

COMPARATIVE ANALYSIS OF STEAM DISTILLED FLORAL OILS OF CACAO CULTIVARS (*Theobroma cacao* L., Sterculiaceae) AND ATTRACTION OF FLYING INSECTS: IMPLICATIONS FOR A *Theobroma* POLLINATION SYNDROME

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Abstract—Steam-distilled floral fragrance oils from nine distinctive cultivars of *Theobroma cacao* L. (Sterculiaceae) in Costa Rica were examined with GC-MS to determine whether or not major differences existed among these cultivars for volatile constituents comprising 50% or more of the samples. The cultivars selected for floral oil analyses were chosen to represent diverse cultivars having supposedly different genetic backgrounds and histories of artificial selection for agronomic purposes. Cluster analysis revealed two major groupings of cultivars: those with higher molecular weight dominant compounds, and those having lower molecular weight compounds. Additionally, one cultivar, Rim-100, selected from criollo or ancestral-type cacao in Mexico and resembling criollo in the appearance of flowers and fruits, formed an extreme group having the highest molecular weight profile for major volatile compounds. Based upon these analyses, bioassays using McPhail traps were performed in an abandoned cacao plantation in northeastern Costa Rica during rainy and dry seasons to determine the relative attraction of these oils to flying insects. Bioassays revealed that the Rim-100 cultivar attracted by far the greatest numbers of cacao-associated midges (Diptera: Ceratopogonidae and Cecidomyiidae), as well as stingless bees (Hymenoptera: Apidae: Meliponinae), suggesting that a floral fragrance having high-molecular-weight volatiles is more potent as an attractant to flying insects than floral oils having lower-

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molecular-weight compounds. It is suggested that Rim-100 more closely resembles an ancestral or wild-type cacao than the other cultivars examined, and therefore it is more effective in attracting opportunistic dipteran floral visitors and pollinators than other cultivars in plantation settings. Several of the major volatile compounds found in the floral oils of *T. cacao* and other species of *Theobroma* occur in mandibular and other exocrine glands in various bees, including stingless bees and halictids, known visitors of *Theobroma* flowers. These compounds are particularly present in noncultivated species of *Theobroma* and have much more noticeable fragrances than the seemingly scentless flowers of cultivated *T. cacao* selected for agriculture. It is hypothesized that the floral attraction system of ancestral or wild (noncultivated) *T. cacao* and other species of *Theobroma* may have evolved to attract certain bees as their principal pollinators in natural habitats in the Neotropics, with a floral reward hypothesized as being sociochemicals needed by bees for mating, foraging, territorial defense, etc. Because of the many generations of extensive selection by cloning for desired cultivars, *T. cacao* might have lost much of its original floral attraction system for bees, instead being pollinated opportunistically by dipterans in plantation habitats. This may help to explain why natural pollination in cultivated *T. cacao* is generally very poor relative to observed levels of fruit-set in wild or noncultivated species of *Theobroma*.

Key Words—Floral oils, cacao, *Theobroma cacao*, Sterculiaceae, attraction, Diptera, Ceratopogonidae, Cecidomyiidae, Hymenoptera, Apidae, pollination, 1-pentadecene, *n*-pentadecene.

INTRODUCTION

The pollination of *Theobroma cacao* L. (Sterculiaceae), cocoa or cacao, is mediated in cultivated stands or plantations by various species of midges belonging to the dipteran families Ceratopogonidae and Cecidomyiidae (Dessart, 1961; Glendenning, 1962; Saunders, 1959; Soria, 1970; Posnette, 1944; Kaufman, 1973, 1975a,b; Young, 1983, 1985a,b, and many other references). While various bees, especially Meliponinae (Apidae) and Halictidae, are regular visitors to the often brightly colored and highly fragrant flowers of various noncultivated species of the exclusively Neotropical *Theobroma* (e.g., Aguiar-Falcao and Lieras, 1983; A.M. Young, unpublished observations), the smaller, less conspicuous, and weakly fragrant flowers of cultivated *T. cacao* in plantations are only occasionally visited by bees (e.g., Soria, 1975; Kaufman, 1975a; Young, 1985b). Because the basic floral design is very similar among the 22 recognized species of *Theobroma* (Cautrecasas, 1964), it has been suggested that floral habits in the genus, including cacao, may have originally evolved as a bee pollination system (Erickson et al., 1987). In this context, an ancestral form, or wild-type, cacao may be more effective in attracting flying insects, including bees, than cultivars derived from extensive cloning and artificial selection for agricultural purposes.

