorea dolichocarpa* Sprague et Sandwith was studied at Mabura Hill in Guyana by Tol and Meijdam (1991) and Maas-van de Kamer (1993). During the first and second days of anthesis, the flowers produced a strong banana-like scent, which was especially notable from 1 to 4 pm. As in *A. brevipes*, floral scent was dominated by aliphatic esters. In *A. dolichocarpa*, ethyl 3-methylbutanoate accounted for 28.1% of the total volatiles, followed by isobutyl acetate with 17.2%. Small beetles, principally Nitidulidae species, which usually feed on fruits, and occasionally Staphylinidae, entered flowers in the female or male stages; the insects were released in the afternoon of the second day when the petals dropped. Although thermogenesis has not been observed by the above mentioned authors, flowers of *A. dolichocarpa* investigated by us in the greenhouse of the Botanical Garden of the University of Ulm (Germany), showed temperature elevations in the late afternoon and the evening of the first day, reaching a maximum of 2°C above air temperature.

In *D. asterotricha* (Diels) R. E. Fries the strong ananas-like floral scent attracted several species of *Colopterus*

Table 1
Locality of study, distribution, habitat, and floral traits in *Anaxagorea*, *Duguetia*, *Rollinia*, and *Xylopia* species[a,b]

Species	*Anaxagorea brevipes*	*Anaxagorea dolichocarpa*	*Duguetia asterotricha*	*Rollinia insignis*	*Xylopia aromatica*	*Xylopia benthamii*
Locality of study	Amazonas University of Manaus, and Reserva Ducke, Brazil	Mabura Hill, Guayana	Manaus, Reserva Ducke, Brazil	Amazonas University in Manaus, Brazil	Rio Negro, Manaus, Brazil	Amazonas University of Manaus, Brazil
Distribution	Amazon region and Guianas	Amazon region and Guianas, northeastern Brazil	Amazon region	Amazon region	Neotropics (West Indies, Central and South America)	Amazon region
Vegetation of study site	Forest along creek	Forest	Upland forest	Secondary forest	Secondary forest along river	Campinarana
Altitude of study site (m NN)	70–100	–	80–100	70–100	60	70
Habit	Tree	Tree	Tree	Tree	Treelet	Tree
Height (m)	3.5–9	5.5	8	8–18	2.5–3.5	8–12
Flowering season	September to December	Throughout the year	December to March	December to June	Throughout the year	June to October
Position of flowers	± Horizontal to ± pendant	± Horizontal to ± pendant	Pendant	Pendant	Erect to laterally inclined	± Pendant
Time of anthesis	Diurnal	Diurnal	Diurnal	Diurnal	Diurnal	Nocturnal
Odor	Fruity, banana-like	Fruity; banana-like, acetonic	Fruity, ananas-like	Fruity, sweet	Sweet, aromatic	Fruity
Main flower colour	Cream-coloured	Light-yellow	Yellowish	Yellow	White	Yellowish
Thermo-genesis	+	+ (–)	-	-	-	+
Protogyny	+	+	+	+	+	+
Pollinators	Nitidulidae (94%) (*C. planus*, *C. ruptus*, *C.* spp.), Staphylinidae (6%)	Nitidulidae (99%), Staphylinidae (< 1%)	Nitidulidae (100%) (*C.* spp.)	Nitidulidae (99%) (*C.* spp.), Staphylinidae (< 1%)	Thysanoptera (80%), Nitidulidae (16%), Staphylinidae (4%)	Nitidulidae (95%), Staphylinidae (5%)

[a] Based on Fries (1959), Maas and Westra (1992), Webber (1996), and Gottsberger (1999).
[b] *C.* = *Colopterus*; — = no information available.

Table 2
Chemical composition of the floral scent in six Annonaceae species[a,d,e]

