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# The power of odor clues from invasive floral species on appetitive responses of honey bee: attractiveness as a factor of success in French Pyrénées

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## ► To cite this version:

Frank Weber, Benoît Boachon, Sylvie Baudino, Sylvie Guillerme, Martin Giurfa. The power of odor clues from invasive floral species on appetitive responses of honey bee: attractiveness as a factor of success in French Pyrénées. Club Neuroéthologie, Réunion Annuelle 2024, May 2024, Albi (81), France. hal-04875937

**HAL Id: hal-04875937**

**<https://hal.science/hal-04875937v1>**

Submitted on 9 Jan 2025

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## 1 INTRODUCTION

- Two invasive alien plant species (IAS), the **Himalayan balsam (*Impatiens glandulifera* Royle)** and the **Japanese knotweed (*Reynoutria japonica* Houtt.)**, show widespread dispersion patterns in the French Pyrenees.
- Pollinator choice – in particular of honey bees - may contribute to IAS invasive success via **attractive signalling** and rich nectar and pollen rewards.
- Here, we evaluated the **bees' appetitive response to the principal volatile compounds (VOCs) emitted by *I. glandulifera* and *R. japonica*** to determine if they are particularly attractive for honey bees, and thus contribute to their reproductive success.

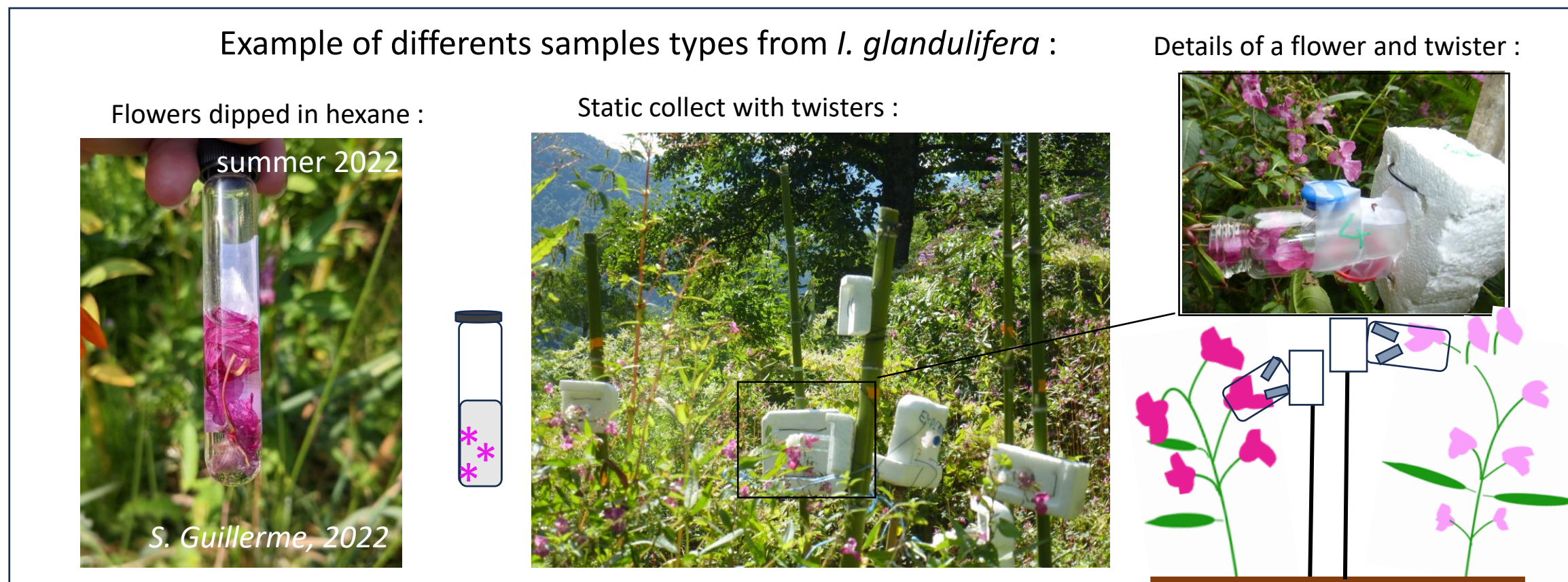
## 2. MATERIAL AND METHODS

### A. Sampling flowers and extracting volatiles compounds

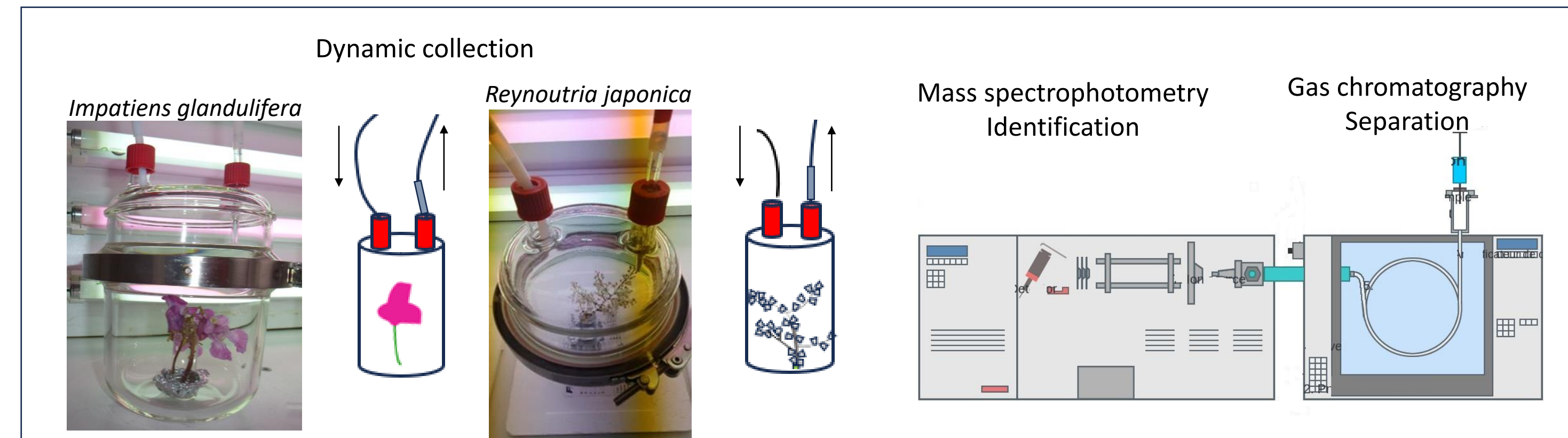
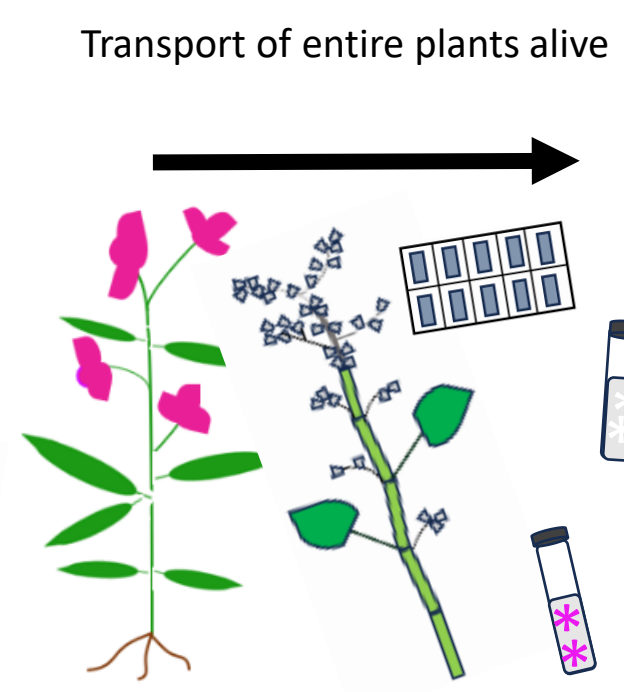
#### 1. Flowers samples collected in the field

