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ANALYSIS OF THE FLORAL FRAGRANCE OF *PLATANTHERA STRICTA*

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Key Word Index—*Platanthera stricta*; Orchidaceae; floral fragrance; trapping; GC; GC/MS; lilac aldehydes; lilac alcohols; α-pinene; monoterpenes; aromatic alcohols and aldehydes.

Abstract—The qualitative and quantitative composition of the floral fragrance of *Platanthera stricta* was determined by trapping fragrance components on charcoal or Tenax adsorbents in the field. Analysis (GC-MS) showed the fragrance to consist largely of lilac aldehydes, lilac alcohols and other monoterpene alcohols, monoterpene hydrocarbons (of which α-pinene is the predominant constituent), and aromatic aldehydes and alcohols. Rates of emission of fragrance among mature inflorescences varied greatly (*ca* 50–0 μg/hr/inflorescence). Neither charcoal nor Tenax alone effectively trapped the full range of floral fragrance compounds. When Tenax was used as the trapping agent, lilac aldehydes, lilac alcohols, aromatic aldehydes, aromatic alcohols, and some of the monoterpenes were recovered, but α-pinene, a major component of the fragrance, was recovered only in trace amounts. By contrast, α-pinene and lilac alcohols were effectively recovered from charcoal, but aromatic aldehydes and alcohols were poorly recovered, and lilac aldehydes decomposed.

INTRODUCTION

Floral fragrances, together with visual cues, are the primary means by which plants attract potential pollinators. Reviews of the chemistry of floral fragrances and the analytical techniques used in determining their composition are available [1, 2]. The purpose of the present study was to identify the floral fragrance compounds produced by the slender bog orchid *Platanthera stricta* Lindley (Orchidaceae) as part of a more general study of the pollination biology of this species [3]. *Platanthera stricta* is a member of a morphologically diverse and taxonomically difficult species-complex occurring in northwestern North America [4]. It has been reported both as being scentless [4] and that a scent emitted by the flowers of *P. stricta* induced foraging behavior in *Empis* and *Rhamphomyia* (Diptera: Empididae) species [3], two of this orchid's several pollinators. Nilsson [5–7] has examined the floral fragrance chemistry of two Eurasian–Mediterranean species of *Platanthera*, *P. bifolia* (L.) Rich. and *P. chlorantha* (Custer) Reichb. and their hybrids. He trapped fragrance constituents on Porapak Q and subjected the eluants to GC-MS analysis.

RESULTS AND DISCUSSION

The floral fragrance of *P. stricta* consists largely of monoterpene aldehydes and alcohols, monoterpene hydrocarbons, and aromatic aldehydes and alcohols (Table 1). Three monoterpene aldehydes (compounds **18–20**) were the predominant fragrance constituents trapped on Tenax. They were tentatively identified as lilac aldehydes [stereoisomers of 2-(1′-formyl)ethyl-5-methyl-5-vinyltetrahydrofuran] by comparison of their MS with data of ref. [8]. Lilac aldehydes were absent from the charcoal-trapped samples which contained considerable quantities of three compounds (**10**, **13** and **14**) which were not present in the Tenax-trapped samples. It was not possible to elucidate the structures of these compounds by mass spectroscopy. Lesser quantities of two lilac alcohols [stereoisomers of 2-(1′-hydroxymethyl)ethyl-5-methyl-5-vinyltetrahydrofuran; **27** and **28**] were trapped from some plants on Tenax and charcoal. An additional lilac alcohol (**23**) was trapped from one plant with charcoal. The structures of these compounds were determined by comparison of their mass spectra with data of refs [9, 10]. α-Pinene was a major constituent of some of the charcoal-trapped samples, whereas it was present in only trace quantities in the Tenax-trapped samples. Benzaldehyde, β-pinene, myrcene, limonene, benzyl alcohol, linalool, and 2-phenylethanol were observed in at least trace quantities in charcoal and Tenax-trapped samples. Salicylaldehyde, verbenone, and two 3,7-dimethyloctatriene isomers were observed in some of the Tenax-trapped samples but not in any of the charcoal-trapped samples (Table 1). The 3,7-dimethyloctatriene isomers were identified by comparison of mass spectra with literature values [9, 11] whereas the identity of each of the completely characterized compounds was established by mass spectral comparison [9, 11] and by comparison of its R_t with that of an authentic sample. Small quantities of various unknowns were also present in both Tenax and charcoal samples (Table 1). Control air samples collected at the study sites on charcoal and Tenax showed no appreciable quantities of trapped volatiles.