Compound	RR_t	Criteria[a]	*A. bre.*	*A. dol.*	*D. ast.*	*R. ins.*	*X. aro.*	*X. ben.*
Fatty acid derivatives								
Alkanes								
Tetradecane	750	a	–	–	1.8	–	–	–
Hexadecane	929	a	tr	tr	0.8	tr	tr	tr
Ketones								
Methyl isobutenyl ketone	206	a	1.7	0.5	–	–	–	–
3-Hexene-2-one	206	a	–	0.1	–	1.2	4.5	–
cis-Jasmone	754	a	–	–	–	–	–	16.0
Alcohols								
3-Hexen-1-ol	253	a	2.9	–	–	–	2.6	–
Esters								
Isobutyl acetate	188	b	–	17.2	–	–	–	–
Isopropyl propanoate	212	a	0.8	–	–	–	–	–
Ethyl 2-methylbutanoate	248	a	52.5	–	–	–	–	–
Ethyl 3-methylbutanoate	249	a	–	28.1	–	–	–	–
Isoamyl ethanoate	272	a	6.9	0.9	–	–	–	–
Propyl butanoate	290	a	tr	–	–	–	–	–
Isobutyl isobutanoate	305	a	–	5.6	–	–	–	–
Prenyl ethanoate	311	a	0.4	–	–	–	–	–
Ethyl tiglate	317	a	–	0.9	–	–	–	–
Unknown aliphatic ester m/z: 145, 116, 103, 85, 74, 57	335	b	4.8	–	–	–	–	–
n-Butyl isobutanoate	346	a	–	0.5	–	–	–	–
Isoamyl *n*-propanoate	361	a	0.7	–	–	–	–	–
Ethyl hexanoate	388	a	–	1.4	–	–	–	–
Isobutyl 2-methylbutanoate	392	a	tr	–	–	–	–	–
Ethyl 3-hexanoate	393	b	–	7.2	–	–	–	–
cis-3-Hexenyl ethanoate	395	a	0.9	–	–	–	–	–
Isobutyl isovalerate	396	a	–	6.7	–	–	–	–
cis-3-Hexenyl propanoate	485	a	0.1	–	–	–	–	–
Isopentyl 3-methylbutanoate	494	a	–	0.6	–	–	–	–
Isobutyl hexanoate	533	a	–	3.2	–	–	–	–
Benzenoids								
Ethylbenzene[b]	262	b	–	–	–	2.2	0.1	0.4
p-Xylene[b]	270	a	–	–	–	3.2	0.1	0.9
o-Xylene[b]	292	a	–	0.1	–	1.9	0.1	0.6
1-ethyl-2-methyl-Benzene	357	b	–	tr	0.6	0.9	–	0.1
Benzaldehyde	360	c	–	tr	–	tr	0.9	0.2
Trimethylbenzene	366	b	–	–	–	0.3	–	0.1
Unknown benzenoid *m/z*: 120, 105, 91, 79, 65	376	b	–	tr	–	0.4	–	0.1
Unknown benzenoid *m/z*: 120, 105, 91, 85, 79, 65	391	b	–	–	5.0	2.7	–	0.6
Unknown benzenoid *m/z*: 120, 105, 91, 79, 65	419	b	–	–	–	0.1	–	0.2
Benzyl alcohol	429	c	–	tr	–	–	2.3	–
Unknown benzenoid *m/z*: 134, 119, 105, 91, 79	446	b	–	–	–	0.2	–	0.1
1,4-diethyl-Benzene	451	b	–	tr	–	0.3	–	0.1
Methylbenzoate	490	c	–	–	–	–	–	38.6
2-Phenylethyl alcohol	509	c	–	–	–	–	61.4	–
Benzyl acetate	551	a	–	–	–	–	0.1	–
2-Phenylethyl acetate	633	a	–	–	–	–	2.8	–
2-(4-Methoxyphenyl) ethanol	731	b	–	–	–	–	0.2	–
Isoprenoids								
Monoterpenes								
α-Pinene	332	c	0.2	0.1	4.2	1.5	0.5	0.2
Sabinene	369	a	–	–	–	–	tr	–
β-Pinene	376	c	tr	tr	–	–	0.4	–

(*continued on next page*)

Table 2 (*continued*)

