Development of semiochemical slow-release formulations as biological control devices against aphids

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Post-doc

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2. Evolutionary Ecology and Genetics Group, Earth and Life Institute, Catholic University of Louvain, Belgium
SOLAPHID project (WALEO 2)

Funding from the Belgium Walloon Region
(2006-2011)

“Biotechnologies related to the industrial production of insects used in biological control”

5 teams: chemistry – formulation – entomology – chemical ecology - industrial production
Summary

General introduction

Objective

The choice of semiochemicals and their origin

How to analyse and quantify semiochemicals?

How to purify semiochemicals?

How to formulate semiochemicals?

Is the formulation efficient?

Conclusions and perspectives
General introduction
The aphid problem

Damages to crops: virus and disease transmitter

Economical and agricultural problem

Pesticide control is limited
- resistance of pest insects
- non species-specific
- unsafe for environment and human health

Biological control
Biological control as pest management strategy

“The use of natural enemies to reduce the damage caused by a pest population”

Attraction of aphid natural enemies
Aphid tritrophic system

1st level
Host plant

2nd level
Aphids

3rd level
Predators

Parasitoids

➡️ Chemical communication: semiochemicals
Semiochemicals

Plant – insect – insect chemical communication signals

- Pheromones
  - alarm
  - sex
  - aggregation
  - trail
  - host marking
  - ...

- Allelochemicals
  - allomones: + emitting species
  - kairomones: + receptor species
  - synomones: + emitting, + receptor

A same molecule can act as a pheromone and as an allelochemical substance
Objective
Global objective

To develop natural semiochemical slow-release formulations as biological control devices attractive towards aphid natural enemies

Which semiochemicals?
- Natural origin?
  - Purification?

Which formulation?
- Analysis and quantification?
- Efficiency?
  - Release?
  - Attractiveness?
The choice of semiochemicals and their natural origin
**E-β-farnesene**

- Aphid alarm pheromone

- Kairomone: attraction of aphid predators (*Episyrphus balteatus De Geer*)\(^2-4\) and aphid parasitoids (*Aphidius ervi* Haliday) \(^5-6\)

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1 Bowers et al., 1972
2 Francis et al., 2005
3 Verheggen et al., 2008
4 Verheggen et al., 2009
5 Du et al., 1998
6 Powell et al., 2003
E-β-caryophyllene

Sesquiterpene (C\textsubscript{15}H\textsubscript{24})

- Reducer of aphid reproduction\textsuperscript{1}
- Attractive towards aphid parasitoids (A. ervi Haliday)\textsuperscript{2}

\textsuperscript{1} Tomova et al., 2005
\textsuperscript{2} Sasso et al., 2009
Natural matrix for sesquiterpenes

→ Essential oils

- Matricaria chamomilla L. (Asteraceae): E-β-farnesene

- Nepeta cataria L. (Lamiaceae): E-β-caryophyllene
**Essential oil characterisation : Gas chromatography**

*Chromatography*: a technique for separating the components of a mixture (liquid or gas) on the basis of differences in their affinity for a stationary (solid or liquid) and a mobile phase (liquid or gas)

*Gas chromatography*

- mixture : gas (headspace or vaporisation of a liquid)
- stationary phase : liquid or polymer in capillary column
- mobile phase : gas (inert carrier)
Essential oil characterisation: Gas chromatography

1. Sample Injector
2. Column
3. Detector
4. Chromatogram

Program of T°: optimisation of the separation of the components of the mixture

He, H₂ or N₂

Most common: Mass spectrometer, FID
Gas chromatography: Ultra Fast GC >> Classic GC

Ultra Fast GC

Classic GC
Gas chromatography: Ultra Fast GC ≻ Classic GC

- Ramp of T°: 100 – 1200° C/min
- Column: 2 – 5 m, 0.1 mm ID

→ Time for 1 analysis < 5 min

- Ramp of T°: 10-30° C/min
- Column: 10 – 30 m, 0.25-0.32 mm ID

→ Time for 1 analysis > 35 min
Essential oil characterisation

Matricaria chamomilla L. (originated from Nepal)

GC-MS (identification)

Fast GC-FID (quantification)

<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-β-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)-α-farnesene</td>
<td>1506</td>
<td>8.3</td>
</tr>
<tr>
<td>5</td>
<td>α-bisabolol oxide B</td>
<td>1649</td>
<td>4.4</td>
</tr>
<tr>
<td>6</td>
<td>α-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>α-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>

Essential oil characterisation

*Nepeta cataria* L. (originated from Canada)

<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>E-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>

GC-MS

Fast GC-FID
How to analyse and quantify semiochemicals?

