PERGAMON

Phytochemistry 60 (2002) 611–617

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Emission of floral volatiles from *Mahonia japonica* (Berberidaceae)

Joanne M. Picone[a], Hazel S. MacTavish[b,*], Robin A. Clery[c]

[a]*Department of Agricultural Sciences, Imperial College, University of London, Wye, Ashford, Kent TN25 5AH, UK*
[b]*ADAS Consulting Ltd, ADAS Arthur Rickwood, Mepal, Ely, Cambridgeshire CB6 2BA, UK*
[c]*Quest International, Ashford, Kent TN24 0LT, UK*

Received in revised form 4 March 2002

Abstract

Flowering *Mahonia japonica* plants were subjected to controlled environments and the floral volatiles emitted from whole racemes (laterals) were trapped by Porapak Q adsorbent and analysed by GC-FID. An experiment with photoperiods of 6 and 9 h at constant temperature (10 ± 1 °C) demonstrated that photoperiod was the stimulus for enhanced emission of most volatiles. Small quantitative differences in emitted fragrance composition were observed between light and dark periods and between plants acclimatised to different photoperiods. Maximum rates of emission occurred in the middle of the light period; aromatic compounds (benzaldehyde, benzyl alcohol and indole) displayed a more rapid increase and subsequent decline compared with monoterpenes (*cis*- and *trans*-ocimene and linalool). When the photoperiod was extended from 6 to 9 h, maximum rates of emission continued throughout the additional 3 h. Total emission (μg/h) of volatiles was 2-fold greater in the day-time (DT) (39.7 μg/h) compared with the night-time (NT) (19.8 μg/h) under a 6 h photoperiod and was not significantly different from total emission under a 9 h photoperiod.

Keywords: *Mahonia japonica*; Headspace; Volatiles; Flower; Floral volatiles; Photoperiod

1. Introduction

The genus *Mahonia* (Berberidaceae) contains approximately 70 species of evergreen shrubs native to East Asia, North and Central America (Brickell, 1996). A number of species of *Mahonia* have been extensively studied for their pharmacologically active components: the roots of *M. aquifolium* contain alkaloids with relaxant, antioxidant and antifungal properties (McCutcheon et al., 1994; Misik et al., 1995; Sotnikova et al., 1997). *Mahonia* species are also grown for their floral fragrance, but quantitative analysis of this has been unavailable in the literature until recently (Clery et al., in press). *Mahonia japonica* is a popular garden shrub with a scent similar to Lily-of-the-Valley (muguet, *Convalaria majalis*); in the UK it flowers in winter, producing bell-shaped yellow flowers borne on horizontal racemes approximately 20 cm long.

Research has illustrated 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); 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). Day-time maxima and circadian control of rhythmicity was observed in *Rosa hybrida* L. cv. Honesty and *Antirrhinum majus* (Helsper et al., 1998; Kolosova et al. 2001). Nocturnal emission of floral volatiles has generally been observed to be controlled by an endogenous (circadian) clock, whereas diurnal emission may be more influenced by prevailing light and temperature conditions (Hansted et al., 1994). Rhythms in emission from flowers are hypothesised to result from petal movement, but since rhythmic emission is also observed in flowers in which there is an absence of petal movement (Helsper et al., 1998; Kolosova et al., 2001) this view is too simplistic to explain this phenomenon.

In this study, emission of the recently identified volatiles comprising the characteristic fragrance of *M. japonica* flowers (Clery et al., 2002) was examined under controlled conditions at 10 ± 1 °C and photoperiods of 6 and 9 h, throughout 48 h ($n = 3$). Porapak Q was used as

* Corresponding author. Fax: +44-1354-694-488.
E-mail address: hazel.mactavish@adas.co.uk (H.S. MacTavish).