Compound	RR_t	Criteria[a]	*A. bre.*	*A. dol.*	*D. ast.*	*R. ins.*	*X. aro.*	*X. ben.*
Myrcene	381	a	–	–	–	–	0.4	–
P-Cymene	420	a	0.1	tr	5.5	0.4	0.1	0.2
Limonene	425	c	0.3	–	14.1	2.3	0.1	0.5
1,8-Cineole	429	a	tr	–	–	–	–	–
Nerol	606	a	–	–	–	–	3.7	–
(L)-Carveole	619	a	–	–	–	–	0.1	–
Sesquiterpenes								
β-Copaen	740	a	0.2	0.2	2.7	1.5	0.1	0.1
β-Caryophyllene	777	a	–	–	1.4	0.3	0.1	–
α-Caryophyllene	805	a	–	–	1.1	0.1	0.1	–
γ-Muurolene	817	a	0.1	–	–	tr	tr	tr
Valencene	824	a	–	–	2.1	0.3	0.2	–
α-Muurolene	834	a	–	–	–	0.1	0.1	tr
γ-Cadinene	848	a	0.1	tr	0.5	0.1	0.1	tr
δ-Cadinene	852	a	tr	tr	0.2	0.1	0.1	tr
γ-Gurjunene	884	a	–	–	–	0.1	tr	tr
Unknown sesquiterpene *m/z*: 204, 189, 161, 147, 133, 119, 105, 91, 77, 69, 55	922	b	tr	–	–	–	–	–
Nitrogen compounds								
Unknown nitrogen compound *m/z*: 131, 102, 81, 69, 57	248	b	10.4	8.5	–	–	–	–
N-methyl-aniline	462	a	–	0.2	–	–	–	–
Indole[a]	672	a	–	–	–	–	9.5	–
Miscellaneous								
Naphthalene[c]	581	a	0.1	0.1	0.4	56.2	1.8	22.3
Unknowns								
m/z: 131, 102, 85, 69	245		1.9	–	–	–	–	–
m/z: 131, 115, 101, 85, 71	271		6.5	–	–	–	–	–
m/z: 120, 105, 91, 77, 65, 51	440		–	–	12.2	–	–	–
m/z: 109, 95, 83, 71, 57	589		tr	tr	2.8	0.8	tr	0.3
m/z: 136, 121, 105, 91, 79, 67	605		–	–	–	–	3.7	–
m/z: 165, 148, 133, 119, 105, 91, 79, 67	673		–	–	–	–	–	9.8
m/z: 141, 115, 89, 70, 58	677		–	tr	1.5	0.2	tr	–
m/z: 149, 135, 107, 97, 83	796		0.1	0.1	1.5	tr	tr	–
m/z: 220, 205, 189, 177, 161, 145, 131, 123, 91	831		0.1	tr	4.7	tr	0.1	tr
m/z: 203, 189, 176, 161, 149, 135, 121, 111, 97, 85, 71, 57	897		–	0.1	1.3	1.1	0.1	tr
m/z: 208, 195, 167, 149, 131, 119, 111, 97, 85, 71, 57	966		–	0.1	2.2	1.0	0.1	tr
m/z: 221, 191, 178, 165, 143, 128, 115, 105, 91, 77, 63	985		–	0.2	2.1	tr	–	–
m/z: 212, 197, 169, 155, 141, 104, 89, 76, 56	993		tr	tr	0.6	tr	tr	tr
Fatty acid derivatives			72.5	81.4	2.6	1.2	7.1	16
Benzenoids			0	0.1	5.6	12.2	68.0	42.0
Isoprenoids			1.0	0.3	31.8	6.8	6.1	1.0
Nitrogen compounds			10.4	8.7	0.0	0.0	9.5	0.0
Miscellaneous			0.1	0.1	0.4	56.2	1.8	22.3
Unknowns			8.6	0.5	28.3	3.2	4.0	10.1
Total of all compounds			92.6	91.4	69.3	79.6	96.5	91.4

[a] Compound identification criteria: a = comparison of MS and retention time with published data; b = comparison of MS with published data; c = identity confirmed by comparison of MS and retention time of authenticated standard.

[b] Xylene and ethylbenzene might be of anthropogenic origin.

[c] Napthalene might be of anthropogenic origin.

[d] Average relative amounts (in%) of floral volatiles emitted by *Anaxagorea brevipes* (*A. bre.*), *A. dolichocarpa* (*A. dol.*), *Duguetia asterotricha* (*D. ast.*), *Rollinia insignis* (*R. ins.*), *Xylopia aromatica* (*X. aro.*), *Xylopia benthamii* (*X. ben.*). The compounds are listed according to class and relative retention time order (RR_t).

[e] tr = trace amounts (< 0.1%). Unknowns were included when present in over 1.0% in any sample.

(Nitidulidae), which entered the floral chamber in the first day of anthesis and so might have acted as pollinators before they were released when the petals dropped in the early morning of the second day of anthesis. The Chrysomelidae did not enter the flowers and fed only on the petals (Webber, 1996). In *D. asterotricha* the main scent compounds were the monoterpenes limonene (14.1%), *p*-cymene (5.5%), and α-pinene (4.2%).

The floral ecology of *R. insignis* R. E. Fries is similar to that described by Webber (1981b) for *R. mucosa* (Jacq.) Baill. In the afternoon of both days a fruity sweet odor attracted several unidentified species of *Colopterus* (Nitidulidae) and occasionally some Staphylinidae. The beetles remained inside the floral chamber until its desintegration in the male phase. Naphthalene dominated the scent in *R. insignis* (56.2%).

