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Mediterranean island endemic *Arum pictum* emits isomycorene as a major component of floral scent

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Abstract

The west Mediterranean *Arum pictum* L.f., endemic from Corsica, Sardinia and Balearic Islands, is the only autumn-flowering *Arum* species. This species is pollinated by deceptively attracting coprophilous and saprophilous Diptera and staphylinid Coleoptera. Four populations from Sardinia and Corsica were studied. The inflorescence produces heat during anthesis from the appendix and stamens; the appendix is able to increase its temperature twice within the same day, a trait unique in *Arum*. *A. pictum* floral scents likely mimic horse/donkey dung by releasing several dung-related volatile organic compounds (VOCs: *p*-cresol, skatole, or indole). Surprisingly, the main floral VOC was 2,6-dimethylocta-1,3,7-triene or isomycorene.

Keywords: Araceae, floral thermogenesis, *p*-cresol, skatole, indole, 2,6-dimethylocta-1,3,7-triene

French version

***Arum pictum*, endémique des îles méditerranéennes, émet de l'isomyocorène comme composant volatil floral majoritaire**

Résumé

Arum pictum L.f., espèce insulaire endémique de la Corse, de la Sardaigne et des îles Baléares, est la seule espèce d'*Arum* à floraison automnale. Cette espèce est pollinisée en dupant des Diptères coprophiles et saprophiles et des Coléoptères staphylinidés. Nous avons étudié quatre populations de Sardaigne et de Corse. L'inflorescence produit de la chaleur durant l'anthèse au niveau de l'appendice et des étamines. L'appendice est capable d'augmenter sa température deux fois dans la même journée, un trait unique pour les *Arum*. Les parfums floraux d'*A. pictum* imitent probablement le crottin de cheval ou d'âne en libérant plusieurs composés organiques volatils liés aux crottins (COV: *p*-crésol, scatole ou indole). Étonnamment, le principal COV floral était le 2,6-diméthyl-octa-1,3,7-triène ou isomyocorène.

Mot-clés: Araceae, thermogenèse florale, *p*-crésol, scatole, indole, 2,6-diméthyl-octa-1,3,7-triène

1. Introduction

The *Arum* genus is composed of 29 described species [1-3] but estimated to be comprised of 43 species [4]. Regardless of the genus diversity, one stable subdivision within the genus, based on morpho/ecological characters and molecular data, consistently groups all *Arum* species separately from *Arum pictum*. *A. pictum* is the only species of the subgenus *Gymnomesium* (L.f.) Schott, which appears to be sister to all other *Arum* species [1,2,5,6]. Thus, *A. pictum* appears to be a unique taxon within the genus *Arum*, endemic to West Mediterranean islands: Corsica, Sardinia, Tuscan Archipelago (Montecristo) and Balearic Islands [1]. This species presents some distinguishing characters such as (1) autumn flowering (versus spring for all other species) and (2) leaves produced after or simultaneously with inflorescences (versus before for all other species, [2]). For all of the above cited reasons, it has been proposed to elevate the subgenus *Gymnomesium* to the rank of a monospecific genus [4]. Since a proper taxonomical synonymy has not yet been published, the name *Arum pictum* will be used in this manuscript.

The inflorescence of *A. pictum* consists of a spadix (*e.g.* reproductive spike) and a spathe (*e.g.* modified bract). The anthesis lasts two days and is protogynous preventing spontaneous self-pollination [7]. The spathe, which completely encloses the spadix during floral development, opens in its upper half during anthesis to reveal the sterile appendix of the apical part of the spadix. This appendix produces heat and releases scent for pollinator attraction. The basal half of the spathe forms a pollination chamber around the basal part of the spadix, which bears unisexual flowers, stamens and ovaries. Attracted insects are trapped on the first day of anthesis during the female stage when they slip and fall into the floral chamber, and eventually pollinate the female flowers. On the next morning, during the male stage, they are dusted with pollen before being able to leave the pollination chamber. After pollination in autumn, red berry-like fruits develop throughout the winter as an infructescence that mature by the following spring [7].

The deceptive pollination strategy of *A. pictum* has been studied more than a decade ago in one Corsican population [7]. In summary, the anthesis cycle of *A. pictum* occurred during the day, attracting mainly sphaerocerid flies (Brachycera) and staphylinid beetles early in the morning and late in the afternoon of the first day. The pollen was released from the anthers early in the morning of the second day before insects depart. *A. pictum*'s thermogenic cycle matched the anthesis cycle and the pollination events with an original and unique, bimodal temperature pattern of the appendix (early morning ~8:00 am and late afternoon ~07:30 pm), contrary to the unimodal pattern found in all other studied *Arum* species [7,8]. As mentioned earlier, the main pollinators of *Arum pictum* were sphaerocerid flies (Brachycera) and staphylinid beetles and not psychodid, chironomid or

ceratopogonid flies (all nematoceran) known to be the main pollinators of *Arum* species in West Europe [8,9,10,11,12,13]. Most of the *Coproica* (Sphaeroceridae) flies captured by *A. pictum* inflorescences were females (more than 70%) suggesting that the floral scent of *A. pictum* may have sex-specific properties and/or may mimic to the fly oviposition sites [7]. *A. pictum* is characterised by a dung odour close to horse/donkey dung, described as pungent [1]. The floral scent was described as been dominated by 5 volatile compounds namely: benzyl alcohol, indole, dihydroocimene, ocimene and skatole [7]. The comparative study of the volatiles compounds (VOCs) emitted by four different types of dung showed that the scent of the horse dung was dominated by *p*-cresol, β -citronellene, and 4-methyl-3-heptanone [14]. Other notables VOCs were dihydrolimonene, *cis*-pinane, indole, skatole, and various alkanes [14].

The aim of this paper is to document some regional aspects of the pollination ecology of *Arum pictum* across the island of Sardinia (Italy) in comparison to Corsica. We measured the floral thermogenic patterns, collected the floral volatiles during the most scented phase of the anthesis according to human nose, and the insects trapped within pollination chambers of inflorescences from several populations.

2. Materials and Methods

2.1 Study species and populations

Arum pictum L. fil. is a Tyrrhenian island endemic, found on Sardinia, Corsica, Mallorca, Menorca (Diana Corrias, 1982) and Montecristo in the Tuscan Archipelago [15], including some minor islets surrounding the large islands. In Sardinia, it is distributed in all sectors of the islands [16], usually below 1000 m a.s.l., but sometimes above [17, 18]. The plant thrives on every kind of soil, but prefers shady or semi-shady habitats under the canopy of evergreen trees and shrubs [19] in the thermo- and meso-Mediterranean bioclimatic belts [20]. This species is not threatened in Italy and its conservation status is considered as LC [21].

