



Emission of Volatiles From Brown Boronia Flowers: Some Comparative Observations

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Extensive research has focused on the concentration of aglycones within brown boronia (*Boronia megastigma*) flowers, however emission of volatiles into the headspace above these flowers is not well documented. Using solid-phase microextraction (SPME) to trap volatiles and GCMS analysis, we observed 23 volatiles in the headspace above buds and flowers throughout flower maturation, above dissected floral organs and above whole plants held for 36 h under either continuous light, continuous dark or 12 h light: 12 h dark: 12 h light treatments. Fully-opened flowers emitted the most complex mixture of volatiles and in the greatest quantity, with a rapid decline in senescent flowers. Caryophyllene, humulene and bicyclogermacrene declined as flower buds matured; β -ionone increased. From the individual floral organs, emission from the petaline anthers comprised 38 % of total emissions from the (calculated) 'whole flower', with 27 % contributed by the petals and 10.5 % by the stigma. Monoterpenes dominated the headspace from the calyx; dodecyl acetate, methyl jasmonate and (Z)-n-heptadec-8-ene were relatively predominant in emissions from the androecium. β -Ionone, the major floral volatile in brown boronia, dominated volatiles emitted from the stigma (87 %). However, the relatively tiny petaline anthers, active in pollen production and high in carotenoids, contributed the greatest overall amount of β -ionone to emission from the whole flower. There were three different patterns in emission of volatiles from plants in response to different light conditions: (1) emission patterns identical irrespective of light environment, with maximum emission in the 'endogenous' dark period, i.e. when the plant would normally have been in the dark (α -pinene); (2) similar emission in all treatments, with an increase and decline over a period of 26 h (5-acetoxy linalool, cyclic β -ionone, dodecyl acetate and (Z)-n-heptadec-8-ene); and (3) emission in all treatments but enhanced in the dark, with a 27.5 h period in some cases (cyclic β -ionone endoperoxide, dihydro β -ionone, β -ionone, and 'total volatiles'). Preliminary evidence is presented for endogenous control of emission of a number of volatiles such as α -pinene, with perhaps diurnal control of others such as β -ionone.

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Key words: *Boronia megastigma*, brown boronia, SPME, headspace, floral volatiles, β -ionone.

INTRODUCTION

Boronia megastigma Nees. (brown boronia, family Rutaceae) is an endemic Australian shrub grown commercially in Tasmania, Australia for production of a highly valued floral extract used in the flavouring industry. Approximately 160 compounds have been identified in the absolute including β -ionone and its derivatives, methyl jasmonate, dodecyl and other long chain acetates, (Z)-heptadec-8-ene, 8-hydroxylinalyl esters, cinnamates, tiglamides and 3-hydroxymegastigm-7-en-9-one (Davies and Menary, 1983; Weyerstahl *et al.*, 1994, 1995). The headspace above boronia flowers has been less well documented: 2,6-dimethyl-3(E),5(Z),7-octatrien-2-ol and its 5(E) isomer were identified in volatiles trapped by charcoal (Kaiser, 1991), and six typical floral volatiles from different floral organs were analysed quantitatively using a cryotrap GCMS technique (MacTavish and Menary, 1997b).

Much research has focused on the rhythmic nature of emission of volatiles from some flowers with either diurnal

or nocturnal maxima (Matile and Altenburger, 1988; Altenburger and Matile, 1990; Loughrin *et al.*, 1990; Jakobsen and Olsen, 1994). For example, emission of benzyl acetone from flowers of *Nicotiana attenuata* is barely detectable during the day but increases dramatically in the evening (Baldwin *et al.*, 1997) and maximum emission from flowers of *Ribes nigrum* occurs in the middle of the photoperiod, with the cyclical nature of emission ceasing under constant light (Hansted *et al.*, 1994). Nocturnal emission of floral volatiles is more often found to be controlled by an endogenous, or circadian clock than diurnal emission which may be more influenced by prevailing light and temperature conditions (Hansted *et al.*, 1994). To demonstrate a circadian rhythm three conditions should be met: (1) the rhythm should continue under constant environmental conditions; (2) the period of the rhythm should not be exactly 24 h; and (3) the phase of the rhythm should change under different environmental conditions (Jones and Mansfield, 1975). In some flowers, for example *Brassica napus*, rhythms in emission of particular volatiles such as sabinene and limonene were observed, but no patterns were observed for linalool (Jakobsen *et al.*, 1994).

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Lack of rhythmicity for linalool was also observed in *Hesperis matronalis* flowers (Nielsen *et al.*, 1995). Rhythms in emission from flowers were hypothesized to result from petal movement, but no evidence of this has been found (Helsper *et al.*, 1998). Furthermore, the complexity of changes in emission in response to excision from the plant has been studied in some species. Mookherjee and co-researchers (1986, 1989) have paid much attention to the detrimental effects on floral volatiles of picking flowers, however Helsper *et al.* (1998) noted no significant quantitative or qualitative differences between cut roses and roses still attached to the plant, although maximum emissions from cut roses occurred 1 d later than those from intact rose flowers.

