Comparative Attractiveness of Floral Fragrance Oils of "RIM" and "Catongo" Cultivars of Cacao (*Theobroma cacao* L.) to Diptera in a Costa Rican Cacao Plantation[1]

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ABSTRACT

Three serial dilutions (100 ppm, 10 ppm, 1 ppm), each replicated three times, of steam-distilled, chloroform-extracted floral fragrance oils of two distinctive self-compatible cultivars, RIM-100 and Catongo, of *Theobroma cacao* Linnaeus (Sterculiaceae), or "cacao", were used in McPhail traps in an abandoned Costa Rican cacao (Matina) plantation during the rainy season, to determine comparative levels of attractiveness to small-bodied Diptera, especially potentially cacao-pollinating midges. Chloroform-distilled water solution was used to inoculate six control traps. All 24 traps were suspended in trees (mostly cacao) only weakly flowering and censused daily for six successive days to determine insects attracted to the floral oil inoculations. Floral oils were refreshed on the third day of the bioassay. Although Cecidomyiidae, especially *Aphidodiplosis triangularis* (Felt) and to a lesser extent, *Mycodiplosis ligulata* Gagné, were more than ten times as abundant (for a total of 11 species) than Ceratopogonidae, representative species of the latter group, especially *Dasyhelea* sp. 1, *grisea* group, indicate that steam-distilled floral oils of both cultivars attract potential pollinating agents of cacao. Females of suspected pollinators, *M. ligulata* and *D.* sp. 1, *grisea* group, dominated the samples, consistent with similar sex ratio patterns observed in cacao flowers for these species. Several other families of Diptera, including the Phoridae known to pollinate the allied genus *Herrania* in Costa Rica, were each represented by one or a few individuals. Diptera, with one exception, were absent from the control traps. Collectively the data indicate that the floral frarance oils of both cultivars of cacao examined actively attract Diptera, including groups containing known natural pollinating agents of *T. cacao*. Further study is required to determine the degree of chemical similarity (for volatile substances) in the fragrance oils between these two cacao cultivars, which in turn may help to explain the observed patterns in field bioassays.

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COMPENDIO

En un terreno de cacao "Matina" abandonado de Costa Rica se hicieron pruebas durante la temporada de lluvias para comparar la atracción de tres concentraciones progresivas (100 ppm, 10 ppm, 1 ppm) de las esencias de las flores de *Theobroma cacao* Linnaeus (Sterculiaceae) para los dípteros pequeños, especialmente los que puedan efectuar la polinización del cacao. Las esencias que se usaron en trampas McPhail fueron de los clones autocompatibles de cacao RIM-100 y Catongo. Estas esencias se extraen con destilación a base de cloroformo. El experimento se repitió tres veces. En seis trampas testigo se colocó agua destilada y cloroformo. Las veinticuatro trampas se colocaron en las ramas de árboles, especialmente de cacao con pocas flores. Se censaron las trampas por seis días sucesivos para determinar cuales insectos experimentaban la atracción a las esencias de las flores. El tercer día del experimento se renovaron las esencias. Los insectos que predominaron fueron los Cecidomyiidae, especialmente *Aphidodiplosis triangularis* (Felt) pero también se contaron *Mycodiplosis ligulata* Gagné. Las 11 especies de esta familia abundaron diez veces más que los Ceratopogonidae. Sin embargo entre las varias especies de Ceratopogonidae abundaron las *Dasyhelea* sp. 1, grupo *grisea* y su presencia indica la atracción a las esencias destiladas de las flores de los dos clones de cacao para los dipteros que pueden efectuar la polinización. Las hembras de *M. ligulata* y *D.* sp. 1, grupo *grisea* predominaron en las muestras y se cree que estos son algunos de las especies que efectúan la polinización del cacao. La proporción de números de hembras a machos de estas especies en las trampas corresponde a las proporciones de estos dipteros en las flores del cacao. También en las trampas se encontraron un solo individuo o unos pocos individuos de varias otras familias de los Diptera e incluso los Phoridae que se sabe que efectúan la polinización de *Herrania* en Costa Rica. *Herrania* es un género de la misma familia que el cacao. No se encontraron sino una sola especie de dipteras en las trampas testigo. En suma los datos indican que las esencias de las flores de los dos clones de cacao RIM-100 y Catongo atraen los Diptera e incluso los grupos de dipteros que se sabe que efectúan la polinización de *T. cacao*. Sin embargo se necesitarán análisis precisos para determinar las propiedades en común de las sustancias volátiles en las esencias de las flores de estos dos clones de cacao. Los resultados de este análisis explicarán las observaciones realizadas en este experimento y en otros. otros.

