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THE FRAGRANT FLORAL OILS OF *TOVOMITA* SPECIES

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Key Word Index—*Tovomita* spp. ; Guttiferae ; pollination ; male euglossine bees ; chemistry.

Abstract—The filaments of the stamens of flowers of the dioecious genus *Tovomita* produce droplets of fragrant oils. In *T. macrophylla* it was observed that the oil is collected by male euglossine bees acting as pollinators. The bees settle down on the anthers of the male flowers and the stigmas of the female flowers respectively to collect the oil with their forebarsitarsi. The oil analyses of four species from the Reserva Ducke (Manaus, Central Amazonia) were investigated revealing that the composition is different for all four species, possibly to attract selectively different euglossine species and avoid interspecific cross-pollination.

INTRODUCTION

The neotropical genus *Tovomita* Aubl. comprises about 80 species of dioecious trees often with buttresses or stilt roots. The taxonomy and biology of *Tovomita* is rather poorly known. The genus has a distribution from Central America, Amazonia and along the Brazilian Atlantic rain forests southwards to Rio de Janeiro state. Like other Guttiferae (Clusiaceae) most tissues of the plants contain yellow or whitish latex. The flowers are small to medium-sized and have numerous stamens (males) or staminodes (females) respectively of similar morphology. The filaments are generally rather stout and the anthers small. The petals are often recurved during anthesis.

Very little is known about the population structure of *Tovomita* species and sympatric occurrence, especially in "terra firme" forests. Plants of the "igapó" species *Tovomita macrophylla* (Poepp. and Endl.) Walp. were observed to grow rather close to each other (Bittrich, V. and Amaral, M. C. E., unpublished results). The collections for the Flora of the Reserva Ducke near Manaus, an area of "terra firme" forest of ca. 100 km^2, have revealed that several *Tovomita* species may occur sympatrically in a rather small area. Up to now 16 species have been found, but only one or a very few collections for each species were made, suggesting a low population density (Bittrich, V., in preparation). Some bees, as for example euglossines, however, are very strong fliers and well-known as long-distance pollinators of tropical plants [1], so that a low population density would not impede successful pollination. As generally more than one *Tovomita* species flowers at the same time of the year, the problem for the plants, however, is how to avoid interspecific cross-pollination.

Floral scents are typical for animal-pollinated plants, but generally lacking in flowers pollinated by birds. Different scents often attract different groups of pollinators and thus are part of the so-called floral syndromes [2]. While the attractive agent is mostly different from the reward (often pollen or nectar), in some flowers fragrant oils are secreted, which not only attract the pollinators but also represent the reward for them. This phenomenon seems to be restricted, however, to male euglossine bees as pollinators. Flowers offering such fragrant oils are especially known from Orchidaceae [3] where more than 600 species produce fragrant oils attracting euglossine males. It was also observed, as a rare phenomenon, in a couple of other angiosperm families [4, 5], but never in a member of the Guttiferae. Like other floral scents, the fragrant oils are generally a mixture of several volatile organic substances. Studies on orchids have shown that the composition of the oils is species-specific and plays an important role for the specificity of euglossine visitors and thus as a reproductive isolating mechanism between sympatric species [6, 7].

We have investigated the chemical composition of fragrant oils of the stamens of four species of *Tovomita* collected in the Reserva Ducke near Manaus, with the objective of comparing the chemical composition between *Tovomita* species to detect differences that would suggest an isolating function between sympatric species flowering at the same time of the year.

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RESULTS AND DISCUSSIONS

The flowers of all species investigated in the field are diurnal and last one day. On the day of anthesis the filaments of the stamens (male flowers) or staminodes (female flowers) produce tiny droplets of a fragrant oil. In *Tovomita macrophylla*, we have observed that male bees of one *Euglossa* sp. land on the stamens of the male flowers and on the large stigmas of the female flowers respectively. While sitting on the anthers or stigmas, respectively, the bees collect the fragrant oil with their forebarsitarsi and transfer the oil afterwards, while hovering close to the flowers, to the hindlegs. This process is generally repeated a couple of times before the bees leave the flower. Sometimes two or more bees compete for the same flower. As bees land on the stamens and collect the oil while sitting on the anthers, their whole sternum is dusted with the dry pollen. Landing afterwards on the large stigmas of the female flowers and collecting the oil from the staminodes while sitting on it, pollination is guaranteed.