Heuskin S. et al., 2009, J. Chrom. A, 1216, 2768-2775
Quantification of semiochemicals: various steps

1. Quantification with internal standard

2. Optimisation of analytical method: resolution of compounds

3. Validation of analytical method:
   - calibration curve
   - evaluation of validation criteria according to norms
Quantification with internal standard

Why an internal standard?
- to avoid the problem of variation of injected volume in GC with autosampler

How to add an internal standard?
- in reference solutions to construct calibration curve: the same concentration of IS in all the levels of concentration of analytes
- in routine samples at a known concentration
Quantification with internal standard

Which internal standard?

- Compound of the same family than the analytes
- Retention time of IS close to the retention time of analytes
- Response factor close to 1:
  \[ F = \left( \frac{\text{Area}_A \cdot \text{Conc}_{IS}}{\text{Area}_{IS} \cdot \text{Conc}_A} \right) \]
- Not naturally present in the routine sample

⇒ Here : IS = longifolene
Optimisation of the analytical method

Ultra Fast GC analysis

→ Good resolution of peaks in less than 5 min.

\[ R_s = 2(t_{R\,E-\beta-caryophyllene} - t_{R\,longifolene}) / (W_{longifolene} - W_{E-\beta-caryophyllene}) \]

\[ R_s = 1,65 > 1,5 \rightarrow \text{OK} \]
Analytical validation

Objective of an analytical method for quantification:

To be able to quantify the more precisely the routine samples

\[ x_i \leftrightarrow \mu_T \]

Results \hspace{1cm} True value
Analytical validation

Objective of a validation:

To give to the laboratory the guarantees that the results are within acceptance limits

\[ |x_i - \mu_T| < \lambda \]

Bias

\( \lambda = \) acceptance limits
Calibration curve

Peak area of EBF / Peak area of IS
Concentration EBF / Concentration IS

Calibration curve of E-β-farnesene

$y = 0.9558x + 0.0053$
$R^2 = 0.9999$

Blank

5 concentrations * 3 replicates
Analytical validations

1. « Classic » validation

ISO 5725, GLP standard operating procedures :
criteria validated 1 by 1

2. « Accuracy profile » validation

Guidelines of the SFSTP* :
Total error concept : combination of systematic and random errors

Accuracy = Trueness + Precision

*SFSTP = Société Française des Sciences et Techniques Pharmaceutiques
1. « Classic » validation

<table>
<thead>
<tr>
<th></th>
<th>$E$-β-Farnesene</th>
<th>β-Caryophyllene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range (µg/µl)</td>
<td>0.008–0.100</td>
<td>0.080–1.000</td>
</tr>
<tr>
<td>Equation of the</td>
<td>$y = 0.9592x - 0.0028$</td>
<td>$y = 0.9558x + 0.0053$</td>
</tr>
<tr>
<td>calibration curve</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r^2$</td>
<td>0.9998</td>
<td>0.9999</td>
</tr>
<tr>
<td>Reduced residual</td>
<td>2.668</td>
<td>1.866</td>
</tr>
<tr>
<td>(Grubb’s test)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accuracy of</td>
<td></td>
<td></td>
</tr>
<tr>
<td>calibration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>curves (%)</td>
<td>99.19</td>
<td>99.86</td>
</tr>
</tbody>
</table>

- **Linearity**
  - > 0.996
  - < 2.75

<table>
<thead>
<tr>
<th>Internal standard</th>
<th>Longifolene</th>
<th>Longifolene</th>
<th>Longifolene</th>
<th>Longifolene</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD (pg)</td>
<td>2.38</td>
<td>2.40</td>
<td>1.79</td>
<td>0.74</td>
</tr>
<tr>
<td>LOQ (pg)</td>
<td>4.76</td>
<td>4.80</td>
<td>3.58</td>
<td>1.48</td>
</tr>
</tbody>
</table>

