PERGAMON

Phytochemistry 51 (1999) 211–214

PHYTOCHEMISTRY

Floral volatiles of the sweet pea *Lathyrus odoratus*

Alexander E.A. Porter[a,*], D. Wynne Griffiths[b], Graeme W. Robertson[b], Roy Sexton[a]

[a]*Department of Biological and Molecular Sciences, University of Stirling, Stirling FK9 4LA, UK*
[b]*Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK*

Received 23 October 1998; received in revised form 23 November 1998

Abstract

The volatile components of the floral fragrances from three sweet pea cultivars have been determined using polymer entrainment and solvent elution, combined with gas-chromatography–mass spectrometry. A total of 48 compounds were detected in quantifiable amounts, 41 of which were common to all three cultivars. The most abundant compounds were consistently found to be (*E*)-β-ocimene and linalool.

Keywords: *Lathyrus odoratus*; Leguminosae; Sweet pea; Flowers; Volatiles; Phenylacetaldehyde; Monoterpenes; β-Ocimene; Linalool; Sesquiterpenes; (*E*)-α-bergamotene

1. Introduction

As implied by its botanical name the sweet pea (*Lathyrus odoratus*) produces flowers with an intense, pleasant fragrance and due to this fact is primarily grown for the cut flower market. Although numerous formulations which emulate the aroma of sweet pea flowers are available (Dumont, 1938; Anonis, 1988) there appears to have been no detailed analysis of the chemical composition of the volatiles released from cut flowers. Unsuccessful attempts to identify the major aroma constituents using steam distillation have been reported (Anonis, 1988), however the maceration process utilised in steam distillation can result in the release and possible enzymatic modification of internally derived compounds and also in chemical modifications due to oxidation and thermal rearrangements. It has been recently suggested (Raguso, & Pellmyr, 1998) that the most satisfactory methods for determining true floral volatile profiles are based on the use of headspace collection combined with solvent elution.

Modern sweet pea cultivars have been perceived by the public to have reduced levels of fragrance. However, no information is currently available as to whether this is due to changes in the total amount of volatiles released by the flowers or to changes in the chemical constituents of the floral bouquet. Similarly, recent investigations on the vase life of sweet pea flowers (Sexton, Porter, Littlejohn, & Thain, 1995) have revealed that some postharvest treatments may significantly prolong the period over which the flowers continue to release detectable volatiles. Clearly in order to attain a better understanding of the fundamental mechanisms involved, detailed analyses of the constituent volatiles are required.

Consequently, the objective of this initial study was to identify, using headspace analysis, the major volatiles present in the floral bouquet of cut sweet peas.

2. Results and discussion

The compounds detected in quantifiable amounts from the head space of the flowers from the three sweet pea cultivars are listed in Table 1. In all cultivars the predominant class of compounds were identified as monoterpene hydrocarbons which on

* Corresponding author. Tel.: +44-1786-467-779; fax +44-1786-464-994.

E-mail address: a.e.a.porter@stir.ac.uk (A.E.A. Porter)

0031-9422/99/$ - see front matter
PII: S0031-9422(98)00755-9

Table 1
Volatile compounds identified in the floral headspace of three sweet pea cultivars; a means identity based on published mass spectrum, b identity confirmed by coelution with authenticated standard or b[a] using secondary standard; nd means not detected and tr present in trace amounts