Small beetles were also observed visiting the flowers of *T. macrophylla*, taking up oil from the filaments, sometimes cutting into them with their mandibles. As they always stayed at the base of the filaments and never touched the anthers, they have to be considered as oil thieves and not pollinators.

The oil was collected by absorption on highly pure filter paper and stored in amber vials. The oil was extracted with solvent and analyzed by GC/MS (Table 1). The identification process relied on retention index and mass spectra comparison (Wiley/NBS mass spectral library of the GC/MS data system). When the retention index [8] was not available and/or not compatible with the mass spectrum, the identification was obtained by co-injection of a synthetic standard compound obtained from commercial starting materials and short path synthesis.

The analyses of the three *Tovomita* oils (Table 1) revealed that the main constituents are terpene alcohols. Germacrenol and cubebol are responsible for 84.2% of the oil mixture of *T. rubella* and, except for eugenol, the remaining constituents are sesquiterpenes. Germacrenol is again the major sesquiterpene alcohol (41.8% of relative abundance) of *T. amazonica* oil but unlike that from *T. rubella* this oil contains a greater variety of hydrocarbon sesquiterpenes and also several minor aromatic constituents including indole (0.9%). Dihydrophytol (91.6%) is the only terpene present in *T. acutiflora* along with long chain hydrocarbons and its identification relied on a synthetic standard coinjection. The synthetic standard was obtained by catalytic hydrogenation of a commercial sample of phytol. *Tovomita* aff. *grata* oil was also investigated and has three main constituents (37.7%) possessing mass spectra compatible with some oxygenated terpenes of the literature [8] but the reported relative retention indices [8] were not compatible. Thus we are now working on synthetic standards to improve identification of these compounds. Owing to the uncertainty of the identification, this analysis has not been included in Table 1. Nonetheless there is an obvious difference in oil composition of the four *Tovomita* species which might be responsible for selective attraction of euglossine bees.

Based on the fact that a hexadecanol derivative attracts male euglossine bees [10], we suggest that 3,7,11,15-tetramethyl-1-hexadecanol (dihydrophytol) is responsible for the pollinator attraction (male euglossine sp.) of *Tovomita acutiflora*. Field experiments using a synthetic standard will be undertaken on our next visit to Reserva Ducke in Manaus, Central Amazonia.

CONCLUSIONS

Our observations of visits of male euglossine bees to the flowers of *Tovomita macrophylla* to collect fragrant oils represent the first reported case for this family and further the flower morphology and the behaviour of the male euglossine bees on *Tovomita macrophylla* leaves no doubt that they are pollinators. The significant diversity of molecular and structural distribution profiles of the chemical composition of these oils suggest that these oils may attract different species of male euglossine bees and thus impede interspecific cross-pollination.

EXPERIMENTAL

Methods and plant material

Observations of the pollination of *Tovomita macrophylla* were made on several days during the flowering season in an "igapó" forest of a tributary of the rio Tarumã-açú, in the vicinity of Manaus (Central Amazonia). The staminal oil of the four studied *Tovomita* species occurring in the Reserva Ducke near Manaus were directly collected from filaments of fresh flowers using highly purified filter paper. Voucher specimens of the species are deposited in the Herbaria INPA and UEC: *Tovomita acutiflora* M.S. and Barros G. Martiz (voucher # J.E.L.S. Ribeiro 1840 *et al.*), *T. amazonica* (Poepp. and Endl.) Walp. (voucher # J.E.L.S. Ribeiro 1745 *et al.*), *T.* aff. *grata* Sandwith (voucher # J.R. Nascimento 517) and *T. rubella* Planch. and Triana (voucher # A. Vicentini 1081).