The pollination ecology of *Arum pictum* was studied in October 2018 in three populations from northern Sardinia (Italy). The two first populations were located in north-western Sardinia, namely: near the lake Baratz (Sassari, GPS coordinates: 40.676729, 8.225554) and Gutierrez (Alghero, GPS coordinates: 40.625107, 8.273025) at almost sea level (24 m a.s.l.), in the thermo-Mediterranean dry bioclimatic belt, the first on aeolian sand, the second on Mesozoic limestone. Both populations grew in *Pinus pinea* and *P. halepensis* plantations, but the first falls in the potential *Juniperus phoenicea* vegetation, whereas the second in potential *Olea europaea* vegetation. The third population was located in north-eastern Sardinia at Diga del Liscia (Sant'Antonio di Gallura-Luras,

GPS coordinates: 41.001154, 9.144749, named Diga afterwards) at an elevation of 242 m a.s.l., corresponding to a meso-Mediterranean dry bioclimatic belt. This population grew on acidic soil (on schists and granites) in an open wood of *Quercus suber*. In autumn 2018, these three *Arum* populations were large with more than 100 individual plants. For a broader geographical comparison, one south-west Corsican population was also studied in October 2018, located in Coti-Chiavari (GPS coordinates: 41.793473, 8.769976, named Coti afterwards) on the south bank of the Ajaccio Gulf in a former penitentiary area. This population, large of ~60 individual plants, grows in an open wood of *Quercus ilex*.

2.2 Thermogenesis and anthesis

Thermogenesis in *A. pictum* was studied by measuring the temperatures of 8 inflorescences belonging to two different Sardinian populations (Baratz in the west and Diga in the east). During the entire anthesis, temperatures were recorded every 10 min from two different zones of the spadix (the appendix and the male flowers) with simultaneous measurements of the ambient air, using type-K probes connected to Digi-Sense® DualLogR® thermocouple thermometers (Testo model 175T3®). The female flowers (*e.g.* gynoecia) are known to be non-thermogenic [7] and were thus not recorded. The two probes recording the spadix temperatures were inserted about 5 mm deep into either the appendix or the stamen zone. Ambient air temperature was recorded near the studied inflorescence in the shade (*e.g.* under an *Arum* leaf). Twice per day (*e.g.* in the morning and in the afternoon) the monitored inflorescences were observed for insect presence and floral observations.

2.3 Floral scent analyses

Floral volatiles were collected during the morning between 09:00–10:30 am in the four studied populations from Coti (N=9 inflorescences) between October 4th and 7th, Gutierrez (N=3) on October 13th, Baratz (N=2) on October 14th, and Diga (N=4) on October 19th. Floral scent samples were collected *in situ* from scented inflorescences that were enclosed within Nalophan (NA colourless, calibre 90, ETS Charles-Frères, Saint-Etienne, France) bags (opened in their upper part) to create a headspace. The emitted volatile organic compounds (VOCs) were adsorbed by Solid Phase Micro Extraction (SPME) Fibers (StableFlex™ SPME Fiber, 65 µm PDMS-DVB Coating, Supelco, Darmstadt, Germany) for 20 min, then stored at 5 °C until analysis by thermal desorption. To identify possible ambient contaminants, control samples were simultaneously collected from an empty bag to each scent sample, following the same protocols described above.

The trapped volatiles were analysed by combined gas chromatography-mass spectrometry (GC-MS). The SPME fibers were manually injected and desorbed into the split/splitless injector of a

Clarus 580 Perkin-Elmer directly coupled to a Clarus SQ8S Perkin Elmer TurboMass detector (quadrupole). The gas chromatographer was equipped with two fused-silica capillary columns (50 m x 0.22 mm, film thickness 0.25 μm), a non-polar BP-1 (polydimethylsiloxane) and a polar BP-20 (polyethylene glycol). The oven temperature was programmed from 60 to 220 $^{\circ}\text{C}$ at 2 $^{\circ}\text{C min}^{-1}$ and then held isothermal at 220 $^{\circ}\text{C}$ for 20 min; injector temperature at 270 $^{\circ}\text{C}$; carrier gas was helium (0.8 mL min^{-1}); split ratio of 1:15; ionization energy at 70 eV. The electron ionization (EI) mass spectra were acquired over the mass range 40 – 350 Da. The relative proportions of the constituents were expressed as percentages obtained by peak-area normalization, without using correcting factors. Kovats retention indices (RI) were determined relative to the retention times of a series of *n*-alkanes with linear interpolation (Target Compounds software from Perkin Elmer). Compounds were tentatively identified by comparing their mass spectra and retention times to those of authentic standards and from the reference libraries Adams, NIST20, Perkin FF2013, Wiley FFNSC Library 2nd Edition and Wiley RegistryTM 8th Edition.

2.4 Isomycorene NMR identification

In order to confirm the Mass identification of the main unidentified floral VOC, floral scent samples were collected *in situ* from four inflorescences during the peak of thermogenic activity and floral scent emission on the female phase of anthesis. The inflorescences were enclosed within Nalophan (NA colourless, calibre 90, ETS Charles-Frères, Saint-Etienne, France) bags (opened in their upper part) to create a headspace. Sampling was conducted by dynamic headspace, scented air was drawn for 30 min through adsorbent traps connected with silicone tubing to a membrane pump (ASF Thomas Inc, Memmingen, Germany) that worked at a constant flow of 250 mL min^{-1} . The adsorbent traps (10 cm long, 0.5 i.d.), made out of silanized glass cartridges, were filled with a 1:1 mixture of TenaxTM TA (50 mg, 80/100, Macherey-Nagel 706318, Düren, Germany) and CarboxpackTM X (20/40, Supelco 1-0435), fixed in each tube with silanized glass wool plugs.