$a$ Bias (%) between the measured value and the theoretical value.
1. «Classic» validation

**Precision of the method**

<table>
<thead>
<tr>
<th>Concentration (μg/μl)</th>
<th>E-β-Farnesene</th>
<th>β-Caryophyllene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td></td>
<td>0.500</td>
<td>0.500</td>
</tr>
<tr>
<td>Repeatability (RSD, %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.16</td>
<td>0.70</td>
<td>0.43</td>
</tr>
<tr>
<td>3.00</td>
<td>2.82</td>
<td>0.89</td>
</tr>
<tr>
<td>Reproducibility (RSD, %)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Criteria:**
- RSD % < 8%
- RSD % < 6%
- RSD % < 16%
- RSD % < 12%
2. Accuracy profile validation

- **Trueness** - Bias – Systematic error

- **Precision** – Repeatability + Intermediate precision – Random error

- **Accuracy** - Trueness + Precision – Total error
<table>
<thead>
<tr>
<th>Range (ng μl⁻¹)</th>
<th>81.6–1019.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response function (m = 3, n = 3)</td>
<td></td>
</tr>
<tr>
<td>Series 1</td>
<td>Series 2</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0089</td>
</tr>
<tr>
<td>Trueness (n = 3, p = 3)</td>
<td></td>
</tr>
<tr>
<td>Concentration levels</td>
<td>Absolute bias (ng μl⁻¹)</td>
</tr>
<tr>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>2</td>
<td>4.8</td>
</tr>
<tr>
<td>3</td>
<td>0.8</td>
</tr>
<tr>
<td>4</td>
<td>2.9</td>
</tr>
<tr>
<td>5</td>
<td>-6.8</td>
</tr>
<tr>
<td>Precision (n = 3, p = 3)</td>
<td></td>
</tr>
<tr>
<td>Concentration levels</td>
<td>Repeatability (RSD, %)</td>
</tr>
<tr>
<td>1</td>
<td>8.8</td>
</tr>
<tr>
<td>2</td>
<td>3.4</td>
</tr>
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<td>3</td>
<td>2.9</td>
</tr>
<tr>
<td>4</td>
<td>0.8</td>
</tr>
<tr>
<td>5</td>
<td>1.0</td>
</tr>
<tr>
<td>Accuracy (n = 3, p = 3, β = 0.95)</td>
<td></td>
</tr>
<tr>
<td>Concentration levels</td>
<td>β-Expectation tolerance limits (ng μl⁻¹)</td>
</tr>
<tr>
<td>1</td>
<td>[63.9–99.1]</td>
</tr>
<tr>
<td>2</td>
<td>[154.2–181.7]</td>
</tr>
<tr>
<td>3</td>
<td>[333.9–401.9]</td>
</tr>
<tr>
<td>4</td>
<td>[654.3–820.0]</td>
</tr>
<tr>
<td>5</td>
<td>[970.5–1055.0]</td>
</tr>
<tr>
<td>Linearity (n = 3, m = 5, p = 3), N = 45</td>
<td></td>
</tr>
<tr>
<td>Range (ng μl⁻¹)</td>
<td>81.6–1019.7</td>
</tr>
<tr>
<td>Slope</td>
<td>0.9928</td>
</tr>
<tr>
<td>Intercept</td>
<td>3.7050</td>
</tr>
<tr>
<td>( r^2 )</td>
<td>0.9989</td>
</tr>
<tr>
<td>Lower LOQ (ng μl⁻¹)</td>
<td>40.8</td>
</tr>
</tbody>
</table>

\( \lambda = \text{acceptance limits} \)

