

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

Roberta Ascrizzi<sup>1</sup>, Lucia Amadei<sup>2</sup>, Pier Luigi Cioni<sup>1</sup>, and Guido Flamini<sup>1</sup>

<sup>1</sup>Dipartimento di Farmacia, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italy  
(email: roberta.ascrizzi@for.unipi.it, guido.flamini@farm.unipi.it)

<sup>2</sup>Museo Botanico dell'Università di Pisa, Via Luca Ghini 13, 56126 Pisa, Italy

DIPARTIMENTO



FARMACIA

www.farm.unipi.it



Orto e Museo botanico  
Dipartimento di Biologia  
Università degli Studi di Pisa

## 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

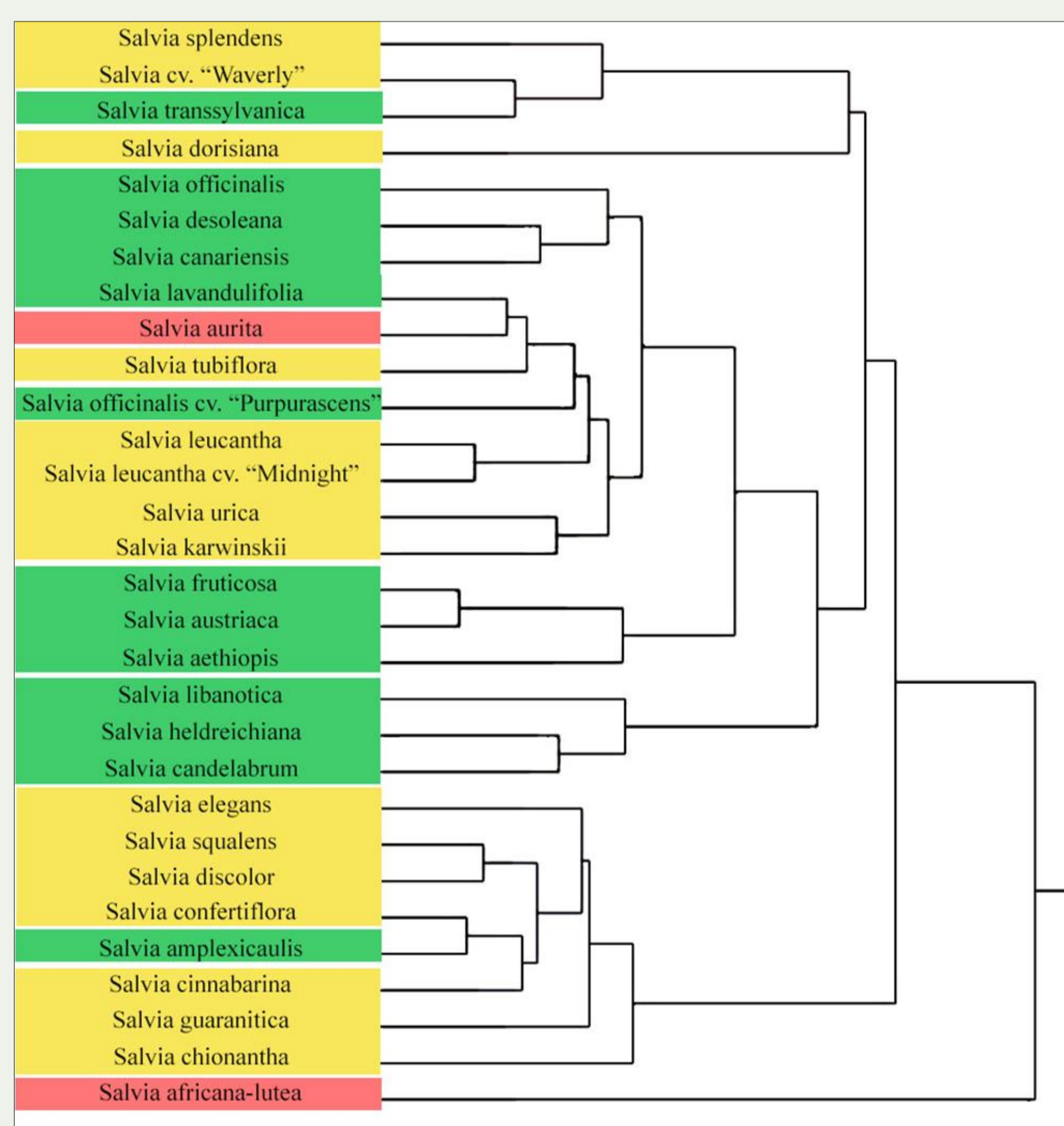
#### Salvia Clade III

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 ( **$\alpha$ - and/or  $\beta$ -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