The considerable differences in composition of the materials collected from the floral fragrance on Tenax or charcoal suggested the following: lilac aldehydes decompose on charcoal during collection and storage of the samples to produce **10**, **13** and **14** and trapping or

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Table 1. Components of the floral fragrance of *Platanthera stricta* and lilac aldehyde decomposition

		Day		Trapped	
	Rt (min)	C_1*	C_9**	C_{-}	C_{15}
1. α-Pinene[1]	11.05	31.4	3.9	t	2.4
2. Benzaldehyde[1]	12.00	t	.	.	t
3. β-Pinene[1]	12.68	1.5	t	t	t
4. Myrcene[1]	13.20	0.8	.	t	t
5. Unknown	13.83	t	.	t	.
6. Unknown	14.54	.	.	.	.
7. Limonene[1]	14.95	4.0	t	t	t
8. Benzyl alcohol[1]	15.20	t	.	.	t
9. 3,7-Dimethyloctatriene isomer[2]	15.35	.	.	.	.
10. Lilac aldehyde decomposition product[2]	15.53	36.4	29.8	t	27.1
11. Salicylaldehyde[1]	15.58	.	.	.	.
12. 3,7-Dimethyloctatriene isomer[2]	15.79	.	.	.	.
13. Lilac aldehyde decomposition product[2]	16.05	18.4	46.2	100.0	47.3
14. Lilac aldehyde decomposition product[2]	16.85	1.9	11.1	t	14.3
15. Linalool[1]	17.98	3.8	3.3	t	3.4
16. Unknown	18.12	.	.	.	.
17. 2-Phenylethanol[1]	18.47	t	.	.	t
18. Lilac aldehyde[2]	19.40	.	.	.	.
19. Lilac aldehyde[2]	19.63	.	.	.	.
20. Lilac aldehyde[2]	20.05	.	.	.	.
21. Unknown	20.51	.	.	.	.
22. Unknown	20.71	.	.	.	.
23. Lilac alcohol[2]	20.95	.	.	.	0.7
24. Uknown	21.00	.	.	.	.
25. Unkown	21.00	.	.	.	.
26. Verbenone[1]	21.19	.	.	.	.
Verbenone and/or lilac alcohol #27	21.19	.	.	.	.
27. Lilac alcohol[2]	21.19	1.6	4.8	t	3.9
28. Lilac alcohol[2]	21.51	0.2	0.9	.	0.7
29. Unknown	21.87	.	.	.	.
Rate of Emission (μg/hr/inflorescence)		26.26	2.08	0.02	11.60

*,**Samples followed by the same number of asterisks were taken from the same inflorescences.
***Verbenone MS obtained from the leading face of the peak, lilac alcohol #27 MS obtained from
[1]Identified by MS and GC retention time.
[2]Identified by MS only.
t = Trace.

desorption efficiencies of other fragrance compounds on charcoal vary greatly from those on Tenax. In particular, α-pinene is more effectively trapped on or desorbed from charcoal than from Tenax and vice versa for the aromatic aldehydes and alcohols. Strong support for both these conclusions was obtained in subsequent experiments.

Instability of the lilac aldehydes on charcoal was investigated by allowing a solution of fragrance compounds in diethyl ether (a combination of samples T_{15} and T_9; Table 1) to evaporate into a container and sampling (see Experimental). The Tenax trap was found to contain lilac aldehydes **18**–**20** but no detectable levels of **10**, **13**, and **14**, whereas, both charcoal traps contained compounds having the same R_t as **10**, **13**, and **14**, but no lilac aldehydes. There was little difference in composition of the material desorbed from each of the charcoal traps. It is unlikely that the lilac aldehydes are themselves artifacts produced by autoxidation of lilac alcohols, considering that aldehydes are more readily oxidized to carboxylic acids than alcohols are oxidized to aldehydes.

Porous trap materials, for example charcoal, Tenax, Porapak Q and Porapak N, vary considerably in their ability to trap volatile organic compounds and in the ease with which trapped materials can be desorbed [12–15]. Recovery efficiencies of the completely identified constituents of *P. stricta* fragrance were measured by sampling vapour containing known concentrations of authentic compounds with Tenax and charcoal traps (Table 2). Recoveries of α- and β-pinene on Tenax were at least two orders of magnitude lower than those on charcoal. Recoveries of the other monoterpenes were also lower on Tenax than on charcoal. On the other hand, recoveries of the aromatic aldehydes and alcohols were much lower on charcoal than on Tenax (Table 2). These results are in good agreement with the low concentration of α-pinene observed in the Tenax-trapped fragrance samples and low concentrations of aromatic aldehydes and alcohols observed in the charcoal-trapped fragrance samples (Table 1).