Accuracy profile

LLOQ

HLOQ
### E-ß-Farnesene

<table>
<thead>
<tr>
<th>Range (ng µL⁻¹)</th>
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<td><strong>Trueness (n = 3, p = 3)</strong></td>
<td></td>
</tr>
<tr>
<td>Concentration levels</td>
<td>Absolute bias (ng µL⁻¹)</td>
</tr>
<tr>
<td>1</td>
<td>−0.1</td>
</tr>
<tr>
<td>2</td>
<td>4.8</td>
</tr>
<tr>
<td>3</td>
<td>0.8</td>
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<td>4</td>
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<td>5</td>
<td>−6.8</td>
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<tr>
<td><strong>Precision (n = 3, p = 3)</strong></td>
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<td>Concentration levels</td>
<td>Repeatability (RSD, %)</td>
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<td>3.4</td>
</tr>
<tr>
<td>3</td>
<td>3.4</td>
</tr>
<tr>
<td>4</td>
<td>0.8</td>
</tr>
<tr>
<td>5</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Accuracy (n = 3, p = 3, β = 0.95)</strong></td>
<td></td>
</tr>
<tr>
<td>Concentration levels</td>
<td>β-Expectation tolerance limits (ng µL⁻¹)</td>
</tr>
<tr>
<td>1</td>
<td>[63.9–99.1]</td>
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</tr>
<tr>
<td>5</td>
<td>[970.5–1055.0]</td>
</tr>
</tbody>
</table>

**Linearity (n = 3, m = 5, p = 3), N = 45**

- **Range (ng µL⁻¹)**: 81.6–1019.7
- **Slope**: 0.9928
- **Intercept**: 3.7050
- **r²**: 0.9989

**Lower LOQ (ng µL⁻¹)**: 81.6
**Lower LOD (ng µL⁻¹)**: 40.8

**Linearity of the method**
How to purify semiochemicals from essential oils?
Purification of components: chromatographic techniques

Solid-Liquid chromatography

1. Essential oil in the head of the column
2. Beginning of the elution with solvent
3. Elution process
4. Collection of the semiochemical of interest
Purification of components: chromatographic techniques

Solid-Liquid chromatography

- Mixture: liquid – essential oil
- Stationary phase: solid – silicagel
- Mobile phase: liquid – solvent of elution

Goal: To obtain highly purified semiochemicals without solvent

Evaporation of solvent of elution
**Choice of the solvent of elution**

*By thin layer chromatography*

- Choice of solvent based on:
  - Best separation of compounds on silica
  - Importance of solvent boiling point

*N-pentane (36°C)*
**Essential oil fractionation**

*By liquid column chromatography*

**Preliminary tests**

Small scale liquid column chromatography

1 ml essential oil deposited on 11 g dried silicagel

↓

Elution with n-pentane

↓

Collection of fractions (1.5 ml)

↓

Fast GC analysis

Dilution
Matricaria chamomilla fractionation

<table>
<thead>
<tr>
<th>Elution volume (ml)</th>
<th>% EBF</th>
<th>% Germacrene D</th>
<th>% E,E-α-farnesene</th>
<th>% monoterpene</th>
<th>% chamazulene</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 10,5 (F0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10,5 - 16,5 (F1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>16,5 - 22,5 (F2)</td>
<td>0 - 82</td>
<td>7,8 - 26</td>
<td>3 - 5</td>
<td>47 - 2</td>
<td>0</td>
</tr>
<tr>
<td>22,5 – 51 (F3)</td>
<td>86,3 - 76</td>
<td>4 - 1,4</td>
<td>5,7 - 22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>51 – 72 (F4)</td>
<td>72 - 56</td>
<td>1,4 - 1,6</td>
<td>22 - 33</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>72 – 90 (F5)</td>
<td>55 - 33</td>
<td>1,6</td>
<td>33 - 41</td>
<td>0</td>
<td>0,5 - 16</td>
</tr>
</tbody>
</table>
**Essential oil fractionation**

**Solvent evaporation at 40°C: recoveries of E-β-farnesene**

<table>
<thead>
<tr>
<th></th>
<th>Water bath</th>
<th>Büchi evaporator at atmospheric pressure</th>
<th>Büchi evaporator under vacuum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>98.73 %</td>
<td>96.30 %</td>
<td>92.47 %</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>0.35 %</td>
<td>0.94 %</td>
<td>3.43 %</td>
</tr>
<tr>
<td><strong>RSD (%)</strong></td>
<td>0.36 %</td>
<td>0.98 %</td>
<td>3.71 %</td>
</tr>
<tr>
<td><strong>Time</strong></td>
<td>More than 4h.</td>
<td>30 min.</td>
<td>10 min.</td>
</tr>
</tbody>
</table>