Compound	Retention time (min)	RRI[a]	Total area (%)				Basis identity
			Royal Wedding (early)	Royal Wedding (late)	Diana	Old Time	
3-Methylbutanal	10.9	696	0.4	1.2	0.7	0.5	a
Methyl butanoate	12.5	747	0.3	0.3	1.9	0.3	a
3-Methyl-1-butanol	14.5	811	0.1	0.5	0.7	0.1	a
3-Methyl-3-buten-1-ol	14.6	814	0.3	0.2	0.2	0.1	a
3-Methyl-2-buten-1-ol	16.1	863	0.3	0.3	0.5	0.2	a
3-Methyl-1-butyl acetate	18.1	927	tr	0.1	tr	tr	a
α-Thujene	18.4	936	tr	tr	nd	nd	a, b
α-Pinene	18.8	949	0.6	1.0	0.9	0.2	a, b
Methyl hexanoate	19.8	981	0.5	0.6	3.7	0.4	a
3-Methyl-2-buten-1-yl acetate	20.0	988	0.5	0.4	0.3	tr	a
Sabinene	20.8	1013	1.4	2.2	1.9	0.3	a, b
β-Myrcene	21.0	1020	1.2	1.4	1.1	0.7	a, b
(*Z*)-β-ocimene	22.6	1071	6.7	5.0	2.3	7.3	a, b
(*E*)-β-ocimene	23.2	1090	35.3	22.9	27.8	46.5	a, b
Undecane	23.5	1100	0.6	1.1	0.5	0.6	a
Benzaldehyde	23.9	1113	0.2	0.1	0.1	tr	a, b
Rose oxide	26.2	1187	0.1	0.1	0.1	nd	a
Linalool	26.7	1203	20.7	16.6	23.6	26.2	a, b
Phenylacetaldehyde	26.9	1210	6.5	5.0	3.6	2.9	a, b
Benzyl alcohol	27.3	1223	0.4	0.4	0.1	tr	a, b
Methyl benzoate	27.5	1229	0.4	0.6	0.4	0.1	a
Citronellal	28.5	1261	0.1	0.4	nd	0.1	a
Tridecane	29.7	1300	0.6	0.9	0.6	0.3	a
Decanal	29.9	1307	0.1	0.3	0.9	0.1	a
Methyl salicylate	30.9	1344	0.1	0.1	0.2	tr	a, b
β-Citronellol	31.0	1348	0.3	1.3	0.2	0.1	a, b
Nerol	31.2	1356	5.1	10.1	5.9	3.3	a, b
Geraniol	31.9	1381	5.9	6.5	4.5	5.4	a, b
(*Z*)-citral	32.3	1396	0.1	0.3	nd	tr	a, b
α-Cubebene	32.5	1404	0.3	0.5	nd	nd	a, b
(*E*)-citral	33.2	1430	0.1	0.3	nd	0.1	a, b
2-Phenoxyethanol	33.3	1433	0.3	0.1	0.5	tr	a, b
(*Z*)-α-bergamotene	33.4	1437	0.3	0.6	0.4	0.1	a
Neryl acetate	33.8	1452	1.6	2.9	0.6	0.3	a, b
β-Cubebene	34.1	1463	0.6	0.8	nd	nd	a
Geranyl acetate	34.4	1474	1.4	1.0	0.3	0.4	a, b
(*E*)-α-bergamotene	34.9	1493	2.5	4.3	6.8	1.3	a, b[a]
Pentadecane	35.1	1500	0.3	1.1	0.7	0.4	a
Unidentified sesquiterpene	35.3	1508	0.6	1.4	1.3	0.3	a
β-Caryophyllene	35.5	1517	1.0	2.3	1.8	0.1	a, b
Unidentified sesquiterpene	35.6	1521	0.1	0.2	0.2	0.1	a
Benzyl-3-methylbutanoate	35.8	1529	0.3	1.0	1.6	0.2	a
Unknown	36.1	1542	0.2	0.3	1.5	0.2	
α-Humulene	36.5	1558	0.4	0.5	0.3	0.2	a, b
Bicyclosesquiphellandrene	37.2	1588	0.7	1.3	nd	nd	a
β-Sesquiphellandrene	37.5	1600	0.3	1.0	1.2	0.2	a, b[a]
Unknown	39.6	1688	0.2	0.5	0.2	0.2	
Heptadecane	39.9	1700	0.1	0.1	0.2	tr	a

[a] RRI means relative retention index based on constituent alkanes and the experimentally determined retention time of 4.8 min for pentane.

average accounted for over 41% of the total volatiles. Within this group, the acyclic compounds predominated with cyclic monoterpenes accounting for less than 2% of the total volatiles. The major monoterpene hydrocarbons were identified as (*Z*) and (*E*)-β-ocimene, with the latter isomer being most abundant. (*E*)-β-ocimene has previously been reported as the major constituent of floral scents from other leguminous flowers including faba beans (*Vicia faba*) (Sutton, Keegans, Kirk, & Morgan, 1992) and alfalfa

(*Medicago sativa*) (Loper, & Berdel, 1978). It has also been found in the floral bouquet of red clover (*Trifolium pratense*) (Buttery, Kamm, & Ling, 1984) but was not detected in white clover flowers (*Trifolium repens*) (Jakobsen, & Olsen, 1994). Although considerable differences were observed in the relative amounts of (*E*)-β-ocimene released by the three cultivars, it should be noted that large differences were also observed between the early and late season samples of Royal Wedding. This would suggest that environmental and/or developmental stages may have a significant effect on volatile production. Similar seasonal fluctuations in (*E*)-β-ocimene production have been reported (Loper, & Berdel, 1978) for alfalfa flowers. Additionally in a study (Neilsen, Jakobsen, Friis, Hansen, & Møller, 1995) of the volatiles released by *Hesperis matronalis* flowers considerable interplant variations were observed. In most samples (*E*)-β-ocimene and linalool were reported as the major constituents of the floral volatiles but some inflorescences did not emit these compounds at all.

