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# ASYNCHRONOUS RHYTHMS IN THE EMISSION OF VOLATILES FROM *HESPERIS MATRONALIS* FLOWERS

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**Key Work Index**—*Hesperis matronalis*; Cruciferae; dame's violet; flower fragrance; rhythmic emission; monoterpenes; benzyl acetate; attractants.

**Abstract**—Twelve monoterpenes and five aromatic compounds were identified from *Hesperis matronalis* flowers *in situ*. The most abundant compounds were 1,8-cineole, *E*-β-ocimene, linalool, and benzyl acetate. These four compounds were released asynchronously by the flowers during a photoperiod of 15 hr light and 9 hr darkness: 1,8-cineole was released mainly during the light period, *E*-β-ocimene mainly near the switch from light to darkness, and benzyl acetate mainly during the dark period. Linalool was not released rhythmically. Release rates of monoterpenes were higher at 20° than at 15°, while release rates of the aromatic compounds were not significantly different at these temperatures. The role of flower volatiles in host plant finding by a specialized *Hesperis* feeding weevil, *Ceutorhynchus inaffectatus*, is discussed.

## INTRODUCTION

The genus *Hesperis* (Cruciferae) contains about 30 biennial or perennial species which are found mainly in east Mediterranian areas [1, 2]. *Hesperis matronalis* L. is the only species growing in northern and western Europe where it was introduced before the 16th century [1]. In Denmark it is a rare plant [3] (Nielsen, unpublished) growing mainly in semi-shaded habitats in open woods, along hedges, railroads or streams, often close to cultivated areas. It is a rather conspicious plant, 40–100 cm high with large white, pink or purple flowers. Especially in the evening, the flowers release a strong, 'violet-like' fragrance [1] as expressed in many common names like: dame's violet (English), night violet and evening star (translation of German and Danish names).

*H. matronalis* is the only host plant of a specialized weevil, *Ceutorhynchus inaffectatus* Gyll. (Coleoptera: Curculionidae) in northern and western Europe [4, 5]. This weevil seems to be very efficient in locating its rare host plant. Small plant patches covering less than 5 $m^2$ are often colonized even when separated by several kilometers from other large populations (Nielsen, unpublished). Attractants from the flowers seem to be important for colonization, since the weevils are attracted to flowers, but not to leaves of *H. matronalis* in olfactometer as well as field assays (unpublished results). Feeding occurs on leaves as well as on flowers and flower buds. Females oviposit in the young pods and the larvae feed on the seeds [4].

The present study was initiated in order to study host plant colonization by *C. inaffectatus*. Volatiles emitted from flowers of *H. matronalis* were identified and the effect of some environmental factors (light–dark cycle temperature) on emission rates were investigated. A comparison was made between plants with white and pink flowers from the same plant population, since the white flowers often seemed to have a more 'resinous' fragrance than the pink flowers.

## RESULTS

Monoterpenes and aromatic esters and alcohols were the predominant compounds in the fragrance emitted from *H. matronalis* flowers (Table 1). Monoterpenes were emitted from flowers *in situ* as well as from detached flowers while the aromatic compounds were emitted mainly from flowers *in situ* (Table 1). Large inter-plant variations were observed in the composition of the fragrance, e.g. in the emission of *E*-β-ocimene and linalool which were predominant compounds in most samples, but some inflorescences did not emit these compounds at all (Table 1). No significant differences were found in the composition of the fragrance from white and pink flowers (*t*-test performed on data for all individual compounds listed in Table 1; $P > 0.05$).

Many compounds were released in a rhythmic manner during a 24 hr period. Emission rates of six monoterpenes, β-pinene, sabinene, myrcene, limonene, 1,8-