0031-9422/02/$ - see front matter
PII: S0031-9422(02)00119-X

absorbent, with a solvent extraction step followed by GC analysis. The use of plants with a racemic flowering pattern, such as *Mahonia*, means that developmental stages from buds to senescent flowers are sampled simultaneously. Emissions from such stages differ (Helsper et al., 1998; MacTavish et al., 2000), as may rhythmic emission patterns, depending on how the environmental cues are perceived by flowers and buds. Therefore, one would expect different responses compared with observations of a single flower.

2. Results and discussion

2.1. GC–MS analysis

The complete list of volatiles identified in the headspace above *M. japonica* flowers is presented in Table 1, including many monoterpenes and phenylpropanoid/benzoid derivatives. The predominating volatiles were *cis*- and *trans*-ocimene, linalool, citronellol, benzaldehyde, benzyl alcohol and indole. Indole has the greatest overall impact on the scent due to its low odour perception threshold. Volatiles may also have arisen from the sepals, buds, stems, and senescent flowers within the sampling vessel, and leaves external to the vessel, since the seal was not airtight. Blank samples of headspace above plants in the same room were also examined; significant levels of volatiles were not observed in such samples.

2.2. Effect of photoperiod on volatile emission

This experiment, using Porapak Q as adsorbent, examined the effect of photoperiod (6 or 9 h) on emission of volatiles at 10 ± 1 °C. The experiment was conducted at the natural flowering time of *M. japonica*, with an ambient day length of approximately 8 h and average daytime temperatures of approximately 10 °C. In this experiment, volatiles contributing to each sample were collected over successive 3 h intervals.

Twelve major volatiles emitted in the day-time (DT) and night-time (NT) were separately pooled for each photoperiod treatment to determine differences in the fragrance composition between light and dark periods or as a result of the different photoperiods (Table 2). Differences in fragrance composition were most pronounced between DT and NT emissions under the 6 h

Table 1
Composition of the volatiles emitted into the headspace above *Mahonia japonica* flowers (% of total) (from Clery et al., 2002)

RRI	Component	% RPA	RRI	Component	% RPA
			1176	Ethyl benzoate	Tr
901	Heptanal	0.1	1196	Naphthalene	Tr
930	α-Thujene	Tr	1197	α-Terpineol	Tr
954	Camphene	Tr	1203	Methyl salicylate	Tr
964	Benzaldehyde	4.1	1206	Decanal	0.2
977	Sabinene	Tr	1222	(*Z*)-Cinnamic aldehyde	Tr
986	6-Methyl-5-hepten-2-one	Tr	1228	α-Terpinyl methyl ether	Tr
1033	Limonene	Tr	1230	Citronellol	6.8
1038	Benzyl alcohol	14.0	1236	3-Phenylpropyl alcohol	Tr
1039	(*Z*)-β-Ocimene	3.5	1256	Geraniol	1.3
1048	Phenylacetaldehyde	Tr	1272	3,5-Dimethoxytoluene	Tr
1049	(*E*)-β-Ocimene	57.0	1279	(*E*)-Cinnamic aldehyde	0.1
1063	γ-Terpinene	Tr	1278	Citronellyl formate	Tr
1069	Octanol	Tr	1283	Methyl neranate	Tr
1076	*cis*-Linalool oxide furanoid	Tr	1304	Indole	2.6
1080	Benzyl formate	Tr	1300	Tridecane	Tr
1092	*trans*-Linalool oxide furanoid	Tr	1314	(*E*)-Cinnamic alcohol	0.1
1094	2-Nonanone	Tr	1326	Methyl geranate	0.4
1100	Methyl benzoate	0.1	1354	Methyl anthranilate	Tr
1101	Linalool	2.2	1350	Benzyl butyrate	Tr
1104	Nonanal	0.1	1355	Citronellyl acetate	Tr
1114	*cis*-Rose oxide	0.5	1378	3-Phenylpropyl acetate	Tr
1119	2-Phenyl ethanol	0.5	1394	Methyl cinnamate	Tr
1132	*trans*-Rose oxide	0.1	1395	Benzyl 2-methylbutyrate	Tr
1131	(*E*,*Z*)-*allo*-Ocimene	0.3	1396	Isobutyl phenylacetate	Tr
1133	(*E*,*E*)-*neo*-*allo*-Ocimene	0.1	1457	(*E*)-Geranyl acetone	Tr
1156	Citronellal	0.1	1458	(*E*)-β-Farnesene	0.1
1167	Benzyl acetate	Tr	1600	Hexadecane	Tr
1168	Phenylpropanal	Tr	1614	Tetradecanal	Tr
1172	Propiophenone	Tr	1776	Benzyl benzoate	Tr