After collection, the four traps were eluted with deuterated chloroform (150 $\mu\text{L CDCl}_3$) and pooled together to attain a sufficient quantity for the NMR analysis. Nuclear Magnetic Resonance spectra were recorded on a Bruker AVANCE 400 Fourier Transform spectrometer (Wissembourg, France) operating at 400.132 MHz for ^1H and 100.623 MHz for ^{13}C , equipped with a 5 mm probe, in CDCl_3 , with all shifts referred to internal tetramethylsilane (TMS). The ^1H -NMR spectrum was recorded with the following parameters: pulse width (PW) 4.3 μs ; relaxation delay 1 s, acquisition time: 2.6 s for 32 K data table with a spectral width (SW) of 6000 Hz; accumulation scans: 32. The ^{13}C -NMR spectrum was recorded with the following parameters: PW= 4 μs (flip angle 45 $^{\circ}$); acquisition time: 2.7 s for 128 K data table with a spectral width of 25 000 Hz (250 ppm); accumulated

scans: 512. For the 2D spectra, sequences from Bruker Topspin™ (Bruker, Wissembourg, France) library (DEPT, COSY, HMBC) and gradient-enhanced sequences were used.

2.5 Visiting entomofauna

Arthropods attracted to the inflorescences and temporarily trapped in the pollination chamber were collected in ethanol 60 % with an aspirator in three populations Coti (N=26 inflorescences), Baratz (N=30) and Diga (N=8). Arthropods per inflorescence were counted and identified at the order rank (and family level for Staphylinidae and Braconidae). Based on known pollinators in the genus *Arum*, we grouped non-insect arthropods plus scarce insects (Formicidae, Blattoidea, Lepidoptera, Hemiptera) in the category, “Others” [8].

3. Results and discussion

3.1 Thermogenesis and anthesis

The timing and intensity of the temperature peaks varied among inflorescences due to asynchronous flowering, inflorescence size, and physiology differences but the daily thermogenic pattern (*e.g.* sequence) was the same between individuals and populations. The appendix and the male flowers appeared to be responsible for heat production in inflorescences of *Arum pictum* but the two regions of the spadix were not synchronized with each other resulting in a complex thermogenic pattern (Figure 1). The first temperature rise, caused by the male flowers, was recorded the night before the opening of the spathe from 6:30 pm until noon the next day (Figure 1 - sta1). Between 09:00–10:00 am, the temperature of the flowers reached a maximum of 23.2 °C, on average, 7 °C warmer than ambient air (Table 1). Later, on the same day, a second thermogenic phase occurred. The stamen temperature increased again between 3:30 and 9:30 pm (Figure 1 - sta2) coinciding with the spathe opening (upper half). The temperature of the male flowers reached a maximum of 19–24 °C between 6:00–8:00 pm, which was, on average, 3.5 °C warmer than ambient air (Table 1). In the same evening, the stamen temperature increased again and with greater intensity between 9:30 pm and 3:30 pm on the following day (Figure 1 - sta3). The temperature of the flowers reached a maximum of 22–30°C between 01:30–04:30 am, which was, on average, 11.4 °C warmer than ambient air (Table 1). The stigmas looked wet with turgescient papillae (*e.g.* receptive) in early morning. The appendix started heating up about the same time the male flowers reached maximum temperatures (sta3), of 28–33 °C between 06:10–09:20 am (Figure 1 – app1), corresponding to a maximum temperature difference of 9–20 °C above ambient air (Table 1). Insects were attracted to the scented inflorescences and eventually slipped or walked down inside the pollination chamber.

Note that the stamen's temperature increased again during the appendix thermogenic peak to a tissue temperature of 30-31 °C at 08:20-08:35 am representing a maximum temperature difference of 8.5–9.5 °C above ambient air (Figure 1). Afterwards, both stamen and appendix temperatures declined within two hours to reach ambient temperature at around noon. A third thermogenic phase began at the end of this first day of flowering: the appendix started heating up at about 1:40–4:00 pm (Figure 1 – app2), while the male flowers started heating up around 3:00–6:00 pm (Figure 1 – sta4). Insects could also be attracted in the afternoon during this second scented phase but in somewhat lower numbers. Appendix temperature quickly reached a maximum temperature around 21–30 °C between 5:00–7:30 pm (4.7–12.7 °C above ambient air). After 6:00–7:00 pm, the appendix temperature decreased gradually until reaching ambient level between 9:20 pm and midnight (Figure 1). The male flowers' temperature was still increasing and reached a maximum temperature of 21–26 °C (3.75–9 °C above ambient air, Table 1) around 00:30–03:00 am on the morning of the second day (Figure 1 – sta4). Later during the morning, the pollen was released from the mature stamens and the trapped insects were able to leave the pollination chamber. After, the temperature declined, returning abruptly to ambient level between 10:30 am and noon.

The floral thermogenic pattern of *Arum pictum* individuals in Sardinia appeared to be consistent with the thermogenic pattern obtained from Corsican plants (see Figures 1B & 1C in [7]). A complex thermogenic pattern resulting from non-synchronized heat productions by two different regions of the spadix (*e.g.* the appendix and the male flowers) appears to be the rule in the genus *Arum sensu lato* [8] as it has been previously documented in *Arum maculatum* [22], *A. italicum* [23] and *A. concinatum* [24]. Floral heat production in *Arum* results from an alternative mitochondrial respiration, the cyanide-insensitive pathway, catalysed by the alternative oxidase (AOX) resulting in heat dissipation instead of the ATP production [24,25]. The thermogenic pattern should be considered as a floral trait such as the colour, the shape or the scent as the floral temperature peaks are synchronised with the main anthesis events in relation to the pollination process. The timing of insect attraction (*e.g.* scent emission and appendix temperature peak) occurred during the early morning and at the end of the afternoon. Morning temperature floral peak has been reported in some *Arum* species from East Mediterranean such as *A. dioscoridis*, *A. creticum* and *A. palaestinum* [24]; whereas in other species such as *A. italicum*, *A. maculatum*, *A. rupicola* and *A. jacquemontii* the temperature appendix peak has been recorded during the first evening or night of anthesis (review in [8]). The main originality of the thermogenic pattern in *Arum pictum*, in contrast with all other documented *Arum* species, is that the appendix is able to increase its temperature twice within the same day in a manner similar to fertile male flowers [7,8,23,24]. This double temperature increase may be related to maximize day insect attraction in autumn, a potentially very temperamental season.

3.2 *Isomycorene identification*

The major VOC was identified as 2,6-dimethyl-1,3,7-octatriene (Figure 2, supplementary material) by comparison of the proton and carbon chemical shifts values with those reported in the literature [26].

¹H NMR (CDCl₃, 400 MHz): 6.14 (d, H3); 5.77 (ddd, H7); 5.62 (dt, H4); 4.94-5.00 (m, 2H, H8); 4.87 (bs, 2H, H1); 2.22 (m, H6); 2.11 (m, 2H, H5); 1.83 (s, Me, H9); 1.00 (d, Me, H10).