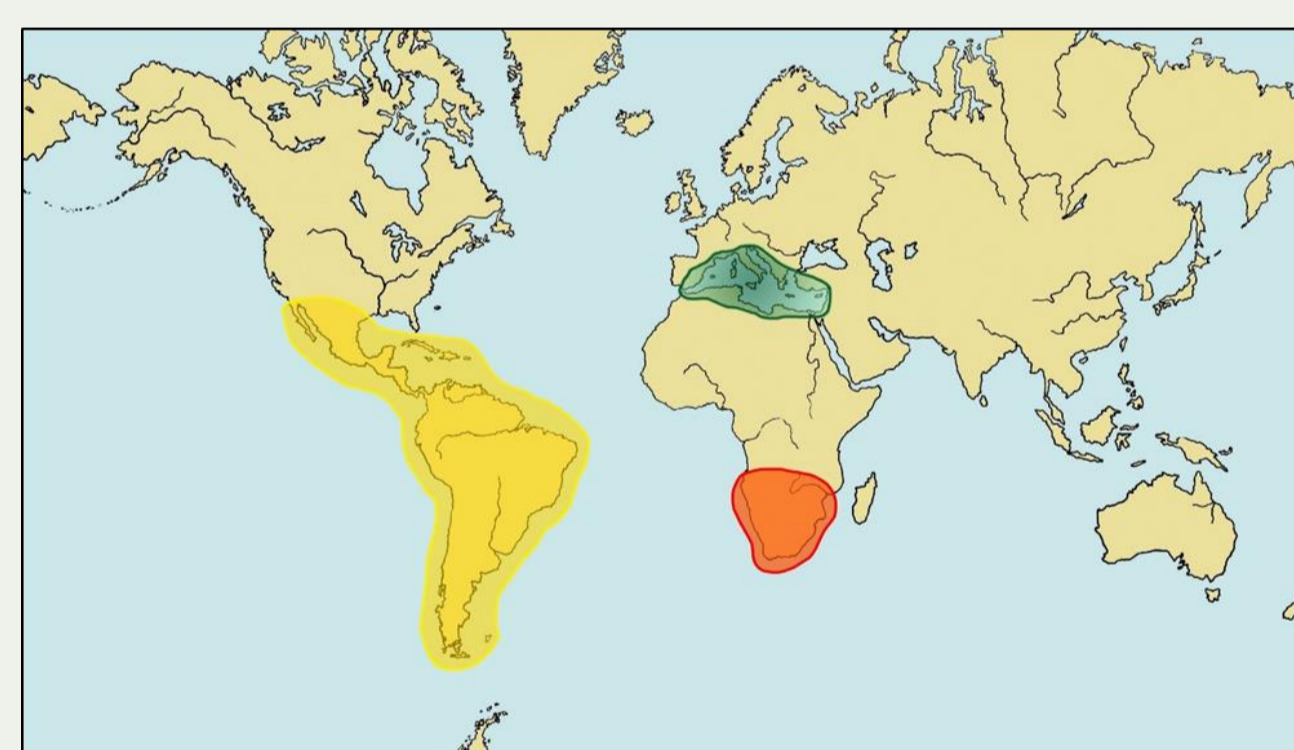


Hierarchical cluster analysis of leaves' VOCs

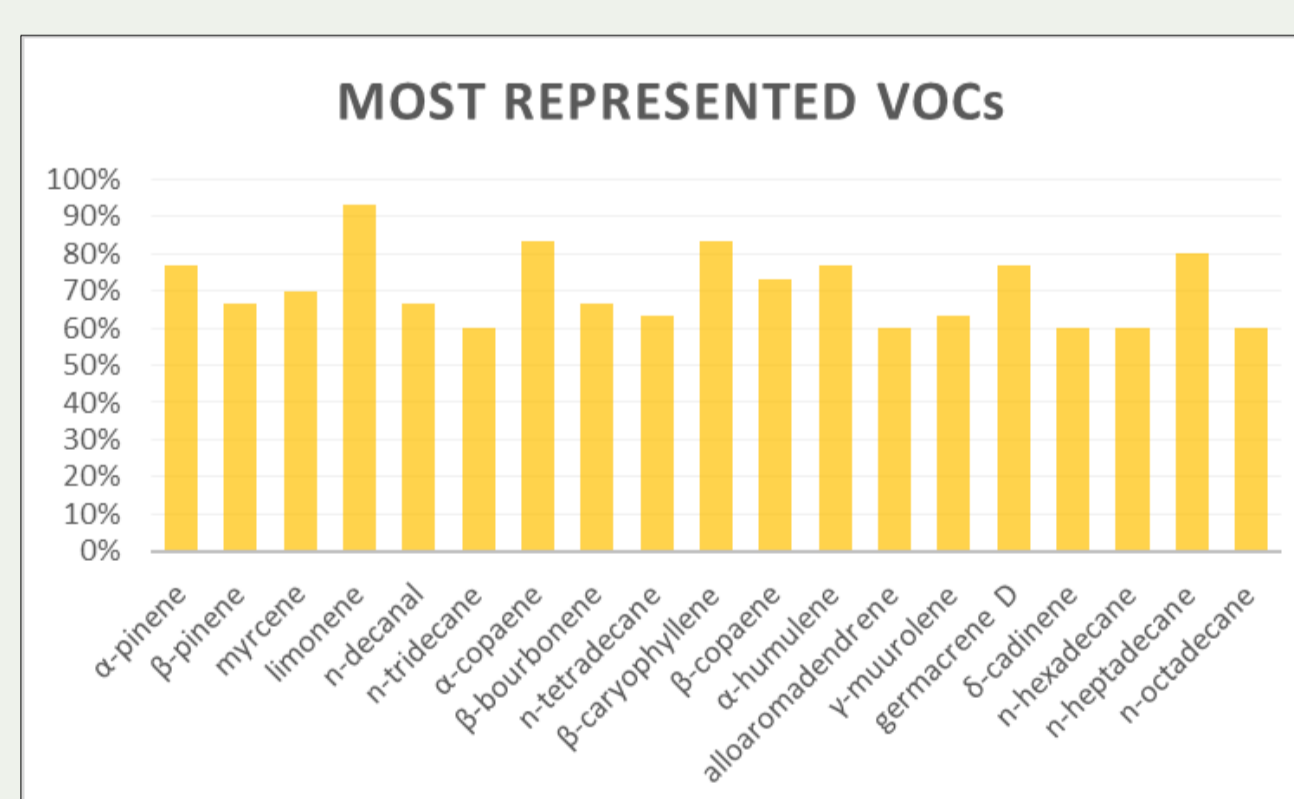
### HCA OF LEAVES VOCs EMISSION