Compromise between analyte recovery and evaporation time
**Essential oil fractionation**

*Flash chromatography*: higher scale under pressure

- **Reduced time**

  10 ml essential oil deposited on 110 g dried silicagel

  Elution with n-pentane under pressure ($N_2 = 0.5$ bar)

  Collection of concentrated fraction + solvent evaporation

  **Dilution**

  Fast GC analysis

- **Solvent-free purified semiochemicals**
**Essential oil fractionation**

**Flash chromatography**

→ Highly purified semiochemicals

### Matricaria chamomilla

<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>

### 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>
How to formulate semiochemicals?

Heuskin et al., 2012, Pest Manag. Sci., 68, 127-136
Formulation criteria

- Natural and biodegradable matrix
- Protection of semiochemicals over time $\gg$ oxidation
- Sufficient release rate of semiochemicals over time
- Attractive towards aphid predators and/or parasitoids

Alginate gel beads
Alginate

\[ \beta\text{-D-mannuronate (M) (Poly M segment)} \]

\[ \alpha\text{-L-guluronate (G) (Poly G segment)} \]

Poly MG segment
Gelling process of alginate

G segment

M segment

« Egg-box » structure

Organisation
Formulation of alginate bead

Formulation optimisation in terms of semiochemical encapsulation capacity and texturometry, considering:

- Type of alginate (M/G – molar mass)
- Alginate concentration
- Type of cross-linker ion
- Cross-linker ion concentration
- Maturation time

For details: see Heuskin et al., 2012, Pest Management Science, 68, 127-136
Formulation of alginate bead

- Semiochemical + sunflower oil + α-tocopherol (oil phase)
- Alginate solution
- Oil phase
- Water phase
- Homogenization (24000 rpm, 20 s.)
- O/W emulsion
- Dried alginate beads (Ø 2mm)
- Drying
- CaCl₂ Suspension (maturation)
Characterisation of alginate bead

« Semiochemical – oil » dispersion in the alginate network

CLSM imaging of a dried (Aw=0.42) E-β-farnesene alginate bead
Protection efficiency of beads towards sesquiterpenes

E-β-farnesene

E-β-caryophyllene

Heuskin S. et al., JPBA, 2010, 53, 962-972
Is the formulation efficient...
… in terms of semiochemical release?
Volatile collection system

Activated charcoal filter

Adsorbent (HayeSep Q) cartridge

Solvent elution + IS quantification (Fast GC)

Teflon box with semiochemical alginate beads

Pump
Volatile collection system

Specifications and performances

- Boxes and tubing in Teflon >> adsorption of semiochemicals
- Activated charcoal filters: air purification
- Sampling + security cartridges ➔ breakthrough
- Total volume of eluting solvent: 4 x 250 µL n-hexane/cartridge
- Mean recovery of elution: 94.5 % ± 4.2 %
Release rate of semiochemicals

Laboratory controlled conditions:
- Temperature: 20°C
- Relative humidity: 65%
- Air flow: 0.5 L/min
Influence of abiotic factors on semiochemical diffusion

Temperature – Relative humidity – Air flow

Preliminary experiments

<table>
<thead>
<tr>
<th>Experimental test</th>
<th>Relative humidity (%)</th>
<th>Airflow (L/min)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N° 1</td>
<td>25</td>
<td>0.05</td>
<td>20</td>
</tr>
<tr>
<td>N° 2</td>
<td>25</td>
<td>0.50</td>
<td>20</td>
</tr>
<tr>
<td>N° 3</td>
<td>25</td>
<td>1.00</td>
<td>20</td>
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<tr>
<td>N° 4</td>
<td>75</td>
<td>0.50</td>
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</tr>
<tr>
<td>N° 8</td>
<td>100</td>
<td>0.50</td>
<td>20</td>
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</table>
Semiochemical diffusion coefficient estimation

Diffusion in a sphere (Cranck, 1975):

\[
\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp\left(-Dn^2\pi^2t / a^2\right)
\]