Table 1. Composition of the fragrance emitted from flowers of *Hesperis matronalis* at 20°

| Compound | Retention times (min) | Flowers *in situ* Mean (%) | Flowers *in situ* Range* (%) | Detached flowers Mean (%) |
|---|---|---|---|---|
| Monoterpenes | | | | |
| α-Pinene | 10.0 | 7.6 | 0.2–27.6 | 4.9 |
| β-Pinene | 13.2 | 1.7 | 0.0– 6.2 | 2.3 |
| Sabinene | 13.8 | 5.4 | 1.0–22.4 | 6.5 |
| Myrcene | 15.7 | 1.6 | 0.0– 4.3 | 5.3 |
| Limonene | 17.7 | 0.4 | 0.0– 2.1 | 1.7 |
| 1,8-Cineole | 18.1 | 10.2 | 4.4–25.2 | 17.8 |
| *Z*-β-Ocimene | 19.5 | 3.3 | 0.0–14.5 | 2.5 |
| *E*-β-Ocimene | 20.5 | 23.6 | 0.1–70.2 | 44.3 |
| *Z*-linalool oxide | 30.4 | 0.6 | 0.0– 2.3 | 0.6 |
| *E*-linalool oxide | 32.5 | 2.7 | 0.0– 4.6 | 0.7 |
| Linalool | 35.3 | 19.9 | 0.0–45.0 | 9.8 |
| α-Terpineol | 42.2 | 2.0 | 0.0– 6.4 | 0.4 |
| Aromatic compounds | | | | |
| Phenylacetaldehyde | 40.4 | 0.7 | 0.0– 4.0 | 0.1 |
| Benzyl acetate | 43.8 | 10.7 | 0.7–17.5 | 2.3 |
| Phenylethyl acetate | 47.5 | 1.3 | 0.0– 3.9 | 0.3 |
| Benzyl alcohol | 49.4 | 5.0 | 0.3– 9.8 | 0.3 |
| Phenylethyl alcohol | 50.9 | 3.0 | 0.0– 9.7 | 0.2 |

*The range expresses differences between inflorescences in daily emission of volatiles—not differences between samples collected at different times of the day.

cineole, and *Z*-β-ocimene were significantly higher during the light than during the dark period at 20° (Table 2; Fig. 1). Emission of these compounds was more or less synchronized with maximum emission rates in the middle of the period. *E*-β-ocimene on the other hand showed maximum emission rates during the last 6 hr of the light period and the first 3 hr of the dark period (Fig. 1). Differences between the maximum release rates (6 hr before to 3 hr after switching off the light) and the minimum release rates (last 3 hr in darkness and first 3 hr in the light period) were significant ($P < 0.05$). For other monoterpenes, e.g. α-pinene, α-terpineol, linalool and the two linalool oxides, no significant rhythmicity was observed (Table 2). The emission rates of two aromatic alcohols and their acetates were significantly higher during the dark than during the light period (Table 2; Fig. 1). These compounds were virtually absent in samples collected during the day, but especially benzyl acetate was one of the predominant compounds in the fragrance emitted at night.

The composition of the fragrance was similar at 15° (data not shown) and 20° (Table 1). Rhythmic emission of the monoterpenes was less conspicuous at 15° than at 20°. A significant rhythmicity at 15° could be demonstrated only in 1,8-cineole, although other compounds (e.g. *E*-β-ocimene; Fig. 1) tended to have emission maxima and minima at the same times during the photoperiod at both temperatures. A rhythmic emission of the aromatic compounds was evident (and significant, $P < 0.05$) at both temperatures (Fig. 1). Emission rates of several monoterpenes were higher at 20° compared to 15°, while there

Table 2. Influence of light-darkness cycle* on emission rates (ng flower$^{-1}$ hr$^{-1}$) of volatiles from flowers of *Hesperis matronalis* at 20°

