Planta (1994) 192:365–371

Planta

Influence of climatic factors on emission of flower volatiles in situ

Henrik Byrial Jakobsen[1], **Carl Erik Olsen**[2]

[1] Department of Plant Biology, Plant Physiology and Anatomy Laboratory, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark
[2] Chemistry Department, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

Received: 7 April 1993 / Accepted: 23 July 1993

Abstract. A device has been developed for determining the influence of temperature, irradiance and relative air humidity on the emission of flower volatiles in situ. The compounds emitted from flowers of *Trifolium repens* L. are mainly products of cinnamic-acid metabolism. Phenethyl acetate was the dominant compound in the fragrance picture. Additionally, a number of sesquiterpenes were identified in the emissions. All compounds were emitted in a rhythmic manner with a maximum at 7–12 h after the light is switched on. Temperature had a strong effect on the the quantity of fragrance in the headspace. Emission at 10°C was significantly lower than at 15°C and 20°C. This difference can be attributed to a temperature effect on the secretion of volatiles rather than on the evaporation rate of volatiles. Light influenced fragrance emission significantly, the most intense emission being noted at high irradiances. No effect of relative humidity on fragrance emission could be detected. The composition of the fragrance picture was not influenced by the climatic factors. Emission was controlled by the light and dark intervals rather than by the endogenous clock.

Key words: Flower fragrance – Fragrance emission (light, temperature, humidity) – Rhythm (fragrance emission) – *Trifolium* (fragrance emission)

Introduction

The fragrance emitted from entomophilous flowers is distinctly different from that released from other parts of the plant. Furthermore, differences among fragrances emitted from the sepals, petals, gynoecium, anthers and pollen have been demonstrated (Dobson et al. 1987, 1990; Knudsen and Tollsten 1991). The origin and function of volatile emission has been subject to extensive discussion. It has been shown that flower volatiles, acting after diffusion through the air, affect a wide spectrum of organisms in the plant sphere. Fragrance emitted by night-pollinated flowers is conspicuously essential whether pollination is performed by bats (Howell 1977), mice (Lumer 1980) or nocturnal moths (Brantjes 1978). Several investigations have shown that diurnal pollinators use flower volatiles as orientation clues (Fægri and van der Pijl 1980; Metcalf 1987).

Apart from acting as synomones, volatiles function as kairomones. Flower volatiles are excellent cues for insect herbivores in their attempt to locate host plants for feeding or oviposition (Metcalf 1987). On the other hand, several of the compounds isolated from flowers have been shown to serve as deterrents against herbivores (Levin 1973; Rodriguez and Levin 1976; Wood 1982). Pellmyr and Thien (1986) suggested that floral volatiles originate from compounds which initially functioned as general herbivore deterrents. Insects which could bypass this repellent barrier were able to use the fragrance as an orientation clue. If these insects contributed to pollination of the flower a mutual adaptation could arise. This co-adaptation could include emission of fragrance during the period when the potential pollinators are active. Indeed, recent investigations have shown that several night-pollinated and day-pollinated species emit fragrance in a rhythmic fashion. Maximum emission coincides in all cases with the flight period of potential pollinators (Matile and Altenburger 1988; Loughrin et al. 1990, 1991).

It has been suggested that qualitative and quantitative changes in fragrance emission may indicate flower readiness for visitation or availability of nectar (Robacker et al. 1983, 1988) or pollen (Dobson et al. 1990) to pollinators. Observations in the field support this hypothesis. We have noticed that when approaching *Trifolium repens* flowers, a bee will often hover for a few seconds in front of a floret before entering or rejecting it without landing (see also Heinrich 1979). It is evident that the bee cannot visually judge the degree of pollen or nectar availability in the *T. repens* flower as the anthers and nectary are hidden behind the petals, which have to be parted before

Correspondence to: H.B. Jakobsen; FAX: (45) 28 33 33

nectar and pollen become visible. This behaviour has been observed in other plant species as well (Wetherwax 1986; Dobson et al. 1990). Whether the rejection or acceptance is caused by changes in the composition of emitted fragrance (Heinrich 1979; Robacker et al. 1983, 1988) or by terpene markings applied to the petals by visiting bees (Free and Williams 1983; Wilhelm and Pflumm 1983; Giurfa and Núñez 1992), it seems evident that fragrance is a key factor in the biology of pollination. An understanding of the possible signals inherent in changes of fragrance composition and intensity rests on a thorough knowledge of the emission as it occurs in a non-pollinated flower under the various changeable climatic conditions which are believed to affect the emission.