At any given time of day, total floral emissions varied

products trapped on charcoal (samples C_1–C_{15}) or Tenax (samples T_3–T_{21}) during the day, at dusk, and at night.

% Composition on charcoal				Trapped on Tenax							
Dusk		Night		Day			Dusk		Night		
C_3^*	C_{11}^{**}	C_5^*	C_{13}^{**}	T_3	T_7	T_9	T_{15}	T_{19}	T_5	T_{17}	T_{21}
51.9	t	32.9	t	t	t	t	t	t	t	t	t
.	.	.	t	3.9	t	t	t	t	t	t	t
t	.	0.9	t	t	t	t	.	.	.	.	.
t	.	.	.	1.4	t	.	.	.	.	.	.
.	.	.	.	.	t	.	.	.	.	.	.
.	.	.	.	.	t	.	.	.	.	.	.
t	.	4.6	t	2.6	t	t	t	t	t	t	t
.	.	.	.	0.4	t	t	t	t	t	t	t
.	.	.	.	0.5	t	t	t	.	t	t	.
20.0	32.2	41.4	42.9	.	.	.	.	.	.	.	.
.	.	.	.	0.3	t	.	.	.	.	.	.
.	.	.	.	0.3	t	t	.	.	.	.	.
28.1	t	20.2	47.3	.	.	.	.	.	.	.	.
t	t	t	9.8	.	.	.	.	.	.	.	.
t	67.7	t	t	2.7	t	.	t	t	t	t	t
.	.	.	.	0.7	.	6.8	.	.	.	.	.
.	.	.	.	0.7	2.0	7.1	.	.	.	t	.
.	.	.	.	54.0	69.0	52.0	72.0	t	78.1	54.2	t
.	.	.	.	13.4	5.3	13.0	28.0	t	21.9	21.7	t
.	.	.	.	6.8	11.8	12.2	t	t	t	11.5	t
.	.	.	.	0.7	9.3	2.8	.	t	.	2.3	t
.	.	.	.	.	2.8	6.1	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	0.1	.	.	.	.	.	.	.
.	.	.	.	.	t	.	.	.	.	.	.
.	.	.	.	.	t	.	.	.	.	.	.
.	.	.	.	3.9***	.	t	.	.	.	10.3	t
.	.	t	t	.	.	.	.	.	.	.	.
.	.	.	.	0.7	.	.	.	.	.	.	.
.	.	.	.	6.9	.	.	.	.	.	.	.
2.11	1.11	1.20	1.26	53.59	6.53	4.85	2.40	0.00	39.35	1.95	0.00

the rear face.

widely among individual plants. Total emissions (μg/hr/inflorescence) trapped on charcoal varied between 26.26 and 0.02 during the day, 2.11 and 1.11 at dusk and 1.20 and 1.26 at night. For Tenax-trapped samples the corresponding figures were 53.59–4.85 (day), 2.40–0.00 (dusk), and 39.35–0.00 (night) (Table 1). These variations occured in spite of the fact that all sampled inflorescences were of similar size and stage of maturity. Such high quantitative variation in fragrance emissions between individual plants does not appear to have been previously observed. It may explain why *P. stricta* has been reported as scentless [4], but the ecological significance of the variation is unexplained and mysterious, because it might be expected that individuals with low emissions would have low evolutionary fitness and, consequently, would be selected out of the population. Conceivably, fragrance emission of *P. stricta* occurs in pulses, since no information was obtained concerning the emission rate of any individual plant over the entire course of its blooming time. In most cases, different individual plants were sampled at the various times of day, but in two cases (samples from individuals C_1, C_3 and C_5; C_9, C_{11}, and C_{13}, Table 1) the same inflorescences were sampled sequentially during the day, at dusk, and at night. Both of these orchids showed a trend for lower rates of emission at dusk and night than during the day (Table 1). Overall, day emissions trapped on charcoal and Tenax averaged 14.96 $\pm$ 19.14 (s.d., $n = 7$) μg/hr/inflorescence, whereas those of dusk and night samples averaged 1.41 $\pm$ 1.09 ($n = 4$) and 8.75 $\pm$ 17.12 ($n = 5$) respectively. These average rates of emission were not significantly different (ANOVA $F = 0.91$, $p > 0.5$). Therefore, although the present study found no conclusive evidence for quantitative diurnal variation in floral emissions of *P. stricta*, it is possible that further studies with large sample sizes would reveal such variation. In addition, no consistent differences in chemical composition were observed between day, dusk, and night samples (Table 1).