INTRODUCTION

Steam-distilled floral fragrance oils from freshly-open flowers of *Theobroma* species, including *T. cacao* Linnaeus (Sterculiaceae), "cacao," are chemically complex in terms of volatile substances (4) and have been shown to attract a range of small-

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bodied Diptera in Costa Rica (20). Given the generally accepted view that some Ceratopogonidae (Diptera), especially species of *Forcipomyia* and allied genera, are the most important pollinators of cacao in commercial plantations (1, 5, 7, 8, 9) and the intense floral visitation by Cecidomyiidae, the role of floral oils as effective attractants for pollinating insects deserves further study. This paper reports on the attraction of Diptera, principally Cecidomyiidae and Ceratopogonidae, to steam-distilled floral oils of two cultivated varieties of *T. cacao* in a Costa Rican cacao plantation. Since different cultivars of *T. cacao* vary greatly in the yields of cacao beans (2, 3), observed differences in levels of attractiveness of floral oils from different cultivars to potential pollinating insects may contribute to our overall understanding of how natural pollination affects commercial yield.

MATERIALS AND METHODS

From 30 June through 6 July 1987, the attraction of flying insects to serial dilutions of steam-distilled floral fragrance oils were bioassayed of the "Catongo" and "RIM-100" cultivars of *T. cacao* in an abandoned plantation of "Matina" cacao (Fig. 1) bordering the La Lola experimental farm, near Siquirres (10°06'N; 83°20'W; elev. approx. 50 m), Limon Province, Costa Rica. As with previous bioassays of *Theobroma* floral fragrances at this locality (20), 18 randomly-distributed experimental and six control McPhail traps (Fig. 1) were used in the present study. The experimental design consisted of testing two cacao cultivars with three serial dilutions (100 ppm of fragrance oil extracted in chloroform and mixed with distilled water, 10 ppm and 1 ppm) and three replicates of each, in addition to the six control traps (10% solution of chloroform and distilled water). 20 of the 24 trees used for suspending traps (1.0-1.5 m above the ground) were cacao, and a census was made of the abundance of flowers on these trees during the study. All traps were filled with approximately 3 cm of distilled water mixed with soap to trap insects. Traps were individually emptied each morning (usually 08:00-10:00) by pouring the water through coffee filter paper on a strainer. All insects were collected into vials of alcohol for sorting. On the morning of the third day, i.e., half-way through the biossay, the traps were re-inoculated with fresh floral oils (experimentals) or chloroform-distilled water (controls). Voucher specimens were later identified and sorted further for taxonomic identification by entomologists at the U.S. National Museum.

