Floral Odor of *Arum italicum*

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ABSTRACT

Volatile compounds emitted by the appendix of *Arum italicum* Mill. in the South of France were analysed. Three chemotypes were found. The first was rich in fatty acid derivatives (about 75%) but was found in only one individual and needs to be confirmed. A second profile (4 individuals) showed a high proportion (57–84% of the blend) of monoterpenes (β-citronellene and 3,7-dimethyl-1-octene, its reduced chemical form). The third profile (2 individuals sampled twice) was rich (79–85% of the blend) in sesquiterpenes, particularly two isomers (γ and β) of caryophyllene. Moreover *p*-cresol and 2-heptanone were also present in the blend. Further work is needed to resolve whether these odor differences are different chemotypes of *Arum italicum*, temporal variation during the flowering season or analytical and experimental biases.

KEY WORDS

arum, pollinators, odor, Araceae, attractants.

INTRODUCTION

Pollinator attraction in *Arum* is mainly olfactory, and insects are attracted by volatile compounds emitted by the inflorescence. *Arum* species are known to have, in general, a foul odor close to faeces or urine. This odor is emitted by the appendix, the distal sterile portion of the spadix, which represents the main odoriferous and thermogenic organ in *Arum* (Kite *et al.*, 1998). The compounds produced by the appendix are also present in the odor composition of the insect laying site (Kite, 1995), thus the appendix odor might mimic the laying sites and could lure the insects. In contrast, in the floral chamber (i.e. the spathe base surrounding the flowers) a sweet odor is produced (Kite, 1995; Kite *et al.*, 1998). The role that this plays in the biology of pollination is not yet known, but it is possible that it stimulates activity in the insects in the chamber, thus increasing the chance of flower pollination.

In England, the main pollinator of *Arum maculatum* L. is a midge: *Psychoda phalaenoïdes* but almost exclusively the females (Lack & Diaz, 1991). A recent study showed that females of *P. phalaenoïdes* are also the main pollinators of *Arum italicum* (subsp. *italicum* and *neglectum*) in England (Diaz & Kite, 2002), but in South of France (e.g. Toulouse), the main pollinators of *Arum italicum* (subsp. *italicum*) are different species from those in England namely, *Psychoda crassipenis* and *P. pusilla* (Albre *et al.*, 2003). Females of a chironomid, a "mosquito" (*Smittia pratorum*), were the second most abundant insects captured by *A. maculatum* and *A.*

italicum in England but were less efficient pollinators (Diaz & Kite, 2002).

The principal compounds in the odor of *A. maculatum* are: 2-heptanone, indole, *p*-cresol, (E)-caryophyllene, a few monoterpenes and two unidentified sesquiterpenes (Kite, 1995; Kite *et al.*, 1998; Diaz & Kite, 2002). Three of these compounds: indole, *p*-cresol and 2-heptanone, were responsible for the odor of faeces or urine in *A. maculatum* (Kite, 1995). Moreover *p*-cresol was found in great quantities with hydrocarbons in the odor composition of cow dung, the laying sites of *P. phalaenoides* (Kite, 1995), explaining the inflorescence attraction to *Psychoda* females. This hypothesis has been proved experimentally by trapping *Psychoda* to odoriferous baits containing *p*-cresol, 2-heptanone or indole, or any combination of these three molecules (Kite *et al.*, 1998). The more attractive molecule on its own seems to be *p*-cresol, but the blend of the three was the most attractive.

The odor composition of *A. italicum* differs from *A. maculatum*. The main compounds were: 1-decene, methyl butyrate, an unidentified sesquiterpene, β-citronellene and a related compound (Diaz & Kite, 2002). Notice that among these compounds, three are absent from the odor of *A. maculatum*: 1-decene, β-citronellene and the related compound. Moreover, the odor of *A. italicum* does not contain indole. Finally, the only compounds in common in the odors of the two species are: an unidentified sesquiterpene, *p*-cresol and 2-heptanone (Diaz & Kite, 2002).

Although the main compounds present in the odor of *A. italicum* are different from those emitted by *A. maculatum*, these two species have the same pollinator in England. Thus females of *Psychoda phalaenoïdes* are responsive to both odors. The main compound in common to both *Arum* species is the *p*-cresol, consequently, this molecule could be the main attractant for *P. phalaenoïdes*. In Toulouse, the captured species by *A. italicum* are *P. crassipenis* and *P. pusilla* (Albre *et al.*, 2003). Their laying site may be humid decaying (vegetal) matter (Vaillant, pers. comm.).