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**.

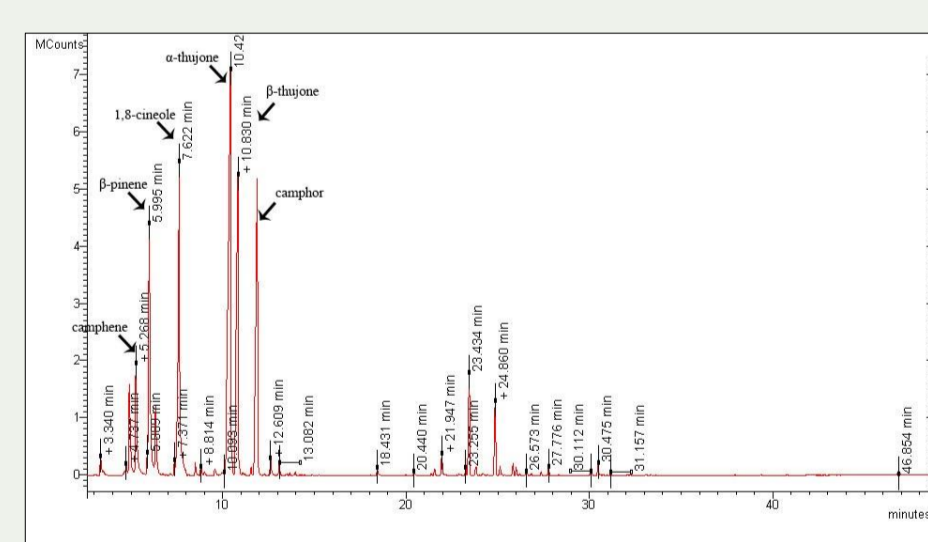
The groups we identified also seem to reasonably match the clades distribution proposed by Walker et al.