GC and GC/MS

HP-5990/5970 system equipped with J & W Scientific DB-5 fused silica capillary column (30 m × 0.25 mm × 0.25 μm); column temp. from 60 to 240° at 3° min^{-1} for integrating purposes. GC: injector and detector temperatures 220 and 285°, respectively; H_2 as carrier gas, flow rate 1.2 ml min^{-1}, split mode. Injection vol., 0.5 μl of about 10 mg of staminal oil in EtOAc. The retention indices were obtained by co-

Table 1. Percentage composition of the oils exuded by the stamens of *Tovomita* species

Compounds	RR_t exp.	RR_t lit.[8]	*T. rubella*	*T. acutiflora*	*T. amazonica*
Indole	1288	1288	—	—	0.9
2-Methoxybenzoic ac., methyl ester	1336	—	—	—	1.0
α-Cubebene	1349	1351	t	—	—
Eugenol	1353	1356	0.7	—	0.6
α-Copaene	1377	1376	—	—	t
β-Cubebene	1390	1390	t	—	—
β-Caryophyllene	1420	1418	—	—	0.8
(*E*)-β-Farnesene	1459	1458	3.2	—	24.8
Alloaromadendrene	1462	1461	—	—	0.4
γ-Gurjunene	1471	1473	—	—	t
Germacrene D	1481	1480	1.7	—	2.6
Eremophilene	1489	—	—	—	0.8
Epicubebol	1494	1493	0.5	—	—
Bicyclogermacrene	1496	1494	2.1	—	12.5
Cubebol	1515	1514	39.1	—	t
δ-Cadinene	1524	1524	1.4	—	0.9
Cadina-1,4-diene	1533	1532	0.7	—	—
Elemol	1550	1549	—	—	1.7
Germacrene D-ol	1576	1574	45.1	—	41.8
Viridiflorol	1589	1590	0.8	—	—
2,6-Dimethoxy-4-(2-propenyl)phenol	1602	—	—	—	2.0
1-Epi-cubenol	1628	1627	0.7	—	—
T-Cadinol	1641	1640	0.8	—	—
α-Cadinol	1655	1653	0.9	—	0.5
Hedycariol	1671	1666	—	—	1.5
Nootkatone	1803	1800	—	—	6.0
Eicosane	2000	2000	—	t	—
Hexadecyl acetate	2008	—	—	t	—
Dihydrophytol	2078	—	—	91.6	—
Heneicosane	2100	2100	—	1.7	—
Docosane	2200	2200	—	2.3	—
Tricosane	2300	2300	—	2.3	—
Tetracosane	2400	2400	—	2.1	—
Total			97.5	100.0	98.8

RR_t exp. = Relative Retention Index calculated applying Van den Dool and Kratz equation Ref. [9]; t = traces.

injecting the oil sample with a C_{11}-C_{24} normal hydrocarbon mixt. GC/MS: carrier gas, He; temp. program the same as that for the GC experiments, MS, 70 eV, scanning speed 0.84 scan sec^{-1} from m/z 40 to 550.

The oils were analyzed by GC and GC/MS and identifications were made on the basis of standard compound co-injection (synthetic material characterized by IR, ^1H and ^{13}C NMR spectra) and comparison of retention indices [8] as well as by computerized matching of the acquired mass spectra with those stored in the Wiley/NBS mass spectral library of the GC/MS data system and other published mass spectra.

Dihydrophytol

Phytol (from Fluka, 0.5 g, 1.7 mmol) in EtOAc (5 ml) was treated with Pt/C (50 mg) in the presence of H_2 (30 psi), at room temp. for 2 h. The reaction mixt. was filtered through a pad of Celite, and the solvent was evaporated under vacuum. GC of the crude reaction revealed the formation of two compounds (1 : 1). Purification by silica gel CC eluting with hexane–EtOAc–MeOH (8 : 1.5 : 0.5) yielded 50 mg of pure dihydrophytol (1 : 1 mixt. of (3*RS*,7*R*,11*R*)-3,7,11,15-tetramethyl-1-hexadecanol) and 450 mg of a mixt. of dihydrophytol and the other subproduct. The ^1H NMR, ^{13}C NMR and mass spectral data were identical to those previously reported [11].

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