¹³C NMR (CDCl₃, 100 MHz) : 144.09 (C7); 142.16 (C2); 134.14 (C3); 128.90 (C4); 114.40 (C1); 112.60 (C8); 39.90 (C5); 37.78 (C6); 19.50 (C9); 18.72 (C10).

EI-MS 70 eV, *m/z* (rel.int): 136 (1), 121 (12), 107 (12), 79 (57), 77 (10), 81 (100), 55 (27), 53 (22), 41 (22), 39 (16), 40 (11).

Kovats retention Indices: non-polar (BP-1): 960; polar (BP-20): 1119.

To the best of our knowledge, 2,6-dimethylocta-1,3,7-triene, also known as isomycorene or alpha-menthrene, was obtained by palladium-catalysed telomerization of isoprene [26]. It has been recently reported as a minor VOC emitted by scentless flowers of *Tillandsia bandensis* [27]. However, we have a doubt concerning the assignments of isomycorene and cosmene in [27]. The authors reported isomycorene with a Kovats retention index value of 1017 and cosmene with a Kovats retention index value of 968 (DB-5MS). Indeed, the Kovats retention index value of 1017 appears more adequate for cosmene isomers which are reported to elute after ocimenes (3,7-dimethyl-octatrienes) [28]. On the other hand, the Kovats retention index assigned to cosmene (RI: 968 DB-5MS) appears to be more appropriate for isomycorene according to our data (RI: 960; BP-1). Then either these two compounds have been reversed or they have been misidentified [27].

3.3 Floral scent composition

In all samples, isomycorene (see above) was the main floral VOC representing between 43 % and 87 % of the floral scent composition. Its corresponding alcohol the 2,6-dimethyl-1,3,7-octatrien-6-ol (synonym hotrienol) has been tentatively identified in small amounts in a few samples.

The floral scent of the Corsican population of Coti was mainly composed of seven VOCs representing 87-99 % of the total blend (Table 2), namely: isomycorene (average 72 %), *p*-cresol (8 %), β-citronellene (6 %), indole and skatole (3% each), unknown1 and unknown4 (2 % each). The floral scent of the west Sardinian population of Baratz was also mainly composed of seven VOCs representing 98-100% of the total blend (Table 2), namely: isomycorene (average 62 %), *p*-cresol

(23 %), β -citronellene (4 %), 3-methylbutanoic acid (4 %), skatole (3 %), indole (1 %), and unknown1 (1 %). The floral scent of the west Sardinian population of Gutierrez was also mainly composed of seven VOCs representing 98-100 % of the total blend (Table 2), namely: isomycorene (average 71 %), *p*-cresol (17 %), 3-methylbutanoic acid (3 %), β -citronellene and unknown3 (2 % each), skatole and 3-methyl-1-butanol (1,2% each). Finally, the floral scent of the east Sardinian population of Diga was composed of only four main VOCs representing 96-99 % of the total blend (Table 2), namely: isomycorene (average 82 %), β -citronellene (9 %), *p*-cresol (6 %), and unknown1 (1 %).

Contrary to the previous published floral scent of *A. pictum*, we found noticeable differences. Among the five major VOCs found previously from Corsican inflorescences [7], two have been recovered in the present study, namely indole and skatole. The three others were not identified in any of our samples both from Corsica or Sardinia [7], (Table 2). The isomycorene was probably mistakenly identified as ocimene, because the two compounds have similar framework and differed by a double bond, the first is the 2,6-dimethyl-1,3,7-octatriene whereas the second is the 3,7-dimethyl-1,3,6-octatriene. Also, *p*-cresol was probably mistakenly identified as benzyl alcohol, and β -citronellene, synonym of dihydromyrcene, as dihydroocimene. No Kovats retention indexes were published and several tentative compound identifications were performed by manual mass spectra interpretations [7].

Arum pictum is characterised by a dung odour close to horse/donkey dung [1]. Several bad smelling VOCs were identified from the floral odour of *A. pictum*, most related to dung (as *p*-cresol, skatole, or indole) or fermentation/cheese (3-methyl-1-butanol, 3-methylbutanoic acid) in combination with floral VOCs (β -citronellene, 3,6-dimethyl-1-octanol, germacrene D). Interestingly several major VOCs found in the floral scent of *A. pictum* were identified as important VOCs emitted by horse dung such as *p*-cresol, β -citronellene, indole or skatole [14]. On the other hand, the major VOC, 4-methyl-3-heptanone, from horse dung was not identified in the floral blend of *A. pictum* [14]. Such diverse VOC combination is probably linked to the deceptive pollination strategy in *Arum* in order to attract insects associated to dung and/or fermenting matters [8,9,11,12,13].

3.4 Visiting entomofauna

The visiting entomofauna was dominated in all populations by Diptera, representing 69-78 % when considering all the arthropods but 80-89 % when considering only insects (Table 3). Secondary insect visitors were Coleoptera, particularly Staphylinidae (rove beetles, 4-16 %). The average number of insects trapped per inflorescence varied among populations, with fewer in Baratz (3.9) than in Coti (6.4) and Diga (6.6). Interestingly, the floral visitors in the populations of Coti and Baratz were both highly dominated by Brachycera flies (respectively 75 and 80 %) with Staphylinidae (respectively 10

and 16 %) being secondary visitors (Table 3). On the other hand, while the floral visitors in the population of Diga were mainly Diptera, Nematocera were attracted in greater proportions (68 %) and Brachycera were less frequent (25 %).

In a previous study, the main pollinators of *Arum pictum* were sphaerocerid flies (Brachycera) and staphylinid beetles [7]. Although we did not identify the Diptera to family level, many of the Dipteran Brachycera were sphaerocerids, confirming the same kind of visiting insect community in *A. pictum* inflorescences from north-west Sardinia (Baratz) and south-west Corsica (Coti and Sagone in [7]). On the other hand, the population of north-east Sardinia (Diga) presented a different visiting insect community dominated by Nematocera (Table 3). Psychodid and Chironomid flies (Nematocera) are known to be the main pollinators of other European deceptive *Arum* species [8,9,10,11,12,13]. Interestingly, the floral scent in the population of Diga was the one with the lowest proportion of dung associated VOCs (~ 6.5 % in average) compared to the three other studied populations (14-28 % in average; Table 2).