#### 2. Transfert to the laboratory

#### 3. Laboratory sample processing



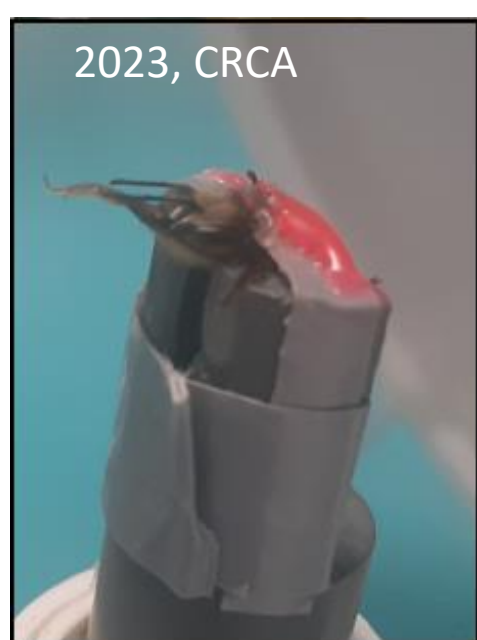
During the flowering period (summer) we collected either flower parts or entire plants for further analysis in the laboratory (see 3.).



Samples obtained were analyzed using **gas chromatography (GC) coupled with mass spectrophotometry (MS)** in order to **identify and quantify the main volatile compounds (VOCs) emitted by these flowers.**

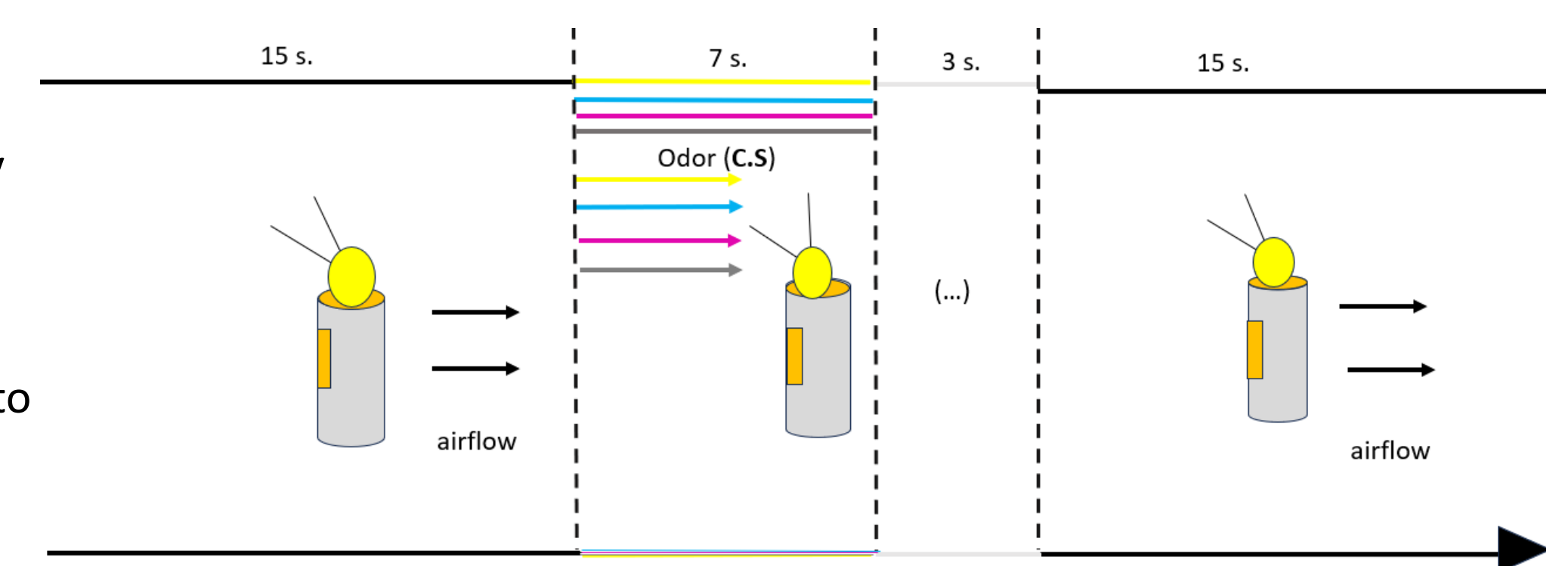
### B. Behavioral assay for testing odor responsiveness in bees

