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Release of volatiles during the flowering period of *Hydrosme rivieri* (Araceae)

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Abstract

The release of volatile compounds from a flower of *Hydrosme rivieri* was recorded during the whole flowering period (7 days). The quantities of six odour components (dimethyl disulphide, dimethyl trisulphide, *n*-alkanes C_{10}, C_{11}, C_{12} and C_{13}) forming the main part of emanating volatiles were plotted versus time. *n*-Alkanes started to emanate 3 days before the release of dimethyl disulphide and dimethyl trisulphide (the components with a rotting meat odour). © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Hydrosme*; *Amorphophallus*; Araceae; Dynamic headspace; Inflorescence odour; Dimethyl disulphide; Dimethyl trisulphide

1. Introduction

Inflorescence of *Hydrosme rivieri* Eng. (*Amorphophallus rivieri* Durieu) (Araceae) produce an odour resembling rotting meat with carrion smell. There are several literature reports on the chemical basis of this smell. In 1928, Klein and Steiner detected trimethylamine and ammonia (Klein & Steiner, 1928; Steiner, 1929). Within an extensive study of production of volatile amines at anthesis of several Arum lily species, Smith and Meeuse (1966) detected many compounds by means of paper chromatography. Beside ammonia and trimethylamine, the presence of isoamylamine, isobutylamine, ethylamine, methylamine, 2-aminoethanol, agmatine, histamine and skatole was described in the odour of *Hydrosme rivieri* (Smith & Meeuse, 1966). With the development of new concentration techniques (Golovnya, 1982; Jursík, Stránský & Ubik, 1991) and in the combination with gas chromatography–mass spectrometry, the odour components can be detected with high sensitivity and reliability. Recently, using a head space technique Kite and Hetterschieid (1997) obtained and analysed odours of 18 species of *Amorphophallus* and 2 species of *Pseudodracontium* (Araceae) flowers in order to compare the chemical composition of their odour.

Release of volatiles from the inflorescence of *H. rivieri* goes on for several days. Our intention was to find out whether the amounts of the odour components are the same during the flowering period or whether their quantities and proportions change with time. Results of the analyses are presented in this paper.

2. Results and discussion

For the concentration of odour components, a method of capturing into a solvent (Jursík et al., 1991) was chosen. This technique seemed to be the more suitable than the sorption techniques for its simplicity and reproducibility from the point of view of the quantitative evaluation of the compounds of interest.

The inflorescence was kept in a glass cylinder (Fig. 1) at constant outer temperature (20°C) during the whole flowering period. The temperature inside the flower was not recorded during the experiment.

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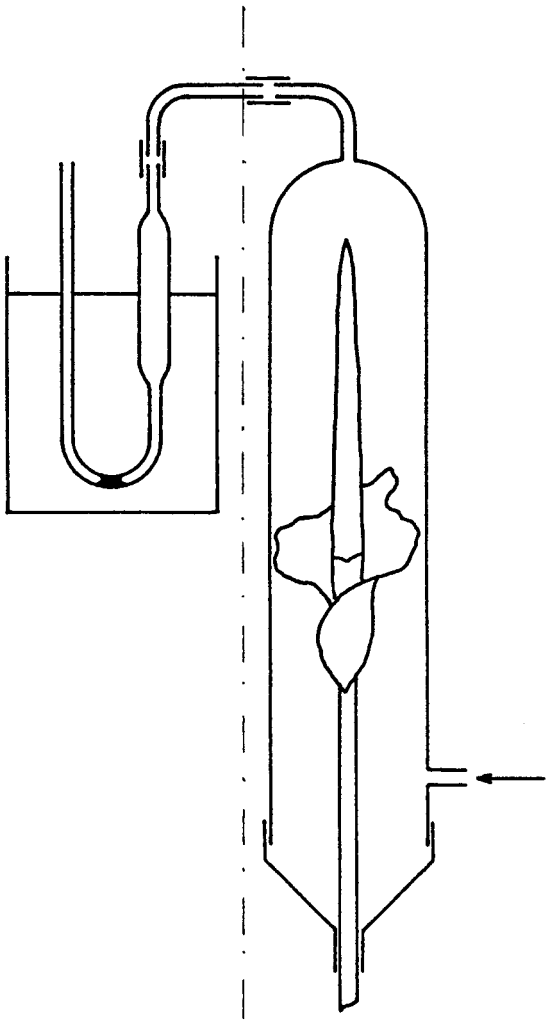

Fig. 1. The part of the trapping device with U-tube and cylinder with flower (*Hydrosme rivieri*).

