Selbyana 5(3-4): 291-295, 1981

FLORAL FRAGRANCE ANALYSIS IN *ANGULOA*, *LYCASTE* AND *MENDONCELLA* (ORCHIDACEAE)

Norris H. Williams[1], *John T. Atwood*[1] *and Calaway H. Dodson*[2]

It has been established for some time that fragrance components of many orchids play a primary role in attracting male euglossine bees (Dodson, 1962; Dodson *et al.*, 1969; Dressler, 1967, 1968a, 1968b; Vogel, 1963: Williams and Dodson 1972). Fragrances in some orchids are known to be more important than visual cues for pollinator attraction, especially over long distances. Some of the components serve to attract pollinators, while others apparently either modify the attracting fragrance or behave as repellents. Since several orchids are known to possess only one or a few pollinators, it has been suggested that unique fragrances attract specific pollinators. The fragrance components of known euglossine-pollinated orchids are mostly terpenoids and aromatic compounds.

Fragrance analyses in three species of *Anguloa*, three species of *Lycaste*, and one species of *Mendoncella* are reported in this study. The three genera are believed to be related and are included in the subtribe Zygopetalinae (Dressler, 1974). *Anguloa* includes about 10 species distributed in the tropical Andean redion of South America, *Lycaste* includes about 45 species (Willis, 1973) distributed over most of tropical America, and *Mendoncella* includes three species distributed from Mexico to Brazil (Hawkes, 1963). Only one report exists on fragrance analysis in the Zygopetalinae (Hills *et al.*, 1968).

A few observations are available on the pollinators of *Anguloa* and *Lycaste* (Dodson, 1962, 1966, 1967; van der Pijl & Dodson, 1969). There are no observations on pollination in *Mendoncella*, but it is presumed to be pollinated by male euglossine bees. Some data are available on the chemical attractants of euglossine bees (Dodson, 1970; Hills *et al.*, 1972; Williams & Dodson, 1972).

MATERIALS AND METHODS

Detailed information on sampling and measuring procedures are given in Hills *et al.* (1972). Flowering material of the following species was examined: *Anguloa cliftonii* Rolfe, *A. clowesii* Lindl., *Lycaste aromatica* (Grah.) Lindl. (two clones), *L. ciliata* (Pers.) Veitch, *L. cruenta* Lindl., and *Mendoncella grandiflora* (A. Rich.) Hawkes. The plants were grown either in the greenhouse at Fairchild Tropical Garden or in the slathouse at the University of Miami, Coral Gables, Florida.

Flowers were sampled at the time of maximum odor production, which was normally at least three days after opening. Flowers were sampled out-of-doors or in the greenhouse to prevent contamination from extraneous compounds present in laboratory air. In order to concentrate floral fragrance the flowers were placed in a Plexiglas box usually between 7:00 and 9:00 a.m. The boxes were designed to enclose the whole inflorescence without severing it from the plant. After an equilibration time of at least 30 minutes, 10 ml samples of the saturated air were taken with a gas-tight syringe and analyzed by gas-liquid chromatography. A 6-foot x 1/4-inch O.D. Carbowax 20M column was used at three temperatures: 70° C, 130° C, and 160° C. Floral fragrance components were determined by comparison of relative retention

[1] Department of Biological Science, Florida State University, Tallahassee, Florida 32306, U.S.A.
[2] The Marie Selby Botanical Gardens, 800 S. Palm Avenue, Sarasota, Florida 33577, U.S.A.

TABLE 1. Percentages of fragrance compounds in injected samples of three species of *Anguloa*. *

Peak Number		Temperature (°C)	Relative retention Time	*Anguloa cliftonii*	*Anguloa clowesii*	*Anguloa uniflora*
1		70	0.39		0.2	
2	alpha-pinene	70	0.63	<u>21.3</u>	<u>17.9</u>	<u>43.8</u>
3	beta-pinene	70	1.00	3.2	2.1	t
4		70	1.19	6.1		
5		70	1.30		0.98	0.21
6	cineole	70	1.80	<u>46.1</u>	<u>25.9</u>	<u>52.9</u>
7		70	2.26	2.6		
8		70	2.72			0.19
9	carvone	130	1.32		<u>52.8</u>	0.21
10		130	1.78	<u>20.6</u>		

* Underlined percentages indicate major components.

times of the unknown compounds to relative retention times of known standard compounds. Positive determination of some of the compounds was confirmed either by analyzing samples on 10% Lac 446 columns, or by other techniques discussed by Hills *et al.* (1972).