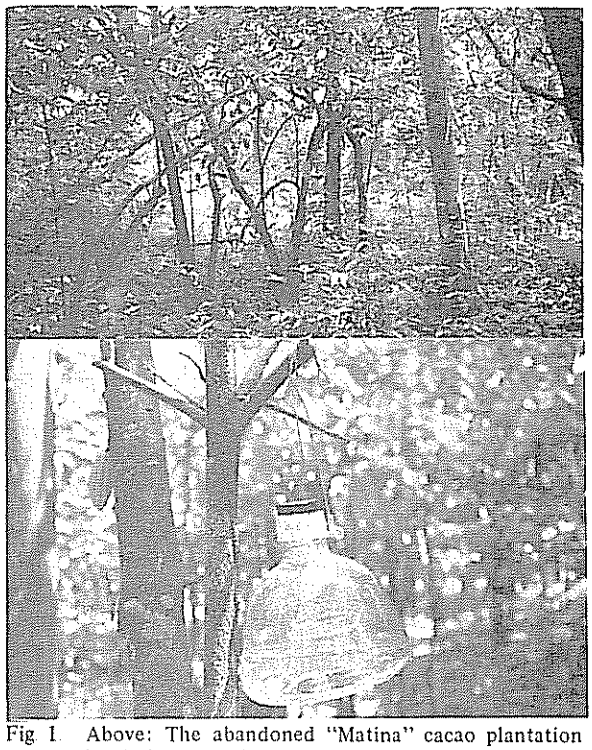

Fig. 1. Above: The abandoned "Matina" cacao plantation bordering La Lola experimental farm in Costa Rica, where the bioassay of cacao floral fragances oils was conducted during July 1987. Below: One of 24 Mc Phail traps used for bioassaying floral oils in the field.

The bioassay was conducted in the rainy season but during a week of fairly dry, sunny weather with intermittent showers. Based upon comparative morphological studies of flowers belonging to different cultivars of cacao underway at the present time and previously noted information, it was decided to compare the attractiveness of Catongo, a Forastero-derived cultivar characterized by comparatively average floral size, and unpigmented peduncle, sepals, ligules and staminoids, with RIM-100, a Criollo-derived, strongly pigmented (reddish) floral type, noted for its uniquely large size. Both cultivars are reported to be self-compatible (2, 3). Although analyses of the floral fragrance oils from these cultivars are incomplete, given the observations of very significant differences in floral structures related to natural pollination between these cultivars, it was suspected that the bioassays would reveal differences in the numbers and kinds of Diptera attracted in the field.

RESULTS AND DISCUSSION

The RIM cultivar floral fragrance oil attracted about twice the number of species of Cecidomyiidae as Catongo, although one species, *Aphidodiplosis triangularis* (Felt), comprised about 70% and 88% of the total samples for each floral oil, respectively (Table 1). For all cecidomyiids, the 100 ppm concentration of RIM floral oil attracted the greatest quantity of midges, whereas the 10 ppm-inoculated traps of Catongo attracted the greatest number of midges for that cultivar (Table 1). Only two individuals (*A.*

Table 1. Attraction* of Cecidomyiidae (Diptera) to replicated serial dilutions of steam-distilled floral oils from two distinct cultivars (clones), "RIM" and "Catongo," of *Theobroma cacao* Linnaeus (Sterculiaceae) during a rainy season bioassay in an abandoned cacao plantation in Costa Rica.

Species	Total numbers of midges in traps:**				Sex ratio
	100 ppm	10 ppm	1 ppm	Total	(F:M)
			"RIM cultivar"		
Aphidodiplosis triangularis (Felt)	38	6	22	66	30:36
Mycodiplosis ligulata Gagné	7	0	0	7	6:1
Ledomyia sp. 1	3	3	2	8	6:2
Coquilletomyia sp.	1	0	0	1	0:1
Trisopsis sp.	0	0	1	1	1:0
Cecidomyiidi sp. 1	0	2	0	2	2:0
Cecidomyiidi sp. 2	2	2	1	5	4:1
Cecidomyiidi sp. 3	0	0	2	2	1:1
Cecidomyiidi sp. 4	0	0	1	1	1:0
Cecidomyiidi sp. 5	0	0	1	1	1:0
Cecidomyiidi sp. 6	0	1	0	1	1:0
Total midges	51	14	30	95	53:42
%*A. triangularis*	69.47%				
			"Catongo cultivar"		
Aphidodiplosis triangularis (Felt)	22	33	17	72	27:45
Mycodiplosis ligulata Gagné	0	1	0	1	1:0
Ledomyia sp. 1	1	1	0	2	1:1
Ledomyia sp. 2	0	2	0	2	0:2
Bremia sp.	0	1	0	1	1:0
Cecidomyiidi sp. 1	1	3	0	4	4:0
Total midges:	24	41	17	82	34:48
%*A. triangularis*	87:80%				
			Controls ("blanks")		
Aphidodiplosis triangularis (Felt)	–	–	–	2	1:1

* Data summed for 24 McPhail traps, consisting of 18 "experimentals" and six "controls" (chloroform-distilled water blanks), for six successive days, with experimental traps re-inoculated with fresh floral oil distillate, and controls accordingly, on the third day of census