RRI = relative retention index; % RPA = % relative proportion of total area; Tr = trace amount (< 0.1% RPA).

photoperiod treatment. The proportional contribution of *cis*-ocimene, *trans*-ocimene and geraniol to the fragrance composition was significantly higher in the DT compared to the NT, with an observed 20–60% increase. Conversely, the contribution of benzaldehyde, benzyl alcohol and phenylethyl alcohol was significantly lower in the DT compared with the NT by 25–43%. The magnitude of the changes in fragrance composition between DT and NT were not sufficient enough to markedly change the quantitative ranking of the fragrance components. Interestingly, these small yet significant differences in fragrance composition were not evident in emissions of plants under a 9 h photoperiod.

Increasing the duration of the photoperiod by three hours significantly affected the contribution of some components to fragrance composition. cis-Ocimene made a significantly greater contribution to fragrance composition when acclimatised to a 9 h photoperiod, regardless of whether it was DT or NT. *Trans*-methyl geraniate also made a significantly greater contribution to fragrance composition in the DT, whereas benzaldehyde, benzyl alcohol and phenylethyl alcohol all made a significantly lower contribution during the NT when acclimatised to a 9 h photoperiod. It remains undetermined whether these small quantitative differences in fragrance composition result in a detectable qualitative difference in the scent.

The average rate of emission of total volatiles (μg/h) from flowers in the DT and NT of the different photoperiods were compared (Table 3). Under both photoperiods, emission throughout the DT (6 h, 40 μg/h; 9 h, 42 μg/h) was significantly higher ($P < 0.05$) and approximately twice that observed during the NT (6 h, 20 μg/h; 9 h, 18 μg/h). Emission of volatiles was sustained at high levels for the additional three hours of light exposure in plants acclimatised to a 9 h photoperiod and was significantly different to the observed decrease in emission from plants acclimatised to a 6 h photoperiod over the same 3 h period ($P < 0.05$). Within this experiment, *M. japonica* flowers emitted higher levels of volatiles in response to light, for as long as the light stimulus was present, in confirmation of observations on the effect of photoperiod on emissions from vegetative tissues (Charron et al., 1996). The limitations to continued higher emission throughout an even more prolonged photoperiod by *M. japonica* are not yet elucidated.

The emission pattern of monoterpenes, aromatic compounds and total volatiles throughout 48 h in both the 6 and 9 h photoperiod treatments are illustrated (Fig. 1A–F). The DT increase in emission of *trans*-ocimene is marked during both light cycles, with smaller maxima, reflecting the lower concentration, observed for*cis*-ocimene and linalool (Fig. 1A). Emission of aromatic compounds was characterised by a sharper emission maxima occurring at the mid-point of the photoperiod (Fig. 1B). By comparing the 3–6 pm sample (the additional 3 h of light) in both the 6 and 9 h photoperiod treatments, one can observe the enhanced emission of monoterpenes and aromatic compounds in response to the extended light period (Fig. 1A–E). Hansted et al. (1994) observed DT maxima in several monoterpenes emitted from *Ribes nigrum* flowers; diurnal maxima have also been observed in emission of the

Table 2
Differences in emission of the 12 main volatiles between DT and NT in flowers exposed to either 6 or 9 h photoperiods, expressed as a % of total emission. Results are expressed as mean (%)±S.D. ($N >/= 6$)[a]