In vegetative tissues such as the needles of *Pinus elliottii*, increasing the light intensity did not directly influence emission rates of five monoterpenes, however increasing the temperature exponentially increased the emission of volatiles (Tingey *et al.*, 1980). Dement *et al.* (1975) proposed that the monoterpene volatilization rate from *Salvia* depended on both the vapour pressure and the monoterpene pool in the tissue; the former is linearly related to temperature, hence the result found by Tingey *et al.* (1980). In flowers, high levels of irradiance may raise the temperature in the petal considerably, subsequently increasing rates of emission of floral volatiles (Hansted *et al.*, 1994; Jakobsen and Olsen, 1994). Increased emission of floral volatiles also occurs in response to stress (Heath and Manukian, 1994). The concentration of volatiles in extracts from brown boronia flowers is affected by incident sunlight: at 75% full sunlight the concentrations of α -pinene and limonene in the floral extract were reduced while concentrations of β -ionone and dodecyl acetate increased (Plummer *et al.*, 1998).

Despite recent progress in the research of floral volatiles, fundamental aspects of the physiology, genetics, ecology and evolution of floral volatiles remain poorly understood (Raguso and Pellmyr, 1998). Floral odours play an important role in attracting pollinators; this so-called synomonal role for floral volatiles is suggested by the high correlation between their emission and the activity of a plant's pollinators (Jakobsen and Olsen, 1994; Baldwin *et al.*, 1997). It has been suggested that fragrance compounds active in pollinator attraction are likely to be emitted from the flowers only (Pellmyr *et al.*, 1987). The pollinating vector(s) for *B. megastigma* have not been identified to our knowledge.

Solid-phase microextraction (SPME) has been shown to be a simple, effective and sensitive sampling method, suitable for quantitation of flavour and fragrance compounds, providing a linear response to concentrations covering four orders of magnitude (Arthur and Pawliszyn, 1990; Louch *et al.*, 1992; Steffen and Pawliszyn, 1996). This is the first reported use of SPME to trap boronia volatiles, and the first time emission from different developmental stages and organs and the effect of different light conditions on emission of volatiles from brown boronia flowers have been studied.

MATERIALS AND METHODS

Plant material and treatment

Clonal plants of brown boronia (*Boronia megastigma* Nees) were developed by the University of Tasmania and grown in pots containing a 1:1 sand:peat bark mixture. Flowers for the developmental study were selected by development stage (MacTavish and Menary, 1997a), excised from a number of clonal plants and immediately placed in the body of disposable syringes to enable minimization and quantitation of the (static) headspace volume ($n = 1$). The SPME fibre was immediately placed into the open tip of the syringe and exposed for 30 min in the dark at 20°C. For this experiment, 21 stage i (very small) buds were held in 1 ml of headspace; 36 stage ii (small) buds in 3 ml; 28 stage iii (medium) buds in 4 ml; 19 stage iv (large) buds in 5 ml; 50 stage v (open flowers) in 40 ml; and 16 stage vi (senescent flowers from which at least one petal had abscised) in 4 ml. Peak areas for each compound after GCMS analysis were calculated per bud per ml of headspace.

For the comparison of emission from different organs, 50 stage v (open) flowers were excised from the same plants as above and were dissected into petals (four per flower), stigmas (one per flower), sepaline (non-functional) anthers and filaments (four per flower), petaline anthers and filaments (four per flower) and the remaining calyx/nectary (one per flower) ($n = 1$). Descriptions of each floral organ in terms of weight and dimensions have previously been reported by MacTavish and Menary (1997b). The dissected parts were sampled in the order above, with 30 min (static) SPME adsorption times at 20°C. Blank injections after adsorption from empty syringes were also made. In addition, volatiles from the first sample were adsorbed a second time at the completion of the experiment to determine if the time since dissection had affected the volatiles; there were no apparent qualitative or quantitative differences.

For the comparison of volatiles emitted from plants in different light environments, three plants of one clonal type were selected for similar habit (50 cm height) and flower maturity index by visual assessment. At the completion of the experiment, all flowers were excised from the plants, weighed, and the percentage of open flowers determined: the fresh flower weight per plant was 18.3 ± 2.3 g; 95–100% of all flowers had opened. The effect of flower maturity index and flower senescence on *in situ* concentrations of volatiles has previously been documented (MacTavish and Menary, 1997a). Immediately prior to the experiment, plants were held outside with average day temperatures of 12–14°C, and 12 h days beginning at 0600 h. Plants were brought into the laboratory (19°C) and placed into experimental conditions at 0800 h on the day of the experiment; conditions were: (A) continuous light for 36 h; (B) continuous dark for 36 h; and (C) 12 h light:12 h dark:12 h light cycles. For sampling, plants exposed to light were placed in a 27 l glass vessel receiving light from 2×60 cm \times 18 W Crompton white fluorescent lights held 45 cm from plants. For sampling, plants in the dark were placed in 27.5 l stainless steel vessels with several layers of shade cloth over the open top, the plants were kept in a

darkened room during and between sampling. Throughout the experiment, thermologgers placed at the base of plants were used to monitor the temperature, which remained at $19^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. In addition, it is important to note that plants were placed inside the sampling vessels for 30 min in every 90 min throughout 36 h: in between sampling, plants were removed from the sampling vessel and were not enclosed. Samples were taken in the order A-B-C throughout the experiment ($n = 1$); the experiment represents repeated measures taken from three individual plants each in different light environments. Samples of the headspace above a plant without flowers and a pot without a plant were taken to quantify volatiles emanating from non-floral tissues and apparatus.