| Compounds | Light Mean ± s.e. | Darkness Mean ± s.e. | *P*† |
|---|---|---|---|
| Monoterpenes | | | |
| α-Pinene | 44.5 ± 11.90 | 42.6 ± 12.47 | 0.1052 |
| β-Pinene | 11.2 ± 1.97 | 7.2 ± 1.51 | 0.0017[a] |
| Sabinene | 26.9 ± 4.12 | 19.1 ± 3.19 | 0.0002[a] |
| Myrcene | 11.3 ± 2.23 | 8.9 ± 1.89 | 0.0232[a] |
| Limonene | 3.5 ± 0.79 | 1.8 ± 0.76 | 0.0045[a] |
| 1,8-Cineole | 59.4 ± 5.13 | 44.9 ± 5.49 | 0.0001[a] |
| *Z*-β-Ocimene | 17.3 ± 3.33 | 10.1 ± 2.25 | 0.0003[a] |
| *E*-β-Ocimene | 178.5 ± 44.16 | 150.6 ± 38.55 | 0.1827[a] |
| *Z*-Linalool oxide | 5.4 ± 2.88 | 7.2 ± 3.65 | 0.1894 |
| *E*-Linalool oxide | 19.6 ± 4.61 | 17.8 ± 5.07 | 0.3195 |
| Linalool | 172.5 ± 61.53 | 195.6 ± 66.72 | 0.1394 |
| α-Terpineol | 15.0 ± 4.67 | 15.2 ± 4.39 | 0.9120 |
| Aromatic compounds | | | |
| Phenylacetaldehyde | 0.4 ± 0.35 | 15.3 ± 8.74 | 0.1049[a] |
| Benzyl acetate | 12.6 ± 3.56 | 160.3 ± 40.60 | 0.0020[a] |
| Phenylethyl acetate | 3.0 ± 1.86 | 23.2 ± 8.89 | 0.0168[a] |
| Benzyl alcohol | 8.7 ± 2.08 | 60.2 ± 14.47 | 0.0022[a] |
| Phenylethyl alcohol | 8.5 ± 3.27 | 44.4 ± 13.33 | 0.0060[a] |

*Light emission is the mean emission rates during a 9 hr period from 3–12 hr after the light was switched on; darkness emission is the mean emission during the 9 hr dark period.

†Significance levels according to paried *t*-test; 'a' indicates that a rhythmic emission can be demonstrated by ANOVA (repeated measures design) on data from individual 3 hr samples ($P < 0.05$).

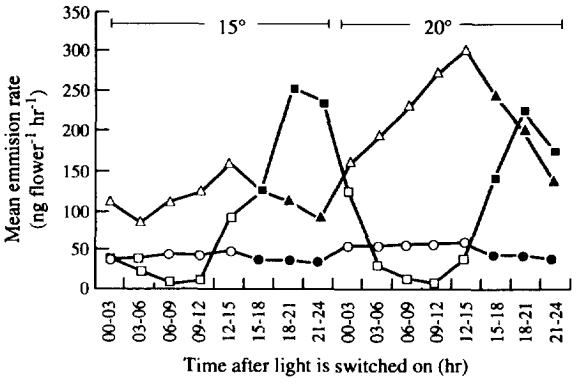


Fig. 1. Rhythmic emission of three volatiles from *Hesperis matronalis* flowers during two 24 hr periods. Data from all plants which had significant emission rates of a given compound ( > 20 ng flower$^{-1}$ hr$^{-1}$ for 24 hr). Symbols: (○) 1,8-cineole, (△) *E*-β-ocimene, (□) benzyl acetate. Shading of symbols indicates samples collected during the dark periods.

Table 3. Influence of temperature on daily emission rate (μg flower$^{-1}$ day$^{-1}$) of volatiles from flowers of *Hesperis matronalis*

| Compounds | 15° Mean ± s.e. | 20° Mean ± s.e. | *P** |
|---|---|---|---|
| Total volatiles | 13.0 ± 1.96 | 17.1 ± 3.40 | 0.0470 |
| Monoterpenes | 9.4 ± 1.36 | 13.3 ± 2.55 | 0.0281 |
| Aromatic compounds | 3.6 ± 0.73 | 3.8 ± 0.95 | 0.3166 |
| Sabinene | 0.5 ± 0.09 | 0.6 ± 0.09 | 0.0219 |
| 1,8-Cineole | 1.0 ± 0.06 | 1.3 ± 0.13 | 0.0039 |
| *E*-β-Ocimene | 2.7 ± 0.67 | 4.0 ± 0.98 | 0.0452 |
| Linalool | 3.0 ± 0.92 | 4.5 ± 1.58 | 0.0510 |
| α-Terpineol | 0.2 ± 0.04 | 0.4 ± 0.10 | 0.0354 |
| Benzyl acetate | 2.2 ± 0.49 | 1.9 ± 0.46 | 0.3293 |

*Significance levels according to paired *t*-test; no significant temperature effects were found on the emission rates of other volatiles listed in Table 1.

were no significant differences in the emission rates of any of the aromatic compounds at these two temperatures (Table 3; Fig. 1).