Volatile	Fragrance composition (% of total)			
	DT		NT	
	6 h photoperiod	9 h photoperiod	6 h photoperiod	9 h photoperiod
Monoterpenes				
cis-Ocimene	5.9±0.2a,b	6.7±0.5b	4.7±0.5a,c	6.4±0.5c
trans-Ocimene	48.3±3.2a	53.4±7.5	38.9±4.6a	47.4±7.0
Linalool	3.9±2.0	3.0±0.4	4.0±1.6	3.2±0.7
cis-Rose oxide	0.3±0.0	0.3±0.0	0.4±0.1	0.3±0.1
Citronellol	4.5±1.2	4.7±1.3	5.3±1.7	6.1±2.4
Geraniol	0.7±0.1a	0.7±0.4	0.3±0.2a	0.3±0.1
Geranial	0.1±0.1	0.2±0.1	0.1±0.1	0.1±0.0
trans-Methyl geraniate	0.4±0.2b	0.7±0.0b	0.3±0.0	0.4±0.2
Aromatic compounds				
Benzaldehyde	10.8±1.7a	8.7±2.8	14.5±0.6a,c	12.1±0.2c
Benzyl alcohol	19.0±1.3a	15.1±5.5	25.2±2.2a,c	16.8±2.5c
Phenylethyl alcohol	0.4±0.1	0.4±0.2	0.7±0.1c	0.4±0.1c
Indole	5.7±1.6	6.2±1.9	5.6±2.6	6.4±2.3

[a] a, b and c denote significant differences ($P < 0.05$) in the % of total emission: a, between DT and NT under a 6 h photoperiod; b, between 6 and 9 h photoperiod treatments during the DT; and c, between 6 and 9 h photoperiod treatments during the NT.