4. Conclusions

The autumn flowering *Arum pictum* has developed a deceptive pollination strategy combining a dark brown-purple spathe, floral heat production and scent emission mimicking probably horse/donkey dung, and attracting a coprophilous/saprophilous insect community composed mainly of diverse Diptera and staphylinid Coleoptera. Several bad smelling VOCs related to dung (*p*-cresol, skatole, or indole) were reported in notable quantities. But surprisingly the main floral VOC was 2,6-dimethylocta-1,3,7-triene, also known as isomycorene. Its odour is not known but probably not related to dung scent as other documented dimethyl-octatrienes (ocimenes) or acyclic hydrocarbon monoterpenes. Further studies are necessary to better understand the relationship between the floral scent composition in *Arum pictum* and the diversity of the attracted insects as it has been recently done for *A. maculatum* [11,12,13].

Conflicts of interest

The authors declare no conflict of interest.

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Table 1.

Thermogenic data of Sardinian *Arum pictum* recorded from 10 inflorescences. For each temperature peak: corresponding day(s) during the anthesis, mean duration of the thermogenic event (range), mean maximum temperature difference (range), and sum of the temperature differences (range). D: pollination day.

Temperature peaks	Anthesis time (days)	Mean Duration (h) (min – max)	Temp. diff. max (°C) (min –max)
Stamens 1	D-2 / D-1	> 17h25*	6.9 (6.7 – 7)
Stamens 2	D-1	6h10 (4h30 – 8h00)	3.5 (2.5 – 5.1)
Stamens 3	D-1 / D	15h30 (14h20 – 17h30)	11.4 (7.1 – 12.6)
Appendix 1	D	10h56 (8h50 – 12h30)	14.6 (9.3 – 20.2)
Appendix 2	D	8h32 (6h20 – 10h10)	6.7 (4.7 – 12.7)
Stamens 4	D / D+1	17h56 (17h00 – 19h40)	7.5 (3.7 – 9)

*: temperatures were recorded after the temperature increase started.

Table 2.

Floral scent composition as blend relative proportion (%): mean, standard deviation, minimum and maximum from four populations (Corsica: Coti; Sardinia: Baratz, Gutierrez, Diga - see material & methods for full details) of *Arum pictum*. Volatile compounds are classified by increasing order of their Kovats retention index (RI) on the polar column (BP-20), RI on the non-polar column (BP-1) are also given. Those with an average relative proportion above 1% are indicated in bold, and tr those with an average relative proportion below 0.1%.

RI polar	RI non-polar	Name	Coti (N=9)		Baratz (N=2)		Gutierrez (N=3)		Diga (N=4)	
			mean ± stdev	min-max	mean ± stdev	min-max	mean ± stdev	min-max	mean ± stdev	min-max
1030	937	β-citronellene	6.3 ± 3.3	3.4-12	4.4 ± 4.5	1.2-7.6	2.4 ± 2.4	0.5-5.2	9 ± 2.7	5.4-11.1
1119	960	isomyocorene	71.5 ± 11.2	57.5-87.4	62 ± 26.5	43.2-80.7	71.3 ± 12.2	60.1-84.3	82 ± 2.1	79.2-83.9
1156	979	myrcene	0.2 ± 0.2	0-0.4	0.1 ± 0.2	0-0.2	0	0	0.1 ± 0.1	0-0.2
1168	984	unknown1 ⁺	1.8 ± 0.3	1.3-2.3	1.2 ± 1.7	0-2.4	0.2 ± 0.3	0-0.6	1.4 ± 0.7	0.6-2.1
1181	997	unknown2 ⁺	0.9 ± 0.4	0.4-1.6	0.5 ± 0.7	0-1	0	0	0.2 ± 0.3	0-0.5
1205	736	3-methyl-1-butanol*	0	0	0.1 ± 0.1	0-0.1	1.2 ± 1.7	0.1-3.1	0	0
1267	1006	3,6-dimethyl-1,7-octadiene*	0.1 ± 0.1	0-0.2	0	0	0.1 ± 0.1	0-0.2	0	0
1445	1091	unknown3 ⁺	0.2 ± 0.2	0-0.4	tr	0-0.1	2.4 ± 4.2	0-7.2	0	0
1456	1380	unknown4 ⁺	1.8 ± 2.9	0-7.6	0	0	0	0	0.4 ± 0.7	0-1.4
1517	1398	2,6,10-trimethyl-1,5,9-undecatriene*	0.5 ± 0.5	0-1.1	0.1 ± 0.1	0-0.2	0	0	0	0
1604	1086	hotrienol*	0.3 ± 0.3	0-0.9	tr	0-0.1	0	0	0	0
1632	1420	unknown5	0.3 ± 0.3	0-0.7	tr	0-0.1	0	0	0	0
1637	1451	alloaromadendrene	0.5 ± 0.6	0-1.5	0.1 ± 0.1	0-0.1	0	0	0	0
1655	1180	3,6-dimethyl-1-octanol*	0.1 ± 0.2	0-0.4	tr	0-0.1	0.1 ± 0.1	0-0.2	0	0
1666	863	3-methylbutanoic acid*	0.2 ± 0.2	0-0.4	3.7 ± 5.1	0.1-7.3	3.3 ± 1.6	2.3-5.2	0.2 ± 0.2	0-0.5
1701	1472	germacrene D	1 ± 1.4	0-3.6	0	0	0	0	0	0
2079	1052	<i>p</i> -cresol	7.6 ± 4.1	3.3-14.1	22.9 ± 17.6	6.2-39.6	17 ± 12.3	5.4-29.9	5.6 ± 3.2	3.2-10.3
2439	1252	indole	3 ± 2.9	0.6-10.1	1.4 ± 1.8	0.1-2.7	1 ± 1	0.1-1.9	0.2 ± 0.3	0-0.5
2485	1344	skatole	3 ± 2.4	0-6.8	3.4 ± 3.7	0.8-6	1.2 ± 1.5	0.1-3	0.7 ± 0.2	0.6-1.1

*Tentative compound identification

⁺Mass spectra given in the supplementary material

Table 3.

Diversity of arthropods inflorescence visitors from three populations of *Arum pictum*. Total arthropod abundances per order or family.