In *X. aromatica* the female phase extends throughout the first day and the male phase occurs in the early morning (between 6 and 8 am) of the second day. The floral odor was strong, aromatic, and pleasant, and reminiscent of *Convallaria* (Liliaceae) flowers. The species is pollinated by thrips (Thysanoptera) and some nitidulid beetles (Gottsberger, 1999). At the Manaus site, thrips were seen in 80% of the flowers, nitidulids in 16%, and staphylinids in 4% (Webber, 1996). Although *X. aromatica* seems to be mainly pollinated by thrips, we found only low amounts of aromatic aldehydes, which are reported as being especially attractive to thrips (Kirk, 1985). In *X. aromatica* benzenoids accounted for over 68% of the total volatiles and the main benzenoids were 2-phenylethyl alcohol (61.4%), 2-phenylethyl acetate (2.8%), and benzyl alcohol (2.3%). Among the nitrogen compounds, indole (9.5%) was found only in the floral scent of this species.

Flowers of the nocturnal *X. benthamii* opened abruptly at dusk around 6 pm, when the stigmas were shiny and receptive. During this female stage, the interior of the flowers was 2.8°C warmer than ambient air and a floral scent was emitted, which was reminiscent of ripe fruits of *Spondias lutea* (Anacardiaceae). In *X. benthamii* benzenoids accounted for over 42% of the total volatiles, dominated by methylbenzoate (38.6%). As in *R. insignis*, Naphthalene (22.3%) was a main compound in *X. benthamii*. The main compound *cis*-jasmone (16.0%) was only found in *X. benthamii*. After 6 pm, the temperature in the flower and the floral odor constantly diminished until 10 pm, when the floral temperature reached ambient temperature. Throughout the whole next day the floral temperature remained low and the floral odor was weak, and also did not change for the short male phase which started at 4:30 pm with the liberation of pollen and finished between 6:00 and 6:30 pm. The main flower visitors and pollinators were Nitidulidae (95%), and to a lesser extent also Staphylinidae (5%). The visiting insects arrived the first evening between 6 and 9 pm, stayed in the flowers during the next day and were released on the second evening when the flowers disintegrated (Webber, 1996).

It is apparent that the species differ in their main biosynthetic pathways of their floral scent compounds in spite of the fact that their fragrances nearly all share a fruity note (Table 1). Many of the main compounds, especially some of the fatty acid derived volatiles, and some mono- and sesquiterpenes, are described as fruity odors or have been identified as scent compounds in fruits. Fatty acid derived volatiles (e.g. fatty acid esters and hydrocarbons) have been found as major components especially in families of the primitive subclass Magnoliidae, to which the Annonaceae belong (Thien et al., 1975; Bergström et al., 1991; Kite et al., 1991; Jirovetz et al., 1998). In a detailed study of *Magnolia* flower fragrances, species producing methyl esters had fruity odors which were said to attract beetles that also fed on rotten bark and fruits (Thien et al., 1975). Fatty acid esters were also the main compounds in the two investigated species of the genus *Anaxagorea* considered by several authors to be basal within the Annonaceae (e.g. Doyle and Le Thomas, 1997; Zuilen and Koek-Noorman, 1997). In *X. benthamii* fatty acid esters were absent but the fatty acid ketone *cis*-jasmone may be responsible for the characteristic fruity odor of this species. This compound was also found by Thien et al. (1975) as a main compound in *Magnolia grandiflora* L., a species that is pollinated by the beetle *Trichotinus piger* (Thien, 1974). All species emitted a range of mono- and sesquiterpenes in their floral scents that have recently been reported as constituents in the volatile fraction of the dried fruits of *X. aethiopica* (Dunal) A. Rich. (Tairu et al., 1999) and in the essential oils of *Annona muricata* L. fresh fruit pulp (Jirovetz et al., 1998), which both consist mainly of a mixture of mono- and sesquiterpenes (Ogan, 1971; Ayedoun et al., 1996; Jirovetz et al., 1997). Many of the sesquiterpenes found in the studied species have also been found in the floral odors of other Magnoliidae: β-copaene, and β-caryophyllene were reported as floral volatiles in *Chimonantus praecox* L. (Calycanthaceae), a family with a number of features resembling those of the Magnoliaceae and Annonaceae (Zheng et al., 1990). The sesquiterpenes γ-cadinene, δ-cadinene, and γ-gurjunene have been found in the scent of *Magnolia* flowers (Thien et al., 1975). However, all the identified isoprenoids are often found in the floral scent of many plant species (Knudsen et al., 1993). In contrast to the finding that similar, or structurally closely related, floral scent compounds tend to be associated with certain pollination syndromes, as in moth-pollinated species (Knudsen and Tollsten, 1993) or bat-pollinated species (Knudsen and Tollsten, 1995), the five Annonaceae species pollinated mainly by nitidulids and staphylinids showed no overall similarities in their main scent compounds. The variation in the floral scent chemistry of the investigated taxa is even more interesting as our investigation is not only based on one plant family but also on genera of this family with a restricted group of pollinating beetles. The same high variation in floral scent chemistry between beetle-pollinated taxa has been found by Thien et al. (1975) in Magnoliaceae species. If beetle pollination has evolved from more generalized phytophagy (Gottsberger, 1977; Pellmyr and Thien, 1986; Bernhardt and Thien, 1987) and floral scents of some beetle-pollinated plants are evolved as fruit mimics, it seems reasonable that they should show the same diversity as fruit odors. The finding of diverse scent components is in accordance with the descriptions of fragrances in beetle-pollinated species, ranging from disagreeably pungent and cyanide- or nutlike, to pleasantly fruity or spicy (Dobson, 1994). In a recent investigation of floral scent chemistry in beetle-pollinated phytelephantoid palms (Arecaceae), Ervik et al. (1999) also found different main scent compounds in different genera. According to Ervik et al. (1999) scent may play an important role as an isolating mechanism in sympatric phytelephantoid palms. Nevertheless, there is a lack of knowledge of the species of Nitidulidae and Staphylinidae pollinating different Annonaceae species. Future surveys of these beetle-pollinated species should study close interrelationships between single species of beetles and plants due to specific odors of the flowers. For the above mentioned Annonaceae, approached by small beetles, it remains an open question if their flower visitors are more opportunistic pollinators which are attracted by a wide range of volatile compounds or if these interactions are more specific.