RESULTS

Tables 1, 2 and 3 include results from *Anguloa*, *Lycaste* and *Mendoncella* respectively. We believe unidentified compounds with common retention times between species represent the same compounds, but this should be confirmed.

The three species of *Anguloa* sampled have the least complex fragrance profiles with only six detectable components in common (alpha-pinene and cineole) and all contain beta-pinene as a minor component. *Anguloa cliftonii* has a third major component (20.6%) differing from the other species. It also contains two minor components not found in *A. clowesii* or *A. uniflora. Anguloa uniflora* has only two major components comprising more than 96% of the sample, and one unique minor component. The fragrance of *A. cliftonii* contains three components in common with the other anguloas. *Anguloa clowesii* and *A. uniflora* have five components in common, but in different quantities. Carvone is the major compound found in the fragrance of *A. clowesii.*

Lycaste fragrance profiles are more complex than those of *Anguloa*. Between eight and 13 components were detected, but only three were positively determined. Most striking is the difference in fragrance profiles between plants labeled *Lycaste aromatica*. Clone A contains 13 detected components, while clone B has only eight; furthermore one undetermined component comprises 99% of the fragrance, but is absent from other species. The fragrance profiles of clone A and *Lycaste cruenta* appear more similar despite appearance of the flowers, since they have five components in common, two

TABLE 2. Percentages of fragrance compounds in injected samples of four species of *Lycaste*.*

Peak Number		Temperature (°C)	Relative Retention Time	*Lycaste aromatica* clone 'A'	*Lycaste aromatica* clone 'B'	*Lycaste cruenta*	*Lycaste ciliata*
1		70	0.33			2.0	
2		70	0.41	0.1			2.3
3		70	0.46		t	1.4	
4	alpha-pinene	70	0.63	<u>22.6</u>		<u>9.7</u>	
5		70	0.71		t		
6		70	0.76		t		
7		70	1.06	1.2		1.6	
8		70	1.11		t		
9		70	1.16				0.4
10		70	1.33	0.36	t		
11		70	1.48	0.32			
12	cineole ?	70	1.80	<u>7.66</u>			1.1
13		70	2.09				
14		70	2.16		<u>99.0</u>		
15		70	2.29	<u>61.7</u>		<u>14.4</u>	
16		130	0.18				<u>9.0</u>
17		130	0.24				<u>29.3</u>
18		130	0.42	0.66			
19		130	0.55	1.19			
20		130	0.59				<u>46.3</u>
21		130	0.74	0.97		<u>7.6</u>	
22	benzyl acetate ?	130	1.26			<u>8.0</u>	
23	citronellol ?	130	1.33	0.86	<u>0.13</u>		<u>5.2</u>
24	methyl salicylate	130	1.58	0.55			<u>6.3</u>
25		130	2.08			<u>12.5</u>	
26		160	0.86			<u>30.2</u>	
27		160	1.50		0.7		
28	methyl cinnamate	160	1.78	1.88		<u>12.4</u>	

* Underlined percentages indicate major components.

of which are major components.

The fragrance profile of *Mendoncella grandiflora* contains nine detectable compounds, with three positively identified. Its fragrance has few components in common with *Anguloa* or *Lycaste* other then alpha-pinene and beta-pinene.