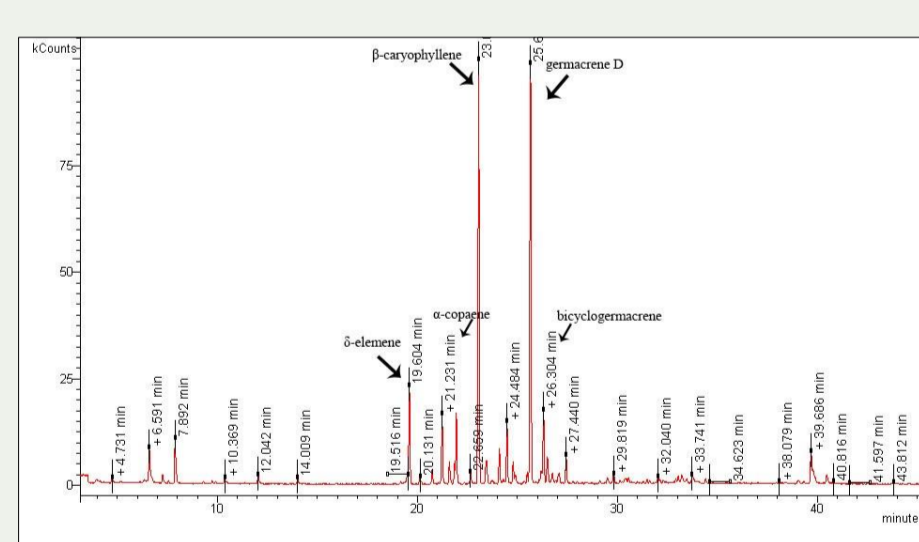
Geographical origin of the collected samples



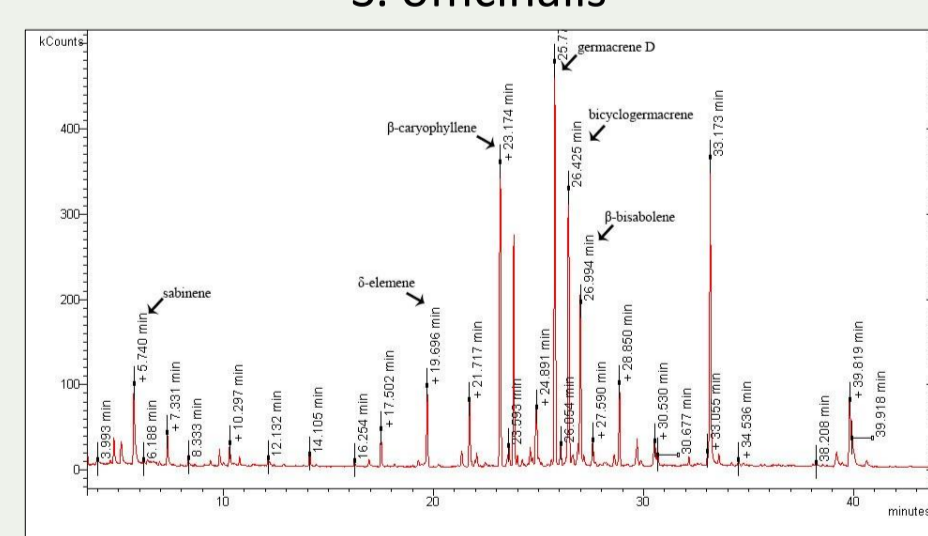
VOCs emitted by the 60% (or more) of the analysed specimens