Capturing started at least one day before the release of volatiles and it continued over the flowering period until no more GC-detectable peaks were present in the obtained sample. Six main components (dimethyl disulphide, dimethyl trisulphide and *n*-alkanes C_{10}, C_{11}, C_{12} and C_{13}) were chosen from the mixture and their content was plotted against time (Fig. 2). The composition of the six components studied did not change during the storage of the samples.

During the first 70 h, only *n*-alkanes C_{11}, C_{12} and C_{13} were released. Their proportions are relatively even and the concentrations show a slightly upward trend. After 3 days, a steep increase of the concentration of the volatiles appeared with a maximum release in time 85 h. Then, the amounts of hydrocarbons sank to their original level (Fig. 2). Dimethyl disulphide and dimethyl trisulphide started to appear after 70 h. The original decrease of their concentrations was followed by a sharp increase reaching maximum in 118 h. Then their concentration was

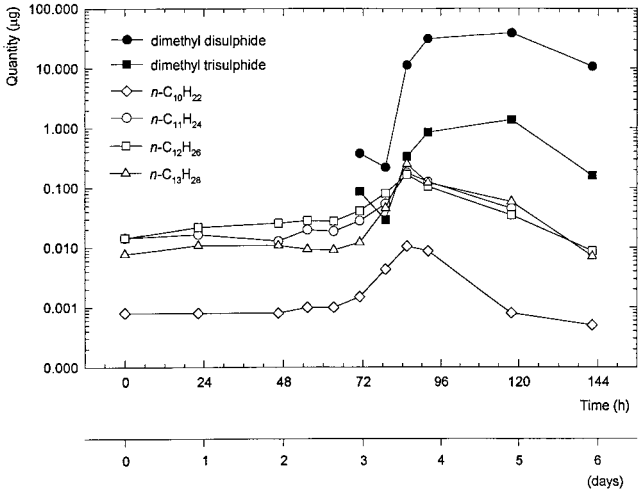

Fig. 2. Quantities of dimethyl disulphide, dimethyl trisulphide and *n*-alkanes C_{10}, C_{11}, C_{12} and C_{13} produced by one inflorescence of *Hydrosme rivieri*; dependence on time.

slowly decreasing until the time 142 h when the release of all volatiles ended. A characteristic intensive smell was noticed after the release of polysulphides had started. It is noteworthy that the *y*-axis in Fig. 2 has a logarithmic scale; thus, the concentration of dimethyl disulphide at its maximum was two to three orders of magnitude higher than that of alkanes.

During the development of the inflorescence, we have measured also the length of spadix and of the whole plant (Fig. 3). Both parameters reached their maximum approximately at the same time when maxi-

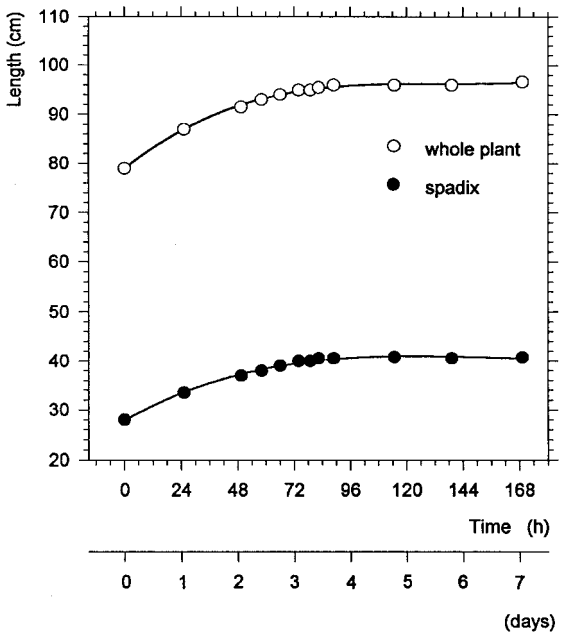

Fig. 3. Dependence of the length of spadix and of the whole plant of *Hydrosme rivieri* on time.

mum of volatiles was released. After 5 days, both the spadix and the whole plant stopped growing.