The composition of the fragrance picture emitted from *T. repens* flowers in situ and the quantitative and qualitative effects of temperature, light and relative humidity (RH) on the composition has not been described previously to our knowledge. However, steam-extraction of *T. repens* leaves and flower heads has been performed (Honkanen et al. 1969; Kameoka et al. 1977). Reports of temperature effects on emission from excised flowers transferred to a syringe chamber during collection have been published (Robacker et. al. 1983, 1988). However, as demonstrated by Mookherjee et al. (1986), prominent changes in scent emission occur rapidly when flowers are detached from the plant. Williams (1982) reported little or no scent emission, as perceived by the human nose, from orchids under greenhouse conditions on cold and overcast days, whereas abundant emission occurred on sunny and warm days. Williams also noted a correlation between odour emission and the activity of euglossine bees, and suggested a selective advantage for the emission of floral fragrance under conditions where pollinators are active.

The aim of the present study has been to develop a device and an assay which make possible qualitative and quantitative descriptions of the influence of temperature, irradiance and relative air humidity on the in-situ rhythmic emission of plant volatiles.

Materials and methods

Plants of *Trifolium repens* (cv. Milkanova, supplied by Danish Plant Breeding Ltd, St. Heddinge, Denmark) were grown in standard soil in 3.5-l pots, watered daily, and once a week supplied with 10 ml of a fertilizer solution (14.3 mg NH_4NO_3, 34.8 mg KH_2PO_4, 7 mg $MgSO_4 \cdot 7H_2O$, 0.075 mg $MnSO_4 \cdot H_2O$ and 0.08 mg H_3BO_3). A growth chamber (2.4 · 3 · 2.2 m) equipped with 23 Philips 400-W HPI/T lamps and 15 standard 100-W light bulbs (Phillips, Eindhoven, The Netherlands), which supplied 570 µmol quanta · m^{-2} · s^{-1}, measured at flower-head level. Low irradiance (280 µmol quanta · m^{-2} · s^{-1}) was achieved by drawing a thin white cotton curtain under the light source. The corresponding irradiance levels measured inside the glass bulb were 520 and 245 µmol quanta · m^{-2} · s^{-1}, respectively.

The device for fragrance collection is illustrated in Fig. 1. Two white clover heads, each with 15–20 open flowers, were guided into a 1.6-l reaction vessel through a 34- to 35-mm socket in the lid. After insertion of a solid Teflon stopper with two grooves allowing room for the flower stems, molten low-temperature-boiling paraffin wax was applied with a Pasteur pipette to the area above the stopper in order to seal the system air tight. Alternatively, Terrostat (Terosan, Heidelberg, FRG) was applied as sealing material. Inlet air was rinsed through a 10-l filter containing granulated charcoal (1–3 mm), which had been regenerated at 200°C under a nitrogen purge for 12 h. Temperature in the vessel was registered by a thermosensor connected to a thermostat which controlled a water pump placed in a water bath with a controlled water temperature. The pump was connected to the vessel cooling chamber. Relative humidity (RH) in the vessel was controlled by a series of eight magnetic valves in the inlet. Four were connected to dry air filtered through 5 l of silica gel (3–5 mm). The other four were connected to a source of saturated air. A probe in the vessel registered the actual RH and a recorder combined with a controller adjusted the opening and closing of the magnetic valves of the inlet. Relative air humidity in the growth chamber and collection chamber was controlled with an accuracy of ± 5%. Teflon tubes and fittings were used throughout the experiment. A constant flow of 350 ml · min^{-1} through the vessel was achieved by a Miniport N 75 KN18 vacuum pump (KNF Neuberger, Freiburg, FRG). Vacuum in the vessel was 1.1 kPa, measured by a glass tube with water inserted into the glass bulb (Fig. 1). Glass tubes (4 mm i.d., length 180 mm) equipped with 100 mg Porapack Q 50–80 mesh (Waters Inc., Milford, Mass., USA) inserted between two de-siloxanized glass-wool plugs were used for collection of volatiles. An eight-channel clock controlling eight magnetic valves inserted between the glass bulb and the pump allowed automatic collection on a series of columns. Unless otherwise specified, the collection time on each column was 3 h. Each 24-h period started at 9 a.m. when the light was switched on. The light period continued till midnight, allowing collection of five samples under light conditions, and three samples in the dark interval. Before insertion of the flower heads, 3-h samples from the empty bulb containing packing material (Terrostat or paraffin) were collected in order to test the air for contaminants. Methyl salicylate (1.12 µg in 10 µl diethyl ether) was injected into the column material as an internal standard (ISTD; Aldrich, Steinheim, FRG). Columns were eluted with redistilled diethyl ether into 100 µl micro vials, followed by careful evaporation of excess ether under nitrogen flow. After use, glass columns were eluted with 20 ml redistilled diethyl ether. The last 100 µl of the eluate was concentrated before gas chromatography (GC) testing for impurities. One microliter of each sam-