Sampling

For sampling, a glass lid was placed on top of each vessel, bearing a small hole into which the fibre assembly was inserted. A solid phase microextraction fibre (SPME: Supelco Co., Supelco Park Bellefonte, PA, USA) coated with polydimethylsiloxane (PDMS, 1 cm long, 100 μm thickness) was used to collect and concentrate volatiles. The SPME device consisted of a retractable fibre enclosed in a sheath. The exposed fibre was pre-conditioned for 1 h at 260°C in the GC injection port. During sampling ($19^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$), after entering the sample container, the fibre was extended, exposing the sorption surface for 30 min. The fibre was then retracted prior to removal from the sample vessel. Volatiles were desorbed from the fibre for 30 s at 200°C into the glass-lined, splitless injector port of the gas chromatograph (GC). An adsorption time of 30 min followed by 30 s desorption were selected as optimal after examining the peak area for β -ionone resulting from adsorption times of 5, 10, 20, 30, 60 and 120 min and desorption times of 30 and 60 s (data not shown). Adsorption times greater than 30 min increased the adsorption of β -ionone; however 30 min was selected to maximize the number of samples within the 36 h period. A desorption time of 30 s was found to be sufficient to desorb all volatiles onto the GC.

Separation and detection

For the developmental and floral organ studies, adsorbed volatiles were desorbed from the fibre onto the Hewlett Packard 5890 Series II GCMS coupled via an open split interface to an HP 5970B mass selective detector, electron impact ionization at 200°C , electron energy 70 eV. Volatiles were separated using an HP-1 cross-linked methyl siloxane column (25 m \times 0.32 mm i.d., 0.52 μm film thickness), pre-conditioned for 2 h at 300°C . The carrier gas was He at 4 ml min^{-1} . The oven temperature programme was 50°C held for 1 min, then $30^{\circ}\text{C min}^{-1}$ to 220°C , then $10^{\circ}\text{C min}^{-1}$ to 290°C and held for 5 min; the injector temperature was 250°C . Volatiles were identified using MS and their retention index by comparison with known MS and Kovats Indices (Weyerstahl et al., 1994, 1995). Volatiles from whole plants under different light regimens were desorbed similarly and analysed by GC and FID using a medium polar, fused

silica capillary column (BP-X5, 25 m \times 0.2 mm i.d., 0.25 μm coating thickness). The carrier gas was N_2 at a flow rate of 2 ml min^{-1} , head pressure 12 psi. The temperature programme was isothermal for 5 min at 30°C , then raised at the rate of $10^{\circ}\text{C min}^{-1}$ to 280°C . The FID detector temperature was 280°C . The peak areas for all peaks were used for calculations, after subtraction of peak areas resulting from adsorption of flower-less plants, plant-less pots and/or blank injections. Major volatiles analysed by the GC-BP-X5 system from SPME-adsorptions above whole plants, standard boronia extract or known standards were tentatively identified by comparison with similar injections on the GCMS-HP-1 system.

RESULTS AND DISCUSSION

Developmental differences

The proportion of each compound in the total emission from each bud stage is presented in Table 1. Volatiles were emitted from buds at even the most immature stage, up to a month prior to flower opening, with caryophyllene, humulene and bicyclogermacrene dominating the headspace at this time; the proportion of these volatiles declined throughout development. At the same time, emission of all volatiles increased: open flowers had the most 'complex' emission, with several compounds emitted only from this stage (v). Both the proportion and quantity of β -ionone emitted from stage iv buds (% of emission per bud \times % of emission from bud stage iv relative to stage v) was at a maximum and subsequently declined. The process of flower senescence, observed as a loss of pigment, fresh and dry matter from flowers and eventually petal abscission, also includes a large reduction in volatile emission (stage vi), however α -pinene, caryophyllene, bicyclogermacrene, dodecyl acetate and (Z)-n-heptadec-8-ene were still emitted at this stage. Interestingly, dodecanol, methyl jasmonate and methyl epijasmonate were not detected in emissions from any stage in this study, which may be due to insufficient flower material present, or a selective effect on volatile emissions of excision from the plant.

Floral organs

Of the (calculated) emission from the 'whole flower', emissions from the petaline anthers (37.6%) and petals (26.7%) dominated (Table 2). This was despite the petaline anthers contributing only 1% of total flower weight (MacTavish and Menary, 1997b); these organs produce pollen and have high concentrations of carotenoids (MacTavish, 1995), potential sources of many norisoprenoids (Enzell, 1985). The bulk of monoterpene emissions, including α - and β -pinene, camphene, myrcene, limonene and *cis* β -ocimene emanates from the calyx, which in this case also included the nectary tissue beneath the stigma. β -Ionone comprised 30% of emitted volatiles from the calyx/nectary, similar in amount and proportion to that emitted from the sepaline anthers; β -ionone dominated emissions from the stigma (87.2%), which was also rich in carotenoids (MacTavish, 1995). It is interesting to note that

TABLE 1. *Headspace volatiles emitted from sequential developmental stages (% of each volatile in headspace per bud or flower)*