In this study, we have identified the volatile compounds emitted by the warm appendices of *Arum italicum* present on the campus of the University Paul Sabatier (Toulouse). Each sampled appendix was covered with a glass vial in order to collect its odor. The volatile compounds were captured *in situ* by headspace on Tenax traps by pumping the air (30 ml/min) during 15 or 30 min. The Tenax was then thermically desorbed at 220°C during 5 min on a gas chromatograph coupled with a mass spectrometer for analysis. The gas chromatograph was equipped with a non-polar column (BPX 5: 50m/0,22 mm/1 µm). The aim of this work was first to identify the molecules responsible for the *Psychoda* attraction to inflorescences of *Arum italicum*. Secondly, our results could be compared to those obtained in England where *A. italicum* is pollinated by another *Psychoda* species.

RESULTS

Between 15 April and 15 May 2002, the odor of 14 appendixes of *Arum italicum* was sampled and analysed, but only 9 collections had enough information (compound quantities) for interpretation.

It is important to note that two chromatograph programs of analysis were used. The second lasts longer and was used because some compounds were not still analysed at the end of the first analysis program (the corresponding samples for each program are noted in Table 1). In program 2, the final temperature reached was higher (280°C instead of 220°C) and it ended with a plateau of 10 min at 280°C.

Thirty-four volatile compounds have been detected. Fourteen of them have not been identified (Table 1), but they were quantitatively secondary compounds as their total fraction represented between 1 and 14 % of the total volatile blend (Table 1).

The twenty identified molecules belong to four chemical classes: Five fatty acid derivatives (alcohols, aldehydes, ketones),

Table 1. Molecules* identified by GC-MS in the volatile fraction emitted by appendices of *Arum italicum* (University Paul Sabatier, Toulouse).

Analysis conditions	Program 1					Program 2			
Trapping during (min)	15	15	15	15	30	15	30	30	30
Time of day	18h15	19h10	19h10	19h05	17h55	18h40	19h20	18h00	18h40
N° individual	1	2	3	4	5	6.1	6.2	7.1	7.2
Alcohols									
2-heptanol	—	14.1	—	—	—	—	—	—	—
Aldehydes									
n-octanal	—	10.7	—	—	—	—	—	—	—
n-nonanal	—	20.2	—	—	—	—	—	—	—
Ketones									
2-heptanone	2.22	29.7	—	20.7	—	1.3	4.15	4.7	5.8
2-nonanone	—	—	—	—	—	—	—	Tr	Tr
Benzenic									
p-cresol	—	—	—	—	—	5.6	15.65	Tr	1.1
Monoterpenes									
3,7-dimethyl-1-octene	34.1	—	56.7	9.9	4.6	—	—	Tr	Tr
2,6-dimethyl-1,7-octadiene (α-citrone)	3.60	—	13.1	5.4	3	—	—	—	—
3,7-dimethyl-1,6-octadiene (β-citronellene)	29.8	5	27.3	57.6	52.5	—	—	3.5	5.1
3,7-dimethyl-2-octene	—	—	—	2.2	Tr	—	—	Tr	Tr
Myrcene	—	—	—	—	Tr	—	—	—	Tr
Nerol	—	—	—	—	—	—	—	—	Tr
2,6-dimethyl-7-octen-2-ol					Tr	—	—	—	—
2,6-dimethyl-2,6-octadiene	—	—	—	—	—	—	—	—	Tr
Sesquiterpenes									
α-cubebene	—	—	—	—	—	Tr	—	Tr	Tr
γ-caryophyllene	13	—	—	—	6.3	10.2	1.7	4.7	30.4

Table 1. Continued.

Analysis conditions	Program 1					Program 2			
Trapping during (min)	15	15	15	15	30	15	30	30	30
Time of day	18h15	19h10	19h10	19h05	17h55	18h40	19h20	18h00	18h40
N° individual	1	2	3	4	5	6.1	6.2	7.1	7.2
β-caryophyllene	15..6	—	—	4.1	30.3	70.7	77.2	79.9	48.8
α-humulene	1.7	—	—	—	Tr	2	Tr	1.1	2
Allo-aromadendrene	—	—	—	—	—	Tr	—	Tr	Tr
δ-cadinene	—	—	—	—	—	Tr	—	Tr	Tr
Not identified	—	14.1	2.9	—	1.8	9	1.3	4.6	5.5
# unidentified compounds	—	3	1	—	2	7	2	6	8

• Composition expressed in percentage of the volatile fraction; —, compound not detected; Tr, compound in small quantity: trace (<1%).

one benzenic compound (*p*-cresol), eight monoterpenes and six sesquiterpenes. Almost all of them have been previously identified in the odor of *Arum italicum* (Kite *et al.*, 1998; Diaz & Kite, 2002).

