

Geographical patterns of *in vivo* spontaneously emitted volatile organic compounds in *Salvia* spp.

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DIPARTIMENTO



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INTRODUCTION

STATE OF THE ART

Salvia, with its over 900 species, is the largest genus in the Labiatae family, with a remarkable range of **variation**. The specie has undergone a **geographical radiation** in many areas of the world and, since Bentham's classification (1848), no other study has been made on the new (500+) species. Walker et al. investigated the monophyly of the genus postulated by Bentham analysing two chloroplast DNA regions (rbcL and trnL-F). This study showed the existence of **at least 3 distinct lineages**, in which much of the diversification fits along **biogeographical lines**:

Salvia Clade I

Largely Europe but with one American lineage

Salvia Clade II

America

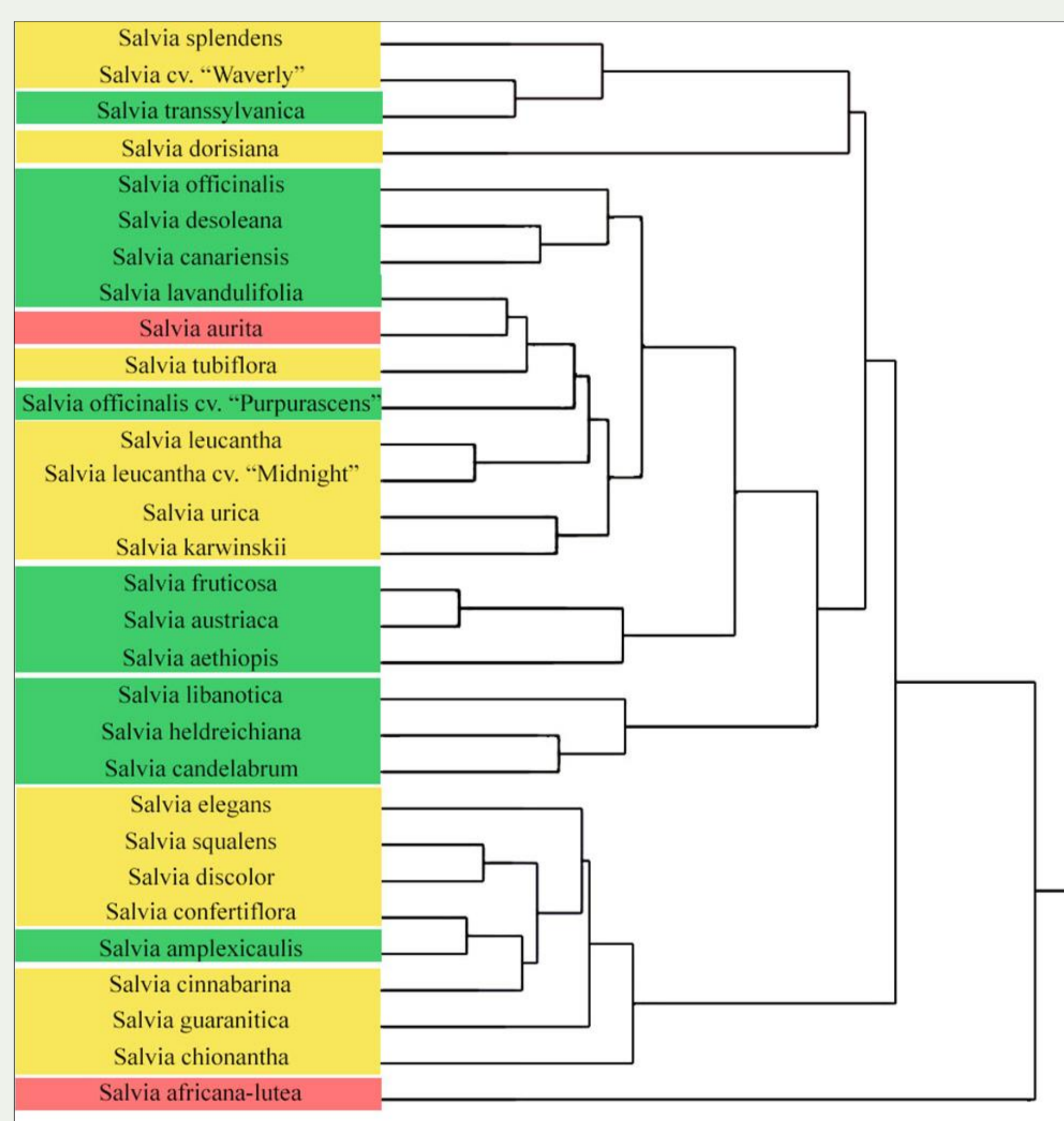
Salvia Clade III

Independent Asian lineage

AIM OF THE STUDY

We investigated 30 species of *Salvia* by means of HS-SPME-GC-MS to evaluate the existence of possible **patterns** in the spontaneous emission of VOCs and to find out possible **parameters** that lead to such patterns. We also investigated the collected leaves samples to assess the presence (or the lack) of thujone (**α - and/or β -thujone**) in the volatile fraction: species with high thujone content are less viable to be used in the food and pharmaceutical industry because of the **neurotoxicity** of these molecules.