However, there is a dearth of observations on the natural pollinators of wild or noncultivated *T. cacao*, especially in its native locality on the eastern, lower slopes of the Andes Mountains. Floral structure in *T. cacao* and related species of *Theobroma* accommodates visitation and effective pollination by medium to small-bodied flying insects (Young et al., 1987a). Steam-distilled whole floral oils from fresh flowers of these species, when bioassayed in the field, attract both dipterans (Ceratopogonidae and Cecidomyiidae) and meliponine (stingless) and halictid bees, albeit in low numbers (Young et al., 1987b, 1988; Young, 1989a). Synthetic analogs of various major volatile constituents of *Theobroma* floral oils attract midges in very low numbers (Young, 1989b). Thus, floral fragrances derived from floral oils undoubtedly function as cues in attracting most visitors and pollinators to flowers of *Theobroma* species. Because cultivated *T. cacao* in plantations consists of many distinctive cultivars or horticultural races, floral oil chemistry and intensity of pollinator attraction may vary considerably among different cultivars, depending upon individual genetic history, degree of cloning, and degree of closeness to a wild-type or ancestral form. In this paper we report for the first time distinctive groupings of several cultivars of *T. cacao*, as determined by patterns of floral fragrance chemistry, and differential attractiveness for flying insects, especially Ceratopogonidae and Cecidomyiidae (Diptera) and stingless bees (Hymenoptera: Apidae: Meliponinae), as assessed in a Costa Rican cacao plantation during rainy and dry season bioassays. These studies suggest that considerable differences exist in the degree to which steam-distilled floral oils from different cultivars of *T. cacao* attract flying insects in a plantation habitat. Further, the steam-distilled floral oil from one cacao cultivar derived from and resembling an ancestral-type cacao from Mexico and Central America is more effective in attracting insects than the other, more derived cultivars examined. Finally, even a relatively primitive cacao cultivar is less attractive to bees relative to reported observations for completely noncultivated species of *Theobroma*. The extensive cloning or asexual propagation of cacao cultivars over long periods of time may have resulted in a breakdown of the original floral attraction-reward system designed to attract flying insects, including effective pollinating insects.

METHODS AND MATERIALS

Analyses of Floral Oils. Fresh flowers for volatile oil extractions were obtained from nine cultivars of *T. cacao* at C.A.T.I.E. in Turrialba (600 m elevation), Cartago Province, Costa Rica. Each of these cultivars was sampled during June of 1988 and 1990. One cultivar, UF613, was sampled at two different time periods, early morning and mid-afternoon, during both years. Time of sample collection for the other cultivars was generally random with respect

to years. Single samples from *T. speciosum* and *T. pentagona* were collected during 1988 and 1990, respectively. About 400 freshly opened flowers were collected for each sample.

Immediately following collection, flowers were subjected to vacuum steam distillation using a 500-ml short-path vacuum distillation unit (KONTES). Flowers were distilled for 1 hr at 100°C, the distillate extracted 3× with dichloromethane, and dried over sodium sulfate. The oil obtained was then frozen.

Frozen samples were transported on ice to the State Hygiene Laboratory at the University of Wisconsin (Madison) and subjected to gas chromatography-mass spectrometry (GC-MS) as previously described (Erickson et al., 1987). Individual components of each sample were qualified by peak areas with normalization to 100. Specific identification of the individual components was determined in a previous study (Erickson et al., 1987).

To examine variability, both between cultivars and species, we elected to compare the relative amounts of the 12 most abundant compounds from the previously described volatile spectra for UF613 (Erickson et al., 1987). The mean contribution for each of these compounds over both years was calculated. Relationships among cultivars and species based upon relative amounts of selected volatile components were analyzed using Euclidean distance and cluster analysis (SAS Institute, 1990). A cluster diagram was constructed using the average linkage clustering algorithm.

