Otpimisation of slow-release formulations as biological control devices

SOLAPHID (WALEO2 convention RW/FSAGX 061/6287 et RW/UCL 051/6067)

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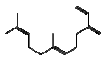
Introduction

E-*β*-Farnesene, the alarm pheromone of many aphid species and *β*-caryophyllene, recently identified as one of the possible component of the aggregation pheromones of the Asian ladybeetles *Harmonia axyridis* Pallas, are considered as two sesquiterpenes attractive for aphids' predators and parasitoids.

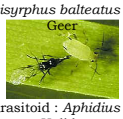
In the present research, alginate gel beads formulations were optimised as semiochemical slow-release devices. The formulations were evaluated in terms of volatiles release capacity, protection efficiency of sesquiterpenes and biological activity towards *Episyrphus balteatus* and *Aphidius ervi*. The sesquiterpenes used in the formulations were obtained from natural sources. They were purified by flash chromatography fractionation of essential oils.

***E*-*β*-farnesene**



***β*-caryophyllene**

Predator : larva of *Episyrphus balteatus* De Geer



Parasitoid : *Aphidius ervi* Haliday

Experimental

Purification of semiochemicals by essential oils flash chromatography

***Matricaria chamomilla* L.**

Compounds	
Sum of monoterpenes	1.3 %
***E*-*β*-farnesene**	**83.8 %**
Germacrene D	1.5 %
Bicyclogermacrene	1.5 %
(*E*,*E*)-*α*-farnesene	11.9 %

***Nepeta cataria* L.**

Compounds	
Sum of monoterpenes	1.2 %
***β*-caryophyllene**	**97.7 %**
α-humulene	1.1 %

The purities of the compounds were determined by fast GC optimised method

Chromatographic conditions

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

Oven :

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**

Alginate gel beads formulations

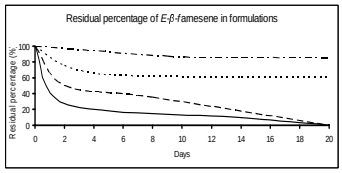
Optimisation of formulations density and encapsulation capacities considering the following parameters:

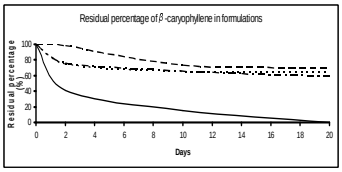
- Alginate type : M/G ratio determined by NMR
- Concentration of calcium chloride and ionic strength
- Concentration of alginate
- Reaction time in $CaCl_2$

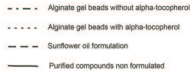
➔ ***optimised formulation (extrusion method)*** : Alginate Sigma Low viscosity (1,5% w/v) mixed with purified semiochemicals, sunflower oil and vitamin E – $CaCl_2$ 0,2M – Ionic strength fixed at 0,5M – Reaction time 48h

Physical and biological efficiency of formulations

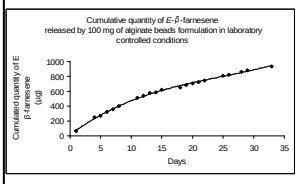
Sesquiterpenes protection

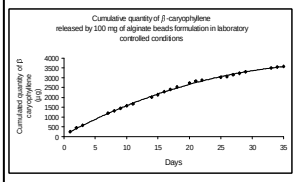






Slow release study





A mathematical modelisation of semiochemicals release is presently in study. The influence of physical parameters is measured (T°, relative humidity, wind speed) for E-β-farnesene and β-caryophyllene formulations.

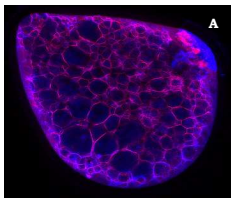
First assays have demonstrated that diffusion doesn't depend on wind speed.

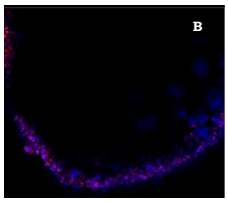
Laboratory controlled conditions :

- Temperature : 20°C
- Sampling air flow : 0.5 L/min
- Relative humidity : 65 %

Slow release autocontrol with relative humidity

Preliminary field and lab tests have demonstrated the stop of release at high relative humidities (a_w). This observation was confirmed by confocal microscopy.





A : Dried beads (a_w : 0.40) B: Wet beads (a_w : 0.99) , at 20°C

Blue : alginate Red : sunflower oil with semiochemicals

The repartition of sunflower oil and semiochemicals is homogenous in dried beads contrarely to wet beads where the oil and the compounds of interest are concentrated at the superficy of the beads. A barrier of water is formed and stop the release of volatiles.

Biological tests

***Episyrphus balteatus* De Geer**

Field experiments : verification of the attraction efficiency of formulations towards Syrphidae. The data were treated with MINITAB (Anova : GLM). *E*-*β*-Farnesene and *β*-caryophyllene beads were compared to beads without semiochemicals.

- *E*-*β*-Farnesene : P = 0.0200 (< 0.05)➔ significative difference*
- *β*-caryophyllene : P = 0.0064 (<0.01) ➔ highly significative difference**

Electroantennography : significative response with the semiochemicals.

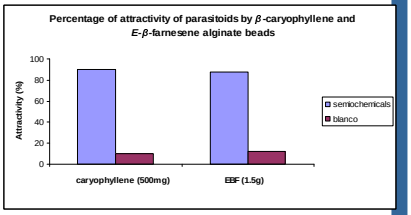
Oviposition tests : more eggs laid with the semiochemicals than with the blanco.

Wind tunnel: lab scale attraction : in progress.

***Aphidius ervi* Haliday**

Olfactometry (2 ways):

Almost 90% of the parasitoids were attracted by the two semiochemicals compared to only 15% for the blanco (beads without semiochemical). However, a quantity of beads three times higher is necessary for the *E*-*β*-farnesene formulation.



Conclusions

- Optimisation of slow release formulations in terms of encapsulation capacities.
- Efficiency of the formulations in biological tests towards predators and parasitoids of aphids.
- Protection properties of alginate beads, adequate slow release devices depending on temperature and relative humidity.
- Other advantages of the formulations are their naturally occurring components and their biodegradability.