#### 1. PER to olfactory stimuli



- Foraging bees were harnessed in individual containing tubes so that only their heads protruded.
- Bees were presented with flower odors delivered by means of a syringe containing a filter paper impregnated with the odorant. Odorants used were: **linalool, geraniol, ambrettolide and hexanol**. The latter was used as a control as it is not emitted by *I. glandulifera* and *R. japonica*.
- We tested the **spontaneous proboscis extension response (PER)** to the four odorants (one group per odorant), which is the **appetitive response of bees in the presence of an attractive stimulus**.
- Each bee was presented with a series of increasing concentration of each odorant to determine if and when they start reacting to the odor. Clean-air presentations were interspersed between odor presentations as a control.

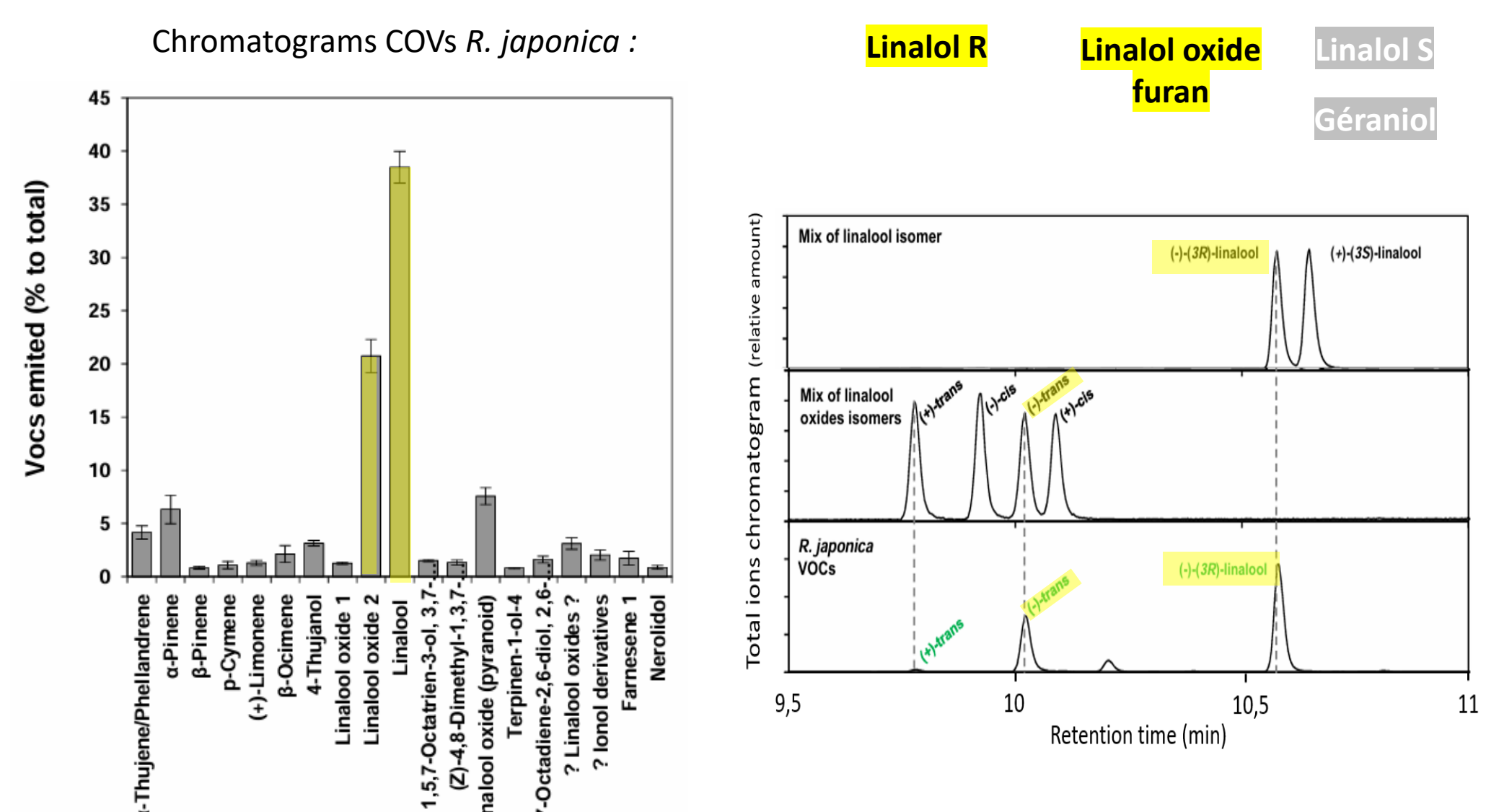
#### 2. Time and action procedure for each bee