No	RI	Volatile	Very small bud (i)	Small bud (ii)	Medium bud (iii)	Large bud (iv)	Open flower (v)	Senescent flower (vi)
1	627	α -pinene	1.8	10.6	28.7	11.3	30.4	33.8
2	648	camphene					0.8	
3	698	β -pinene			4.0	1.2	2.7	
4	726	myrcene		2.5	1.7		0.9	
5	766	unknown (<i>m/z</i> 79, 91, 134)					1.2	
6	791	limonene			1.2		1.1	
7	823	cis β -ocimene					0.4	
8	902	hotrienol				0.6	0.5	
9	928	n-undecane				2.4	0.5	
10	1116	unknown (<i>m/z</i> 85, 119, 134)					1.5	
11	1268	cyclic β -ionone					0.5	
12	1387	β -ionone				0.7	0.5	
13	1404	caryophyllene	39.9	48.3	21.0	9.7	10.5	10.8
14	1448	humulene	11.3	8.9	4.7	1.9	1.5	
15	1454	dodecanol						
16	1465	β -ionone	4.9		2.5	54.7	39.5	12.8
17	1502	bicyclo-germacrene	19.6	25.7	19.0	9.0	3.5	11.4
18	1512	n-pentadecane				2.8	0.9	
19	1627	dodecyl acetate				1.5	0.3	5.2
20	1647	(<i>Z</i>) methyl jasmonate						
21	1683	(<i>Z</i>) methyl epijasmonate						
22	1728	(<i>Z</i>)-n-heptadec-8-ene				3.0	2.3	20.0
23	1757	n-heptadecane						
		Total emission (% of open flowers)	4.7	21.3	17.1	76.6	100	7.6

RI, Retention index.

TABLE 2. *Headspace volatiles emitted from individual floral organs from 50 flowers (% of each volatile in headspace)*

No	RI	Volatile	Calyx/nectary	Petals	Stigma	Sepaline anthers	Petaline anthers*
1	627	α -pinene	39.9	2.8	3.0	0.3	1.6
2	648	camphene	0.9				
3	698	β -pinene	2.4				
4	726	myrcene	1.2				
5	766	unknown (<i>m/z</i> 79, 91, 134)					
6	791	limonene	1.9				0.3
7	823	cis β -ocimene					
8	902	hotrienol	0.8	1.6	2.3		1.0
9	928	n-undecane				0.9	
10	1116	unknown (<i>m/z</i> 85, 119, 134)					
11	1268	cyclic β -ionone		0.7			
12	1387	β -ionone		0.4	0.6		0.2
13	1404	caryophyllene	6.2	2.5	1.4		2.4
14	1448	humulene	1.2	0.4			0.3
15	1454	dodecanol		0.3		1.0	0.6
16	1465	β -ionone	29.9	67.9	87.2	33.7	57.5
17	1502	bicyclo-germacrene	3.9	2.2	0.4	0.4	1.0
18	1512	n-pentadecane		0.55		1.51	0.60
19	1627	dodecyl acetate	1.7	3.2	1.5	8.7	6.6
20	1647	(<i>Z</i>) methyl jasmonate		0.4	0.8	1.9	0.9
21	1683	(<i>Z</i>) methyl epijasmonate				0.9	
22	1728	(<i>Z</i>)-n-heptadec-8-ene	9.2	15.9	2.9	48.1	20.7
23	1757	n-heptadecane		0.5		1.5	0.6
		Total emission (% of whole flower)	11.2	26.7	10.5	14.0	37.6

RI, Retention index.

* Petaline anthers are active in pollen production and high in carotenoids (MacTavish, 1995).

the most comprehensive array of volatiles was emitted from the petals, followed by the petaline anthers which lack only emission of cyclic β -ionone, and emit more dodecyl acetate by comparison. The two anther types, one producing pollen and the other not, differed markedly in total and specific emissions, with the non-functional sepaline anthers emitting less in total, and lacking emission of β -ionone, caryophyllene and humulene, yet emitting methyl epijasmionate. This confirms previous observations in a study using a different volatile entrapment procedure (MacTavish and Menary, 1997b). Overall, the dominance of the floral headspace by the petaline anthers, tissues lacking discrete oil glands (Bussell et al., 1995; MacTavish, 1995), is interesting from an ecological perspective in terms of pollinator attraction, and from a biochemical perspective in terms of localization and compartmentalization of biosynthesis and storage of floral volatiles.

Effect of light on whole plants

Of the 23 compounds detected by GC above flowering boronia plants, nine were tentatively identified by comparison of peak size and retention time between adsorption/injection of a standard boronia product on the different GC and GCMS systems. The peak areas of these compounds were corrected by subtraction of peak areas from volatiles emitted from plants with no flowers and plant-pots with no plant above soil level. These nine volatiles showed three different emission patterns: (1) emission patterns identical irrespective of light environment, with maximum emission in the 'endogenous' dark period, i.e. when the plant would normally have been in the dark (α -pinene, Fig. 1); (2) similar emission in all treatments, with an increase and decline over a period of 26 h, and in most cases no evidence of a second increase during the remainder of the experiment [5-acetoxy linalool (Fig. 2), cyclic β -ionone, dodecyl acetate and (*Z*)-n-heptadec-8-ene]; and (3) emission in all treatments, but enhanced in the dark, in some cases with a period of 27.5 h [cyclic β -ionone endoperoxide, dihydro β -ionone (Fig. 3), β -ionone (Fig. 4), and 'total volatiles' (Fig. 5)].