Population Region Insect Orders \ Sample size	Coti Corsica N=26	Baratz West Sardinia N=30	Diga East Sardinia N=8
Brachycera (Diptera)	126	93	13
Nematocera (Diptera)	10	3	36
Coleoptera	12	1	0
Staphylinidae (Coleoptera)	17	19	2
Braconidae (Hymenoptera)	2	0	2
Others*	51	21	10

*Others represent the sum of unlikely *Arum* pollinators: Collembola (N=8), Acari (N=34), Salticids (N=29), Blattodea (N=1), Hemiptera (N=1), Formicidae (N=4), Isopoda (N=1), Lepidoptera (N=1), Psocoptera (N=1), Thysanoptera (N=2).

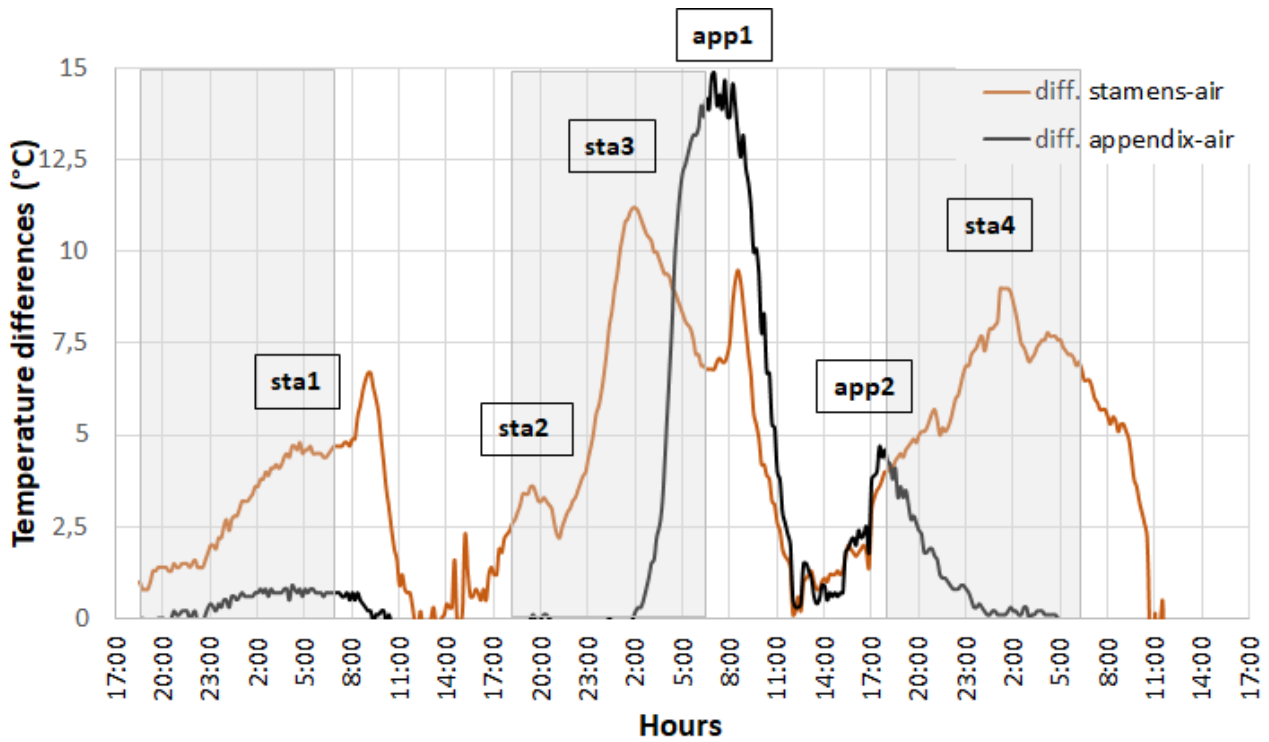


Figure 1.

Temperature difference curves from one inflorescence from Baratz representative of the typical thermogenic pattern in *Arum pictum*: between the appendix and ambient air (black line) and between the male flowers and ambient air (brown line). The grey boxes indicate the night period. The temperature peaks are numbered by chronological order for both the stamens “sta” and the appendix “app”.

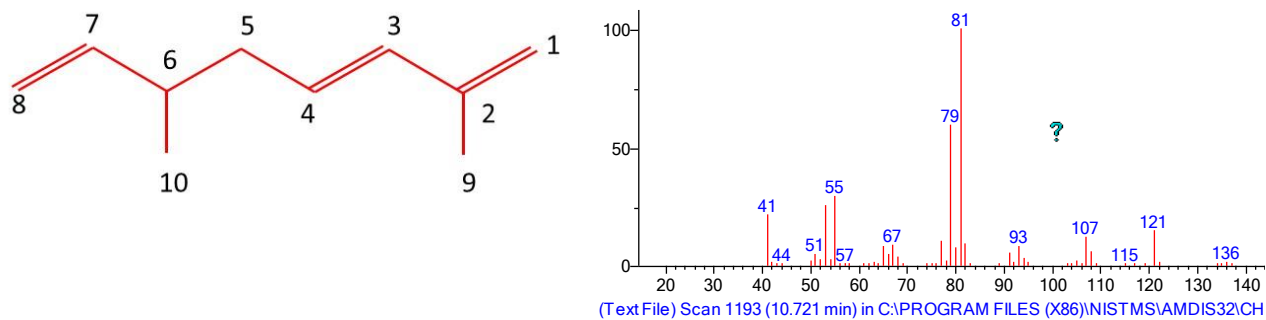
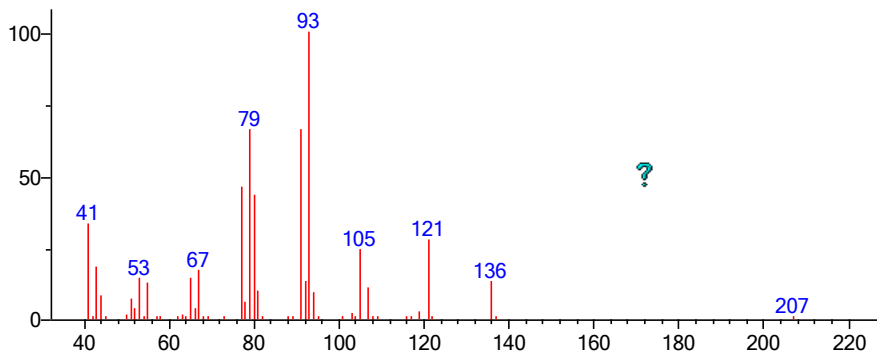


Figure 2.