Using Porapak Q to trap the volatiles, Nilsson [7] found that the fragrance of *P. chlorantha* was dominated

Table 2. Recovery efficiencies (%) of components of the fragrance of *Platanthera stricta* from charcoal and Tenax.

	Concentration*	Charcoal		Tenax	
	($\mu g/l$)	$\bar{x}$†	sd	$\bar{x}$†	sd
α-Pinene	0.27	69.9	5.5	0.6	0.0
Benzaldehyde	0.33	6.9	2.7	54.1	3.7
β-Pinene	0.28	68.6	5.2	0.6	0.2
Myrcene	0.26	40.8	6.2	25.9	1.6
Limonene	0.27	45.0	5.1	17.0	1.7
Benzyl Alcohol	0.33	<0.1		36.2	1.9
Salicylaldehyde	0.37	<0.1		46.2	1.8
Linalool	0.28	49.4	2.7	24.1	0.2
2-Phenylethanol	0.33	14.5	4.4	44.4	3.1
Verbenone	0.32	50.5	6.4	12.1	0.5

*in the vapour phase
†$n = 3$

by lilac aldehydes [termed 'lilac alcohols (m = 168)' in ref. [7] Table 3, but now identified as lilac aldehydes (Nilsson, L. A., personal communication)] and methyl benzoate, plus minor quantities of lilac alcohols, other terpenes, and aromatics. In contrast, he found that the fragrance of *P. bifolia* consisted largely of methyl benzoate and linalool plus minor quantities of other aromatics and terpenes, with no lilac aldehydes or alcohols. The fragrance of *P. stricta* therefore more closely resembles that of *P. chlorantha* than that of *P. bifolia*. On the other hand, methyl benzoate, an important constituent of the fragrances of both Eurasian–Mediterranean species, was absent from the fragrance of *P. stricta* and α-pinene, a major component of the fragrance of *P. stricta*, was detected only in trace amounts in the fragrances of the Eurasian–Mediterranean species. β-Pinene, salicylaldehyde, 2-phenylethanol and verbenone, minor constituents of the fragrance of *P. stricta*, were not observed in those of the Eurasian–Mediterranean species. Besides occurring as major constituents of the floral emissions of *P. chlorantha* and *P. stricta*, lilac aldehydes are minor components of lilac (*Syringa vulgaris L.*) flower oil [8, 10].

EXPERIMENTAL

Floral fragrance compounds from *P. stricta* were isolated by adsorption on charcoal or Tenax, desorbed with solvents, and identified and quantified by GC/MS.

Plant material. Platanthera stricta Lindley plants were identified by J. M. Patt. A voucher specimen (WTU-312362) was deposited at The Herbarium, Department of Botany, University of Washington, Seattle, WA, U.S.A. Sixteen inflorescences of similar size, with at least 75% of their flowers open, each on a separate *P. stricta* plant, were sampled *in situ* in the Soleduck River Valley of Olympic National Park, WA, U.S.A. at two sites (elev. 690 m and 1100 m). Five of the inflorescences (T_3, C_1–C_4; Table 1) were sampled at the 690 m site, 26 June–13 July 1985; and 11 inflorescences (C_9, C_{15}, T_1–T_{21}) at the 1100 m site, 14 July–21 August 1985.

Trapping fragrance compounds on charcoal or Tenax. Fragrance compounds were entrained in the following manner. A 1.5 l glass bottle supported by a ring stand was placed over an inflorescence. After placement, no part of the inflorescence was permitted to come into contact with the inside of the bottle. A black nylon mesh screen was placed above the bottle to shade it from direct sunlight. Glass sampling cartridges (SKC, Inc., Eighty Four, PA, U.S.A.) containing either coconut charcoal (100 mg, SKC 226-01) or Tenax (30 mg, SKC 226-35) were placed in a holding device connected by hose to a portable, battery-powered vacuum pump (85 ml/min, SKC 'Air-Check' Personal Air Sampler, model 222-4). The cartridge holding device, supported by a clamp, was then inserted into the bottom of the glass bottle, which was then loosely sealed with Parafilm so that all volatiles emitted by the inflorescence would be drawn through the sampling cartridge while allowing a steady stream of air (85 ml/mn), maintained by the vacuum pump, to enter the apparatus. The inflorescences were sampled for 6 hr during the day (10:00–16:00 hr) and at night (22:00–04:00 hr) or for 1 hr at dusk (21:00–22:00 hr). The total volume of pumped air, recorded by the pump, was found to be within the range expected for the sampling time in each case. This ensured that no pump malfunction had occurred during sampling. Adsorption efficiencies of charcoal or Tenax are not appreciably decreased at high humidity, as witnessed by their use in purge-and-trap methods [14] of sampling volatiles from aqueous solutions, in which the RH of the carrier gas approaches 100%. In addition, no H_2O condensation was observed in any of the sampling cartridges, even though some H_2O condensation was observed on the insides of the sampling bottles of some of the daytime samples. Therefore it is unlikely that the high quantitative variation in emissions observed between individual plants is an experimental artifact. Control air samples were taken simultaneously with the fragrance samples, using an identical apparatus. After sampling, the cartridges were sealed, stored for *ca* 1 day at ambient temp., then *ca* 1 month at −20°.