DISCUSSION

Pollination of *Anguloa clowesii* by *Eulaema boliviensis* and *A. ruckeri* by *Eulaema cingulata* have been observed (Dodson, 1967). *Euglossa viridissima* and *Euglossa* sp. (reported originally as *E. cordata*) have been observed pollinating *Lycaste aromatica* (Dodson, 1962, 1967). *Euglossa cordata* was

TABLE 3. Percentage of fragrance compounds in injected samples of *Mendoncella grandiflora.*

Peak number		Temperature(° C)	Relative Retention Time	Percent Composition
1	alpha-pinene	70	0.63	50.2
2	beta-pinene	70	1.0	1.3
3		70	1.30	28.0
4		70	1.62	10.8
5		70	1.74	t
6		70	2.20	2.3
7		70	2.65	t
8	citronellal	130	0.39	3.6
9	geraniol ?	130	0.69	1.9
10		130	0.89	1.3

attracted to cineole and methyl cinnamate in Panama, but in Dawa, Guyana it visited cineole and eugenol, but not methyl cinnamate (Williams & Dodson, 1972). The fragrance profile of *Lycaste aromatica* clone A is consistent with pollination observations in Panama by *Euglossa cordata*, but little can be said of clone B, since the major component was undetermined. *Euglossa viridissima* has been observed pollinating *Lycaste consobrina* (Dodson, 1967), but there are no data on fragrance components for the orchid. Nothing is known about pollination of *Mendoncella grandiflora* but all known pollinators of Zygopetalinae have been euglossine bees.

The prevalence of alpha-pinene in *Anguloa*, *Lycaste* and *Mendoncella* is also observed in *Catasetum* (Hills *et al.*, 1972), and is a conspicuous feature of the euglossine syndrome. Since alpha-pinene is known to decrease attraction when combined with known attractants, it is suggested that non-attraction or active repulsion may be an important factor in maintaining pollinator efficiency and/or specificity.

The distinct profiles of *Anguloa* and the yellow-flowered *Lycaste* species suggest that fragrance analysis may be useful in distinguishing biological species in these genera.

ACKNOWLEDGEMENTS

Portions of the work reported here were supported by NSF grants GB-7142, GB-17923, and GB-6409 to CHD.

LITERATURE CITED

Dodson, C. H. 1962. The importance of pollination in the evolution of the orchids of Tropical America. Amer. Orchid Soc. Bull. 31: 525-534, 641-699, 731-735.

———— 1966. Studies in orchid pollination: The genus *Anguloa*. Amer. Orchid Soc. Bull. 35: 624-627.

———— 1967. Relationships between pollinators and orchid flowers. Atas do Simpósio sôbre a Biota Amazônica 5: 1-72.

Dodson, C. H., R. L. Dressler, H. G. Hills, R. M. Adams & N. H. Williams 1969. Biologically active compounds in orchid fragrances. Science 164: 1243-1249.

Dressler, R. L. 1967. Why do euglossine bees visit orchid flowers? Atas do Simpósio sôbre a Biota Amazônica 5: 171-180.

——— 1968a. Pollination by euglossine bees. Evolution 22: 202-210.

——— 1968b. Observations on orchids and euglossine bees in Panama and Costa Rica. Revista Biol. Trop. 15: 143-183.

——— 1974. Classification of the orchid family. Pages 259-279 *in* M. Ospina (ed.). Proceedings of the Seventh World Orchid Conference. Medillin, Colombia.

Hawkes, A. D. 1963. The genus *Mendoncella*. Orquidea (Brazil) 25: 7.

Hills, H. G., N. H. Williams & C. H. Dodson 1968. Identification of some orchid fragrance components. Amer. Orchid Soc. Bull. 37: 967-971.

——— 1972. Floral fragrances and isolating mechanisms in the genus *Catasetum* (Orchidaceae). Biotropica 4: 61-76.

Pijl, L. van der & C. H. Dodson 1966. Orchid Flowers: Their Pollination and Evolution. University of Miami Press, Coral Gables, Florida, 214 pp.

Vogel, S. 1963. Das sexuelle Anlockungsprinzip der Catasetinen-und-Stanhopeen-Blüten und die wahre Funktion ihres sogenannten Futtergewebes. Oesterr. Bot. Z. 110: 308-337.

——— 1966. Parfümsammelnde Bienen as Bestäuber von Orchidaceen und *Gloxinia*; Oesterr. Bot. Z. 113: 302-361.

Williams, N. H. & C. H. Dodson 1972. Selective attraction of male euglossine bees to orchid floral fragrances and its importance in long distance pollen flow. Evolution 26: 84-95.

Willis, J. C. 1973. A Dictionary of the Flowering Plants and Ferns (eighth edition, revised by H. D. Airy Shaw.) University Press, Cambridge.