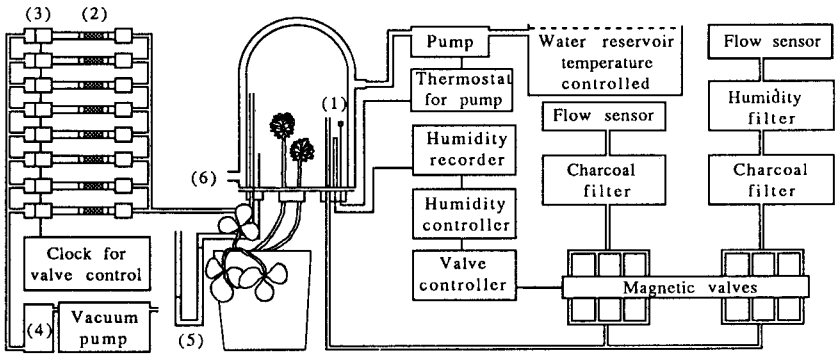

Fig. 1. Device for investigating the influence of climatic factors on the emission of fragrance from flowers in situ. 1, probes for temperature and RH measurement, inlet of filtered air; 2, porapack columns; 3, magnetic valves; 4, flow meter; 5, vacuum indicator; 6, water outlet

ple was injected onto the GC column. Gas chromatography was performed on a Hewlett-Packard (Palo Alto, Cal., USA) HP5890 gas chromatograph equipped with a Chrompack (Middelburg, The Netherlands) WCOT fused silica capillary column (25 m long, 0.25 mm i.d., DF = 1.2 µm liquid phase: Cp-sil 5 CB). Helium was applied as carrier gas, flow rate = 0.9 ml · min^{-1}, head pressure = 110 kPa, splitless purge time = 35 s. Oven program: 2 min at 34°C, followed by 40°C · min^{-1} to 50°C, then 4°C · min^{-1} to 220°C, and finally 10°C · min^{-1} to 250°C. The injection temperature was 185°C and the FID-detector temperature 285°C. Identification of compounds was performed on a JEOL-JMS AX 505W (JEOL Ltd. Akishima, Tokyo, Japan) mass spectrometer (MS). Compounds suggested by the MS-database were verified by comparison of the retention times of authentic reference compounds. Quantification of emitted volatiles was performed after calculation of standard curves and response factors for each compound, based on several samples and a range of concentrations. Quantification of a compound (x) in an unknown sample, was performed on the baiss of the formula: (area x/area ISTD) · response factor for x · amount ISTD in the sample. Statistical analysis of variation, using repeated measures design, was performed in all experiments. Each treatment was repeated five or more times.

Results and discussion

The fragrance of *T. repens* flowers consists mainly of aromatics from metabolism of cinnamic acid (87%). Phenethyl acetate is the most abundant compound emitted, constituting more than half the fragrance (Table 1). Several sesquiterpenes were observed, but only caryophyllene could be followed during the entire 24-h period. It was not possible to discern other sesquiterpenes from the noise signals under dark conditions, as the general level of emission was too low. Identified compounds are listed in Table 1.

To our knowledge, the composition of the fragrance emitted from flowers of *T. repens* in situ has not previously been described. However, 7 of the 16 compounds identified in the present investigation have been isolated from the steam distillate of *T. repens* flower heads. Honkanen et al. (1969) isolated 42 compounds by this method including benzaldehyde, benzyl alcohol, benzyl acetate, 2-phenethyl alcohol, phenethyl acetate and eugenol methylether. Honkanen et al. (1969) did not detect terpenes among the volatiles of the cell extracts. In a qualitative study Kameoka et al. (1977) identified 18 mono- and sesquiterpenes after steam distillation of *T. repens* flower heads. Only one of these compounds, caryophyllene, was detected in the present study.