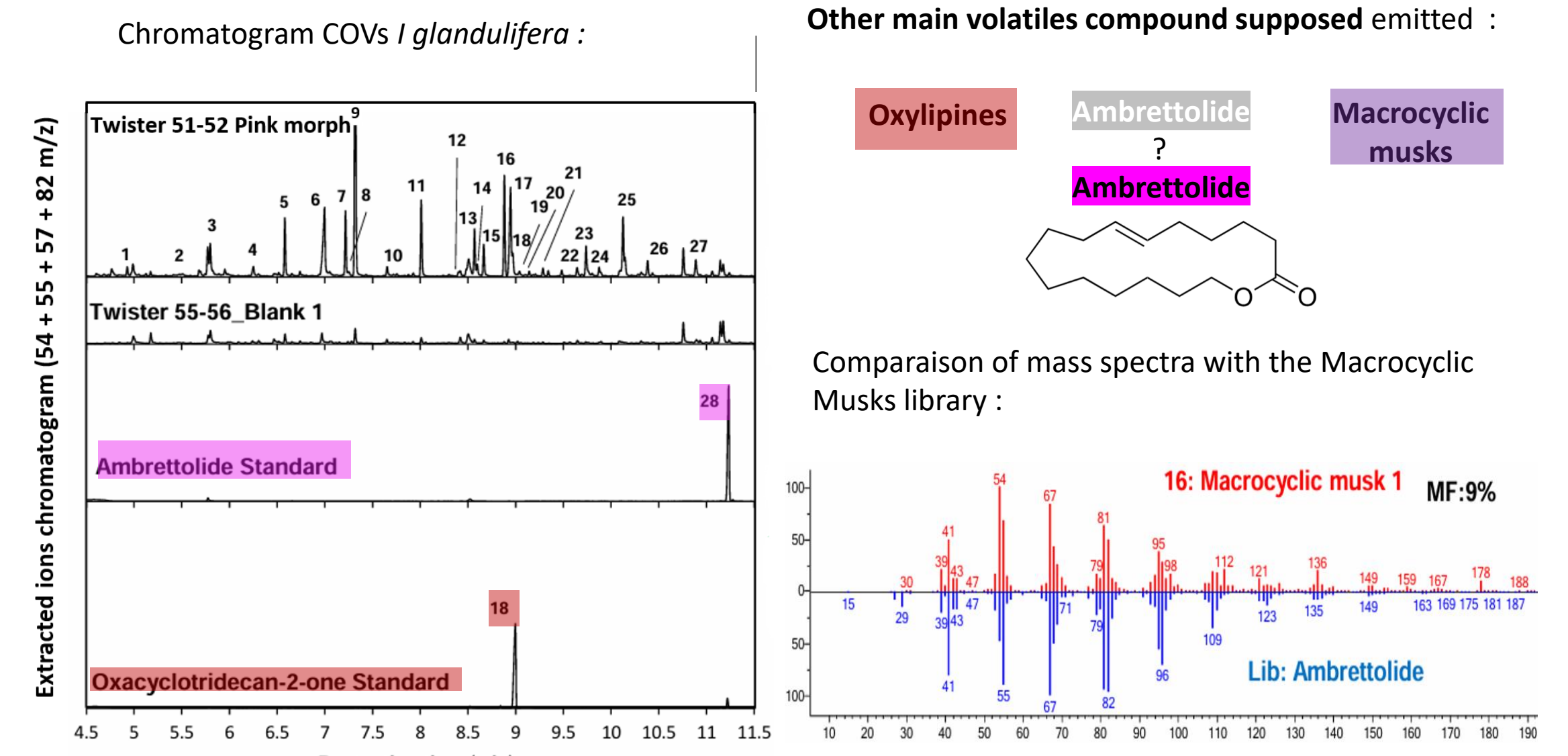
## 3. RESULTS

### A. What are the main volatile compound(s) (COVs) emitted by invasive *I. glandulifera* and *R. japonica* ?

#### 1. Japanese knotweed (*Reynoutria japonica*) fragrance



#### 2. Indian balsam (*Impatiens glandulifera*) fragrance

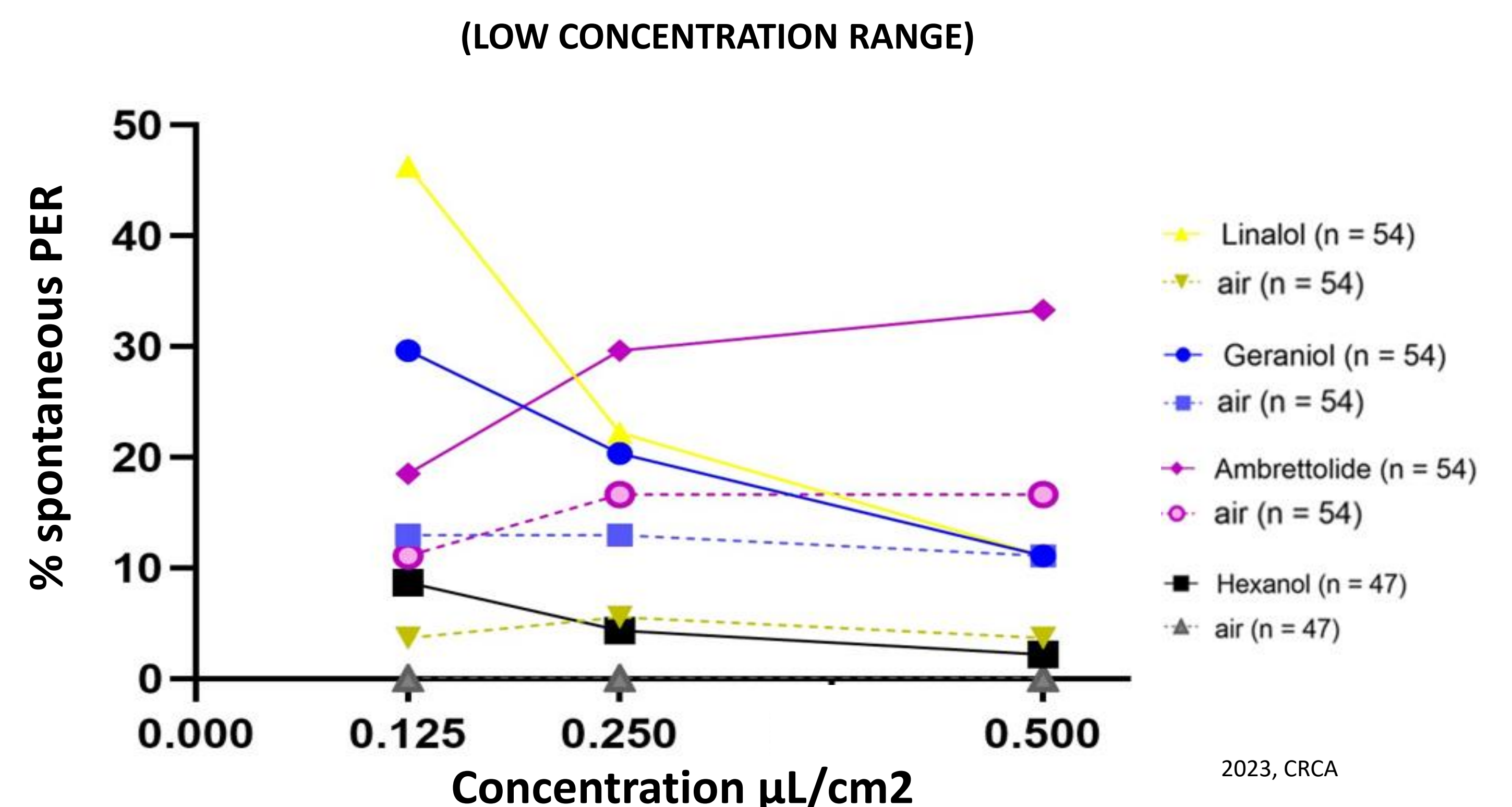
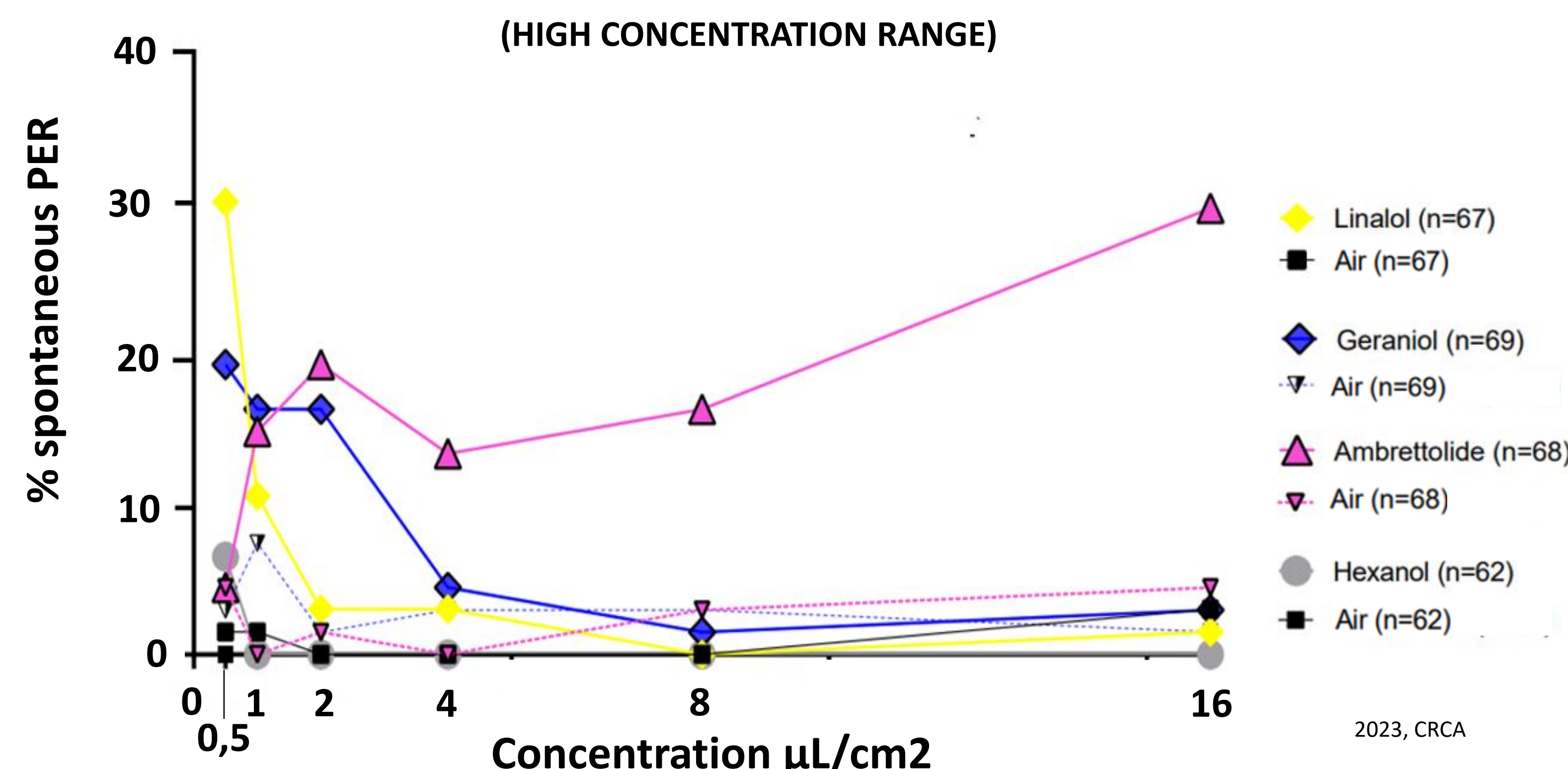