RESULTS AND STATISTICS

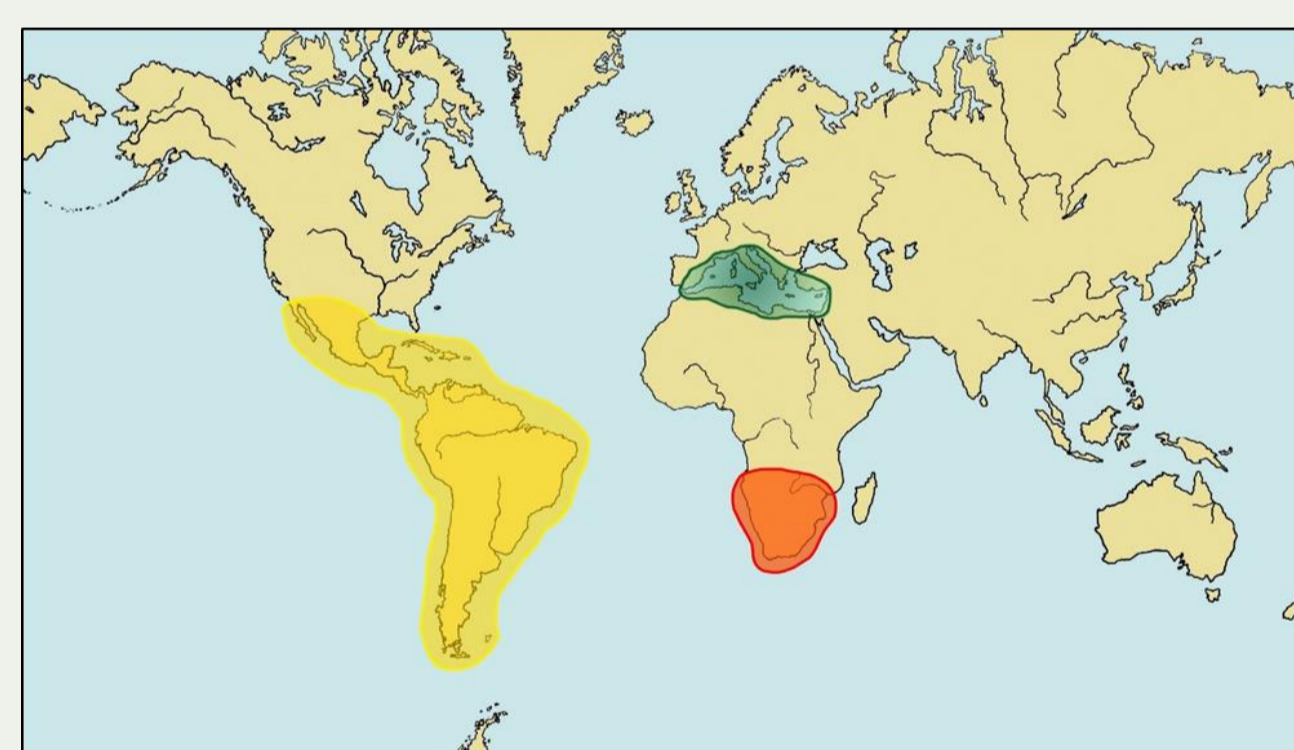


Hierarchical cluster analysis of leaves' VOCs

HCA OF LEAVES VOCs EMISSION

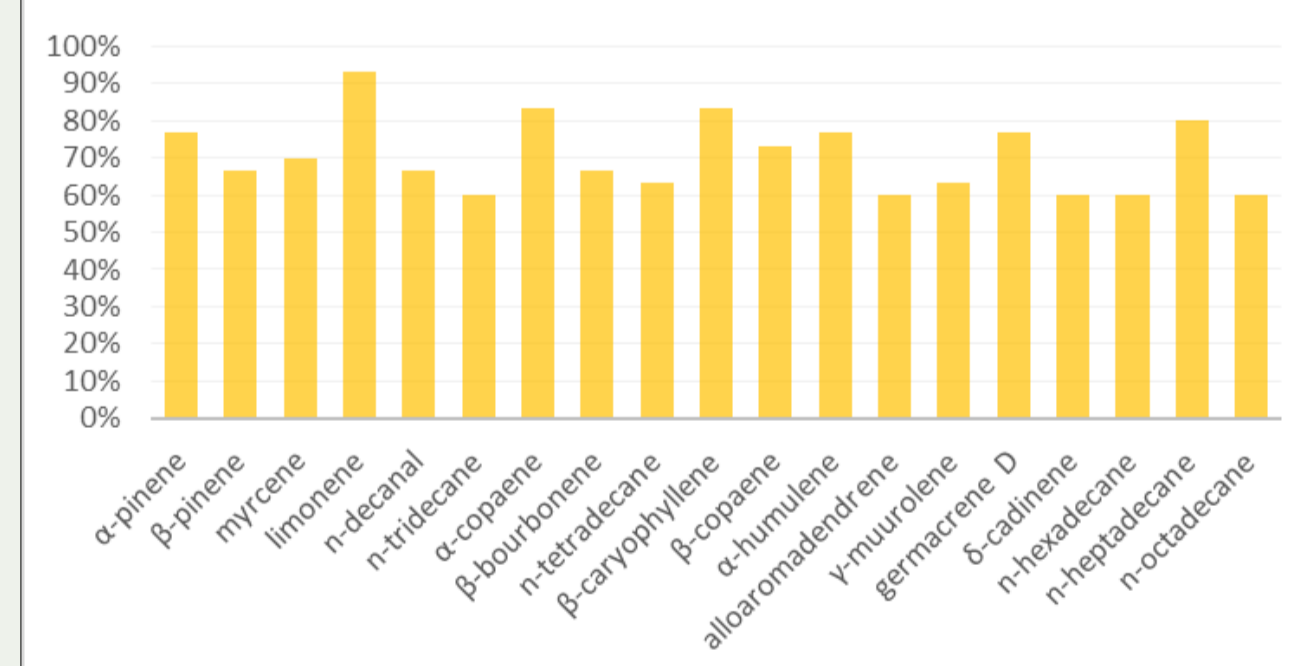
The spontaneous volatile emission profiles **differ significantly according to the geographical origin** of the various species. This permitted to hypothesize that the **environment** plays a fundamental role in *Salvia* secondary metabolites production: the volatile fraction, in particular, represents the reaction of the specimen to the particular local environment (temperature, humidity, latitude, altitude, pollinators, enemies...), making it a **possible chemotaxonomical marker**.

The groups we identified also seem to reasonably match the clades distribution proposed by Walker et al.