All the identified major compounds of *T. repens* flower fragrance were emitted in a rhythmic manner under 15-h photoperiod conditions. Emission was two to three times higher in the middle of the day compared to the dark period ($P < 0.0001$, Table 2 and Fig. 2). The observed time of maximum emission coincided with the flight activity period of *Apis* and *Bombus* spp. which are the primary pollinators of *T. repens* (McGregor 1976).

The composition of the fragrance picture remained fairly constant during a 24-h period. All compounds which could be separated with certainty from the GC noise signals were emitted throughout the period. Two factors caused a minor deviation from the average rela-

Table 1. Identified volatiles from *Trifolium repens* flowers together with their relative contribution to the fragrance picture, and maximum emission of volatiles in relation to photoperiod initiation

	Identification method[a]	Proportion of fragrance	Main peaks of mass spectrum (M^+ in italics)	Maximum emission (h after photoperiod initiation)
Cinnamic-acid metabolism				
Benzaldehyde	MS and GC[b]	Trace	39 51 77 *106*	–
Benzyl alcohol	MS and GC[b]	8–12%	39 51 79 *108*	6–9
Phenethyl alcohol	MS and GC[b]	8–11%	51 65 92 *122*	6–9
Benzyl acetate	MS and GC[b]	5–8%	79 90 108 *150*	9–12
Phenethyl acetate	MS and GC[b]	54–60%	43 91 104	8–9
4-Ethylacetophenone	MS and GC[b]	1–2%	105 117 133 *148*	–
Eugenol methylether	MS and GC[b]	8–11%	107 147 163 *178*	9–12
Terpene metabolism				
Perillene	MS	Trace	41 69 107 *150*	–
β-Bourbonene	MS	Trace	81 123 161 *204*	–
δ-Cadinene	MS	Trace	134 161 189 *204*	–
β-Caryophyllene	MS and GC[c]	2–4%	133 161 189 *204*	12–15
Alloaromadendrene	MS	Trace	133 147 189 *204*	–
β-Cuvebene	MS	Trace	105 119 161 *204*	–
Thujopsene	MS and GC[c]	Trace	119 148 189 *204*	–
Other pathways				
2-Methyl cyclopentanone	MS	Trace	42 55 *98*	–
2-Ethyl 1-hexanol	MS and GC[b]	9–13%	43 57 83	6–9

[a] MS: Compounds suggested by the MS-database. GC: Compounds suggested by the MS-base verified by comparison with the retention time of authentic reference compounds
[b] Reference compound supplied by Aldrich, Steinheim, Germany
[c] Reference compound supplied by Roth, Basel, Switzerland

Table 2. The effect of temperature on day and night emission of volatiles from *T. repens* flowers. Compounds constituting more than 1% of the total fragrance composition are included in the test. Row-numbers with identical lettering are not significantly different at the 5% level

Response factor	*P* model	Emission ($ng \cdot flower^{-1} \cdot h^{-1}$)		
		10° C	15° C	20° C
Night emission	0.236	19.4	28.4	32.5
Day emission	0.012	45.5 a	66.9 b	70.3 b
Total emission	0.043	34.2 a	48.1 b	54.3 b

tive contribution of each compound listed in Table 1. Due to the smaller amplitudes of the emission peaks of eugenol methylether and caryophyllene, these compounds contributed relatively more to night emission than to day emission (Fig. 2). Furthermore, peak emission was not synchronised for compounds originating from different biosynthetic pathways (Table 1, Fig. 2). Emission of caryophyllene from terpene metabolism culminated later in the photoperiod than products from other pathways. Some deviation in the position of maximum emission was observed in most compounds. However, collection of volatiles at 1-h intervals showed a remarkable constancy in position of maximum emission of the dominating compound phenethyl acetate 7–8 h after photoperiod initiation (Fig. 3). The timing of minimum emission varied somewhat, but was usually positioned in one of the last two of the dark intervals (Fig. 2). Variation in timing of maximum emission has been reported previously in other species (Matile and Altenburger 1988; Altenburger and Matile 1990)