Field Bioassays. Based upon the results of the GC-MS analyses of the distilled floral oils, three groupings of these oils, based upon cluster analysis were revealed, providing a rationale for the design of field bioassays. Two field bioassays were established in order to evaluate the comparative attractiveness of representative cultivars from each grouping or cluster to flying insects, including either known or suspected pollinators of cacao. This locality generally experiences annually one moderately intense dry season between January and April (and shorter dry spells at other times) and a lengthy rainy season, which may have profound effects on the population cycles of cacao-pollinating midges (Young, 1983). Therefore, bioassays were scheduled for both rainy and dry seasons in order to obtain an adequate assessment of insect attraction to cacao floral oils. Field bioassays using 100 ppm serial dilution by volume of floral oil in spectral-grade chloroform were conducted in a semiabandoned cacao plantation, Finca La Tigra, La Virgen (10°23'N, 84°07'W; 220 m elevation), Sarapiquí District, Costa Rica, during the dry season (March 7–11) and rainy season (September 14–18) in 1991. The site within this cacao plantation where traps were placed is about 25 m from mixed primary and secondary premontane tropical wet or rain forest (Holdridge, 1966).

Inoculations of McPhail traps were done using carefully sealed and refrigerated samples of the same floral oils used for the analysis described above. Each McPhail Trap (Bennett, 1972) was labeled, using white tape, to identify

the treatment (floral oil type used) and preparation of the traps and subsequent observations on their contents followed that of previous studies (Young et al., 1987a,b, 1988; Young, 1989a,b). Floral oils from three distinct cacao cultivars, Rim-100, Catongo, and UF613, representing the three major groupings detected by the GC-MS analysis, were used in both bioassays. Inoculation of each trap consisted of dripping 2 ml of floral oil solution directly on a 100% cotton ball, using a pipete, suspended inside the McPhail trap. Inoculated traps were suspended in cacao trees at heights between 1.0 and 1.5 m from the ground (Figure 1). In both bioassays, traps were reinoculated on the third day of each five-day bioassay period. The design of each bioassay consisted of six replicates for each of the three floral oils being tested, and two blank or control traps, each of the latter inoculated with 2 ml of 10% chloroform-distilled water mixture. Traps were arranged in the cacao trees in moderate proximity to one another, with a range of 5–10 m of space between adjacent traps.

The 20 traps in each bioassay were emptied each morning by pouring the soapy water from each through a metal strainer lined with a coffee filter. Trapped insects were picked from soaked filter paper using fine forceps and placed into vials of 70% ethanol. Traps were refilled with water daily. Collected insect samples were kept separate for each treatment and date, and later sorted in the laboratory and identified using previously determined voucher specimens.

RESULTS

Analyses of Floral Oils. The mean relative abundance of the major *T. cacao* volatile components, consisting of saturated and unsaturated hydrocarbons, is shown in Table 1. The relative composition of these compounds in UF613 is within the range of previously reported values for this cultivar (Erickson et al., 1987). The most abundant compounds observed in this cultivar were 1-pentadecene and *n*-pentadecane. The mean level of 1-pentadecene was increased in samples of UF613 collected in mid-afternoon (46.9%) compared to samples collected in early morning (26.0%). Relatively high levels of 1-pentadecene were observed in four other *T. cacao* cultivars (SCA6, UF29, UF221, UF668). We were unable to detect 1-pentadecene in samples from Rim-100 and *T. speciosum*. Low levels of 1-pentadecene were observed in the remaining *T. cacao* cultivars and in *T. pentagona*.

Given the relatively high levels of lower-molecular-weight compounds observed in UF613 in this study (Table 1) and in previous studies (Erickson et al., 1987), we elected to conduct a cluster analysis based on the summation of the relative contribution of the five lowest-molecular-weight compounds (tridecane, 1-tetradecene, *n*-tetradecane, 1-pentadecene, *n*-pentadecane). This analysis separated all samples into two major groups, those with relatively high



FIG. 1. Abandoned cacao plantation in Costa Rica used for bioassays of cacao floral oils (above), and McPhail trap used in bioassays (below).