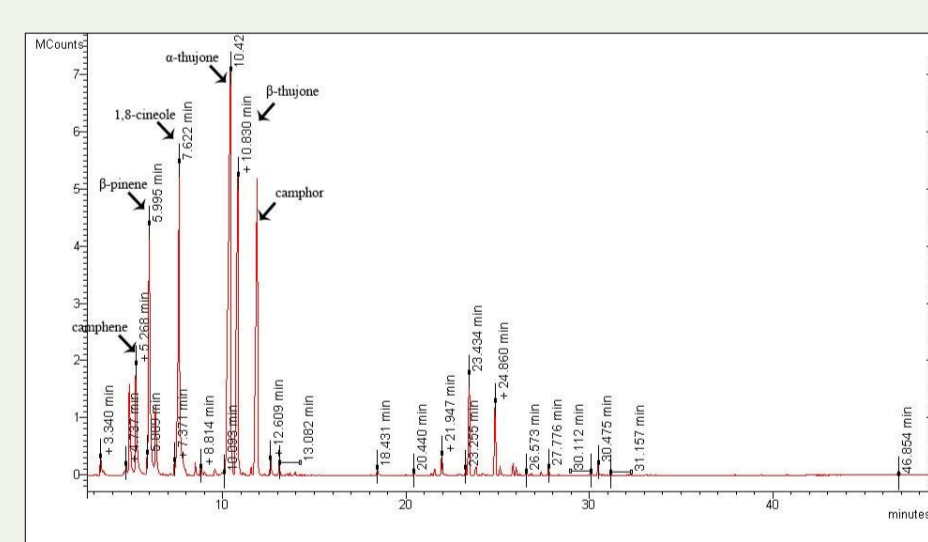


Geographical origin of the collected samples

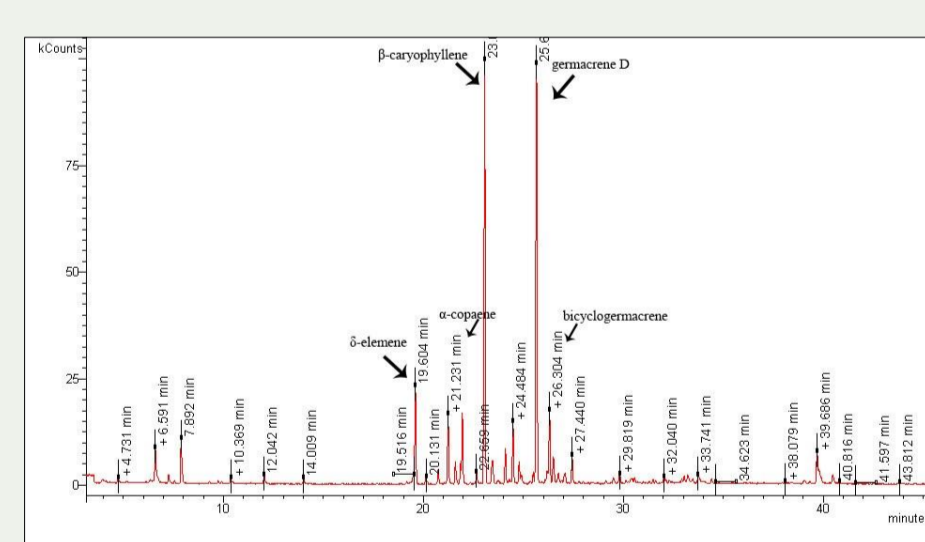
MOST REPRESENTED VOCs



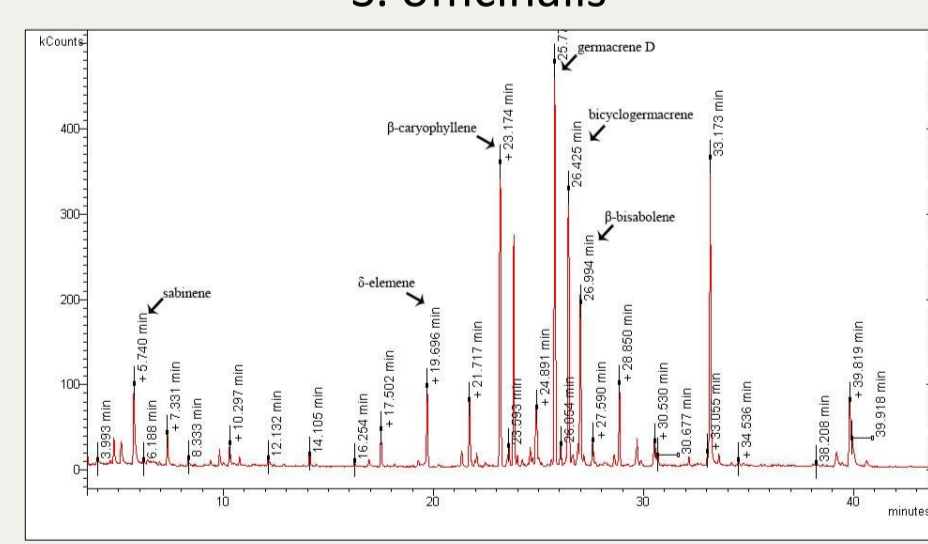
VOCs emitted by the 60% (or more) of the analysed specimens



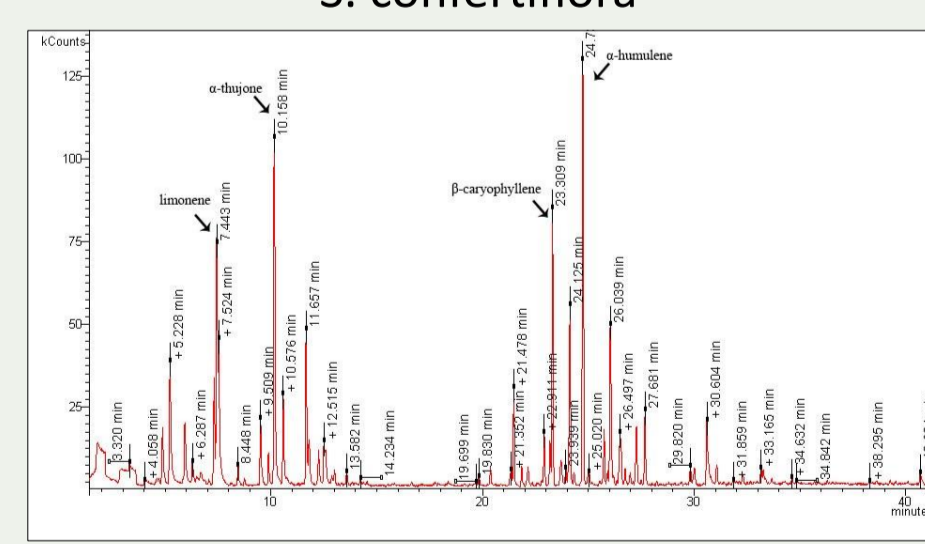
S. officinalis



S. confertiflora



S. cinnabarina



S. lavandulifolia

MATERIALS & METHODS

SAMPLES

30 living leaves samples taken from a collection located at the **Botanical Garden of Pisa**.

HS-SPME-GC-MS

Sampling was carried out for a variable time (30 min – 1 h) with a **PDMS coated fibre**. The GC/EI-MS analyses were performed with a **Varian CP-3800** apparatus equipped with a DB-5 cap. column (30 m x 0.25 mm i.d., film thickness 0.25 mm) and a **Varian Saturn 2000** ion-trap mass detector.

