Biological control formulations incorporating essential oil components

Stéphanie Heuskin, Ir.
Department of Analytical Chemistry, Gembloux Agro-Bio Tech, University of Liege, Belgium.
Stephanie.heuskin@ulg.ac.be

Walloon Region Ministry grant (WALEQ2: SOLAPHID-RW/FUSAGX 061/6287)
Definitions

Biological control of insect pests:

« the use of living organisms (insects or pathogens) to suppress pest populations, making them less damaging that they would otherwise be »


⇒ Semiochemicals
**Definitions**

*Semiaochemicals*: in Greek « semeion » = signal

« Chemicals emitted by living organisms (plants, insects,...) that evoke a behavioural or a physiological response in other individuals »
Semiochemicals

Intraspecific interactions

- Alarm
- Sex
- Aggregation
- Trail
- Host marking
- ...

Interspecific interactions

- Allomones: + emitting species
- Kairomones: + receptor species
- Synomones: + emitting, + receptor

A same molecule can be a pheromone and an allelochemical substance
Aphid problem

Hemiptera: Aphididae
Aphid tritrophic system

1st level

Plant

Vicia fabae (L.)

2nd level

Herbivorous pest insect

Aphid: Megoura viciae (Buckton)

3rd level

Predator

Larva of Episyrphus balteatus (De Geer)

Parasitoid

Aphidius ervi (Haliday)
Volatile compounds

Alarm pheromone
E-β-Farnesene

- Alarm pheromone of aphid species
- Kairomone: attraction of predators (Diptera: Syrphidae) and parasitoids (Hymenoptera: Braconidae) of aphids

Isolated from essential oil of Matricaria chamomilla L. (Asteraceae)
**Matricaria chamomilla essential oil**

*Originated from Nepal (Vossen & Co., Belgium)*

<table>
<thead>
<tr>
<th>No.</th>
<th>Major compounds</th>
<th>Retention index</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E-(\beta)-farnesene</td>
<td>1456</td>
<td>42,6</td>
</tr>
<tr>
<td>2</td>
<td>Germacrene D</td>
<td>1478</td>
<td>2,9</td>
</tr>
<tr>
<td>3</td>
<td>bicyclogermacrene</td>
<td>1494</td>
<td>1,9</td>
</tr>
<tr>
<td>4</td>
<td>(E,E)-(\alpha)-farnesene</td>
<td>1506</td>
<td>8,3</td>
</tr>
<tr>
<td>5</td>
<td>(\alpha)-bisabolol oxide B</td>
<td>1649</td>
<td>4,4</td>
</tr>
<tr>
<td>6</td>
<td>(\alpha)-bisabolone oxide A</td>
<td>1673</td>
<td>4,5</td>
</tr>
<tr>
<td>7</td>
<td>Chamazulene</td>
<td>1715</td>
<td>1,1</td>
</tr>
<tr>
<td>8</td>
<td>(\alpha)-bisabolol oxide A</td>
<td>1735</td>
<td>21,1</td>
</tr>
<tr>
<td>9</td>
<td>Cis-ene-yne-dicycloether</td>
<td>1802</td>
<td>5,9</td>
</tr>
</tbody>
</table>
**β-Caryophyllene**

Sesquiterpene: \( \text{C}_{15}\text{H}_{24} \)

- **Aggregation pheromone** of the Asian lady beetle, *Harmonia axyridis* (Pallas) (aphid predator)


- **Isolated from** essential oil of *Nepeta cataria* L. (Lamiaceae)
**Nepeta cataria essential oil**

Originated from Canada (Essential7, USA)

<table>
<thead>
<tr>
<th>No.</th>
<th>Major compounds</th>
<th>Retention index</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(Z,E)-nepetalactone</td>
<td>1353</td>
<td>8.4%</td>
</tr>
<tr>
<td>2</td>
<td>(E,Z)-nepetalactone</td>
<td>1377</td>
<td>22.5%</td>
</tr>
<tr>
<td>3</td>
<td>β-caryophyllene</td>
<td>1415</td>
<td>58.9%</td>
</tr>
<tr>
<td>4</td>
<td>α-humulene</td>
<td>1465</td>
<td>3.9%</td>
</tr>
</tbody>
</table>
Fast GC analytical method optimisation

Program of T°:
- Initial T°: 40°C; 0.10 min
- Ramp 1: 30°C/min → 95°C
- Ramp 2: 35°C/min → 155°C
- Ramp 3: 200°C/min → 280°C; 0.5 min
- Oven run time: 4.78 min

Ultra Fast Module: Ph5; 0.1 µm film thickness, 5m x 0.1mm I.D.