TABLE 1. MEAN RELATIVE COMPOSITION OF MAJOR VOLATILE COMPOUNDS OBTAINED BY STEAM DISTILLATION OF FLOWERS FROM NINE *Theobroma cacao* CULTIVARS AND FROM *T. pentagona* AND *T. speciosum*, 1988 AND 1990^a

Compound	Cultivar or species												
	Catongo	EET400	Pound7	Rim-100	SCA6	UF29	UF221	UF613a ^b	UF613b	UF688	<i>T. pentagona</i> ^c	<i>T. speciosum</i> ^d	
Tridecane	0.4	0.1	0.1	0.0	0.5	0.5	2.3	2.9	1.5	2.3	0.1	0.1	
1-Tetradecene	2.2	0.2	0.3	0.1	0.4	0.8	1.8	5.9	2.3	0.4	0.0	0.0	
<i>n</i> -Tetradecene	0.6	0.5	0.2	0.0	0.3	0.5	1.1	1.5	1.8	0.2	0.1	0.0	
1-Pentadecene	8.2	6.0	7.0	0.0	32.3	26.9	41.3	46.9	26.0	24.3	11.8	0.0	
<i>n</i> -Pentadecene	3.8	9.0	1.8	0.5	6.8	6.2	13.2	10.0	12.4	9.0	6.4	0.7	
1-Hexadecene	1.4	3.7	0.4	0.0	0.4	3.1	0.9	1.9	0.8	0.4	0.1	0.0	
1-Heptadecene	22.6	24.5	27.0	0.8	19.1	0.3	8.2	13.8	0.5	6.7	8.8	0.0	
Henicosane	0.9	1.3	0.7	2.6	0.5	0.5	0.4	0.1	0.4	1.0	1.7	1.0	
Tricosane	6.0	5.2	1.9	9.9	2.2	0.1	5.2	1.9	2.9	4.9	1.7	9.1	
Tetracosane	0.7	0.7	2.5	5.4	0.4	0.6	0.4	0.2	0.6	0.2	0.7	14.1	
Pentacosane	7.6	5.1	4.4	14.3	3.8	6.0	4.6	1.4	2.0	4.6	3.1	16.2	
Hexacosane	0.0	0.4	0.1	5.4	0.2	0.2	1.0	1.0	0.0	0.0	0.0	14.2	

^aRelative percentages listed as zero represent observed levels of less than 0.1%.

^bUF613a samples collected during early morning; UF613b samples collected during mid-afternoon.

^cSingle sample of *T. pentagona* collected in 1990; single sample of *T. speciosum* collected in 1988.

levels of the lower-molecular-weight compounds and those with relatively low levels of those same compounds (Figure 2).

Variability among the group exhibiting high levels of these compounds probably relates to time of sample collection, as the cultivars with the highest levels represented samples collected in mid- to late afternoon (UF613a and UF221), while cultivars with slightly lower levels represented samples collected from early to late morning (SCA6, UF29, UF613b, UF668).

Variability among the group exhibiting low levels of these compounds appeared to be independent of time of sample collection. This group was, however, separated into two distinct subgroups. One subgroup consisted of those samples in which essentially none of the lower-molecular-weight compounds was observed (Rim-100 and *T. speciosum*). The second subgroup consisted of those samples in which each of the lower-molecular-weight compounds was observed (Catongo, Pound7, EET400, *T. pentagona*), albeit at greatly reduced levels when compared to the high level group.

Field Bioassays of Midges. About four times the number of midges belonging to the Cecidomyiidae were retrieved from all traps combined over the bioassays than midges belonging to the Ceratopogonidae (Table 2). The frequency at which midges are attracted to the Rim-100 floral oil is significantly different (3×11 contingency test, $\chi^2 = 40.50$, $df = 20.00$, $P < 0.01$) (Table 2) from the attraction of midges to the other floral oils bioassayed. Thus, only 23.46% of all midges attracted to floral oils in this study were Ceratopogonidae, and these were represented by only a few species. For Ceratopogonidae and Cecidomyiidae combined, more than three times the number of midges were attracted to Rim-100 cacao floral oil than to Catongo, and more than nine times the number attracted to UF613 (Table 2). Although sample sizes of trapped

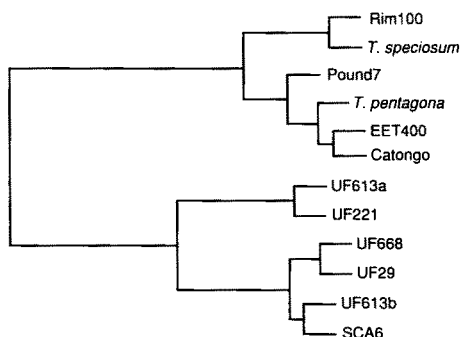


FIG. 2. Cluster analysis based on lower-molecular-weight volatiles representing major compounds observed in steam-distilled volatiles from *Theobroma cacao*, cultivar UF613 (Erickson et al., 1987).