Three acyclic monoterpene alcohols, linalool, geraniol and nerol accounted on average for 34% of the total volatiles detected, with linalool accounting for almost two thirds of the total. This alcohol has been detected in the floral scents of a wide range of plant species (Knudsen, Tollsten, & Bergström, 1993) including alfalfa (Loper, 1972) as well as in the head space of faba bean foliage (Blight, Pickett, Smith, & Wadhams, 1984).

In all three cultivars a number of previously reported aromatic compounds were detected and together accounted for on average 5.5% of the total headspace. These included aldehydes, alcohols and esters, with phenylacetaldehyde being consistently the most abundant. Aromatic compounds have previously been shown to be present at high concentrations in the floral bouquet of other leguminous flowers such as white (Jakobsen, & Olsen, 1994) and red clovers (Buttery et al., 1984).

On the basis of their mass spectral characteristics (*m*/*z* 204, 93) a total of 10 sesquiterpene hydrocarbons were identified which together accounted for between 2.4 and 12.9% of the total volatiles detected. As with ocimene large variations in the relative concentrations were observed between the early and late season sampling again suggesting that further studies are required before meaningful comparisons can be made between cultivars.

In all three cultivars, the predominant sesquiterpene was identified as (*E*)-α-bergamotene and all also contained β-caryophyllene, α-humulene, β-sesquiphellandrene and a further three unidentified sesquiterpenes with retention times of 33.3, 35.3 and 35.6 min. The sesquiterpene (Rt 33.3 mins) was tentatively identified on the basis of its mass spectrum as (*Z*)-α-bergamotene. The remaining two sesquiterpenes common to all cultivars at (Rt 35.3) show EIMS (70 eV), *m*/*z* (rel int): 204 [M^+](8), 189(2), 175(1), 161(31), 147(4), 133(24), 120(23), 109(17), 105(20), 93(55), 91(40), 82(15), 79(26), 77(26), 71(19), 69(100), 67(21), 57(22), 55(33), 53(12), 41(67); and at (Rt 35.6) EIMS (70 eV), *m*/*z* (rel int): 204 [M^+](7), 189(2), 175(2), 161(28), 147(6), 133(24), 120(21), 109(15), 105(23), 93(47), 91(40), 82(16), 79(27), 77(25), 71(18), 69(100), 67(21), 57(24), 55(32), 53(13), 41(73). These mass spectra are virtually identical and closely resemble that of β-sesquiphellandrene (Rt 37.5) and may be positional double bond isomers of this compound.

Three structurally similar sesquiterpenes, identified as α-cubebene (Rt 32.5), β-cubebene (Rt 34.1) and bicyclosesquiphellandrene (Rt 37.2) were detected in the floral bouquet produced by Royal Wedding. These compounds were not detected in the headspace from flowers of the other two cultivars, Diana and Old Times.

During the normal growth of sweet peas in particular, there is a marked change in the intensity of the floral bouquet associated with the opening of the flowers. Thus, although some aroma is evident with the unopened flowers, it is more characteristic of the type of scent associated with the growth of green plants. Analysis of the head space of unopened sweet pea flowers (cultivar, Royal Wedding) revealed that the predominant compound was (*Z*)-3-hexenol, which is a well documented (Visser, van Staten, & Maarse, 1979) component of green leaf volatiles. However all the major mono- and sesquiterpene hydrocarbons detected in the fully opened flowers were identified (data not shown) clearly demonstrating the onset of terpene biosynthesis at this early stage of flower development.

From this study it is clear that, as expected, the volatile profile of sweet pea flowers consists of a complex mixture, with a total of 48 compounds being detected in quantifiable amounts. The profiles of three cultivars were broadly similar with (*E*)-β-ocimene and linalool the predominant compounds. It is also clear that before any meaningful intervarietal comparisons based on the relative concentrations of individual compounds can be made further studies are required to determine how these levels are affected by the developmental stage of flower and plant as well as environmental conditions. It is however, of interest that in this limited survey of only three cultivars, there is evidence for intervarietal variation with respect to sesquiterpene synthesis. This would suggest that a wider survey might possibly open up new avenues for the study of both the genetic control of sesquiterpene synthesis and their contribution to the overall fragrance of sweet pea flowers.