Desorption and GC–MS analysis. Fragrance compounds were desorbed by addition of CH_2Cl_2 (100 μl) for charcoal samples or dry diethyl ether (400 μl) for Tenax samples, at 25° for 1 hr or more. Both solvents contained toluene (5 μl/100 ml) as an int. GC standard. Negligible change in concn of desorbed materials occurred after 1 hr. Desorbed samples were stored in brown glass vials equipped with Teflon caps at −20°. All samples were first analysed by GC using a FID at 250°, a 25 m × 0.31 mm crosslinked 5% phenylmethyl silicone WCOT column (1.0 μm film), 3.3 ml/min He at 12 psi, 1 μl sample, 250° splitless injection, and a 4-step temperature program as follows: 30° (4 min), 9°/min to 80° (4 min), 5°/min to 100° (0 min), 10°/min to 150° (5 min), 15°/min to 250° (1 min). Components were quantified by

comparing their FID peak areas with that of the int. standard, allowing for their individual response factors. Percentage compositions of the fragrances and rates of emission of fragrance (Table 1) were then calculated taking into account the known % recoveries of the individual components (Table 2) and assuming recovery values of 50% (charcoal) and 20% (Tenax) in the case of unidentified and partly characterized components. Two Tenax samples (T_3 and T_7, Table 1), that together contained the full range of Tenax-trapped components were selected, evapd to a small vol. with N_2 and individually subjects to GC-MS using the same column and chromatographic conditions previously described. The EI MS parameters were as follows: scan speed 280 mass units/sec, filament emission current 20 μA, ionization voltage 70 eV, unit mass resolution set at 1000 mass units, mass range scanned 20–400 mass units, source temp. 200°, direct interface temp. 250°. For GC-MS analysis of the charcoal samples, all samples were combined, evapd to a small vol. and the composite was analysed as previously described. Completely characterized fragrance components were identified by comparing both their R_ts with those of authentic compounds and MS with those of lit. values [8–11]. Partly characterized components were identified by MS only. Mass spectra of unidentified and partly characterized components of the fragrance are available from the authors on request.

Decomposition of lilac aldehydes on charcoal. The desorption solutions from Tenax-trapped samples T_{15} and T_9 (Table 1) were combined and evaporated to *ca* 50 μl with N_2 on a watch glass which was then placed in a 1.5 l bottle. The top of the bottle was sealed and the atmosphere in the bottle, containing volatilized fragrance and Et_2O solvent, was simultaneously drawn through one Tenax and two charcoal traps, each connected to a vacuum pump (85 ml/min), for 30 min. Charcoal-filtered air was admitted to the bottle during sampling to compensate for that removed by the sampling system. The traps were sealed and stored at 25° for 20 hr in order to simulate the field sampling and storage protocol. The compounds on the Tenax trap and one charcoal trap were then desorbed and analysed as previously described. The other charcoal trap was stored for a further 2 weeks at −20° before desorption and analysis.

Recovery of monoterpenes and aromatics from Tenax or charcoal. A 5 μl aliquot of a mixture of equal vols of the compounds listed in Table 2 and 7 other volatile organic compounds, was injected through a heated block (180°) with N_2 carrier gas (50 ml/min) into a sealed Plexiglas chamber (920 l) equipped with a fan to equilibrate vapour concns throughout the chamber. Chamber air was then sampled for 200 min with a Tenax and a charcoal sampling cartridge, each connected to a vacuum pump (85 ml/min). Air was admitted to the chamber through a small orifice to compensate for that removed by the pumps. Trapped compounds were desorbed, analysed and quantified as previously described. Recovery efficiencies (Table 2) were then calcd.

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