** Total of three replicates for each experimental treatment for each cultivar tested

triangularis) were found in the floral oil-free control traps (Table 1). Males of *A. triangularis*, the most abundant cecidomyiid species in samples of both cultivars, were more abundant than females, although only slightly so for RIM floral oil (Table 1). But for two of the next most abundant species found in the RIM samples, *Mycodiplosis ligulata* Gagné and *Ledomyia* sp., females were more numerous, even though the number of individuals was very low (Table 1). For all floral oil concentrations studied, both cultivars were fairly well matched in terms of the total number of cecidomyiid midges attracted (Table 1).

Several species of Ceratopogonidae were attracted to floral fragrance oils of both cultivars, with the most abundant species, albeit a very small sample size (representing about 57% of the sample), being *Dasyhelea* sp. 1, *grisea* group appearing in RIM-inoculated traps (Table 2). Interestingly, three species of *Forcipomyia* were attracted to the floral oils tested. The most abundant ceratopogonid, *D.* sp. 1, *grisea* group, was represented only by females (Table 2). Several other families of Diptera, including the Phoridae, were represented by various species attracted to both kinds of floral fragrance oils, and none were found in the control traps (Table 3). For virtually all groups of Diptera found, attraction of floral oils was fairly spread out across different levels of concentration (Tables 1-3). Additionally, one individual of the stingless bee *Trigona amalthea* (Oliv.) (Hymenoptera: Apidae: Meliponinae) was found in the 100 ppm of Catongo floral fragrance oil.

Compared to other times of the year, floral abundance on the twenty cacao trees with traps was low. With a range of 0-213 flowers per tree and six trees with no flowers, the average (X ± S.E.) number of flowers was 52.21 ± 15.77 during the study.

Athough overall Cecidomyiidae were more than ten times as abundant as Ceratopogonidae collectively in all floral oil-inoculated McPhail traps in this study, ceratopogonids, especially *Forcipomyia* species, are considered to be the principal pollinating agents of

Table 2. Attraction* of Ceratopogonidae (Diptera) to replicated serial dilutions of steam-distilled floral oils from two distinct cultivars (clones), "RIM" and "Catongo," of *Theobroma cacao* Linnaeus (Sterculiaceae) during a rainy season bioassay in an abandoned cacao plantation in Costa Rica.

Species	Total numbers of midges in traps:**				Sex ratio
	100 ppm	10 ppm	1 ppm	Total	(F:M)
			"RIM cultivar"		
Forcipomyia genualis (Loew)	0	1	0	1	0:1
F. harpegonata Wirth & Soria	0	1	0	1	0:1
Forciponyia sp	0	1	0	1	0:1
Dasyhelea sp. 1, *grisea* group	1	3	3	7	7:0
Total midges:	1	6	3	10	7:3
			"Catongo cultivar"		
Forcipomyia genualis (Loew)	1	1	0	2	0:2
Dasyhelea sp. 1, *grisea* group	1	0	0	1	1:0
Atrichopogon sp	0	0	1	1	0:1
Total midges:	2	1	1	4	1:3

* Data summed for 24 McPhail traps, consisting of 18 "experimentals" and six "controls" (chloroform-distilled water blanks), for six successive days, with experimental traps re-inoculated with fresh oil distillate and control accordingly, on the third day of census.

** Total of three replicates for each experimental treatment for each cultivar tested.

cacao flowers (12). The exact role of cecidomyiids as major pollinators of cacao remains to be determined, although certain species may be effective pollinators (6). In Costa Rica, *Mycodiplosis ligulata* Gagné is a likely pollinator of cacao flowers (17), and several of the genera and species attracted to cacao flower fragrance oils in the present study, including *Aphidodiplosis triangularis* (Felt), routinely visit cacao flowers in Costa Rica. However, other reports indicate that cecidomyiids may not be pollinating agents of cacao (9). An outstanding feature of the data, also found in a previous study (20), are the very small numbers of insects trapped in these bioassays. It is believed that these abundance patterns of cacao-associated adult Diptera reflect actual population densities of these insects in cacao habitats. Given the observed low abundance of cacao flowers in the study area during the bioassay, it is unlikely that midges were more attracted to flowers than fragrance oils in the traps.