## DISCUSSION

All the volatiles released from *H. matronalis* flowers are widely distributed in plants [6]. Therefore, it is unlikely that the monophagous weevil, *C. inaffectatus* is attracted to its host plant by any single compound in the fragrance, but it may respond to a mixture of several compounds as described in another *Ceutorhynchus* species feeding on oilseed rape [7, 8]. Dependence on any single compound for the attraction of the weevil to its host plant would be an unreliable strategy, since the composition of the fragrance is very variable and some of the predominant compounds, e.g. *E*-β-ocimene and linalool, may be completely absent in the fragrance from some plants (Table 1). A similar variation between plants from the same population has been reported previously in other species [9–11]. Variability in fragrance emission might confuse herbivores and reduce colonization rates [12]. It is assumed that the effect of *C. inaffectatus* is largely unfavourable to the plant, although beetles may also act as pollinators [13].

Changes in volatile emission may occur during the life span of an individual flower and as a result of pollination [14]. These factors were probably unimportant for the inter-plant variability in the present study, since inflorescences always contained 6–14 open flowers of different ages. *H. matronalis* plants with different flower colour are sometimes treated as separated subspecies [2]. The value of flower colour as a taxonomic character in this species seems doubtful since the white and pink flowered plants used in the present study originated from the same natural locality, and no significant differences were found in the fragrance composition of plants with different flower colour.

Emission rates of monoterpenes increased when the temperature was raised from 15 to 20°, while the emission of aromatic compounds was unaffected (Table 3). In *Trifolium repens* flowers which release mainly aromatic compounds, emission rates at 15 and 20° were not significantly different, but emission rates at both these temperatures were higher than at 10° [15].

Three of the major constituents of the fragrance, *E*-β-ocimene, 1,8-cineole and benzyl acetate were emitted in a rhythmic manner during a 24-hr period, while a fourth major compound, linalool, was not. This observation suggests that different mechanisms regulate the emission and/or biosynthesis of these four compounds. One possibility could be that the compounds are produced separately in different flower parts [16]. The inter-plant variation mentioned above adds further evidence to the presence of different regulatory mechanisms for these four compounds since some of them may be lacking in some plants while other compounds may be released at normal rates. Asynchronous emission of volatiles from different biosynthetic pathways have been described in other species [17–19]. Increased emission of aromatic compounds during the night has been demonstrated in *Nicotiana sylvestris* [18].

Most of the monoterpenes identified in the present study as well as benzyl alcohol, phenylethanol and phenylacetaldehyde have been identified from flower fragrances of other Cruciferae [7, 8, 20–22], but α-terpineol, the two linalool oxides, benzyl acetate and phenylethyl acetate have not earlier been reported as flower volatiles from members of Cruciferae. A rhythmic emission of volatiles has not previously been described in flowers of crucifers. Most previous investigations on fragrances from crucifers used abscised plants and are not directly comparable to our investigations of flowers *in situ*. The proportion of aromatic compounds in the fragrance was higher in plants on the root than on abscised flowers (Table 1). Significant differences between growing plants and detached flowers have been documented in other plant species [23]. No sulphur containing compounds or other degradation products of glucosinolates were detected in the

flower fragrances of *H. matronalis*, although they were detected in flower fragrances from other crucifers [7, 20–22]. However, the predominant glucosinolates in *H. matronalis* do not yield volatile isothiocyanates or nitriles [24].

The identification of monoterpenes in flower fragrances of crucifers is also interesting in relation to scent substances found in specialized scales on the wings of male pierid butterflies (Lepidoptera: Pieridae) which feed on crucifers and related species during their larval stages. *E*-β-ocimene has been found in the scent scales of *Hebomoia glaucippe* L. [25] and other monoterpenes have been identified from cabbage white butterflies (*Pieris* spp.) [26–28]. The scent scales are probably important in courtship behaviour [26]. Therefore, the relation between crucifer feeding insects and their host plants may be parallel to the situation in bark beetles, where aggregation pheromones mimic compounds found in the host plants, and sometimes the pheromones are even synthesized from precursors obtained from the plants [29]. Aggregation and sexual pheromones have not yet been described in *Ceutorhynchus* species, but they are present in other weevils [30].