A number of volatiles were emitted, either largely or totally, irrespective of light conditions: α -pinene appeared to have endogenous 'night-time' emission (Fig. 1). Increased night-time concentrations of limonene and β -phellandrene within boronia flower tissues have been observed in previous work (MacTavish, 1995). Emission of 5-acetoxy linalool (Fig. 2) increased and declined over 26 h, irrespective of light, however a second cycle did not begin, perhaps due to constant temperature. A similar pattern occurred in emission of cyclic β -ionone (data not shown). Linalool was not observed in headspace emissions from boronia plants, however it is a component of the solvent-extracted product from boronia flowers (Davies and Menary, 1983).

The third pattern, in which lower levels of emission generally occurred in the light and patterns of emission differed between light treatments, was displayed most notably by dihydro β -ionone (Fig. 3) and β -ionone (Fig. 4). The rapid increase in emission of both volatiles in the dark phase of the alternating light:dark treatment is a clear indication that production and emission of these

volatiles is controlled by light. Preliminary work on diurnal changes in tissue concentrations of particular volatiles also showed relatively higher concentrations of β -ionone at night-time (MacTavish, 1995). The emission of all volatiles pooled together, which also follows this pattern (Fig. 5), is influenced to a great extent by the emission of major volatiles: ionones and dodecyl acetate. Other researchers have also shown the dependence of dihydro- β -ionone emission on radiation (Helsper et al., 1998). The same study found maximum emission of (13) volatiles from rose

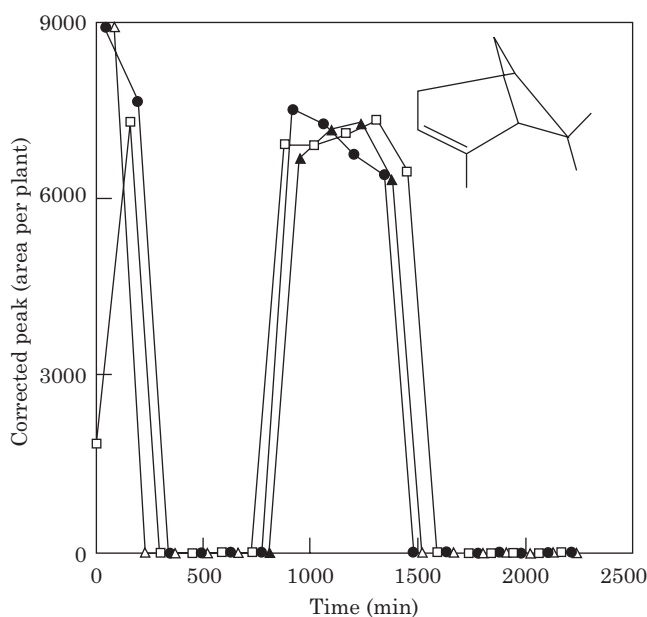


FIG. 1. α -Pinene. Continuous dark (●), continuous light (□), alternating light/dark (△, ▲).

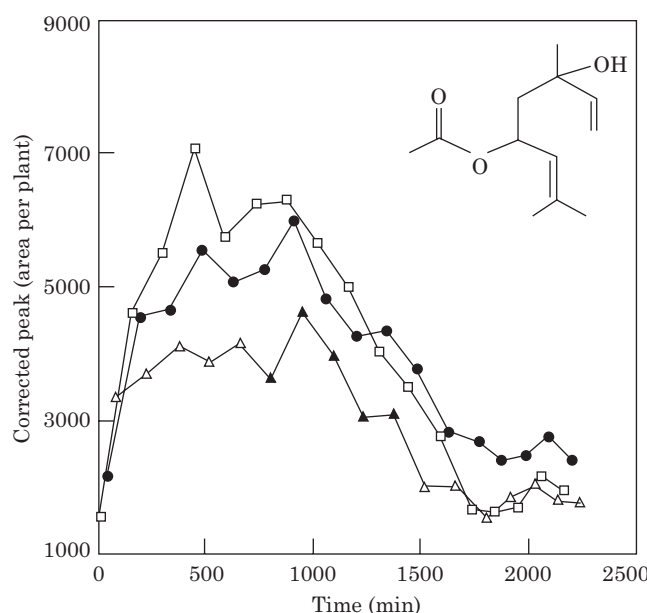


FIG. 2. 5-Acetoxy-linalool. Continuous dark (●), continuous light (□), alternating light/dark (△, ▲).