- \(M_t\) (µg): cumulative mass of semiochemical released at time \(t\)
- \(M_\infty\) (µg): cumulative mass of semiochemical released at time \(\infty\) (supposed to be the total quantity of volatile in the bead at time \(t=0\))
- \(a\) (m): radius of one bead
- \(t\) (s): diffusion time
- \(n\): number of terms in the sum
- \(D\) (m²/s): effective diffusion coefficient of semiochemical
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<tr>
<th>Experimental test</th>
<th>Relative humidity (%)</th>
<th>Airflow (L/min)</th>
<th>Temperature (°C)</th>
<th>Diffusion coefficient for E-β-farnesene (m²/s)</th>
<th>Diffusion coefficient for E-β-caryophyllene (m²/s)</th>
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</thead>
<tbody>
<tr>
<td>N° 1</td>
<td>25</td>
<td>0.05</td>
<td>20</td>
<td>1.98 * 10⁻¹⁴</td>
<td>1.35 * 10⁻¹⁵</td>
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<tr>
<td>N° 2</td>
<td>25</td>
<td>0.50</td>
<td>20</td>
<td>3.40 * 10⁻¹⁴</td>
<td>1.57 * 10⁻¹⁵</td>
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<tr>
<td>N° 3</td>
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<td>1.00</td>
<td>20</td>
<td>3.71 * 10⁻¹⁴</td>
<td>1.23 * 10⁻¹⁵</td>
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<tr>
<td>N° 4</td>
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<td>0.50</td>
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<td>1.23 * 10⁻¹⁴</td>
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<td>N° 5</td>
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<td>N° 6</td>
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<td>1.56 * 10⁻¹⁵</td>
<td>1.33 * 10⁻³²</td>
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<td>N° 7</td>
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<td>6.15 * 10⁻³³</td>
<td>8.26 * 10⁻³³</td>
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<tr>
<td>N° 8</td>
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<td>0.50</td>
<td>20</td>
<td>1.03 * 10⁻³²</td>
<td>9.93 * 10⁻³¹</td>
</tr>
</tbody>
</table>
**Influence of abiotic factors on semiochemical diffusion**

- Most limiting factor: relative humidity ≥ 85%
- Influence of temperature
- Weak influence of air flow
Improvement of the research

- Box-Behnken experimental design (3 factors in 3 levels)
- Water sorption / desorption isotherms on alginate beads
- Evolution of bead diameter with $A_w$

→ Confirmation of the preliminary results

TFE F. Daems (2011), GxABT, ULG
Is the formulation efficient...
... in terms of attractiveness of beneficial insects?
On parasitoids (*Aphidius ervi*): 2-way olfactometer

**On parasitoids (Aphidius ervi): 2-way olfactometer**

Blank vs Alginate beads with semiochemicals

**Graph:**
- **Y-axis:** Attractiveness (%)
- **X-axis:** Caryophyllene and EBF
- **Legend:**
  - Semiochemicals
  - Blanco
- **Annotations:**
  - *** very highly significant difference (P<0.001)
  - N = 30
On Syrphidae: on-field experiments

- 3 crops: beet, horse bean, winter wheat
- \(E-\beta\)-farnesene, \(E-\beta\)-caryophyllene and blank alginate beads
- 1 latin square design per crop
On Syrphidae: on-field experiments

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

- $E$-$\beta$-Farnesene: P-value = 0.0200 (< 0.05) * significant difference
- $E$-$\beta$-Caryophyllene: P-value = 0.0064 (< 0.01) ** highly significant difference
Conclusions and perspectives
Conclusions

1. How to analyse and quantify semiochemicals?
   ➔ Ultra Fast GC method validated

2. How to purify semiochemicals?
   ➔ Flash Chromatography: molecules at high purity

3. How to formulate semiochemicals?
   ➔ Alginate gel beads: formulation optimised and characterised

4. Is the formulation efficient?
   ➔ In terms of release... YES
   ➔ In terms of biological control device... YES
Perspectives or improvements of the research

- Time of degradation and microbiological study of alginate beads outdoors
- Field experiments: maximal distance of attraction; maintaining beneficial insects on field
- At larger scale:
  - automated flash chromatography + solvent recycling system
  - automated alginate bead production system
- Encapsulation of other molecules useful in chemical ecology
Thank you for your attention