TABLE 2. PATTERN OF ATTRACTION OF MIDGES (DIPTERA: CERATOPOGONIDAE AND CECIDOMYIIDAE) TO MCPHAIL TRAPS INOCULATED WITH DISTILLED FLORAL OILS^a FROM THREE CACAO CULTIVARS IN NORTHEASTERN COSTA RICA^b

Midge	Total numbers of midges by treatments ^c				
	Rim-100	Catongo	UF613	Blanks	Total
Ceratopogonidae					
<i>Forcipomyia spatulifera</i> Saunders	4 (f)	0	0	0	4
<i>F. genualis</i> (Loew)	8 (f)	3 (f)	0	0	11
<i>Dasyhelea</i> sp.	2 (f)	0	0	0	2
<i>Atrichopogon</i> sp.	2 (f)	0	0	0	2
Total	16	3	0	0	19
Cecidomyiidae					
<i>Mycodiplosis ligulata</i> Gagné	22 (17f, 5m)	7 (7f)	3 (f)	0	32
<i>Aphodiplosis triangularis</i> (Felt)	10 (8f, 2m)	3 (2f, 1m)	1 (m)	0	14
<i>Clinodiplosis</i> sp.	8 (7f, 1m)	3 (f)	0	0	11
<i>Ledomyia</i> sp.	0	1 (f)	0	0	1
<i>Bremia</i> sp.	0	1 (f)	1 (f)	0	2
<i>Chrybaneura</i> sp.	0	0	1 (f)	0	1
Micromyini	0	0	1 (f)	0	1
Total	40	15	6	0	61
Combined totals	56	18	6	0	80

^aSteam distilled oils from collected *Theobroma cacao* L. flowers in Costa Rica during 1988-1990.

^bTwo field bioassays performed at Finca La Tigra, La Virgen, Sarapiquí District, Heredia Province, Costa Rica, March 7-11, and September 14-18, 1991.

^cSix replicate traps of each cultivar floral oil at a dilution of 100 parts/million, and two blanks or control traps inoculated with similarly diluted chloroform solvent only. Traps suspended over moderate distances on adjacent cacao trees in a semiabandoned plantation adjacent to tropical rain forest.

midges are relatively small, the data exhibit fairly marked patterns of differential attraction of midges to floral oils belonging to three distinctive cacao cultivars, especially evident for the more abundant Cecidomyiidae in the samples. Blank traps in this study lured no midges at all (Table 2).

Almost twice as many species of cecidomyiids were attracted to the lures than ceratopogonids (Table 2), even though the total numbers of both groups of midges trapped in this study remained the same between rainy and dry season bioassays at this locality (Table 3). For the three most attracted species of Cecidomyiidae, 82.46% of the total catches were females (Table 4). For all of these species, the sex ratio for trapped midges was skewed towards females, a pattern especially noticeable in the most attracted species, *Mycodiplosis ligulata* Gagné (Table 4). In addition to midges, a total of four individuals of an un-

TABLE 3. GENERAL PATTERNS OF ATTRACTION OF MIDGES (DIPTERA: CERATOPOGONIDAE AND CECIDOMYIIDAE) TO MCPHAIL TRAPS INOCULATED WITH FLORAL OILS OF *Theobroma cacao* L. CULTIVARS (STERCULICEAE), AND CONTROL (BLANK) TRAPS DURING RAINY AND DRY SEASONS IN A NORTHEASTERN COSTA RICA CACAO PLANTATION^a