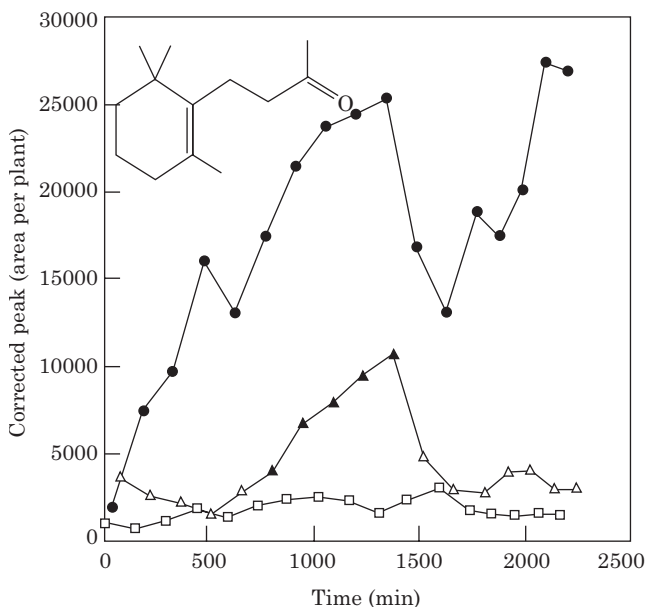


FIG. 3. Dihydro- β -ionone. Continuous dark (●), continuous light (□), alternating light/dark (△, ▲).

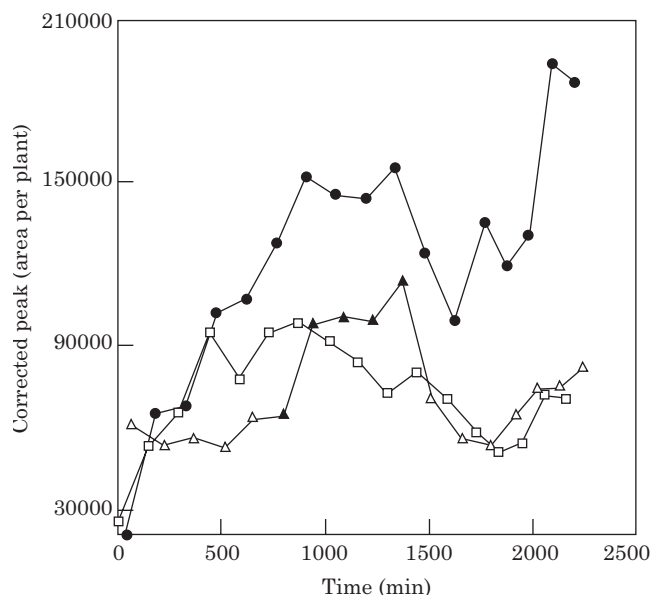


FIG. 5. Total volatiles. Continuous dark (●), continuous light (□), alternating light/dark (△, ▲).

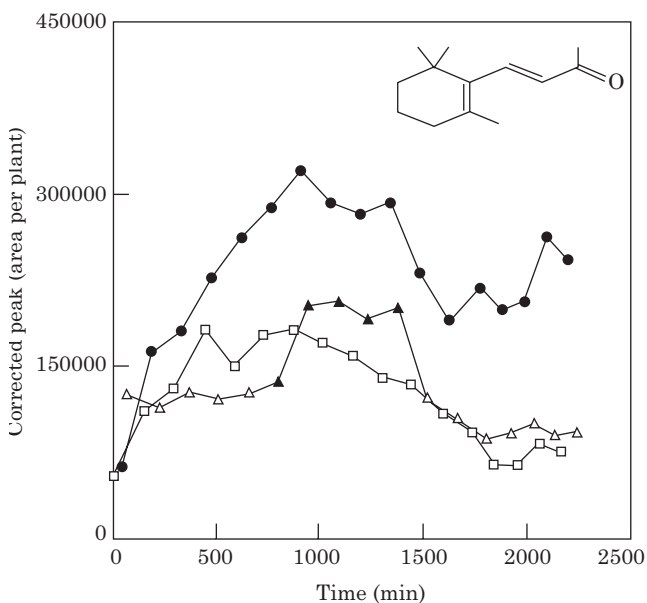


FIG. 4. β -Ionone. Continuous dark (●), continuous light (□), alternating light/dark (△, ▲).

flowers occurred in the light, although the dominance of monoterpenes in rose emissions and the finding of light-enhanced emission of these compounds (Nielsen *et al.*, 1995) may explain this difference when compared with the relatively complex emissions from boronia flowers.

From the emission patterns of several ionone derivatives present in the headspace above boronia flowers it is tempting to speculate on relationships and pathways of conversion. However, without complete identification and further research on light responses and reaction times, it is sufficient to note that in the light, production of cyclic β -ionone appears to be at the detriment of dihydro β -ionone, and that

low levels of cyclic β -ionone correspond with high levels of cyclic β -ionone endoperoxide. The rapid increase of dihydro β -ionone in the dark (Fig. 3) indicates that light-mediated metabolism of β -ionone via oxidative reactions (Gloria *et al.*, 1993; Bosser *et al.*, 1995) may reduce the pool of β -ionone available for metabolism into less oxidized products (Enzell, 1985). There is a decline in β -ionone emission from senescent flowers, concomitant with a decline in other volatiles, suggesting that products of senescence-related carotenoid degradation may differ from those actively produced in flower buds and mature flowers, or even that β -ionone is not in fact formed via this route *in vivo*. The dominance of β -ionone in the headspace above organs richly endowed with carotenoids does, however, suggest a link. It will be interesting to note the response of ionone emission from boronia flowers to light intensity, other oxidative events or even the presence of oxidative inhibitors, and ideally to correlate sequential enzyme-mediated metabolism of carotenoids into ionones and derivatives over time. The plants in our study were relatively 'stressed', having been moved into laboratory and experimental conditions and being moved between sampling vessel at regular intervals throughout the experiment. It has been suggested that plants respond to stress by enhancing emission of volatiles (Heath and Manukian, 1994), and for this reason the results presented herein represent preliminary observations of a limited number of plants in response to different conditions whilst stressed. The reader should be encouraged to study in more detail the vagaries of volatile emission in response to 'quantified' stresses in an otherwise controlled environment. The identification of immediate precursors to emitted floral volatiles is the subject of intense research by other researchers and ourselves, with the role of glycosidically-bound compounds attracting the most speculation. Our studies into precursors to free aglycones in boronia will be