3. Experimental

3.1. Sampling of volatiles

Sweet pea cultivars Royal Wedding, Diana and Old Times (Unwins Seeds, UK) were grown using normal cultural practice in greenhouses and were free from disease or parasitic infestations. In each trapping experiment, 10 freshly cut flowers in a conical flask containing water, were placed in a 2-l glass vessel equipped with a flanged top with four ports, which had previously been cleaned and baked overnight at 200°. Stainless steel columns (89 × 6 mm, Perkin-Elmer, Beaconsfield, UK), containing 250 mg of the 2,6-diphenyl-*p*-phenylene oxide porous polymer Tenax-TA (60–80 mesh), were preconditioned by washing with purified Et_2O and passing oxygen scrubbed helium through them for 16 h at 250°. Entrainment of the volatiles was carried out over a period of 8 h as previously described (Robertson, Griffiths, Macfarlane-Smith, & Butcher, 1993). Following the collection of samples the tubes were eluted with 2 ml of purified Et_2O and the volume of the eluate was reduced to 0.1 ml in N_2 just prior to analysis.

3.2. Gas chromatography/mass spectrometry

The eluted volatiles were analysed using a Hewlett Packard 5989B gas chromatograph/mass spectrometer. Samples of 1 μl were directly introduced into the capilliary column using the cold on column technique to minimise the potential thermal breakdown associated with conventional hot vaporising injectors. Chromatographic separation was achieved using a DB 1701column (60 m × 0.25 mm i.d.; 1.0 μm film; J&W Scientific, Fulsom, CA 95630, USA) with a helium carrier gas flow rate of 1 ml/min in constant flow mode using electronic pressure programming. The oven temperature programme was: 40°, increased at 5°/min to 280° and held isothermally for 5 min. The quadrupole mass spectrometer was scanned over the range 20–300 a.m.u. at 1 scan/s. The combined EI/CI source was used in Electron Impact mode with an ionisation energy of 70 eV and a trap current of 300 μA. Source temperature was held at 250°. GC–MS data were processed using the Hewlett Packard G1034 MA Chemstation. Component identification was carried out using the Wiley 138 K mass spectral database and from retention times and mass spectra of authentic standards when available. For two components (*E*)-α-bergamotene and β-sesquiphellandrene, mass spectral identification was confirmed from analysis of essential oil samples of bergamot (*Citrus bergamia*) and ginger (*Zingiber officinale*) respectively.

References

Anonis, D. P. (1988). *Perfumery and Flavourist*, *13,* 65.

Blight, M. M., Pickett, J. A., Smith, M. C., & Wadhams, L. J. (1984). *Naturwissenschaften*, *71,* 480.

Buttery, R. G., Kamm, J. A., & Ling, L. C. (1984). *Journal of Agricultural and Food Chemistry*, *32,* 254.

Dumont, H. M. (1938). *Soap, Perfumery and Cosmetics*, *11,* 428.

Jakobsen, H. B., & Olsen, C. E. (1994). *Planta*, *192,* 365.

Knudsen, J. T., Tollsten, L., & Bergström, L. G. (1993). *Phytochemistry*, *33,* 253.

Loper, G. M. (1972). *Phytochemistry*, *11,* 1865.

Loper, G. M., & Berdel, R. M. (1978). *Crop Science*, *18,* 447.

Neilsen, J. K., Jakobsen, H. B., Friis, P., Hansen, K., & Møller, C. E. (1995). *Phytochemistry*, *38,* 847.

Raguso, R. A., & Pellmyr, O. (1998). *OIKOS*, *81,* 238.

Robertson, G. W., Griffiths, D. W., Macfarlane-Smith, W. H., & Butcher, R. D. (1993). *Phytochemical Analysis*, *4,* 152.

Sexton, R., Porter, A. E., Littlejohn, S., & Thain, S. C. (1995). *Annals of Botany*, *75,* 337.

Sutton, C. S., Keegans, S. J., Kirk, W. D. J., & Morgan, E. D. (1992). *Phytochemistry*, *31,* 3427.

Visser, J. H., van Staten, S., & Maarse, H. (1979). *Journal of Chemical Ecology*, *5,* 13.