## EXPERIMENTAL

*Plants.* Seeds of *H. matronalis* were collected in a wood near Nyvang (north of Copenhagen) and sown in the field at the agricultural experimental station Højbakkegaard, Taastrup. Plants used for collection of volatiles from detached flowers were left to overwinter in the field, and flowers were collected in June. Plants used for collection of volatiles from flowers *in situ* were transferred in the autumn (in the rosette stage) to 20 l buckets containing a peat–soil mixt. and stored at 4°. After at least 2 months of vernalization, these plants started flowering after being transferred to temps used in the present investigations.

*Chemical analysis.* GC used a fused silica WCOT capillary column (Carbowax 20M; 50 m × 0.32 mm i.d.); carrier gas (He) flow 1 ml $min^{-1}$; injection temp. 200°; FID temp. 250°; oven 70° for 10 min, 70–190° at 3° $min^{-1}$. Inlet split 1:10. GC-MS was performed on a Varian MAT 311 A mass spectrometer with a SS 200 data system or a JEOL-JMS AX 505W mass spectrometer with data system running under Jeol's complement software. MS library suggestions were verified with respect to retention times and mass spectra by running authentic ref. compounds under the same conditions.

*Collection of volatiles from plants* in situ. One inflorescence containing 6–14 flowers was inserted into a 1.6 l glass reaction vessel with the openings turning downwards. Teflon stoppers sealed with Terrostat (Terrosan, Heidelberg) or with molten paraffin were used to keep the system air tight. A constant flow of 350 ml $min^{-1}$ through the vessel was achieved by a vacuum pump. Inlet air was cleaned through a 10 l filter with granulated charcoal. Volatiles were collected on 100 mg Porapak Q 50–80 mesh in glass tubes (4 mm i.d., length 180 mm). A series of 8 tubes was inserted between the vessel with the flower and the vacuum pump. An 8 channel clock controlled the air flow through the tubes by means of magnetic valves. Collection time on each column was 3 hr, and every 3 hr the air flow was directed to a new tube containing Porapak. In this way 8 samples were collected during a 24 hr period, each sample containing the volatiles collected during a 3 hr period. Collections started at 9 a.m. when the light was switched on. Five samples were collected during the 15 hr light period, and 3 samples during the 9 hr dark period starting at midnight. Each plant was sampled in 2 light–dark cycles, first at 15° and next day at 20°. Further details on the device for collection of volatiles from plants *in situ* are given in ref. [15].

Collections took place in a growth chamber equipped with 23 Philips 400 W HPI/T lamps and 15 standard 100 W light bulbs which supplied 570 μmol quanta $m^{-2}$ $sec^{-1}$ measured at the level of the inflorescence. Plants were transferred to the growth chamber at least 3 days before the collection of volatiles began.

Volatiles were eluted from the Porapak with redistilled $Et_2O$ (250 μl) into micro vials. The samples were concd to a small wt (40 mg) in a gentle $N_2$ air stream. An int. standard (4.07 μg 4-methyl-1-pentanol in 5 μl $Et_2O$) was added to the concd samples before 0.5 μl was analysed by GC. Estimates of amounts of the compounds were based on peak areas neglecting individual compound response factors.

*Collection of volatiles from detached flowers.* Individual flowers (40 g fr. wt) were cut from the inflorescences in the field and immediately transferred to a 500 ml glass washing bottle. A gas mixt. (21% $O_2$, 79% $N_2$) was purged over the flowers at 80 ml $min^{-1}$ for 14–20 hr (including a dark period) at ambient light and temp. conditions. The emerging gas was led through 300 mg Porapak Q (50–80 mesh) in a glass tube (0.3 cm i.d.; length 150 mm). The retained volatiles were eluted from the Porapak with 400 mg double distilled $Et_2O$, which was concd and analysed as described above.

*Statistical analysis.* Rhythmic emission was tested by analysis of variance (ANOVA) using repeated measures design [31]. Differences between individual samples was tested by Duncan's multiple range test after ANOVA as described above. All other analyses used common methods.