3. Experimental

3.1. Plant material

Floral characteristics of the six species are summarized in Table 1. Voucher specimens of the investigated plant and beetle species have been deposited in the collections of the Instituto Nacional de Pesquisas da Amazônia, the Universidade do Amazonas in Manaus, Brazil, and the Abteilung Systematische Botanik und Ökologie of the University of Ulm, Germany.

3.2. Pollination and floral ecology

The pollination biology of *A. brevipes*, *D. asterotricha, R. insignis*, *X. aromatica*, and *X. benthamii* was studied in the field around Manaus, Brazil, during the last ten years (see also Webber, 1996; Gottsberger, 1999).

3.3. Volatile sampling

The odor samples were collected in the field in Brazil in and around Manaus during floral biological studies during the years 1994–1998. Only the floral odor of *A. dolichocarpa* was collected from a cultivated individual at the Botanical Garden of the University of Ulm (Germany). Floral scent was collected following the method of Knudsen and Tollsten (1993) with minor modifications. Odor was collected at the phase of most intense scent production with a battery operated membrane pump, scent containing air was sucked through glass cartridges with a 1:1 by weight (300 mg) mixture of Tenax-TA, mesh size 20–40 (2,6-diphenyl-*p*-phenylene oxide), and Carbotrap, mesh size 20–40. The flow rate through the cartridges was ca. 150 ml min^{-1}. Cartridges were conditioned by washing with acetone and heated out at 250°C. After 2–3 h, the adsorbed scent substances were extracted with 1 ml of acetone into glass vials.

3.4. Gas chromatography/mass spectrometry

The samples were analysed by coupled gas chromatography and mass spectrometry (GC–MS) on a Varian Saturn 2000 System, equipped with a 8200 CX autoinjector. The samples (1 μl) were introduced using a 1079 Injector. A nonpolar fused silica GC-column was used (CP-Sil-8 CB-MS 30 m long, inner diameter 0.25 mm, film thickness 0.25 μm). Electronic flow control (EFC) was used to maintain a constant helium carrier gas flow of 0.8 ml min^{-1}. The GC was programmed for 2 min at 60°C, increased by 8°C per min for 35 min, and maintained at 260°C; split ratio 20; injector temperature 200°C; interface heating 175°C; ion trap heating 200°C; mass spectra 70 eV (in EI mode), scan range, 40–650 amu at scan rate of 1 $scan^{-1}$. The GC–MS data were processed using the Saturn Software package 5.2.1. Component identification was carried out using the NIST 98 mass spectral data base (NIST algorithm) and confirmed by comparison of retention times with published data (Jennings and Shibamoto, 1980; Davies, 1990; Adams, 1995). Identification of individual components was confirmed by comparison of both mass spectrum and GC retention data with those of authenticated standards.

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