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Pollination Biology of *Theobroma* and *Herrania* (Sterculiaceae). IV. Major Volatile Constituents of Steam-Distilled Floral Oils as Field Attractants to Cacao-Associated Midges (Diptera: Cecidomyiidae and Ceratopogonidae) in Costa Rica[1]

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ABSTRACT

Several distinctive, commercially-available volatile hydrocarbons and terepenoid substances identified from the floral fragrance oils of *Theobroma* species (Sterculiaceae), especially *T. simiarum* Donn. Smith and *T. cacao* Linnaeus, were bioassayed in "Finca Experimental La Lola" near Siquirres, Limon Province, Costa Rica during dry and wet seasons to determine their individual attractiveness to cacao-pollinating midges. Cecidomyiidae exhibited a slightly greater attraction to terpenoid substances tested, most notably geraniol, limonene, whereas Ceratopogonidae, the principal pollinators of *T. cacao*, were attracted in much lower numbers to both sets of substances. Attraction levels of midges to the volatiles tested are of the same order of magnitude observed for attraction of midges to the whole steam-distilled floral fragrance oils of these *Theobroma* species. Field observations indicate that Cecidomyiidae are attracted to the open flowers of both *T. simiarium* and *T. cacao*, while Ceratopogonidae are mostly attracted to *T. cacao*. The data, while preliminary, suggest that both terpenoid substances and hydrocarbons function as attractants for cacao-pollinating Ceratopogonidae and Cecidomyiidae.

COMPENDIO

En la Finca Experimental La Lola, cerca de Siquirres en la provincia de Limón, Costa Rica, se hicieron pruebas con dos constituyentes volátiles de aceites florales de dos miembros de la familia Sterculiaceae del género *Theobroma*, específicamente de *T. simiarum* Donn. Smith y *T. cacao* Linnaeus para determinar su capacidad para atraer los dípteros que efectúan la polinización del cacao. Este estudio se hizo durante el tiempo seco y la temporada de lluvias. Las Cecidomyiidae mostraron un poco más de atracción hacia las tres sustancias "terpenoid" que se probaron: "geraniol" "linalool" y "limonene" en preferencia a los hidrocarburos. En cambio las Ceratopogonidae, los dipteros que efectúan la mayor parte de la polinización de *T. cacao*, mostraron mucho menos atracción a los dos grupos de constituyentes volátiles que las Cecidomyiidae. Las observaciones registradas en la Finca Experimental La Lola denotan la atracción de las Cecidomyiidae a las flores de *T. simiarum* y de *T. cacao* y la preferencia de las Ceratopogonidae para las flores de *T. cacao*. Estos datos son preliminares, sin embargo parecen indicar que tanto las sustancias "terpenoid" como los hidrocarburos atraen los dípteros Ceratopogonidae y Cecidomyiidae que efectúan la polinización del cacao.

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INTRODUCTION

The steam-distilled floral fragrance oils from various species of *Theobroma* (Sterculiaceae), including *T. cacao* L. or "cocoa," contain major volatile constituents undoubtedly involved in the attraction of midge floral visitors and pollinators since the whole steam-distilled fragrances attract these insects (1, 3, 5, 6, 7, 9). In this paper I report field bioassays of major volatile constituents of *Theobroma* species as studied in an abandoned cocoa plantation in Costa Rica. These data suggest that certain volatile substances are more effective as field attractants for midges than others.

MATERIALS AND METHODS

All bioassays were conducted in the Barker Cacao Forest, an abandoned patch of Matina variety cacao bordering "Finca Experimental La Lola" Madre de Dios, near Siquirres, Limon Province, Costa Rica. This habitat and the bioassay technique (Fig. 1) used are described elsewhere (5, 7, 8, 9). In the present study, two successive bioassays of commercially-available hydrocarbon and terpenoid compounds (Sigma Chemical Company[r]) were conducted between February 11-20, 1988. A third bioassay was conducted June 10-13, 1988. The design of the first two bioassays was as follows: four compounds tested x four serial dilutions (1 000, 100, 10, 1 ppm) x one replicate each, making up sixteen experimental treatments. Additionally, four "blanks" or controls were used for each bioassay. The third bioassay consisted of testing two compounds (1-pentadecene, limonene) at two serial dilutions (100, 10 ppm) with four replicate traps of each (16 experimental traps), and six blanks. Compounds tested in the first bioassay were: n-pentadecane, 1-pentadecene, geraniol, and linalool; in the second bioassay: citral, citronellol, d-limonene, and tricosane. The choice of these compounds was based upon previous analyses of the major volatile constituents of floral fragrance oils steam-distilled from *T. cacao*, *T. simiarum* Donn. Smith and *T. mammosum* Cuatr. & Leon (1). Based upon this information, these bioassays were designed to determine whether or not these compounds, tested singly in water-filled McPhail traps, using standard dilutions (1), would attract cecidomyiid and ceratopogonid midges and which, if any, of the substances is most effective as an attractant for these insects. The results of the first two bioassays determined the design of the third one, since the last focused on two compounds suspected of being effective attractants, based upon previous results.