Ranked abundance of midge species	Numbers of midges in traps			
	Floral oils	Controls	Rainy season	Dry season
Ceratopogonidae				
<i>Forcipomyia genualis</i> (Loew)	11	0	6	5
<i>F. spatulifera</i> Saunders	4	0	2	2
<i>Dasyhelea</i> sp.	2	0	0	2
<i>Atrichopogon</i> sp.	2	0	1	1
Cecidomyiidae				
<i>Mycodiplosis ligulata</i> Gagné	32	0	18	14
<i>Aphodiplosis triangularis</i> (Felt)	14	0	9	5
<i>Clinodiplosis</i> sp.	11	0	5	6
<i>Bremia</i> sp.	2	0	0	2
<i>Ledomyia</i> sp.	1	0	0	1
<i>Chrybaneura</i> sp.	1	0	0	1
Micromyini (undetermined species)	1	0	0	1
Total catches	81	0	41	40

^aRainy season census: September 14-18, 1991; dry season census: March 7-11, 1991.

identified species of stingless bee, *Trigona* sp., were found in the Rim-100 traps.

DISCUSSION

The steam-distilled floral fragrance oil from freshly collected flowers of *T. cacao* cultivar UF613, as determined in a previous study (Erickson et al., 1987), revealed a predominance of the straight-chain hydrocarbons pentadecene and pentadecane. Our cluster analysis of the major volatile constituents, arbitrarily based upon cultivar UF613, for the series of other cultivars examined in this present study, revealed some interesting differences among these cultivars for the five or six most abundant compounds in our samples. Although it cannot be determined from our study, it is likely that the basis for midge attraction to

TABLE 4. PATTERNS OF SPECIFIC ATTRACTIVENESS OF THREE SPECIES OF CECIDOMYIIDAE (DIPTERA) MOST ATTRACTED TO FLORAL OILS OF *Theobroma cacao* L. (STERCULIACEAE) CULTIVARS IN MCPHAIL TRAPS IN A CACAO PLANTATION IN NORTHEASTERN COSTA RICA^a

Midge species	Numbers of midges in traps						
	Rainy season			Dry season			
	Female	Male	Total	Female	Male	Total	Total
<i>Mycodiplosis ligulata</i> Gagné	15	3	18	12	2	14	32
<i>Aphodiplosis triangularis</i> (Felt)	6	3	9	4	1	5	14
<i>Clinodiplosis</i> sp.	5	0	5	5	1	6	11
Total catches	26	6	32	21	4	25	57

^aData shown are for floral oil-inoculated traps only, since no midges were found in the control (blank) traps. In each of two field bioassays, a total of 20 McPhail traps were used, with six replicates for each of the three types of floral oil evaluated for midge attractiveness, and two blanks. Rainy season bioassay: September 14–18, 1991; dry season bioassay: March 7–11, 1991.

distilled floral oils of *T. cacao* is a mixture of several chemical constituents, perhaps a combination of major and minor compounds. Examining the distribution of the major volatiles among the cacao cultivars is a logical way to begin looking for differences among cultivars, but does not comprise a complete analysis. Aside from these abundant compounds, our distilled floral oils contained many minor components (Erickson et al., 1987) comprising less than 1% of the estimated 80 compounds found in *T. cacao* floral oils. The compounds selected for analyses represent 50% or more of the total spectrum of floral oil constituents revealed in our samples.

In spite of steam distillation being more prone to yielding artifacts and possibly underrepresenting lower-molecular-weight compounds, data from our bioassays show that midges are attracted to steam-distilled floral oils of *T. cacao*. Higher-molecular-weight hydrocarbons revealed in our analyses (e.g., penta-cosane and hexacosane) are not very volatile and are probably cuticular waxes on the surface of flowers. Thus the chemical composition of these steam-distilled floral oils is almost certainly not what the midges perceive from intact cacao flowers.

The cacao cultivars examined fall into two major clusters based upon the relative abundance of high- and low-molecular-weight compounds. What is particularly interesting about the Rim-100 cultivar is the complete absence of pentadecene, a major component of the floral oils in several other cultivars, especially those in the UF series, in which lower-molecular-weight compounds prevail. Upon examination of the presence or absence of genetic self-compati-

bility, self-incompatibility, and general regions of derivation for the cultivars examined (i.e., Amazonian Ecuador cultivars vs. Central American-derived cultivars), we could find no definite correlation with the results of this cluster analysis based upon floral oil chemistry (Enriquez and Soria, 1967).