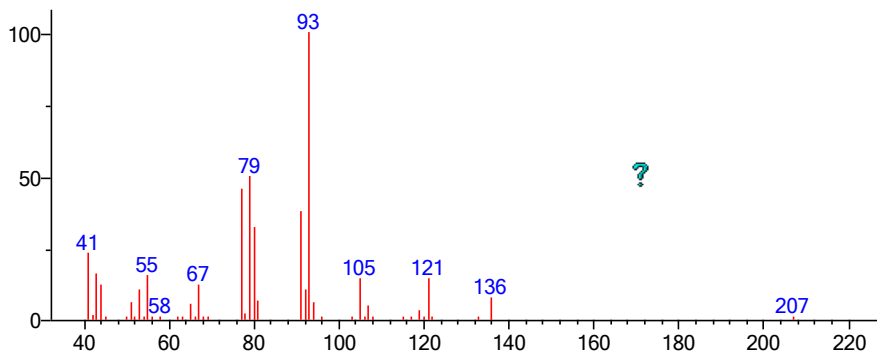
Left. Structure of the 2,6-dimethyl-1,3E,7-octatriene also known as isomycorene. Right. Corresponding mass spectra.

Supplementary material – Mass spectra of the 4 unidentified compounds from Table 2 with the “wrong” best mass library match.



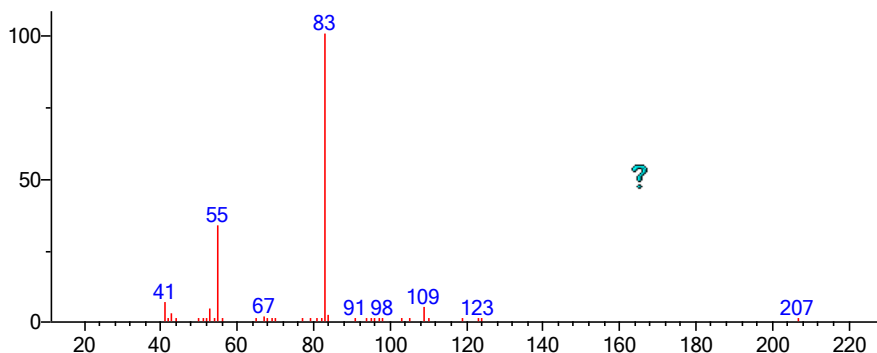
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Unknown 1 (mass library best match: cis- β -ocimene)



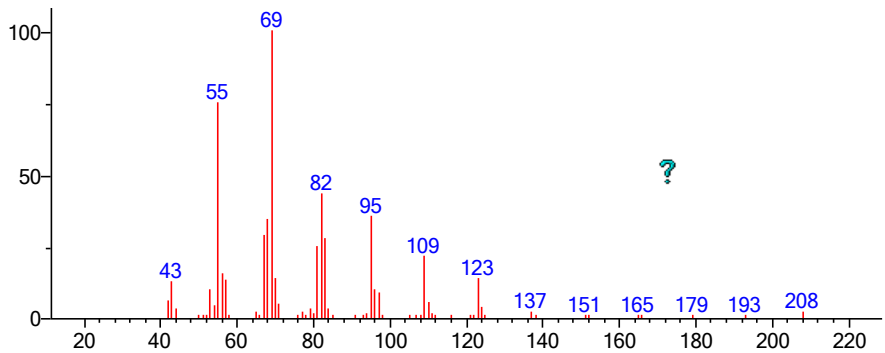
(Text File) Scan 1590 (13.037 min) in C:\PROGRAM FILES (X86)\NISTMS\AMDIS32\CHROMA1

Unknown 2 (mass library best match: Rothrochene)



(Text File) Manual Component in C:\PROGRAM FILES (X86)\NISTMS\AMDIS32\CHROMATO

Unknown 3 (mass library best match: artemisia ketone)



(Text File) Scan 4042 (27.346 min) in C:\PROGRAM FILES (X86)\NISTMS\AMDIS32\CHROMA1
Unknown 4 (mass library best match: citronellyl formate)

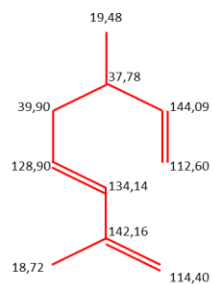
Supplementary material – NMR proton and carbon chemical shift values for isomyocorene comparing our results with published ones.

Isomyocorene
2,6-dimethyl-1,3E,7-octatriene

^{13}C NMR

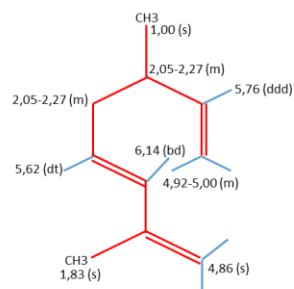


Literature (Jackstell et al. 2007)