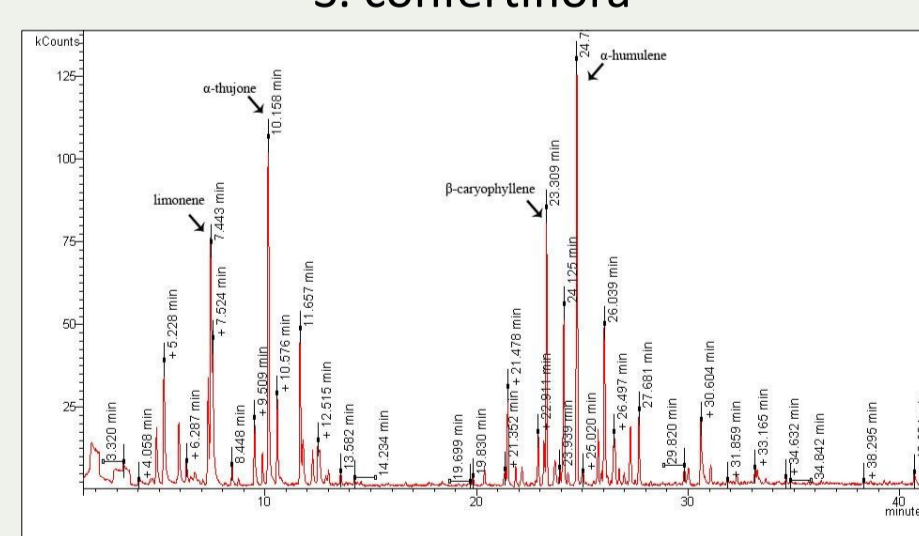
*S. officinalis*



*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

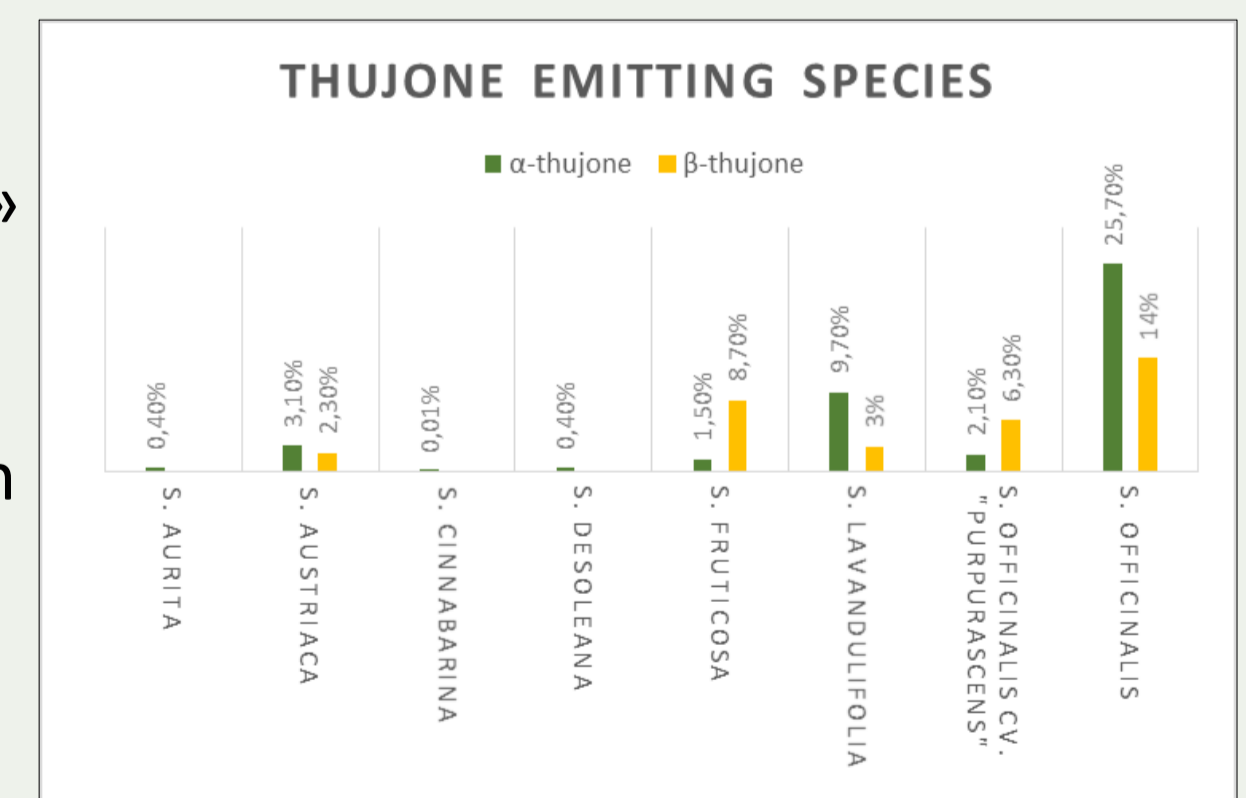
The statistical analyses were carried out with the **JMP software package** (SAS Institute, Cary, NC, USA). The **hierarchical cluster analysis (HCA)** was performed using **Ward's method** with squared Euclidian distances as a measure of similarity.

## THUJONE EMITTING SPECIES

Of all the analysed specimens, eight were found to emit  $\alpha$ - and/or  $\beta$ -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

- Halicioğlu O. et al., 2011. Toxicity of *Salvia officinalis* in a newborn and a child: an alarming report. *Pediatric neurology* 45: 259–60.
- Pelkonen O. et al., 2013. Thujone and thujone-containing herbal medicinal and botanical products: toxicological assessment. *Regulatory toxicology and pharmacology* : RTP 65: 100–7.
- Walker J. B. et al., 2004. *Salvia* (Lamiaceae) is not monophyletic: implications for the systematics, radiation, and ecological specializations of *Salvia* and tribe Menthaeae. *American Journal of Botany* 91: 1115–1125.