Several of the major floral oil constituents available as synthetic analogs, when bioassayed in the field, attract low numbers of both Ceratopogonidae and Cecidomyiidae (Young, 1989a,b), the principal groups of Diptera visiting cacao flowers through the cacao-growing regions of the world (Entwistle, 1972). Differences among *T. cacao* cultivars in floral oil composition may influence the ability of cacao flowers to attract insect visitors, including pollinators. While we were not equipped to perform EAG analysis as a means of screening major volatile constituents for olfactory reception activity (e.g., Gabel et al., 1992), our results nonetheless reveal some interesting differences in levels of insect attraction among cultivars.

Floral oils of *T. cacao*, as examined with UF613 and various species of *Theobroma* in previous field bioassays, attract low numbers of Ceratopogonidae and somewhat higher numbers of Cecidomyiidae (Young et al., 1987a, 1988), as well as other insects (Young et al., 1987b). Although cecidomyiid midges are most likely cacao pollinators (Kaufman, 1973; Young, 1985a), they are not considered as effective pollinators in spite of their relatively high abundance as cacao flower visitors (Brew, 1986; Ibrahim and Jussein, 1987). It is generally considered that ceratopogonid midges, especially *Forcipomyia* (*Euprojoannisia*) sp., are effective pollinators of *T. cacao* in plantation settings (e.g., Saunders, 1959; Hernandez, 1965; Posnette, 1944; Soria, 1970; Winder, 1977; Bystrak and Wirth, 1978).

Our field bioassays confirmed the well-known observation that cecidomyiid midges, especially females, belonging to several genera and species, are attracted to not only cacao flowers, but distilled floral oils from them. Ceratopogonid midges are generally much less abundant at cacao flowers, with only a few species represented, and our bioassay data confirms the same pattern with floral oil traps. Although twice the number of cecidomyiids were trapped during the dry season than in the rainy season in a previous bioassay in Costa Rica (Young et al., 1988), the numbers of these midges remained about the same between dry and rainy seasons in the present study. Because midges are very sensitive to moisture, year-to-year variation in seasonal rainfall patterns, typical for this particular locality, will bring about differences in abundance levels and population dynamics between years. The high abundance of female midges in the traps most likely indicate a behavioral preference in which females seek some reproductive or mating resource from cacao flowers. Female cecidomyiids, especially in the most abundant species such as *M. ligulata* Gagné, are far more numerous at cacao flowers (Young, 1985a-c).

Our data show that cecidomyiid midges, especially *M. ligulata* Gagné, a

probable opportunistic pollinator of cacao (Young, 1985a-c), are strongly attracted to Rim-100 cacao floral oil, moderately attracted to Catongo, and only weakly attracted to the UF series floral oils. Exactly 70% of all midges trapped in the two bioassays combined were obtained from the Rim-100 traps, and about 40% of these belonged to *M. ligulata* Gagné. The three most abundant cecidomyiids, *M. ligulata* Gagné, *Aphodiplosis triangularis*, and *Clinodiplosis* sp., were most frequently trapped with Rim-100 floral oil and were found in previous studies in Costa Rica to be the most abundant species in other bioassays (Young et al., 1987a,b, 1988) and visiting cacao flowers (Young, 1985). About 84% of the ceratopogonids trapped were found trapped with Rim-100 floral oil, and all four species are known or suspected pollinators of *T. cacao* (e.g., Bystrak and Wirth, 1978; Winder, 1977; Young, 1983). In a previous bioassay comparing the attractiveness of Rim-100 floral oil with that of Catongo in another abandoned cacao plantation, more than twice the number of ceratopogonid midges were found in the Rim-100 traps (Young, 1989a).