the subject of other publications; however analysis of all compounds (free, bound and emitted), diurnally, throughout senescence and after harvest may help explain metabolic fluxes and precursor/product relationships.

In a study of several headspace analysis methods, it was concluded that analyses of small samples by headspace entrapment procedures routinely carry a high risk of experimental artifact (Raguso and Pellmyr, 1998); however, SPME was not considered. All the methods examined required the use of solvent extraction of adsorbed volatiles from the adsorbing matrix. SPME is an equilibrium method, therefore once equilibrium has been reached, the concentration of the analytes remains constant, the limiting step being the diffusion of analytes through the system (Steffen and Pawliszyn, 1996). The time required to saturate a 100 µm polydimethylsiloxane (PDMS) coated fibre such as the one used in this study varies from less than 2 min to more than 90 min depending on the volatile compound, the latter fact limiting the quantitation possible using SPME (Matich *et al.*, 1996; Steffen and Pawliszyn, 1996). Most compounds with a Kovats retention index of <1300 on a nonpolar GC column (DB-1) equilibrate in 30 min or less (Bartelt, 1997). The response is linear in the ppb to ppm range when the adsorption duration is standardized (Song *et al.*, 1997). The temperature of the system to be sampled is critical: increasing the temperature increases the signal to noise ratio, as does increasing the fibre exposure time. Our observations of the reproducibility of 'identical' injections suggest that artifacts mainly arose via sampling differences, and these were carefully controlled in this experiment. The adsorption time was 30 min ± 0.5 min, the desorption time was 30 s ± 2 s, although since this latter process was exhaustive, increased times should not alter responses. The main limitations were statistical analysis: limited by analysis time and the effect of phenology on floral volatiles (MacTavish and Menary, 1997a, 1999); and the quantitation of volatiles due to lack of an appropriate internal standard. Improvements in identification of volatiles via GCMS analysis of all samples will be a part of future experiments, as will the use of simultaneous volatile entrapment techniques with differing selectivity.

In short, we have found SPME to be a rapid, precise method for analysis of volatiles emitted into the headspace above boronia flowers *in vivo* and after excision from the plant and dissection. We have used the technique to compare emission of volatiles from successive developmental stages, different floral organs, and from whole plants subjected to different light regimens. We have made preliminary observations that suggest emission patterns of floral volatiles may be regulated both endogenously and by the environment.