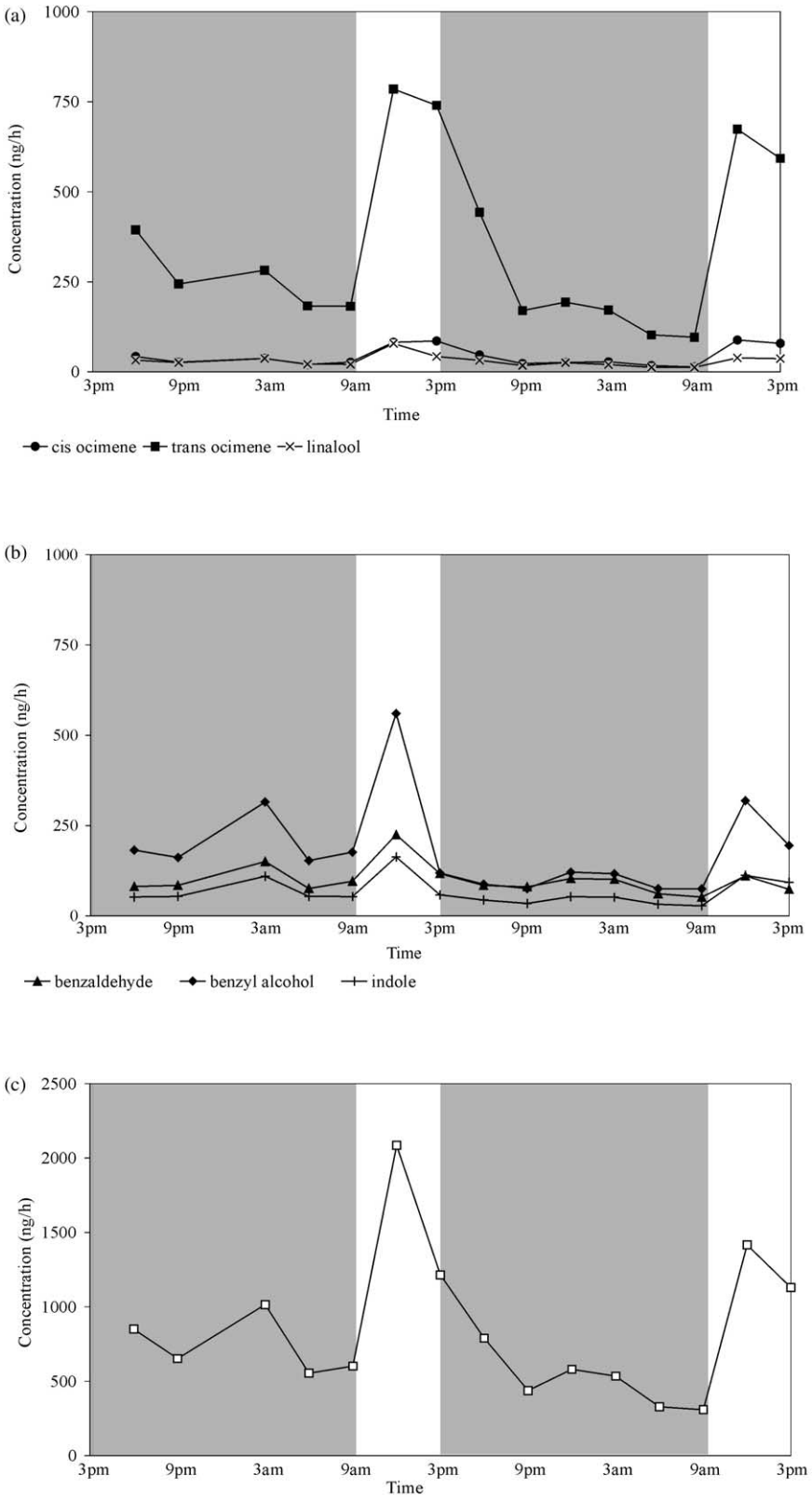
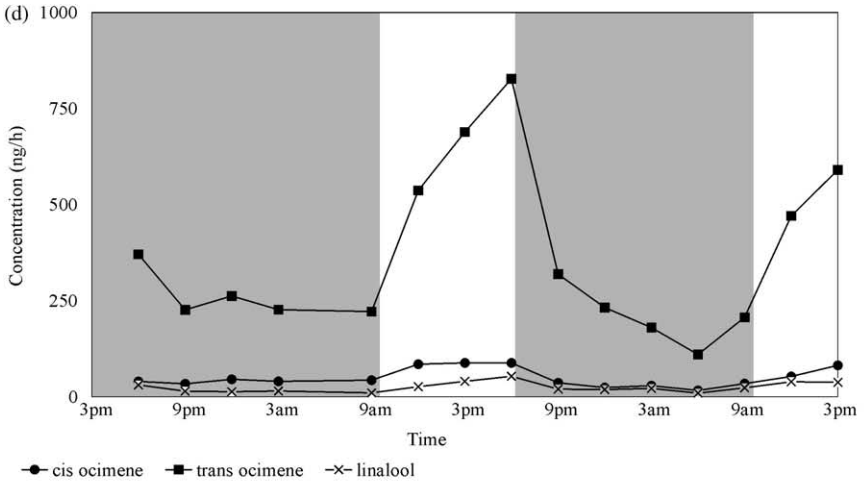
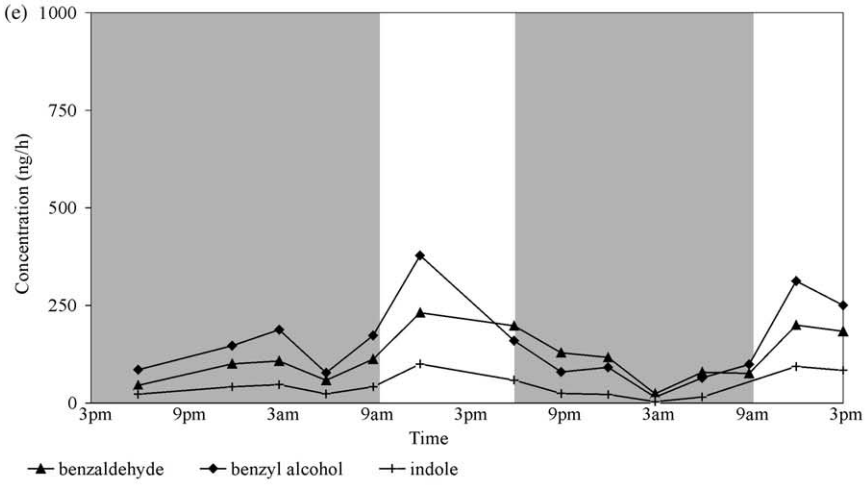