While our study does not allow us to determine precisely why Rim-100 floral oil strongly attracted cacao-associated midges, the unique chemical composition of this floral oil, with higher-molecular-weight components prevailing, may be a key factor in pollinator attraction. Rim-100 was selected in Mexico from a criollo-type cacao, and the flowers and fruits closely resemble criollo (G.A. Enriquez, personal communication). Criollo is considered to be the original wild-type or near wild-type cacao endemic to Mexico and Central America (Cuatrecasas, 1964). If it is assumed that the Rim-100 cultivar of *T. cacao* is closely akin to Central American wild-type or criollo cacao, its floral fragrance composition may resemble that of wild *T. cacao*. Such a floral fragrance might have been characterized by higher-molecular-weight volatile components that provided a stable fragrance cue for pollinators, but with the extensive selective breeding of *T. cacao* cultivators, and their propagation by cloning over many generations, there could have occurred a relaxation of selection pressures favoring a functional floral fragrance. The result would be the production of many cultivars with nonfunctional or only partially functional floral fragrances, since sexual reproduction has essentially been uncoupled and replaced with clonal propagation or asexual reproduction. If true, this could help explain why natural pollination in commercial cacao plantations is generally very poor (Dessart, 1961), since plantations contain an assortment of cultivars propagated chiefly by vegetative means.

Various species of stingless bees (Hymenoptera: Apidae: Meliponinae), and other wild bees (Halictidae), have been trapped with *Theobroma* distilled floral oils, especially *T. simiarum* Donn. Smith. (Young et al., 1987a,b). Cacao floral oils, as seen in previous studies and in the present study, weakly attract stingless bees. The floral oil of *T. simiarum* is rich in terpenoid compounds, unlike the hydrocarbons predominating in *T. cacao* (Erickson et al., 1987).

While large-sized *Trigona* bees routinely visit the flowers of *T. simiarum* and *T. cacao*, smaller-size species function as "pollen thieves" on the small whitish and scentless *T. cacao* flowers (Young, 1985c). The role of *Trigona* and other bees as pollinators of the large, red, highly fragrant flowers of *T. simiarum* remains unstudied. Interestingly, hydrocarbons such as pentadecane and terpenoids such as geraniol and citronellol, major volatile constituents of floral oils among *Theobroma* species (Erickson et al., 1987), are important mandibular gland exudates in various species of bees, including some stingless bees (Blum et al., 1970; Crewe and Fletcher, 1976; Duffield et al., 1984). Whether or not stingless bees and other bees such as halictids may collect these compounds from floral hosts remains to be studied.

Because most cultivars of *T. cacao* have been artificially selected, including Rim-100, they may be largely dysfunctional in attracting the most effective pollinating insects. It has been argued elsewhere (Erickson et al., 1987) that certain bees could be the most effective pollinators or *Theobroma* species, perhaps even *T. cacao*. Certain unusual features of cacao floral morphology in some cultivars (UF613), suggest a dysfunctional condition for attracting pollinators. Such a condition is absent in the flowers of noncultivated species of *Theobroma*, which also tend to have highly fragrant flowers (Young et al., 1984).

Depending upon their genetic histories, which are largely unknown, and the degree of selection, cultivated cacao varieties may represent a wide range of functional to dysfunctional flowers for natural pollination. Even in a cultivar such as Rim-100, which is relatively far more attractive to insects than others bioassayed, as indicated in the present study, some level of dysfunctionality for pollination may still exist. Midge-mediated pollination in cacao plantations is most likely opportunistic and effective primarily over small distances (Yamida, 1991), even though cacao trees in these settings are spaced closely together. Yet purportedly wild *T. cacao* in the Ecuadorian Amazon occurs as small clumps scattered over large areas of tropical rain forest (Allen, 1982 and personal communication). Under these conditions, long-distance movement of pollen among different clumps of *T. cacao* may necessitate strong flying insects such as bees.

Our data suggest that wild *T. cacao* would very likely possess a floral fragrance profile dominated by higher-molecular-weight volatile compounds (given phenotypic similarities of the Rim-100 cultivar to presumed wild cacao), even more so than that what was found in the Rim-100 cultivar examined in the present study. It is predicted that the floral fragrance profile of ancestral or wild *T. cacao*, therefore, would more closely resemble that of the criollo-derived Rim-100 than that of cultivars having low-molecular-weight constituents such as the UF series examined. Thus, what might once have been an ancestral bee-pollination syndrome in wild *T. cacao* centered around the collecting of fragrance compounds needed for mating, territorial defense, and foraging by bees

(Duffield et al., 1984), as a result of artificial selection, has been broken down in cultivated *T. cacao* in which midges prevail as opportunistic pollinators in plantation habitats.

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