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VOLATILE CONSTITUENTS OF *DACTYLANTHUS TAYLORII* FLOWER NECTAR IN RELATION TO FLOWER POLLINATION AND BROWSING BY ANIMALS

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Key Word Index—*Dactylanthus taylorii*; Balanophoraceae; wood rose; flower nectar; aromatic esters; fatty acid esters; hydrocarbons; squalene.

Abstract—The volatile fraction of *Dactylanthus taylorii* flower nectar from male and female plants has been shown to contain squalene as a major constituent. The inflorescence from male plants contained approximately twice the amount of squalene as that detected in the inflorescence of female plants. In addition, the nectar contained C_{21} to C_{31} hydrocarbons and in one of the male plants samples, ethyl and benzyl esters of hexadecanoic and C_{18} to C_{23} polyunsaturated fatty acids as other significant lipid components. The fragrance chemicals in the nectar comprised a relatively simple mixture of butenols, butanols and pentanols, benzyl and phenylethyl alcohols, nerol, geraniol and ethyl esters of benzoic, salicylic and cinnamic acids. The inflorescence from female plants contained approximately five times the amount of benzyl alcohol compared with the amount from the inflorescences of male plants. The importance of the nectar volatile compounds in bat pollination and browsing of *Dactylanthus* inflorescences by possums and ship rats is briefly discussed.

INTRODUCTION

Among the many plant species worldwide threatened with extinction is *Dactylanthus taylorii* Hook.f., (Balanophoraceae) New Zealand's only fully parasitic native flowering plant, known as the 'wood rose' or by the Maori name 'pua o te Reinga' [1]. This dioecious plant exists as a rhizome, which is attached to the roots of forest trees and shrubs. During autumn, from late February to early May, the plant produces a profusion of pink to purple–brown inflorescences. Each inflorescence consists of between ten and forty spadices, and each of these carries about 50 to 400 simple flowers. The male flower consists of a simple pollen-bearing stamen, while the female flower is little more than a pistil [1]. The inflorescences of both sexes produce a quantity of cloudy nectar, approximately 0.5 ml per inflorescence per day and having a fragrance cloyingly sweet, but with a slightly fatty backnote.

Despite the apparent fertility of the plant, it is in serious decline. The flowers have been shown to be pollinated by the New Zealand short-tailed bat, *Mystacina tuberculata*, which itself is a rare New Zealand native mammal [2]. Recently, however, evidence has also shown the flowers to be heavily browsed by the Polynesian rat (*Rattus exulans*, Maori name 'kiore'), to a lesser extent by the ship rat (*Rattus rattus*) and particularly by the introduced possum (*Trichosurus vulpecula*). It is believed that the possum is primarily responsible for the decline of *Dactylanthus* [2, 3].

There have been few chemical studies of species of the Balanophoraceae [4] and apparently none concerned with the essential oils or fragrance compounds. As part of a wide-ranging study of *Dactylanthus* aimed at it's conservation, the chemical nature of the nectar was examined with a view to clarifying questions about pollination of the flowers by the bat, and it's attractiveness to browsing animals. This paper reports the composition of the volatile compounds derived from the nectar isolated from the inflorescences of male and female plants, and discusses the possible relationship of the nectar constituents to pollination and browsing damage.

RESULTS AND DISCUSSION

The nectar volatile compounds were obtained in approximately 0.02% yield from male plants and approximately 0.04% from female plants. The composition of the steam-volatile fraction from *D. taylorii* nectar from the inflorescences of male plants is summarised in Table 1. Among the major compounds identified in the steam-volatile fraction was squalene. Its identity was confirmed by comparison with authentic compound.

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Table 1. Composition of *D. taylorii* inflorescence nectar steam volatile fraction (male plants, Mamaku Plateau)

Compound identification	Retention time (min)	Area (%)
3-Methylbutan-1-ol	7.1	0.1
3-Methylbutan-2-ol	7.4	0.1
3-Methylbut-3-en-2-ol	9.1	0.2
3-(Methylthio)-prop-1-ene	9.7	0.6
3-Methylbut-3-en-1-ol	10.9	0.1
2-Methylbut-2-en-1-ol	11.4	tr*
3-Methylbut-2-enal	11.5	tr
Furan-2-carboxaldehyde	14.8	tr
Ethyl but-2-enoate	15.0	0.3
Nerol oxide	15.5	2.2
Ethyl 3-hydroxybutanoate	17.5	0.8
Ethyl benzoate	21.5	3.7
Nerol	25.5	0.9
Ethyl salicylate	25.6	0.5
4-Methylbenzaldehyde	25.8	tr
Geraniol	26.8	0.9
Benzyl alcohol	27.5	1.1
Phenyl ethanol	28.5	0.5
Heneicosane	33.6	7.8
Ethyl cinnamate	34.1	0.8
Ethyl hexadecanoate	37.1	0.6
Tricosane	38.6	14.1
Tricosene (*Z*)†	38.8	0.9
Tricosene (*E*)†	39.0	1.1
Phenylacetaldehyde	40.3	0.4
Tetracosane	40.5	tr
Benzyl hexadecanoate	41.0	0.8
Pentacosane	42.8	5.6
Pentacosene (*Z*)†	43.2	0.7
Pentacosene (*E*)†	43.4	0.9
Benzyl octadecatrienoate†	44.7	2.2
Hexacosane	44.9	0.3
Benzyl octadecatetraenoate†	45.3	0.7
Heptacosane	46.8	2.9
Heptacosene (*Z*)†	47.1	0.5
Heptacosene (*E*)†	47.3	0.5
Octacosane	49.0	0.1
Benzyl eicosatrienoate†	49.5	1.1
Hexadecanoic acid	51.2	5.1
Nonacosane	51.5	1.2
Nonacosene (*Z*)†	51.6	0.2
Nonacosene (*E*)†	51.7	0.2
Hexadecenoic acid †	51.8	1.4
Benzyl docosadienoate†	53.8	1.3
Squalene	54.2	26.5
Hentriacontane	55.4	0.9
Benzyl tridecatrienoate†	58.4	1.0
Benzyl tridecatetraenoate†	59.6	3.9