In Table 1, three chemical profiles appear clearly. The first profile corresponds to individual #2. It's characterised by a blend composed almost exclusively of fatty acid derivatives (about 75%). A second profile, corresponding to the individuals 1, 3, 4 and 5, shows a high proportion of monoterpenes and particularly of β-citronellene and of 3,7-dimethyl-1-octene, its reduced chemical form (57–84% of the blend). Secondary compounds are sesquiterpenes or 2-heptanone (fatty acid derivative). One thing to notice is that *p*-cresol is absent from the odors belonging to these two groups. The third profile is observed in individuals 6 and 7. Their odor blend is rich in sesquiterpenes, particularly two isomers (γ and β) of caryophyllene (79–85% of the blend). Moreover *p*-cresol and 2-heptanone are also present in the blend. In addition, odor samples of individuals 6 and 7 were made at two different times at 40 min intervals on the same evening (noted 6.1–6.2 and 7.1–7.2 in Table 1). Variations in relative contributions of some compounds in the blend can be observed, but the second odor collections still belong to this chemical profile. For example the relative contribution of *p*-cresol and 2-heptanone tends to increase. In contrast, sesquiterpene variations are not consistent between the two individuals, an increase in one case (individual n°6) and a decrease for the other (n°7).

DISCUSSION

From the 20 compounds identified, three different odor profiles have been identified for the appendix of *Arum italicum*. Only two profiles are considered further as the third one is only based on one collection and needs to be confirmed. Five out of the fourteen samples were not concentrated enough for interpretation due certainly to the short period of odor trapping (15 or 30 min).

The first odor profile was dominated by monoterpenes (particularly β-citronellene) whereas the second profile contained mainly sesquiterpenes (caryophyllenes). Another qualitative difference is that *p*-cresol is absent from the first odor profile but present in the second.

Two hypotheses could explain this result. First, the inflorescence odor could have changed between mid-April and mid-May (the end of flowering period). But pollinators are trapped to *Arum* inflorescences throughout the flowering period and late flowering inflorescences are also pollinated (Albre *et al.*, unpub data).

Second, these two odor profiles may correspond to different chemotypes of *A. italicum*. Consequently the observed differences are of genetic order among different individuals.

Most of the samples of profile 1 were collected during 15 min except #5, and those from profile 2 during 30 min except #6.1. While these two exceptions are not strongly different from the corresponding profile, it may in part explain the relative high proportion of caryophyllene in the blend of sample #5, a compound which is not found in the other "short" samples of profile 1. But the main uncertainty is because the two odor profile correspond to two chromatographic analysis programs, with program 2 longer and warmer. Consequently, the analysis of heavy compounds (particularly the sesquiterpenes) was more precise in the latter case. Further work is needed to give a definitive interpretation of these results.

In comparison to odor analysis from England, fewer molecules (20 instead of 36) were identified (Kite, 1995; Kite *et al.*, 1998; Diaz & Kite, 2002). In fact, samplings in England were performed through the whole night and represent a "cumulative" odor whereas our data are most likely "instantaneous" odors. But the volatile compounds emitted by *A. italicum* in England and in Toulouse belong to the same chemical classes: β-citronellene, and its derivatives were in both cases the major blend constituents of *A. italicum*. The difference may be the high proportion of sesquiterpenes in Toulouse even if sesquiterpenes (one unidentified and caryophyllenes) were also present in the volatile fraction of *A. italicum* from England (Diaz & Kite, 2002). These compounds have been found in *A. italicum* in England but with some differences, 1-decene and methyl butyrate, major compounds in English *A. italicum* were absent in French samples (Diaz & Kite, 2002).

Finally, 2-heptanone was found in almost all the *Arum italicum* studied whereas *p*-cresol was found only when analysis program 2 was used. Thus *p*-cresol may have been undetected in individuals analysed with program 1, as a faster temperature increase may improve *p*-cresol detection on a non-polar column (Kirby, pers. comm.). Further samples are needed to examine this question. These two molecules have been shown to be highly attractive for *Psychoda phalaenoïdes*, the pollinator of *Arum italicum* (and *A. maculatum*) in England (Kite *et al.*, 1998). More studies are necessary to test whether the same molecules are attractive to *Psychoda crassipenis* and *P. pusilla*, the main pollinators of *A. italicum* in Toulouse, especially since their laying sites are supposed to be different (humid rotting vegetation) from the laying site of *P. phalaenoïdes* (i.e. cow dung). The chemical attractants of *A. italicum* in Toulouse may be different volatile compounds than those effective in England.

Another question that needs to be clarified is the temporal odor variation, and samples should be taken at different times of the night not just during the early evening (1800–2000 hours), and also at different times during the flowering season. Finally in order to have data directly comparable with those published in England (Kite *et al.*, 1998; Diaz & Kite, 2002), odor samples accumulated over the whole night should also be collected. Then, it will possible to know if the observed odor differences are due to different chemotypes of *A. italicum*, to temporal variation during the flowering season or to analytical and experimental biases.

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