In the paper recently published by Kite and Hetterschieid (1997), the presence of *n*-alkanes in the odour of *Amorphophallus* and *Pseudodracontium* is not mentioned. However, Borg-Karlson, Englund and Unelius (1994) detected a homological series of *n*-alkanes (C_{10}–C_{19}) in the odour of *Sauromatum guttatum* Schott (Araceae). Occurrence of hydrocarbons in flower fragrances of different species was mentioned by several authors. In the genus *Ophrys*, *n*-alkanes C_{11}–C_{19} are relatively abundant (Borg-Karlson, 1990; Borg-Karlson, Bergström & Groth, 1985; Borg-Karlson, Groth, Ågren & Kullenberg, 1993; Schiestl, Ayasse, Paulus, Erdmann & Francke, 1997). A homological series of *n*-alkanes with nonadecane strongly predominating was reported in *Papaver* flowers (Dobson, Groth & Bergström, 1996). Hydrocarbons (without more specific identification) were described in the floral scent of *Angelica* (Tollsten, Knudsen & Bergström, 1994). Alkanes were also reported by several authors in volatiles of some insect species (Smith, 1974, 1978a,b; Farine, Bonnard, Brossut & Le Quere, 1992a,b; Stránský, Valterová, Ubik, Čejka & Křeček, 1998). Dumelin and Tappel (1977) showed that short-chain hydrocarbons (C_2–C_5) are formed by decomposition of peroxides of polyunsaturated acids. However, longer-chain hydrocarbons originate from fatty acids by loss of $C_{(1)}$ by decarboxylation or decarbonylation (Kolattukudy, 1987; Kolattukudy, Croteau & Buckner, 1976). The role of floral volatiles in insect biology was studied by Dobson (1994). While the compounds with carrion smell present in the Araceae flowers were reported to attract the pollinators (carrion beetles) (Kite & Hetterschieid, 1997), the question of the function of high hydrocarbons in plants and insects odour has not yet been answered.

3. Experimental

3.1. Odour collection

A method of concentration of volatile compounds into a solvent (Fig. 1) was used (Jursík et al., 1991). The inner surface of all glass parts of the device was silanised with a solution of dimethyldichlorosilane in toluene (20% v/v, 20 min). A cylinder containing the inflorescence was kept at laboratory temperature (ca. 20°C). Purified air (flow 15 ml/min) as a carrier gas was passing through the vessel. U-tube (i.d. 3 mm) as a cold trap (−78°C) contained 100 μl of ultra-pure methanol (99.999%). The time of each odour collection was 6 h. The middle of each 6 h interval is marked on x-axis in Figs. 2 and 3. Time of the first concentrate in which the compounds appeared was defined as time 0.

3.2. GC analysis

An HP 5890A gas chromatograph with split–splitless injector and flame ionisation detector was used, injector and detector temperature were 180 and 250°C, respectively. Initial oven temperature was 35°C (5 min), then 2°C/min up to 180°C, carrier gas hydrogen (velocity 50 cm/s at 35°C), split ratio 25:1, injections 2 μl of concentrate. Fused silica capillary column DB-1 (30 m × 0.25 mm × 0.25 μm) was used.

Concentrations of individual components in the mixtures of volatiles were determined from calibration curves of dimethyl disulphide, dimethyl trisulphide (both Aldrich, 98%) and *n*-undecane (Alltech Associates Applied Science Ltd, 99%). The response of the detector (FID) is practically linear ($r^2 = 0.995$ for dimethyl disuphide, $n = 22$; $r^2 = 0.993$ for dimethyl trisuphide, $n = 27$; $r^2 = 0.990$ for *n*-undecane, $n = 20$). Response factor of FID related to *n*-undecane is 2.90 for dimethyl disulphide and 4.95 for dimethyl trisulphide. Curves in Fig. 2 are based on the calibration curves of the above mentioned three standards and on the volume of concentrates trapped in individual samples. The reproducibility of the analytical method was tested with 0.1% solution of *n*-undecane in hexane; 1 μl injected at the split ratio 25:1 gave a response corresponding to 0.0435 ± 0.0017 μg (mean value ± S.D., $n = 12$) of the standard (theory 0.0385 μg).

3.3. GC-MS analysis

A Fisons instruments MD 800 with electron-impact ionisation was used. Injector, interface and source temperatures were 200°C, oven temperature program the same as at GC analysis, carrier gas helium, velocity 26 cm/s at 30°C, split ratio 20:1, fused silica capillary column BPX 5 (30 m × 0.22 mm × 0.25 μm).

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