*tr, trace: < 0.1%.

†Position of double bonds not known.

Although well-known as the major lipid in shark liver oil, squalene has also been isolated from flowers [5] and roots [6] of plants, and is also found in the scent mark of animals such as the tamarin (*Saguinus* spp.) [7]. The nectar volatile fraction also contained low-volatility lipids, such as the saturated and unsaturated C_{21}–C_{31} hydrocarbons and polyunsaturated fatty acid (C_{18}–C_{23}) benzyl esters. Nectar from male and female plants from the Pureora Forest location had almost five times the amount of benzyl alcohol, but only small amounts of the fatty acid benzyl esters compared with the male plant nectar from Mamaku Plateau. Hexadecanoic (palmitic) acid and its ethyl and benzyl esters were also found in the male plant nectar. The presence of the esters was confirmed by synthesis of ethyl hexadecanoate [*cf.* 8], benzyl hexadecanoate and linolenate [9, 10] as standards. All the benzyl esters were identified from their mass spectra, viz., *m/z* 108, benzyl alcohol radical ion, *m/z* 71 and 85 for saturated alkanoate, *m/z* 69 and 81 for di-unsaturated alkanoate and *m/z* 67 and 79 tri-unsaturated alkanoate fragments. The positions of the double bonds in the polyunsaturated alkanoate chain could not be determined.

The presence of compounds such as the hydrocarbons is more suggestive of the lipids associated with epicuticular wax chemistry [11]. However, the source of lipids in nectar is oil glands, known as elaiophors [12]. The above classes of compounds would contribute only weak, fatty odours to the nectar and could possibly behave as a fixative for the more volatile compounds. However, on exposure to air for a lengthy period of time, the polyunsaturated compounds could autoxidise and generate rancid odours that could have attractive properties for animals.

The volatile highly odoriferous compounds identified in *Dactylanthus* flower nectar were mainly the ethyl esters of benzoic, salicylic and cinnamic acids, and it is these compounds which impart the very sweet odour to the nectar. Benzyl and phenylethyl alcohols would add to this, while the terpene derivatives, nerol, geraniol, nerol oxide, and the pentenyl alcohols would impart their own floral and citrus notes. Allyl methyl sulphide would provide an onion or garlic odour quality to the nectar, but this was difficult to discern alone in samples of fresh nectar.

Dactylanthus taylorii flower nectar therefore has a comparatively simple top note odour profile providing a very sweet smell, a type known to be attractive to flower pollinating bats [13]. The lipid components, a chemical class to which some bats are known to be sensitive [14], in the nectar could possibly provide the bat pollinators (and the browsing animals) with essential fatty acids as part of their diet [15] in addition to the glucose, fructose and sucrose in the nectar (Franich, R. A., pers. comm.). The polyunsaturated fatty acids are presented however as benzyl esters rather than the usual glyceryl esters which may be an important factor in the bats being attracted to male plant inflorescences to collect pollen during feeding.

There are many potential reasons for the differences in the composition of the nectar volatile compounds isolated from inflorescences of the male plants from two forest locations. There may be genetic differences in the two populations, differences in soil types or even differences in maturity of the inflorescences.

The information obtained in this study has enabled synthetic nectars to be prepared in order to assess their attractiveness to the short-tailed bat and to the possum

[16]. Synthetic nectars comprising as many of the compounds as possible as well as a selection of individual compounds identified were prepared using commerically-available and synthesised compounds. They have been tested as a potential lures for possums using both captive animals and freely-ranging possums in their forest habitat (Ecroyd, C. E., pers. comm.). The synthetic nectar was also tested for its attractiveness to short-tailed bats as a potential tool for luring the bats to enable their closer study (Ecroyd, C. E., pers. comm.). While the synthetic nectar and individual components, e.g, squalene, did attract possums and ship rats (shown by recording their nocturnal behaviour using video with infrared light), the synthetic nectar was less effective than commerically-available lures based on cinnamon oil. Similarly, both synthetic and isolated natural nectar alone were ineffective as lures for the short-tailed bats unless the bats were within close distance to the lures. Only occasionally during infrared video recordings were short-tailed bats seen consuming synthetic nectar.