Fig. 1. Concentration (ng h^{-1}) of monoterpenes (a,d), aromatic compounds (b, e) and total volatiles (c, f) in headspace above *Mahonia japonica* laterals exposed to 6 h (a–c) and 9 h (d–f) photoperiods under constant temperature (10±1 °C). Shaded and unshaded areas correspond to exposure to darkness and light. Samples represent volatiles collected throughout previous 3 h.





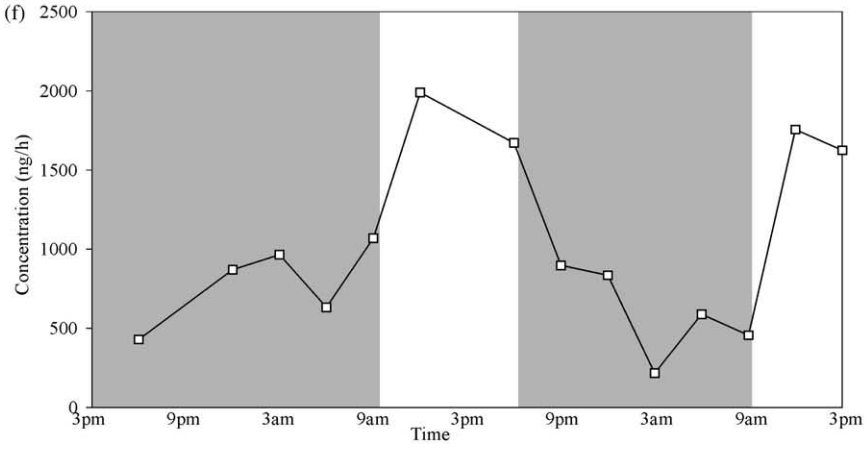


Fig. 1 (*continued*).

Table 3
Differences between total emission of volatiles in DT and NT, average of both 6 and 9 h photoperiod data[a]

Treatment	Average total volatile emission (μg/h)	
	DT	NT
6 h photoperiod	39.7±11.6	19.8±7.2
9 h photoperiod	42.4 ±10.6	18.2±6.9

[a] DT and NT observations are significantly different under both photoperiods ($P < 0.05$).

phenylpropanoid/benzoid derivative methyl benzoate from *Antirrhinum majus* (Kolosova et al., 2001). The major difference between the emission of monoterpenes and aromatic compounds during the extended photoperiod is that emission of monoterpenes was sustained at maximum levels or continued to increase throughout light exposure whereas emission of aromatic compounds decreased prior to the acclimatised light–dark transition. This could indicate that the timing of the light–dark transition is an important factor determining emission patterns for aromatic compounds, whilst photoperiod duration determines the emission of monoterpenes in *M. japonica*.

Loughrin et al. (1991) observed endogenously controlled emission of the phenylpropanoid/benzoid derivatives benzaldehyde, benzyl alcohol and methyl benzoate in *Nicotiana* sp. with nocturnal maxima, whereas emission of monoterpenes was rhythmic only under light/dark entrainment. Endogenous control of methyl benzoate emission has also recently been demonstrated with diurnal maxima in *Antirrhinum majus* (Kolosova et al., 2001). It is therefore possible that the emission of monoterpene and phenylpropanoid/benzoid classes may be differentially controlled in *M. japonica*. This is yet to be confirmed.