Carrier gas: He; 0.5 ml/min

Split ratio: 1:100

Analytical validation
Accuracy profile concept

According to the guidelines of the Société Française des Sciences et Techniques Pharmaceutiques (SFSTP).

E-β-farnesene

β-caryophyllene
- **Trueness**
  - Relative bias (%)
  - Systematic error

- **Precision**
  - 95% β-expectation tolerance limits (%)
  - Random error

- **Accuracy**
  - Total error

The **trueness** expresses the closeness of agreement between the mean value obtained from a series of measurements and the value accepted as the true value.

The **precision** expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same sample. (repeatability – intermediate precision (RSD%))

The **accuracy** expresses the closeness of agreement between the value found and the value accepted as the conventional true value.
Acceptance limits
($\lambda \pm 15 – 25 \%$)

**Essential oil fractionation**

Flash Chromatography: purification of semiochemical compounds

Composition of solvent-free semiochemical enriched fractions:

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of monoterpenes</td>
<td>1.3 %</td>
</tr>
<tr>
<td>E-β-farnesene</td>
<td>84.0 %</td>
</tr>
<tr>
<td>Germacrene D</td>
<td>1.4 %</td>
</tr>
<tr>
<td>Bicyclogermacrene</td>
<td>1.4 %</td>
</tr>
<tr>
<td>(E,E)-α-farnesene</td>
<td>11.9 %</td>
</tr>
</tbody>
</table>

**Matricaria chamomilla**

**Nepeta cataria**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of monoterpenes</td>
<td>1.5 %</td>
</tr>
<tr>
<td>β-caryophyllene</td>
<td>97.4 %</td>
</tr>
<tr>
<td>α-humulene</td>
<td>1.1 %</td>
</tr>
</tbody>
</table>
Semiochemical alginate beads formulation as slow-release devices

- **Semiochemical + sunflower oil (oil phase)**
- **Alginate solution**
- **Oil phase**
- **Water phase**

**Steps:**
1. Homogenization
2. o/w emulsion
3. Peristaltic pump

**Products:**
- Dry alginate beads (Ø 2mm)
- CaCl₂ Suspension (maturation)
Semiochemical alginate beads formulation

→ Optimisation of bead density and encapsulation capacity according to:

- Alginate type: Mannuronate/Guluronate ratio
- Alginate concentration
- CaCl$_2$ concentration
- Reaction time in CaCl$_2$
**Semiochemical alginate beads formulation**

⇒ Protection efficiency of beads towards sesquiterpenes

(Heuskin et al., JPBA 2010)
Release rate measurement: dynamic sampling

- Activated charcoal filter
- Adsorbent (SuperQ) cartridge
  - Solvent elution + IS quantification (Fast GC)
- Teflon box with semiochemical beads
- Pump
**Release rate study**

Laboratory-controlled conditions:
- Temperature: 20°C
- Relative humidity: 65%
- Air flow: 0.5 L/min
Release rate study

Modelisation of release rate according to physico-chemical parameters (T°, RH, wind speed)

→ Preliminary tests: diffusion coefficients estimation
  - no influence of wind speed
  - influence of temperature
  - influence of relative humidity
Biological tests

On parasitoids (*Aphidius ervi*): 2 ways olfactometer

- Alginate beads without semiochemicals (blank)
- Alginate beads with semiochemicals

Air flow
Biological tests

On parasitoids (Aphidius ervi): 2 ways olfactometer

Alginate beads without semiochemicals (blank)

Alginate beads with semiochemicals

*** very highly significative difference (P<0.001)
**Biological tests**

*On Syrphidae: on-field experiments (June – August 2009)*

Dunnett Test (95%) : comparison of attractiveness between semiochemical beads and blank

- *E-β-Farnesene*: P-value = 0.0200 (< 0.05) * significative difference
- *β-Caryophyllene*: P-value = 0.0064 (< 0.01) ** highly significative difference
Conclusions

• Sesquiterpenes of high purity from essential oil origin
• Fast and accurate analytical method
• Semiochemical alginate beads:
  - efficient biological control devices
  - slow-release systems
• Slow-release modelization according to physico-chemical parameters: In progress
Aknowledgments

Walloon Region Ministry grant (Belgium)
(WALEO2: SOLAPHID-RW/FUSAGX 061/6287)

Department of Analytical Chemistry
Prof. G. Lognay

Department of Functional and Evolutionary Entomology
Prof. E. Haubruege & Dr. F. Verheggen

Institute of Condensed Matter and Nanosciences
Ir. S. Lorge
Thank you for your attention