Temperature significantly influences the amount of fragrance emitted from *T. repens* flowers. Initial investigations showed that emission is a function of the prevailing temperature, and that emission is independent of temperature during the previous period (Fig. 2). The sensitivity to changes in temperature is emphasised by the observed higher emission in the last 3-h dark interval at 15°C than in the following light interval at 10°C. During constant temperature conditions, emission in the last dark interval was always lower than in the following light intervals (Fig. 2). On this basis, it was decided to sample each subject (group of flowers) randomly at the three temperatures without insertion of periods for adjustment to the new temperature. The generally prominent amplitudes in fragrance emission intensity show that the observed differences in amount of sampled fragrance at the three temperatures are caused by differences in emission rather than limited evaporation at low temperatures. If evapo-

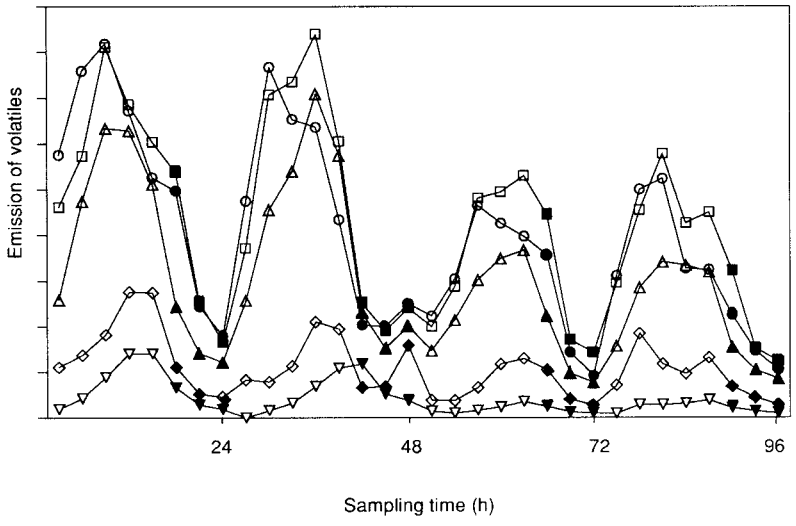

Fig. 2. Emission of five volatiles from *T. repens* flowers in four periods of 24 h. The temperatures were 15°C during the initial 48 h and 10°C during subsequent periods. *Filled symbols* indicate collection in darkness. □—□, 2-ethyl hexanol; ○—○, phenethyl alcohol; △—△, eugenol methylether; ◇—◇, benzyl acetate; ▽—▽, caryophyllene

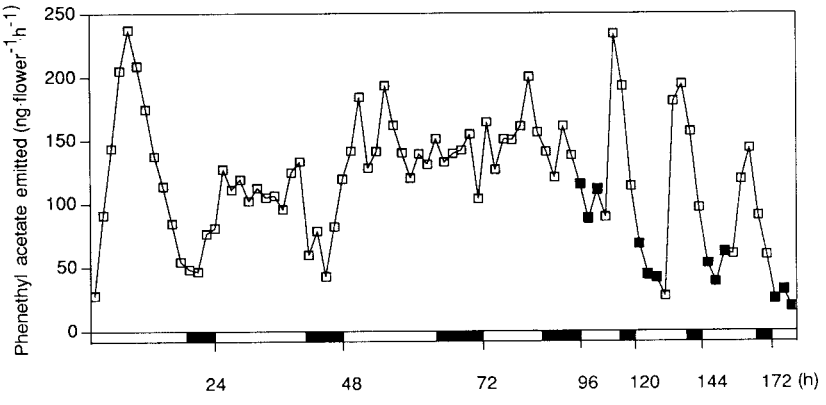

Fig. 3. Emission of phenethyl acetate after transfer from periods with 9-h dark intervals to 96 h in constant light, followed by three entrained 24-h periods with 9-h dark intervals. *Filled symbols* indicate collection in darkness. *Shading on the abscissa* indicates 9-h dark intervals under normal growth conditions

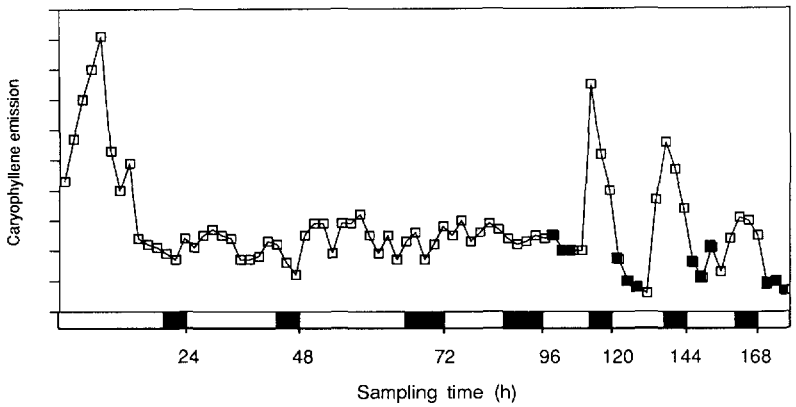

Fig. 4. Emission of caryophyllene after transfer from periods with 9-h dark intervals to 96 h in constant light, followed by three entrained 24-h periods with 9-h dark intervals. *Filled symbols* indicate collection in darkness. *Shading of the abscissa* indicates 9-h dark intervals under normal growth conditions