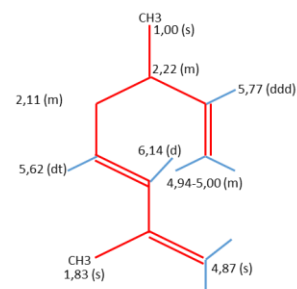


Our results

^1H NMR

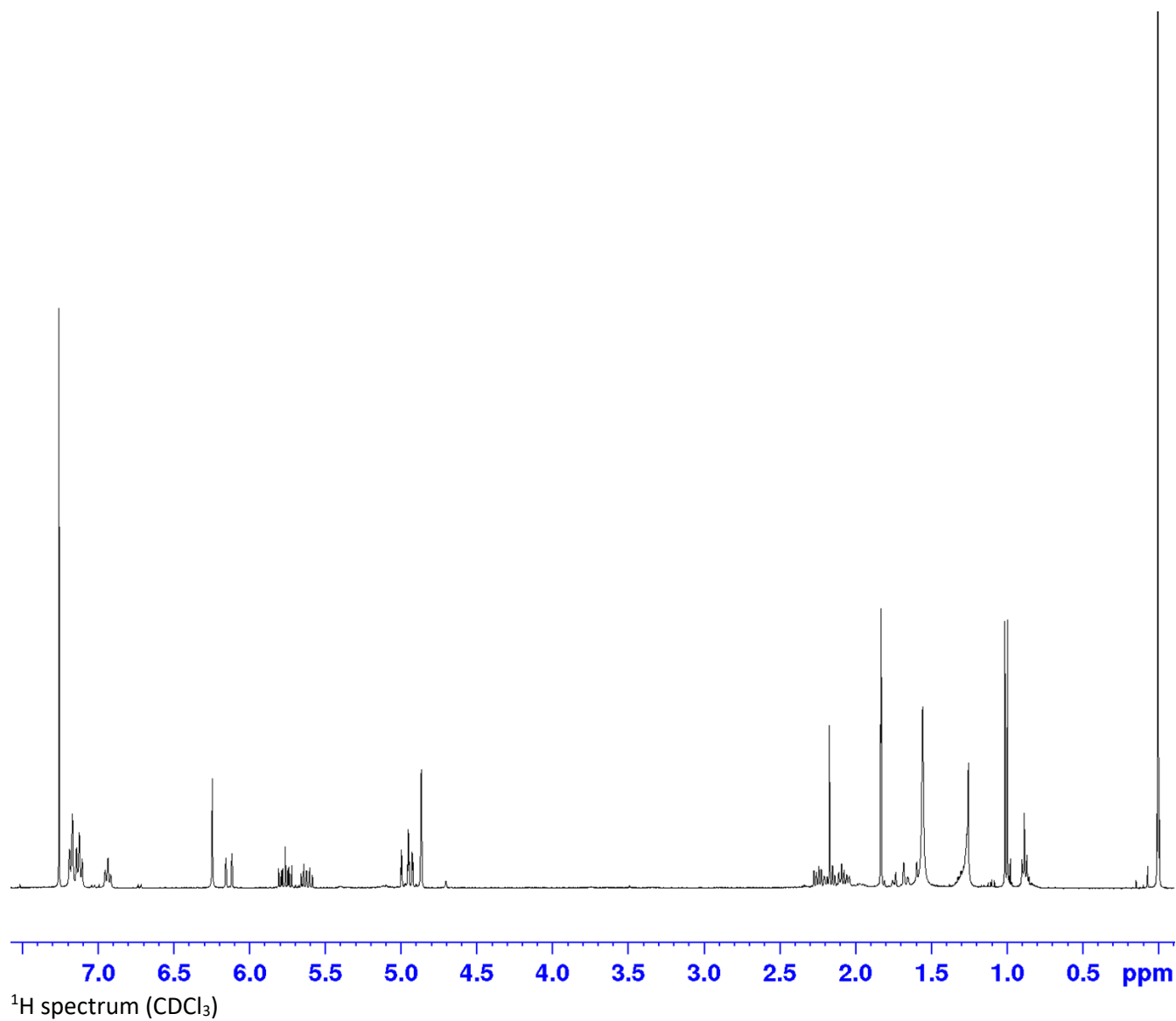


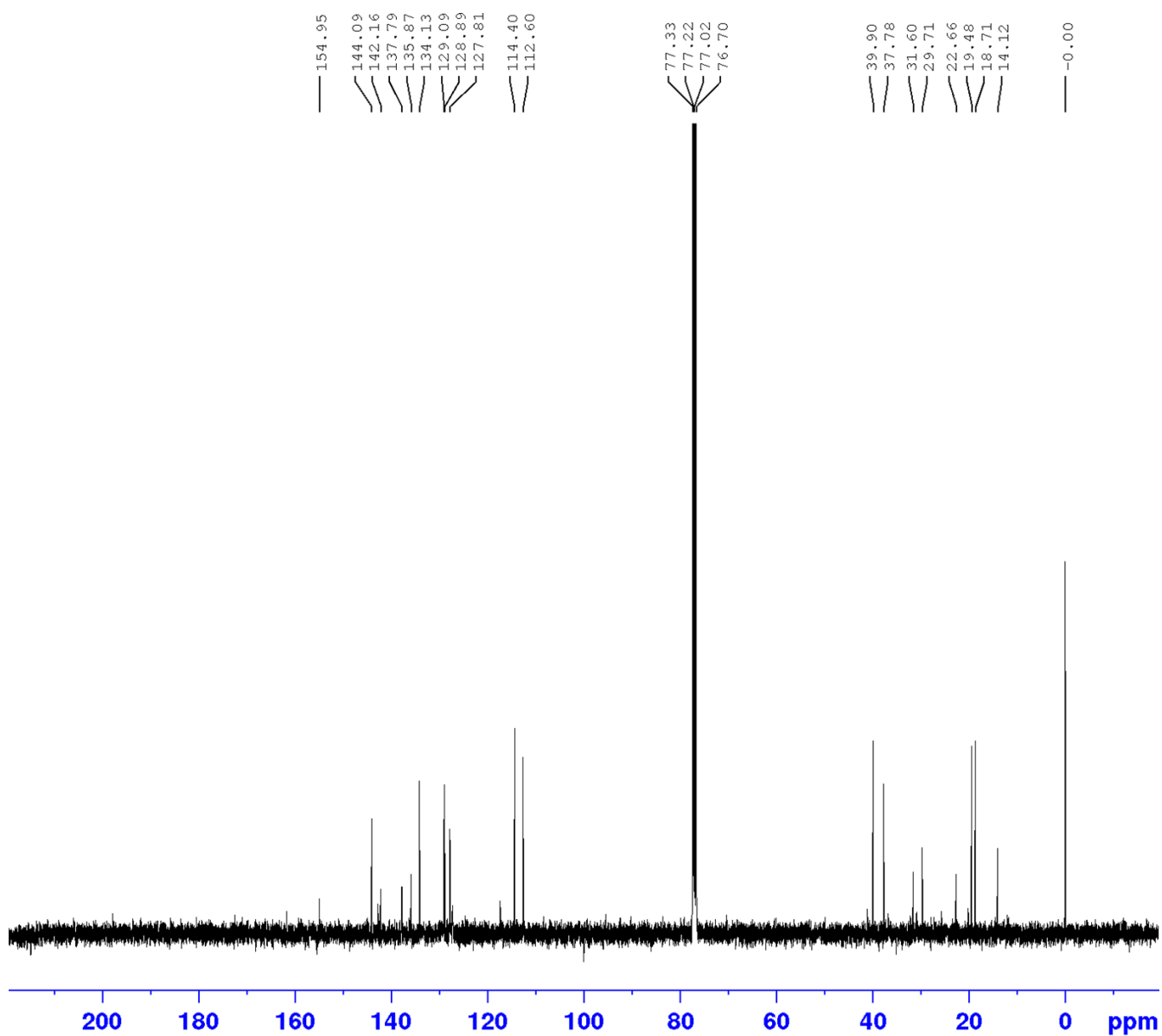
Literature (Jackstell et al. 2007)



Our results

Supplementary material – NMR data for the identification of isomycorene or 2,6-dimethyl-1,3,7-octatriene





^{13}C spectrum (CDCl_3)