EXPERIMENTAL

Plant material. Male and female plants from Pureora Forest (lat. 38°12′, long. 176°01′, 560 m asl) and male plants growing at Mamaku Plateau (lat. 38°30′, long. 175°32′, 560 m asl) in the central North Island of New Zealand were located for nectar collection. Plants were protected from animal damage by wire netting exclosures.

Collection of nectar. Nectar was collected from newly-opened inflorescences of both male and female plants using a glass syringe (approximately 0.5 ml per inflorescence), and stored frozen prior to analysis.

Isolation of nectar volatiles. Pooled collected nectar (5 g) was transferred to a 20 ml round bottom flask, which was connected to one arm of a micro-Likens–Nickerson apparatus [17]. The other arm was fitted with a 2 ml flask containing 1 ml of redistilled CH_2Cl_2 with and without internal standards (naphthalene, 0.254, Δ-3 carene, 0.505 and perhydrosqualene, 0.521 $\mu g\,ml^{-1}$) for quantitation of the aromatic, terpenoid and hydrocarbon compounds respectively identified in the nectar volatile fraction for estimation of yield. The apparatus was set up for high-density solvent recycling. The volatile compounds in the nectar were hydrodistilled for 2 h and extracted into the CH_2Cl_2. A 0.5 ml portion of the CH_2Cl_2 extract was concentrated to approximately 50 μL for GC–MS analysis.

GC–MS analysis. GC: The CH_2Cl_2 extract of the nectar volatiles was analysed using a 25 m × 0.2 mm HP5 fused silica capillary column. The carrier gas was helium at 1 $ml\,min^{-1}$ flow rate. The purged splitless injector was heated at 200°. The column oven was programmed as follows: initial temp. 40°, rate 4° min^{-1}, final temp. 220° held for 15 min. A heated (230°) direct interface and transfer line were used to the mass spectrometer. MS: A quadrupole mass analyser instrument was used operating in electron ionization mode, with 70 eV, 300 μA electron energy, and an ion source temperature of 200 °C. Spectra were acquired each 1.1 s. Reference chemicals were obtained from commercial sources where available. Ethyl [*cf.* 8] and benzyl esters [9, 10] of the fatty acids were synthesised. Squalene was obtained by vacuum distillation of crude shark liver oil to give a fraction giving a single GC peak and characteristic mass spectrum [18].

REFERENCES

1. Warne, K. and Woods, J. (1990) *N Z Geographic* **6**, 115.
2. Hunt, R. (1992) *BBC Wildlife* **10**, 13.
3. Klitscher, K. and Miller, J. (1991) *Growing Today* **4** (7), 28.
4. Paris, R. R., Alexis, M. N., Faugeras, G. and Jacquemin, H. (1978) *Pharm. Acta. Helv.* **53**, 130.
5. Tadashi, A. and Suga, T. (1977) *Phytochemistry* **16**, 1515.
6. Ueyama, Y. and Furukawa, K. (1987) *Nippon Nogei Kagaku Kaishi* **61**, 1577.
7. Belcher, A., Epple, G., Kuederling and Smith, A. B. (1988) *J. Chem. Ecol.* **14**, 1367.
8. Blatt, A. H. (ed.) (1943) *Organic Syntheses Collective* (Vol. II), p. 264.
9. Vowinkel, E. (1966) *Chem. Ber.* **99**, 1479.
10. Klemm, H.-P., Hintze, U. and Gercken, G. (1973) *J. Chromatogr.* **75**, 19.
11. Kolattukudy, P. E. (ed.) (1976) *Chemistry and Biochemistry of Natural Waxes.* Elsevier, Amsterdam.
12. Faegri, K. and van der Pijl. (1979) *The Principles of Pollination Biology* (3rd edn), p. 69. Pergamon Press, Oxford.
13. Baker, H. G. (1961) *Quart. Rev. Biol.* **36**, 64.
14. Schmidt, U. (1987) in *Social Odours in Mammals* (Vol. 1) (R. E. Brown and D. W. Macdonald, eds), p. 220. Clarendon Press, Oxford.
15. Baker, H. G. and Baker, I. (1982) in *Biochemical Aspects of Evolutionary Biology* (M. H. Nitecki, ed.), p. 161. University of Chicago Press, Chicago.
16. Ecroyd, C. E., Franich, R. A. and Tustin, J. R. (1992) Possum Lure. NZ. Patent Appl. No. 242064.
17. Godefroot, M., Sandra, P. and Verzele, M. (1981) *J Chromatogr.* **203**, 325.
18. Hites, R. A. (1992) *Handbook of Mass Spectra of Environmental Contaminants* (2nd edn.), p. 5. Lewis Publ., Boca Raton, FL.