Table 3. The effect of irradiance on emission of volatiles from *T. repens* flowers. Measured at 20° C and 50% RH. Compounds constituting more than 1% of the total fragrance composition are included in the test

Response factor	*P* model	Emission ($ng \cdot flower^{-1} \cdot h^{-1}$)	
		245 µmol quanta $\cdot m^{-2} \cdot s^{-1}$	570 µmol quanta $m^{-2} \cdot s^{-1}$
Night emission	0.280	10.5	16.6
Day emission	0.027	61.2	120.2
Average emission	0.048	39.3	65.9

Table 4. The effect of relative air humidity on day and night emission of volatiles from *T. repens* flowers. Compounds constituting more than 1% of the total fragrance composition are included in the test

Response factor	*P* model	Emission ($ng \cdot flower^{-1} \cdot h^{-1}$)	
		45–55% RH	90–100% RH
Night emission	0.348	19.6	16.6
Day emission	0.136	144.3	96.5
Average emission	0.134	76.0	51.8

ration was the limiting factor at low temperatures, the amplitude would be expected to be insignificant at constant temperatures in periods with light/dark intervals (Fig. 2) and in constant darkness (Fig. 4).

Total emission of fragrance was 58% higher at 20°C than at 10°C (Table 2). All compounds were affected by the change in temperature (Fig. 2). No significant effect was found on night emission, whereas day emission was 54% higher at 20°C than at 10°C (Table 2).

Investigations on temperature influence on fragrance emission from detached flowers transferred to a syringe chamber for accumulation of volatiles have been performed in *Glycine max* (Robacker et al. 1983). Emission of one unidentified compound was higher at 20°C compared to 28°C and 32°C. In contrast, another unidentified compound was higher at 28°C and 32°C than at 24°C. No significant effect of night temperature was noted (Robacker et al. 1983). Quantification was made on the basis of peak height. No information on internal-standard application was given. It has been shown that the composition of emitted fragrance changes significantly when the flowers are detached from the plant (Mookherjee et al. 1986). Furthermore, effects on the quantitative emission may be expected when flowers are detached and transferred to a air-tight syringe chamber. Consequently, these findings on the effect of temperature on fragrance emission are not directly comparable with the in-situ results of the present study.

Irradiance influenced the emission of volatiles from *T. repens* flowers significantly. Higher emission was noted at high irradiance. However, the effect was only noted on emission in light, whereas no effect could be detected on dark emission (Table 3). Effects of light quantity on fragrance emission have received little attention in the literature. However, an evaluation of the influence of irradiance indicates that it may in part be a temperature effect. High irradiance may raise the temperature in the petal tissue considerably. Büdel (1959) reported an up to 10°C higher temperature in the flower than in the air of the environment under light conditions.

The lower emission intensity of floral volatiles in members of the Orchidaceae under low temperature and light intensity noted by Williams (1982) on the basis of human perception are consistent with the results obtained in the present study.

No significant difference in fragrance emission could be detected at the two levels of RH. However, the results obtained in the light indicated a positive effect of low RH on emission ($P = 0.134$, Table 4).

The climatic treatments had similar effects on all emitted compounds. Consequently, no change in the emission picture was apparent when comparing emission under different climatic conditions.