As in previous bioassays (5, 7, 8, 9), traps were emptied each morning for several successive days, and the contents poured through coffee filters to collect trapped insects (Fig. 1). This insect material was then sorted and preserved in 70% ethanol for further taxonomic identifications. As a standard practice, data on flowering was recorded for the cacao trees used for the traps.

RESULTS

During February of 1988, a time of reduced flowering in the Matina cacao compared to June of the same year (Table 1), Cecidomyiidae trapped using fragrance compounds were about five times as abundant as Ceratopogonidae (Tables 2, 3). About

Fig. 1 Testing volatile constituents of cacao floral fragrance in the field. From top to bottom: Barker Cacao Forest where bioassays were conducted; McPhail trap used for testing the attractiveness of volatiles to pollinating midges; method for collecting midges from McPhail Traps by pouring trap contents through filter papers and placing trapped insects into vials.

Table 1. Patterns of floral abundance on trees of *Theobroma cacao* L. (Sterculiaceae), Matina Variety, in The Baker Cacao Forest at Finca Experimental La Lola, near Madre de Dios, Limon Province Costa Rica during separate bioassay studies of floral fragance oils*.

Date	Floral Buds			Open Flowers		
	Total	X S.E.	Range	Total	X S.E.	Range
14 Feb 1988	28	1.75 0.89	0–10	2	0.12 0.08	0–1
12 June 1988	1 774	98.55 27.10	0–387	319	17.72 4.71	0–65

* A total of 20 cacao trees. The trees are tagged so that the same trees are used for every census. For each bioassay of floral fragrance oils, all but two of the McPhail traps were suspended in these tagged trees.

57% of the Cecidomyiidae trapped in two successive bioassays conducted in February, the dry season, were attracted to terpenoid fragrance compounds, especially geraniol and linalool, and about 41% were attracted to the hydrocarbons pentadecane and pentadecene (Table 2). In spite of their very low numbers, almost twice as many ceratopogonids were attracted to terpenoids than to hydrocarbons in the same biossays (Table 3). Attraction of Cecidomyiidae to a terpenoid, limonene, relative to pentadecene, was very high in the wet season study (Table 4. Few if any midges were found in the "blanks" or control traps, indicating a definite attraction of midges to the attractant-inoculated traps (Tables 2, 3). While the overall sex ratio for the three most abundant species of Cecidomyiidae in the February bioassays was skewed to about 60% towards females, the species with the greatest likelihood of being a cacao pollinator, *Mycodiplosis ligulata* Gagne, was 100% female in the attractant-baited traps (Table 5) All ceratopogonids trapped in the February bioassays were females.

DISCUSSION

Young *et al* (5, 7, 8, 9) demonstrated that low numbers of Cecidomyiidae and Ceratopogonidae are

Table 2. Patterns of specific attractiveness for various species of Cecidomyiidae (Diptera) attracted to volatile compounds identified as major constituents of floral fragrance oils from *Theobroma* species*.

Midge species	Total numbers of midges in scented McPhail traps:									
	pentadecane	pentadecene	geraniol	linalool	citral	citronellol	limonene	tricosane	blanks	total
Mycodiplosis ligulata Gagne	6	4	3	1	3	2	2	1	0	22
Aphodiplosis triangularis (Felt)	1	9	2	4	0	0	0	0	0	16
Ledomyia sp.	1	2	2	1	1	5	1	1	0	14
Clinodiplosis sp.	1	0	0	0	0	0	0	0	0	1
Mycodiplosis sp	0	0	1	0	0	0	0	0	0	1
Trisopsis sp.	0	0	1	0	0	0	0	0	0	1
Lestodiplosis sp	0	0	0	1	0	0	0	0	0	1
Stromatosema sp	0	0	1	0	0	0	0	0	0	1
Lestremiinae	0	0	1	0	0	0	0	0	1	2
poss. *Resseliella* sp.	0	0	0	0	0	0	0	0	1	1
Cecidomyiidi (undet.)	1	1	0	1	0	0	0	0	0	3
Total	10	16	11	8	4	7	3	2	2	63

* Bioassays conducted in the Barker Cacao Forest at Finca Experimental La Lola, Madre de Dios, Limon Province, Costa Rica, February 1988

Various other groups of Diptera, represented by 1-2 individuals, include: Culicidae, Sciaridae (*Bradysia* spp.), Chironomidae, Dolichopodidae (*Sciapus* spp., *Chrysotus* spp.)