MULTIVARIATE STATISTICAL ANALYSIS

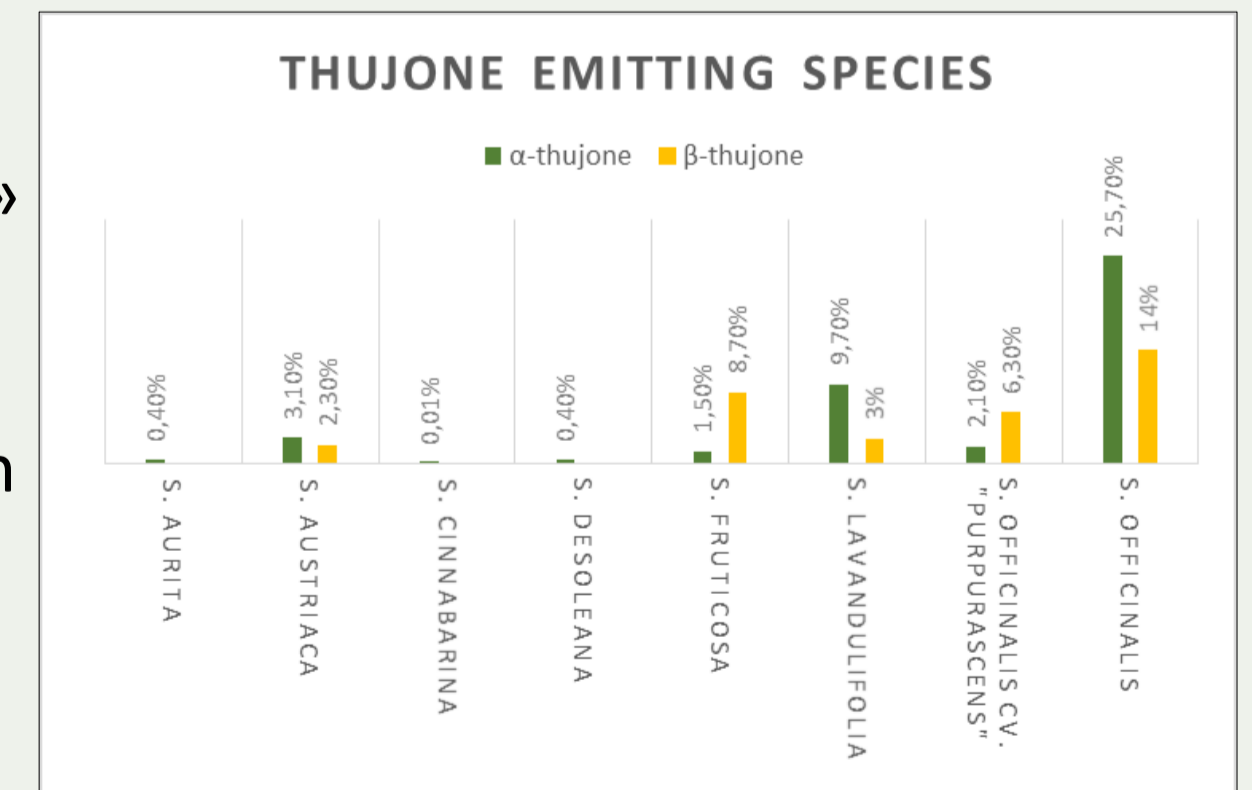
The statistical analyses were carried out with the **JMP software package** (SAS Institute, Cary, NC, USA). The **hierarchical cluster analysis (HCA)** was performed using **Ward's method** with squared Euclidian distances as a measure of similarity.

THUJONE EMITTING SPECIES

Of all the analysed specimens, eight were found to emit α - and/or β -thujone. The four species with the highest percentage of these molecules in their VOCs emission profile are:

1. *S. officinalis*
2. *S. officinalis* cv. «Purpurascens»
3. *S. lavandulifolia*
4. *S. fruticosa*

All of them are plants whose origin is located in the Mediterranean area.



CONCLUSION

OUR FINDINGS

The spontaneous VOCs emission profiles showed a distribution which **significantly matches the geographical origin** of the analysed specimens. The identified cluster also showed a **correspondence with the clades** identified in Walker et al., whose study seems to be confirmed by our results. The volatile fraction of the specimens is a result of the plant adaptation to the environment, thus making it a possible marker of the plants' origin.

POSSIBLE FUTURE ANALYSIS

- Analysis of a wider number of specimens.
- Analysis of Asian specimens.
- Evaluation of essential oils composition patterns.

Bibliography

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