When flowers were transferred to either continuous light or darkness the rhythmic nature of emission ceased. A broad low peak of phenethyl acetate was observed in the 'day' period (9 a.m. to midnight) following the first 24 h of continuous light. A more or less defined drop in emission intensity in the first period, during which darkness would be expected under normal conditions, was sometimes be observed. However, emission in the following periods proceeded at a constant level slightly below

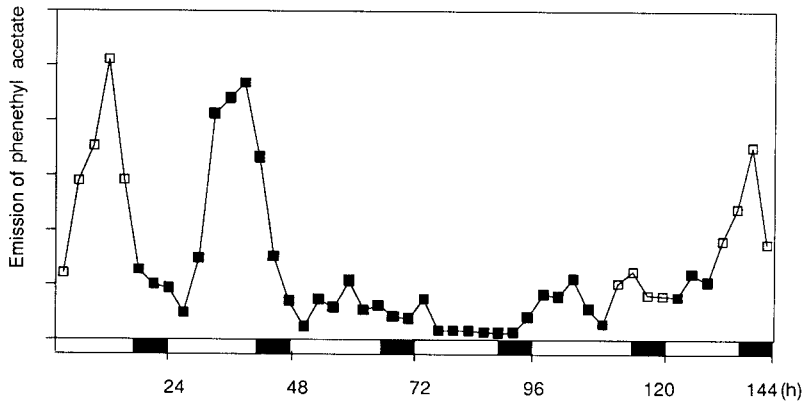

Fig. 5. Emission of phenethyl acetate after transfer from periods with 9-h dark intervals to 96 h in constant darkness, followed by three entrained 24-h periods with 9-h dark intervals. *Filled symbols* indicate collection in darkness. *Shading of the abscissa* indicates 9-h dark intervals under normal growth conditions

that of normal day emission (Fig. 3). A similar pattern was observed for caryophyllene under constant light. However, the level of emission in constant light was slightly above the normal night emission (Fig. 4). Insertion of entrained light/dark periods following periods of constant light, induced an adjustment to the new conditions within two normal periods (Figs. 3, 4)

When flowers were transferred to continuous darkness, emission proceeded in a normal manner during the subsequent period. However, emission in the following periods stabilised at the level of the night emission in normal light/dark intervals (Fig. 5). When entrained light periods were applied following the periods of constant darkness, emission adjusted to the new position of the light period within two 24-h periods (Fig. 5).

Three conditions should be met in order to demonstrate a true endogenous rhythm of fragrance emission (i) The rhythm should continue under constant environmental conditions. (ii) The period of circadian rhythm should not be exactly 24 h. (iii) It should be possible to shift the phase of the free-running rhythm by changing the environmental conditions (Jones and Mansfield 1975). Rhythmic emission of fragrance from *T. repens* flowers does not continue under constant environmental conditions. It is therefore concluded that the timing of the rhythmic emission in *T. repens* is determined by the position of the light and dark intervals in the period rather than by the endogenous clock. The investigations performed so far have shown a circadian nature of emission in species with nocturnal emission, whereas emission in diurnal species appear to be independent of the biological clock (Altenburger and Matile 1988; Altenburger and Matile 1990; Loughrin et al. 1991). We have shown that fragrance emission in *Brassica napus* and *Ribes napus*, which emit most fragrace in the light period, follow a emission pattern under constant environmental conditions very similar to that described for *T. repens* in the present paper (data not shown).

It is evident that the climatic effects on scent emission in *T. repens* are of a quantitative rather than qualitative nature. The emission picture is remarkably uniform under the conditions tested and remains so for several periods under normal day/night conditions. From this platform, it should be possible to investigate the effects of pollination on scent emission, the application of volatiles by visiting insects and stress emission in response to herbivore visitation.

The authors wish to thank Mette Sørensen and Niels Midtgaard for technical assistance. Thanks to Hugo Fritzen from Danish Plant Breeding Ltd, St. Heddinge, Denmark, for providing plant material. We are grateful to P. Friis, Chemistry Department, H. Martens, Department of Plant Biology, both The Royal Veterinary and Agricultural University, Copenhagen, Denmark, for constructive criticism and to P. Lakos for editorial assistance. The research was supported by the Danish Agricultural and Veterinary Research Council (Grant no. 13–4178 and 13–4580–1), The Danish Seed Testing Board and Stiftelsen Hofmansgave.