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## REFERENCES

1. Hegi, G. (1986) *Illustrierte Flora von Mitteleuropa.* Band IV, Teil 1, V. Paul Parey, Berlin.
2. Tutin, T. G., Burges, N. A., Chater, A. O., Edmond-

son, J. R., Heywood, V. H., Moore, D. M., Valentine, D. H., Walters, S. M. and Webb, D. A. (eds) (1993) *Flora Europaea* **1**, 2nd Edn. Cambridge University Press, Cambridge.
3. Hansen, K. (ed) (1981) *Dansk Feltflora.* Nordisk Forlag, Copenhagen.
4. Dieckmann, L. (1972) *Beitr. Entomol.* **22**, 3.
5. Hoffmann, A. (1954) Coleoptères Curculionides II. *Faune de France* **59**, 487.
6. Knudsen, J. T., Tollsten, L. and Bergström, G. (1993) *Phytochemistry* **33**, 253.
7. Evans, K. A. (1992) in *Proc. 8th Int. Symp. Insect–Plant Relationships* (Menken, S. B. J., Visser, J. H. and Harrewijn, P., eds), p. 107. Kluwer, Dordrecht.
8. Evans, K. A. and Allen-Williams, L. J. (1992) *J. Chem. Ecol.* **18**, 1641.
9. Moya, S. and Ackerman, J. D. (1993) *Nord. J. Botany* **13**, 41.
10. Patt, J. M., Rhoades, D. F. and Corkill, J. A. (1988) *Phytochemistry* **27**, 91.
11. Tollsten, L. and Bergström, G. (1993) *Nord. J. Botany* (in press).
12. Visser, J. H. (1986) *Ann. Rev. Entomol.* **31**, 121.
13. Gottsberger, G. (1977) *Plant Syst. Evol.* Suppl. **1**, 211.
14. Tollsten, L. and Bergström, J. (1989) *Nord. J. Botany* **9**, 359.
15. Jakobsen, H. B. and Olsen, C. E. (1994) *Planta* **192**, 365.
16. Dobson, H. E. M., Bergström, G. and Groth, I. (1990) *Isr. J. Botany* **39**, 143.
17. Altenburger, R. and Matile, P. (1990) *Planta* **180**, 194.
18. Loughrin, J. H., Hamilton-Kemp, T. R., Andersen, R. A. and Hildebrand, D. F. (1990) *Phytochemistry* **29**, 2473.
19. Loughrin, J. H., Hamilton-Kemp, T. R., Andersen, R. A. and Hildebrand, D. F. (1991) *Physiol. Plant* **83**, 492.
20. Blight, M. M., Hick, A. J., Pickett, J. A., Smart, L. E., Wadhams, L. J. and Woodcock, C. M. (1992) in *Proc. 8th Int. Symp. Insect–Plant Relationships* (Menken, S. B. J., Visser, J. H. and Harrewijn, P., eds), p. 105, Kluwer, Dordrecht.
21. Robertson, G. W., Griffiths, D. W., MacFarlane Smith, W. and Butcher, R. D. (1993) *Phytochem. Anal.* **4** (in press).
22. Tollsten, L. and Bergström, G. (1988) *Phytochemistry* **27**, 4013.
23. Mookherjee, B. D., Trenkle, R. W., Wilson, R. A., Zampino, M., Sands, K. P. and Mussinan, C. J. (1988) Flavors and Fragrances: A World Perspective (Lawrence, B. M., Mookherjee, B. D. and Willis, B. J., eds), *Devel. Food Sci.* **18**, 415. Elsevier, Amsterdam.
24. Larsen, L. M., Nielsen, J. K. and Sørensen, H. (1992) *Entomol. Exp. Appl.* **64**, 49.
25. Hayashi, N., Nishi, A., Murakami, T., Maeshima, K., Komae, H. and Sakao, T. (1985) *Z. Naturforsch.* **40C**, 47.
26. Bergström, G. and Lundgren, L. (1973) *Zoon* Suppl. **1**, 67.
27. Hayashi, N., Kuwahara, Y. and Komae, H. (1978) *Experientia* **34**, 684.
28. Kuwahara, Y. (1979) *Appl. Entomol. Zool.* **14**, 350.
29. Hughes, P. R. (1974) *J. Insect Physiol.* **20**, 1271.
30. Blight, M. M., Pickett, J. A., Smith, M. C. and Wadhams, I. J. (1984) *Naturwissenschaften* **71**, 480.
31. Cody, R. P. and Smith, J. K. (1987) *Applied Statistics and the SAS Programming Language*, 2nd Edn. Elsevier, New York.