Emission of monoterpenes and aromatic compounds from *M. japonica* is controlled by diurnal changes in light levels. The co-occurrence of endogenous (circadian) rhythms has not yet been established. A more detailed investigation into the effect of light and temperature on primary reactions within vegetative and floral tissues and the effect of this on fragrance emission are the obvious next stage of research on this species.

3. Experimental

3.1. Plant material and experimental conditions

Uniform *M. japonica* plants, produced in the Netherlands, were purchased from a plant centre in the UK. The potted plants were approximately 50 cm in height, each bearing several racemes (laterals) with flower buds, mature and senescent flowers. The plants were acclimatised for 7 days with daily watering to a constant 10±1 °C with either 6 or 9 h photoperiod (natural light supplemented with 200 μmol/m^2/s provided by a tubular sodium lamp with increased blue light, 400W SON-T AGRO, mounted in a Philips SG140 luminaire) in custom-built controlled environment growth cabinets (external dimensions: 207 × 114 × 100 cm; growth area 0.75 m^3; height 1 m). The day length and temperature were selected for their similarity with ambient environmental conditions at the time of the experiment, i.e. the natural flowering period for *M. japonica* in the Northern hemisphere. Three plants were placed in each cabinet, representing 3 replicates for each treatment.

3.2. Collection of headspace samples

One flowering raceme on each plant with approximately 40 flowers, of which 50% had reached anthesis, were gently placed within the sampling enclosure, with the base of the raceme being open to the atmosphere. Glass sampling enclosures (50×200 mm; 160 cm^3) were fused at one end, with a sample point, comprising an open glass arm and screw-thread nylon adaptor, at either side of the fused tip (Glass Forming, Kent, UK). Traps for adsorbing volatiles comprised glass tubes (2.2 mm id, 6 mm od ×100 mm) with fire-polished ends (Cambridge Glassblowing, UK) packed with approximately 100 mg Porapak Q 50/80 mesh (Supelco) between two plugs of pesticide grade glass wool (Supelco). Traps were preconditioned with 5 ml acetone (AR grade; Merck) and dried with high purity nitrogen gas. The traps were inserted into the nylon adaptor on the sampling tube and were connected by polypropylene tubing to a Capex 2DC suction pump, pulling ambient air through each trap at a measured 50 ml min^{-1} (Charles Austen Pumps Ltd, Surrey, UK). Samples were collected for 3 h periods throughout 48 h. Volatiles were eluted from traps in the opposite direction to air flow with 0.2 ml acetone containing tetradecane as an internal standard. The recoverability of desorbed volatiles and internal standard were examined using a synthetic mixture of typical *Mahonia* volatiles prepared by Quest International. Samples were analysed by GC–FID, the 12 most significant volatiles were selected for comparison throughout the experiment.

3.3. GC analysis

3.3.1. GC–MS system

Compounds were identified by GC–MS (MS database, Quest International) and confirmed by retention times of pure reference compounds. A Varian 3400 GC equipped with an OPTIC injector and coupled to a Finnigan ITS40 ion trap mass spectrometer was used. The column was an HP ultra2 (5% diphenyl, 95% dimethyl-polysiloxane) 50 m × 0.2 mm, with film thick-

ness 0.33 μm (Cat. No. 19091B-105). Carrier gas: He at 1.6 ml min^{-1}; oven temperature program: 50–270 °C at 2 °C min^{-1}. Injector temperature: 250 °C; injection volume: 0.5 μl.