Table 3. Patterns of specific attractiveness for various species of Ceratopogonidae (Diptera) attracted to volatile compounds identified as major constituents of floral fragrance oils from *Theobroma* species.

	Total numbers of midges in scented McPhail traps:									
Midge species	pentadecane	pentadecene	geraniol	linalool	citral	citronellol	limonene	tricosane	blanks	total
Forcipomyia cinctipes group	1	0	0	0	0	0	0	0	0	1
F. (Warmkea) louriei (Macfie)	0	0	0	0	0	0	0	1	0	1
F. (Rhynchoforcipomyia) brachyrhynchus Wirth & Waugh	0	0	0	0	0	0	0	1	0	1
F. (Thyridomyia) spp	0	0	0	0	0	0	1	0	0	1
Dasyhelea mutabilis group	0	1	0	0	0	0	0	1	0	2
D. borgmeieri Wirth & Waugh	0	0	0	0	1	0	0	0	0	1
D. grisea group	0	1	0	0	0	0	0	0	0	1
Dasyhelea spp	1	0	0	0	0	0	0	0	0	1
undet. genus & species	0	0	0	0	0	0	0	2	0	2
Totals	2	2	0	0	1	0	1	5	0	11

routinely attracted to steam-distilled floral fragrance oils at various concentrations in the Barker Cacao Forest at La Lola. The observed abundance levels in the present study, using synthetic analogs of major identified volatile substances (as revealed by gas chromatography and mass spectrometry (1)) as attractants in McPhail Traps, are very similar to numbers of midges trapped using the fragrance oil extracts. This is to say that some of the individual substances used, such as pentadecene, pentadecane, limonene and linalool, are individually as effective in attracting cacao-pollinating midges as the whole floral extracts. While terpenoid substances are more typical of floral fragrance oils from *T. simiarum* Donn. Smith and *T. speciosum* Wild., and hydrocarbons more characteristic of *T. cacao* L. and *T. mammosum* Cuatr. & Leon (1), both sets of volatiles function in the attraction of Cecidomyiidae and Ceratopogonidae, as revealed by the present study. Both pentadecene and pentadecane were observed to attract various Diptera in La Lola in a previous study using Pherocon IC traps rather than McPhail traps (8).

Field observations in La Lola indicate that Cecidomyiidae are floral visitors to *T. simiarum*, while Ceratopogonidae are much less abundant at the flowers of this species. Yet both groups of Diptera are attracted to the flowers of *T. cacao*. The role of some species

Table 4. Patterns of specific attractiveness for various species of Cecidomyiidae (Diptera) attracted to pentadecene and limonene, two major volatiles found in *Theobroma* floral fragrance oils*.

	Number of midges in traps:		
Midge species	pentadecene	limonene	total
Mycodioplosis ligulata Gagne	1	9	10
Aphodiplosis triangularis (Felt)	0	7	7
Lestodiplosis spp (sensu lato)	1	0	1
Cecidomyiidi (undet.)	1	0	1
Totals	3	16	19

* Bioassay conducted in June 1988, early phase of the rainy season at the study locality

All *M. ligulata* trapped were females; four females and three males, respectively, for *A. triangularis*.

of Cecidomyiidae as effective pollinators of cacao requires confirmation through further field studies (2, 4). Furthermore, adult midges of certain species of both Cecidomyiidae and Ceratopogonidae collected from the flowers of *T. cacao* are mostly female (4, 9), suggesting a sex-related differential attraction of these insects to cacao. Midge species attracted to *Theobroma* flowers possess mouthparts adapted for piercing and chewing pollen grains, and female midges may obtain egg-building nitrogenous substances from pollen. The data presented here suggest that while both hydrocarbon and terpenoid substances in *Theobroma* floral fragrance oils undoubtedly play a role in attracting pollinating midges, subtle-to-considerable differences in the relative amounts of these two sets of volatile substances, as reported in Erickson *et al.* (1), may determine whether Ceratopogonidae, Cecidomyiidae, or other Diptera (such as Phoridae) are the principal floral visitors and pollinators of a particular species of this genus.

Table 5. Sex ratio patterns for midges (Diptera: Cecidomyiidae) attracted to volatile compounds of *Theobroma* floral fragrance oils.*

Midge species	Number of midges in traps: female	male	total
Mycodiplosis ligulata Gagne	22	0	22
Aphodiplosis triangularis (Felt)	4	12	16
Ledomyia spp.	5	9	14
Totals	31	21	52

* All but three individuals of the 11 Ceratopogonidae trapped in the bioassays were females

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