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LITERATURE CITED

- Altenburger R, Matile P. 1990. Further observations on rhythmic emission of fragrance in flowers. *Planta* **180**: 194–197.
- Arthur CL, Pawliszyn J. 1990. Solid-phase microextraction with thermal desorption using fused silica optical fibers. *Analytical Chemistry* **62**: 2145–2148.
- Baldwin IT, Preston C, Euler M, Gorham D. 1997. Patterns and consequences of benzyl acetone floral emissions from *Nicotiana attenuata* plants. *Journal of Chemical Ecology* **23**: 2327–2343.
- Bartelt RJ. 1997. Calibration of a commercial solid-phase microextraction device for measuring headspace concentrations of organic volatiles. *Analytical Chemistry* **69**: 364–372.
- Bosser A, Paplorey E, Belin J-M. 1995. A simple way to (±)-dihydroactinidiolide from β-ionone related to the enzymic co-oxidation of β-carotene in aqueous solution. *Biotechnology Progress* **11**: 689–692.
- Bussell BM, Considine JA, Spadek ZE. 1995. Flower and volatile oil ontogeny in *Boronia megastigma*. *Annals of Botany* **76**: 457–463.
- Davies NW, Menary RC. 1983. Volatile constituents of *Boronia megastigma* flowers. *Perfumer and Flavorist* **8**: 3–8.
- Dement WA, Tyson BJ, Mooney HA. 1975. Mechanism of monoterpene volatilisation in *Salvia mellifera*. *Phytochemistry* **14**: 2555–2557.
- Enzell C. 1985. Biodegradation of carotenoids—an important route to aroma compounds. *Pure and Applied Chemistry* **57**: 693–700.
- Gloria MBA, Grulke EA, Gray JI. 1993. Effect of type of oxidation on β-carotene loss and volatile products formation in model systems. *Food Chemistry* **46**: 401–406.
- Hansted L, Jakobsen HB, Olsen CE. 1994. Influence of temperature on the rhythmic emission of volatiles from *Ribes nigrum* flowers *in situ*. *Plant, Cell and Environment* **17**: 1069–1072.
- Heath RR, Manukian A. 1994. An automated system for use in collecting volatile chemicals released from plants. *Journal of Chemical Ecology* **20**: 593–608.
- Helsper JPF, Davies JA, Bouwmeester HJ, Krol AF, van Kampen MH. 1998. Circadian rhythmicity in emission of volatile compounds by flowers of *Rosa hybrida* L. cv. Honesty. *Planta* **207**: 88–95.
- Jakobsen HB, Olsen CE. 1994. Influence of climatic factors on emission of flower volatiles *in situ*. *Planta* **192**: 365–371.
- Jakobsen HB, Friis P, Nielsen JK, Olsen CE. 1994. Emission of volatiles from flowers and leaves of *Brassica napus* *in situ*. *Phytochemistry* **37**: 695–699.
- Jones MB, Mansfield TA. 1975. Circadian rhythms in plants. *Science Progress Oxford* **62**: 103–125.
- Kaiser R. 1991. Trapping, investigation and reconstitution of flower scents. In: Müller PM, Lamparsky D, eds. *Perfumes—art, science and technology*. London: Elsevier Applied Science, 213–250.
- Louch D, Motlagh S, Pawliszyn J. 1992. Liquid-coated fused silica fibers. *Analytical Chemistry* **64**: 1187–1199.
- Loughrin JH, Hamilton-Kemp TR, Andersen RA, Hildebrand DF. 1990. Volatiles from flowers of *Nicotiana sylvestris*, *N. otophora* and *Malus × domestica*: Headspace components and day/night changes in their relative concentrations. *Phytochemistry* **29**: 2473–2477.
- MacTavish HS. 1995. *Factors affecting yield and composition of floral extract from Boronia megastigma* Nees. PhD thesis, University of Tasmania, Australia.
- MacTavish HS, Menary RC. 1997a. The effect of flower maturity and harvest timing on floral extract from *Boronia megastigma* (Nees). *Annals of Botany* **80**: 299–303.
- MacTavish HS, Menary RC. 1997b. Volatiles in different floral organs, and effect of floral characteristics on yield of extract from *Boronia megastigma* (Nees). *Annals of Botany* **80**: 305–311.
- MacTavish HS, Menary RC. 1999. Floral extract from four clones of *Boronia megastigma* (Nees) throughout flowering. *Journal of Horticultural Science and Biotechnology* **74**: 47–52.
- Matich AJ, Rowan DD, Banks NH. 1996. Solid phase microextraction for quantitative headspace sampling of apple volatiles. *Analytical Chemistry* **68**: 4114–4118.

- Matile P, Altenburger R. 1988.** Rhythms of fragrance emission in flowers. *Planta* **174**: 242–247.
- Mookherjee BD, Trenkle RW, Wilson RA. 1989.** Live vs dead. Part II. A comparative analysis of the headspace volatiles of some important fragrance and flavour raw materials. *Journal of Essential Oil Research* **2**: 85–90.
- Mookherjee BD, Trenkle RW, Wilson RA, Zampino M, Sands KP, Mussinan CJ. 1986.** Fruits and flowers: live vs dead—which do we want? In: Lawrence BM, Mookherjee BD, Willis BJ, eds. *Flavors and fragrances: A World perspective. Proceedings of the 10th International Congress of Essential Oils, Fragrances and Flavors*. Washington, DC: Elsevier, 415–424.
- Nielsen JK, Jakobsen HB, Friis P, Hansen K, Moller J, Olsen CE. 1995.** Asynchronous rhythms in the emission of volatiles from *Hesperis matronalis* flowers. *Phytochemistry* **38**: 847–851.
- Pellmyr O, Bergstrom G, Groth I. 1987.** Floral fragrances in *Actaea*, using differential chromatograms to discern between floral and vegetative volatiles. *Phytochemistry* **26**: 1603–1606.
- Plummer JA, Wann JM, Orifici R, Spadek ZE. 1998.** Effects of photon flux density on photosynthesis, growth, flowering, and oil content in boronia. *Australian Journal of Agricultural Research* **49**: 791–797.
- Raguso RA, Pellmyr O. 1998.** Dynamic headspace analysis of floral volatiles: a comparison of methods. *Oikos* **81**: 238–254.
- Song J, Gardner BD, Holland JF, Beaudry RM. 1997.** Rapid analysis of volatile flavor compounds in apple fruit using SPME and GC/Time of flight mass spectrometry. *Journal of Agricultural and Food Chemistry* **45**: 1801–1807.
- Steffen A, Pawliszyn J. 1996.** Analysis of flavor volatiles using headspace solid-phase microextraction. *Journal of Agricultural and Food Chemistry* **44**: 2187–2193.
- Tingey DT, Manning M, Grothaus LC, Burns WF. 1980.** Influence of light and temperature on monoterpene emission rates from slash pine. *Plant Physiology* **65**: 797–801.
- Weyerstahl P, Marschall H, Bork W-R, Bilk R. 1994.** Megastigmanes and other constituents of the absolute of *Boronia megastigma* from Tasmania. *Liebigs Annalen der Chemie* 1043–1047.
- Weyerstahl P, Marschall H, Bork W-R, Bilk R, Schneider S, Wahlburg W-C. 1995.** Constituents of the absolute of *Boronia megastigma* Nees. from Tasmania. *Flavour and Fragrance Journal* **10**: 297–311.