References

Altenburger, R., Matile, P. (1988) Circadian rhythmicity of fragrance emission in flowers of *Hoya carnosa* R. Br. Planta **174**, 248–252

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

Brantjes, N.M.B. (1978) Sensory responses to flowers in night-flying moths. In: The pollination of flowers by insects (Linnean Soc. Symp. Ser. 6), , pp. 13–19, Richards, A.J., ed. Academic Press, London

Büdel, A. (1959) Das Mikroklima der Blüte in Bodennähe. Z. Bienenforsch. **4**, 131–140

Dobson, H.E.M., Bergström, J., Bergström, G., Groth, I. (1987) Pollen and flower volatiles in two *Rosa* species. Phytochemistry **26**, 3171–3173

Dobson, H.E.M., Bergström, G., Groth, I. (1990) Differences in fragrance chemistry between flower parts of *Rosa rugosa* Thunb. (Rosaceae). Israel J. Bot. **39**, 143–156

Free, J.B., Williams, I.H. (1983) Scent-marking of flowers by honeybees. J. Apic. Res. **22**, 86–90

Fægri, K., van der Pijl, L. (1980) The principles of pollination Biology. Pergamon Press, Oxford

Giurfa, M, Núñez, J.A. (1992) Honeybees mark with scent and reject recently visited flowers. Oecologia **89**, 113–117

Heinrich, B. (1979) Bumblebee economics. Haward University Press, Cambridge Massachusetts London

Honkanen, E., Moisio, T., Karvonen, P. (1969) Studies on the volatile flavour substances in some clover species. Suomen Kemistilehti **42**, 448–451

Howell, D.J. (1977) Time sharing and body partitioning in bat-plant pollination systems. Nature **270**, 509–510

Jones, M.B., Mansfield, T.A. (1975) Circadian rhythms in plants. Sci. Prog. Oxf. **62**, 103–125

Kameoka, H., Wang, C.-P., Tokimitsu, K. (1977) Terpenoids in the essential oil from the flower of *Trifolium repens* L. Agric. Biol. Chem. **41**, 1785–1786

Knudsen J.T., Tollsten, L. (1991) Floral scent and intrafloral scent differentiation in *Moneses* and *Pyrola* (Pyrolaceae). Plant Syst. Evol. **177**, 81–91

Levin, D.A. (1973) The role of trichomes in plant defence. Quart. Rev. Biol. **48**, 3–15

Loughrin, J.H., Hamilton-Kemp, T.R., Andersen, R.A., Hildebrand, D.F. (1990) Volatiles from flowers of *Nicotina sylvestris*, *N. otophora* and *Malus* x *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 *Nicotina sylvestris* and *N. suaveolens*. Physiol. Plant. **83**, 492–496

Lumer, C. (1980) *Blakea chlorantha* (Melastomaceae): A rodent pollinated plant in the neotropics. Bot. Soc. Am. Misc. Ser. Publ. **158**, 68

McGregor, S.E. (1976) Insect pollination of cultivated crop plants. Agriculture Handbook No. 496. Agricultural Research Service. United States Department of Agriculture, Washington, D.C.

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

Metcalf, R.L. (1987) Plant volatiles as insect attractants. Crit. Rev. Plant Sci. **5**, 251–301

Mookherjee, B.D., Trenkle, R,W., Wilson, R.A., Zampino, M., Sands, K.P., Mussinan, C.J. (1986) Fruits and flowers: Live vs dead – which do we want. In: A world perspective. Proc. 10th Int. Congress of essential oils, fragrances and flavors, Washington, USA

Pellmyr, O., Thien, B.T. (1986) Insect reproduction and floral fragrances: Keys to the evolution of the Angiosperms. Taxon **35**, 76–85

Robacker, D.C., Flottum, P.K., Sammantaro, D., Erickson, E.H. (1983) Effects of climatic and edaphic factors on soybean flowers and the subsequent attractiveness of the plant to honey bees. Field Crop Res. **6**, 267–278

Robacker, C.R., Meeuse, B.J.D., Erickson, E.H. (1988) Floral aroma. How far will plants go to attract pollinators. Bioscience **38**, 390–398

Rodriguez, E., Levin, D.A. (1976) Biochemical parallelisms of repellants and attractants in higher plants and arthropods. In: Recent Adv. Phytochem. **10**, 214–270

Wetherwax, P.B. (1986) Why do honeybees reject certain flowers. Oecologica **69**, 567–570

Wilhelm, K.T., Pflumm, W. (1983) Über den Einfluss blumenhafter Düfte auf das Duftmarkieren der Sammelbiene. Apidologie **14**, 183–190

Williams, N.H. (1982) The biology of orchids and euglossine bees. In: Orchid biology, reviews and perspectives II, pp. 119–171, Arditti, J., ed. Cornell University Press, Ithaca London

Wood, D.L. (1982) The role of pheromones, kairomones and allomones in the host selection and colonization behavior of bark beetles. Annu. Rev. Entomol. **27**, 411–446