- Linalool and geraniol identified as main COVs for *R. japonica*.** Geraniol only in plant tissues (not emitted) and linalool as main VOC (specifically the R-linalool (40% total of VOCs) and linalool oxide furan (20%).

- Ambrettolide as main COV for *I. glandulifera* ;** 2<sup>nd</sup> measurements identified derivates of macrocylic musk molecules and oxylipines.

### B. Do bees respond spontaneously to odor compounds emitted by *I. glandulifera* and *R. japonica* ?

#### SPONTANEOUS RESPONSES RATE TO ODORANTS



- PER to odorants was recorded using two orders ranges of concentrations of magnitude (from 0.5 to 16 μL/cm<sup>2</sup> and from 0.125 to 0.5 μL/cm<sup>2</sup>).
- Bees showed a high level of spontaneous PER to geraniol and linalool (from 20% to 50%) in both concentration ranges and upon the very first odor presentation. This shows that these odorants have a **high attractiveness for foragers**.
- Across presentations and concentration increase, responsiveness to linalool and geraniol decreased in an habituation-like fashion.**
- Presentation of ambrettolide, on the contrary, resulted in a progressive increase of responsiveness along concentrations in a sensitization-like manner.** Although the responses recorded do not follow the same pattern as those for geraniol and linalool, they show a clear sensitizing, **appetitive value for the bee**. Ambrettolide could have a natural reinforcing value as PER responses follow a pattern comparable to that observed upon stimulation with increasing sucrose concentrations.
- Responses to the control odorant hexanol were either extremely low or null.

## 4. DISCUSSION

- The study of the fragrance of two invasive flower species reveals the presence of odorants with intrinsic appetitive value, which either elicit high level of spontaneous appetitive responses upon the first presentation (geraniol, linalool) or trigger responses similar to sensitization via a reinforcing stimulus (ambrettolide). These two strategies converge with the different color signalling adopted by these two species: while *R. japonica* displays colors that are perceptually similar to those of envionring native species, *I. glandulifera* exhibits salient colors (in the majority of its morphs) combined with a rich nectar offer.
- .. These results indicate that **IAS benefit from olfactory cues to enhance their attractiveness and thus enhance their competitive success in the rich floral pollination market of the French Pyrenees.**