



## Dimorphism in inflorescence scent of dioecious wild grapevine



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### ABSTRACT

Wild grapevine (*Vitis vinifera* subsp. *sylvestris*) is the dioecious ancestral form of grapevine, from which the domesticated cultivars have derived (*V. vinifera* subsp. *vinifera*). Little is known about the floral scent compounds of wild grapevine that is considered as being partly insect pollinated. The knowledge of volatiles released by male and female inflorescence may contribute to the understanding of the pollination biology of this endangered taxon. Inflorescence scents of male and female individuals were collected by dynamic headspace and analysed by thermal desorption-GC/MS. A total of 17 compounds of C5-branched chain alcohols, aliphatics, aromatics, and terpenoids were identified with benzyl alcohol being most abundant in both sexes. Eight of the compounds were sex-specific and differences in 1,2-dimethoxybenzene were most obvious. This aromatic compound was a main constituent in the scent of females (30%), but it did not occur in males. Some of the main compounds of the scent samples are known to be detected by beetles (Cerambycidae) or attract sweat bees (Halictidae) and honey bees (*A. mellifera*), all well-known inflorescence visitors in wild grapevine. The data presented here are an important step in understanding the chemical communication between wild grapevine and its inflorescence visitors/potential pollinators.

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## 1. Introduction

Wild grapevine (*Vitis vinifera* subspecies *sylvestris* (Gmelin) Hegi [Vitaceae]) is considered as the ancestral progenitor of *V. vinifera* L. cultivars (grapevine) which are economically among the most important crops, mainly used for grape, wine and vinegar. Wild grapevine is listed as Endangered in the IUCN red list (IUCN, 1997) due to anthropogenic action, such as spreading of exotic pathogens and improper management of natural environments/habitats (Garfi et al., 2013 and references therein). Little is known about the sexual reproduction of wild grapevine, though this taxon could represent a resource for future breeding programs of cultivated grapevines. This is because the extensive use of only a few clones of cultivated grapevines has drastically reduced their genetic diversity, increasing the risk of epidemic diseases (Garfi et al., 2013). Self-

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pollination by wind seems to be the primary mode of pollination in cultivated grapevine (Martin et al., 2009; Lückner et al., 2004), though detailed studies are missing. Indeed, also insects may be involved in sexual reproduction, and these pollinators are potentially attracted by volatile organic compounds (VOCs) released by the inflorescences (Lückner et al., 2004; Martin et al., 2009; Barbagallo et al., 2014).

In contrast to monoecious cultivated grapevine, wild grapevine is dioecious. In this and other dioecious *Vitis* taxa (Carmona et al., 2008) insects are involved in cross-pollination and the effect of insects on pollination may even exceed the effect of wind [e.g., in *Vitis coignetiae* (Kimura et al., 1998)]. Carmona et al. (2008) observed bees as pollinators of dioecious wild grapevine plants in an Australian germplasm collection and they reported that scent was more attractive than visual stimuli in attracting these insects.

Branties (1978) reported that the inflorescences of the wild grapevine are visited by Cerambycidae, Mordellidae and several other beetles which worked on the anthers, by Halictidae bees that collected pollen on their venter, and by Syrphidae flies which licked the anthers. *Apis mellifera* was also found visiting the inflorescences. Branties (1978) speculated that the inflorescence scent was the stimulus attracting these insects. The inflorescences in male and female wild grapevine plants emit quite strong scents to the human nose (P. Zito, personal observation). However, despite several papers have been published on the volatile composition of inflorescences of different cultivars of grapevine (e.g. Martin et al., 2009; Barbagallo et al., 2014; Matarese et al., 2014), little is known about inflorescence scents of their ancestral progenitor. In fact, studies on VOCs of wild grapevine inflorescences are limited to compounds that elicit electrophysiological responses in antennae of insect grapevine pests (Schmidt-Büsser et al., 2011; von Arx et al., 2011). Thus, a description of the whole blend is missing so far.

In the present study we report the headspace volatile composition of male and female wild grapevine inflorescences and specifically ask: 1) Do inflorescences of female and male plants emit the same or different amounts of scent? 2) Is the qualitative and semi-quantitative scent pattern the same between the sexes or do females and male inflorescences emit different scent bouquets?

## 2. Materials and methods

### 2.1. Volatile collection

Inflorescence scent from male and female wild grapevine individuals was collected in May 2014 from plants cultivated at the experimental station of the Institute of Biosciences and BioResources (IBBR-CNR), Collesano, Palermo–Italy. The genotypes used in the present study were originally collected in the wild from a population of plants growing in the Madonie Mountains (Garfi et al., 2013). For each sex, eight different plants in full bloom were sampled using a dynamic headspace method. On each plant, one inflorescence was enclosed for 3 min in an oven bag (size: 20 × 13 cm, CUKI® cofresco s.p.a.). Subsequently, the emitted scent was trapped for 2 min in an adsorbent TD (thermal desorption)-tube (Varian Inc. ChromatoProbe quartz microvials; length: 25 mm, inner diameter: 1.75 mm) filled with 1.5 mg of Tenax-TA 60–80 and 1.5 mg of Carbotrap B 20–40 (both Supelco) using a membrane pump (G12/01 EB, Rietschle Thomas, Puchheim, Germany) with a flow rate of 200 mL/min. In the same way, scent of leaves was collected as negative control (blank). After sampling, the TD-tubes were stored at –20 °C until chemical analysis.

### 2.2. Chemical analysis

The VOCs trapped in the TD-tubes were analysed by GC/MS using an automatic thermal desorption system (TD-20, Shimadzu, Japan) coupled to a Shimadzu GC/MS QP2010 Ultra equipped with a ZB-5 fused silica column (5% phenyl polysiloxane; 60 m, i.d. 0.25 mm, film thickness 0.25 µm, Phenomenex). The samples were run with a split ratio of 1:1 and a constant helium carrier gas flow of 1.5 mL/min. The GC oven temperature started at 40 °C, then increased by 6 °C/min to 250 °C and held for 1 min. The MS interface worked at 250 °C. Mass spectra were taken at 70 eV (EI mode) from *m/z* 30 to 350. GC/MS data were processed using the GCMSolution package, Version 2.72 (Shimadzu Corporation 2012). Identification of compounds was carried out using the NIST 11, Wiley 9, FFNSC 2, and Adams (2007) databases, and the database available in MassFinder 3. To determine the absolute amount of scent emitted from an inflorescence, known amounts of terpenoids, aliphatics, and aromatics were injected into the GC/MS system; mean peak areas of these compounds were used to determine the total amount of scent (see Dötterl et al., 2005).

### 2.3. Statistical analysis

A Mann–Whitney U-Test was calculated in STATISTICA 7.1 to test for differences in the total absolute amount of scent trapped from female and male inflorescences. We also compared the scent profiles between the sexes using both qualitative (presence/absence of compounds) and semi-quantitative (relative amount of compounds with respect to total peak area) approaches. To determine pairwise qualitative and semi-quantitative similarities, we calculated the Sørensen and Bray–Curtis indices, respectively. Based on the obtained similarity matrices, we performed analyses of similarities (ANOSIM, 10,000 permutations; see e.g., Milet-Pinheiro et al., 2015) to test for differences in scent bouquet between females and males. Non-metric multidimensional scaling (NMDS), based on the Bray–Curtis similarity matrix, was used to graphically display the

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