3.4. GC–FID system

A Hewlett Packard 6890 GC equipped with a FID, on-column injector, autosampler, and an HP5 column (Cat. No. 19091-J102, 5% diphenyl, 95% dimethyl polysiloxane) 25 m × 0.2 mm, film thickness 0.33 μm, was used. Carrier gas: H_2 at 2 ml min^{-1}; oven temperature program: 50–280 °C over 22 min, held at 280 °C for 7.5 min. Injector temperature: 250 °C, detector temperature: 280 °C. Injection volume: 0.5 μl.

Quantitative peak estimation was achieved by comparison with the internal standard, an FID response factor of one was assumed for all components. The concentrations of volatiles were also corrected for any differences in flow rates or sample collection times.

Acknowledgements

The authors wish to thank Quest International for preparation of a blend of synthetic chemicals similar to the composition of *Mahonia* fragrance for calibration of sampling equipment.

References

Altenburger, R., Matile, P., 1990. Further observations on rhythmic emission of fragrance in flowers. Planta 180, 194–197.

Baldwin, I.T., 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.

Brickell, C. (Ed.), 1996. A-Z Encyclopaedia of Garden Plants. Royal Horticultural Society, Dorling Kindersley, London.

Charron, C.S., Cantliffe, D.J., Wheeler, R.M., Manukian, A., Heath, R.R., 1996. Photosynthetic photon flux, photoperiod, and temperature effects on emissions of (*Z*)-3-hexenal, (*Z*)3-hexenol, and (*Z*)-3-hexenyl acetate from lettuce. Journal of the American Society for Horticultural Science 121, 488–494.

Clery, R.A., MacTavish, H.S., Picone, J.M., 2002. Identification of volatiles in headspace emitted from *Mahonia japonica* flowers. Journal of Essential Oil Research, in press.

Hansted, L., Jakobsen, H.B., Olsen, C.E., 1994. Influence of temperature on the rhythmic emission of volatiles from *Ribes nigrum* flowers *in situ*. Plant, Cell and Environment 17, 1069–1072.

Helsper, J.P.F.G., Davies, J.A., Bouwmeester, H.J., Krol, A.F., van Kampen, M.H., 1998. Circadian rhythmicity in emission of volatile compounds by flowers of *Rosa hybrida* L. cv. Honesty. Planta 207, 88–95.

Jakobsen, H.B., Olsen, C.E., 1994. Influence of climatic factors on emission of flower volatiles *in situ*. Planta 192, 365–371.

Loughrin, J.H., Hamilton-Kemp, T.R., Andersen, R.A., Hildebrand, D.F., 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.

Loughrin, J.H., Hamilton-Kemp, T.R., Andersen, R.A., Hildebrand, D.F., 1991. Circadian rhythm of volatile emission from flowers of *Nicotiana sylvestris* and *N. suaveolens* flowers. Physiologia Plantarum 83, 492–496.

Kolosova, N., Gorenstein, N., Kish, C.M., Dudareva, N., 2001. Regulation of circadian methyl benzoate emission in diurnally and nocturnally emitting plants. Plant Cell 13, 2333–2347.

MacTavish, H.S., Davies, N.W., Menary, R.C., 2000. Emission of volatiles from brown boronia flowers: some comparative observations. Annals of Botany 86, 347–354.

McCutcheon, A.R., Ellis, S.M., Hancock, R.E.W., Towers, G.H.N., 1994. Antifungal screening of medicinal plants of British Columbian Native peoples. Journal of Ethnopharmacology 44, 157–169.

Matile, P., Altenburger, R., 1988. Rhythms of fragrance emission in flowers. Planta 174, 242–247.

Misik, V., Bezakova, L., Malekova, L., Kostalova, D., 1995. Lipoxygenase inhibition and antioxidant properties of protoberberine and aporphine alkaloids isolated from *Mahonia aquifolium*. Planta Medica 61, 372–373.

Sotnikova, R., Kettmann, V., Kostalova, D., Taborska, E., 1997. Relaxant properties of some aporphine alkaloids from *Mahonia aquifolium*. Methods and Findings in Experimental and Clinical Pharmacology 19, 589–597.