The highly skewed ratio of both *M. ligulata* (Cecidomyiidae) and *Dasyhelea* sp. 1, *grisea* group (Ceratopogonidae) in favor of females observed in the traps, is consistent with direct observations of these species in cacao flowers (15, 17). Female ceratopogonid midges are considered to be the chief pollinating agents of cacao (1) and female cecidomyiids may also pollinate cacao flowers more than males. *Dasyhelea* species are routinely found in cacao flowers in Brazil and elsewhere in the New World tropics (10) and the apparent close association of these midges with the culturing of cacao warrants further examination (9, 13). Hernandez (5) reported that *Dasyhelea* midges pollinate cacao flowers in Costa Rica. The observed attraction of ceratopogonids and cecidomyiids to steam-distilled floral fragrance oils of cacao (20) demonstrates that cacao flowers emit fragrant volatiles attractive to natural pollinating agents, although other floral cues are most certainly involved in the pollinator association with cacao flowers (19). Since we have used distilled oils in the bioassays (20); (present study), undoubtedly our inoculates include those volatiles emitted from cacao flowers.

The observed attraction of several other families of Diptera, and the general absence of other insect groups, further suggests a floral-pollinator syndrome adaptive to Diptera. Although stingless bees regularly visit cacao flowers in the Neotropics, they are not considered to be pollinators of cacao (14). Of particular interest is the attraction of phorids, such as *Megaselia* sp., known pollinators or *Herrania* (16), a genus closely related to *Theobroma*. Phorids have been captured in the immediate vicinity of cacao flowers in Costa Rica (18).

Soria, Silva and Chapman (11), examining several different cultivars of cacao including the nonpigmented Catongo, concluded that floral pigmentation did not influence rates of natural pollination by *Forcipomyia* midges in Costa Rica. The results of the present study indirectly confirm this conclusion since no major differences in the levels of attraction between

Table 3. Attraction* of Diptera, various families, to replicated serial dilutions of steam-distilled floral oils from two distinct cultivars (clones), "RIM" and "Catongo," of *Theobroma cacao* Linnaeus (Sterculiaceae) during a rainy season bioassay in an abandoned cacao plantation in Costa Rica.

Species	Total numbers of midges in traps:**			
	100 ppm	10 ppm	1 ppm	Total
		"RIM cultivar"		
Sciaridae				
Bradysia sp . *coprophila* group	0	1	0	1
Phoridae, undet genus & species	1	0	0	1
Chironomidae				
Orthocladinae	0	0	1	1
Psychodidae, undet genus & species	2	0	0	2
Empididae, undet genus & species	1	1	0	2
Culicidae				
prob. *Culex* sp	0	0	2	2
Aedes sp	0	0	1	1
Trichoprosopon sp	1	0	0	1
undet genus and species	1	1	2	4
Total dipterans:	6	3	6	15
		"Catongo cultivar"		
Sciaridae				
Bradysia sp , *corprophila* group	2	0	1	3
Phoridae				
Megaselia sp	2	0	0	2
undet genus & species	0	1	1	2
Psychodidae, undet genus & species	1	0	0	1
Empididae, undet genus & species	0	0	1	1
Culicidae				
prob. *Culex* sp.	0	0	1	1
Total dipterans:	5	1	4	10

* Data summed for 24 McPhail traps, consisting of 18 "experimentals" and six "controls" (chloroform-distilled water blanks), for six successive days, with experimental traps re-inoculated with fresh floral oil distillate, and controls accordingly, on the third day of census

** Total of three replicates for each cultivar tested

floral fragrance oils of two very distinctive cacao cultivators, including Catongo, for both Ceratopogonidae and Cecidomyiidae, were found in the bioassay. Of interest, however, was the observed greater taxonomic diversity of Cecidomyiidae attracted to RIM floral oil. Further understanding of these observations awaits the analyses of floral oil differences among cacao cultivars and a clearer understanding of the genetic derivations of cacao cultivars.

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