

Bioherbicides development based on essential oils

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CONTENT



1. Introduction
2. Selection
3. Modes of action
4. Formulation
5. Efficacy trials
6. Conclusions

Urban and Integrated Plant pathology laboratory



- Diagnostic and surveillance
- Plant-microbiome interactions
- Urban agriculture
- Biological control methods

Supporting R & D of companies



40 (inter)national programs on bioprotection

2 professors

500 publications

30 researchers

7 patents

5 technicians

4 Spin off companies

<http://www.phytopathologie.be>

<http://www.agricultureurbaine.be>

Selection of effective microbial strains



1991

Candida oleophila (strain O)



Efficient against wound parasites
(*B. cinerea* and *P. expansum*)

Micro-organism application followed by *B. cinerea* inoculation

Non treated control inoculated with *B. cinerea*



Registration and commercialisation of *C. oleophila* strain 0 by LESAFFRE (AGRAUXINE)



US submission : 2007
US registration : 2009

EU submission : 2005
EU registration : 2013
(inclusion in annex 1)
No RML

National Registration
UK, France, Austria, Netherlands, Italy

Production by
Agrauxine - Lesaffre company



Distribution

for postharvest protection for banana, citrus, apples and pears

Evolution of weed control practices



- Herbicide-resistant of weeds
- Retailer and consumer reluctance to chemical residues and public concern for environmental safety
- Limitation and withdrawal of authorized active ingredients (Glyphosate, Diquat,...)
- Development of novel practices (e.a. Mechanical weeds killing) or products (Pelargonic acid)
- Development of new marketing labels

10 years of R&D on essential oils as plant protection tools

- 20 – 100 molecules
- Classification based on main compounds :
 - Terpenic EOs
 - Monoterpen: $C_{10}H_{16}$
 - Harboring various chemical functions
 - Ex: Alcohol, phenol,...
 - Aromatic EOs
 - Phenyl propanoide
 - Harboring various chemical functions
 - Ex : Aldehyde



Essential oils are natural substances (botanicals)

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Pre-selection considering plant protection market and EO market



- Non-selective Herbicides
 - Chemicals– Glyphosate, Diquat, Carfentrazone, Hormones
 - Biologicals – Pelargonic Acid, Acetic Acid
- Professional & Non-Professional
 - Organic
 - Conventional

Pre-selection considering plant protection market and EO market

- Among 3000 EOs, pre-selection of 91 EOs for **fungicidal** and **herbicidal** activities based on :



- Literature
- Composition
- Majority of the chemical families (Terpens, phenol, alcohol)
- Cost
- Availability

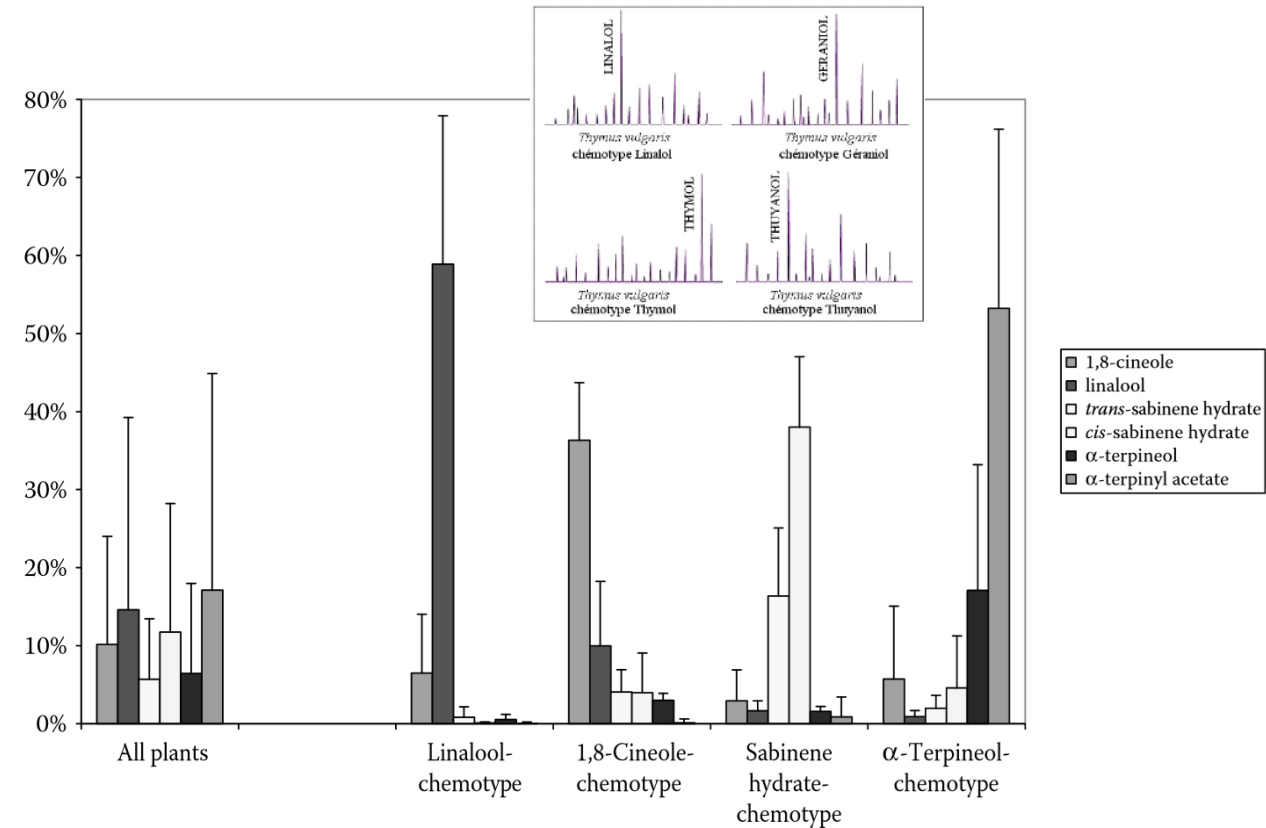
Pre-selection considering plant protection market and EO market

Chemotype

Chemotype = intraspecific variation
Chemotype of *Thymus vulgaris*



Selection of our suppliers
according to the right chemotype
and its stable composition



Source graph : Baser, K. H. C., & Buchbauer, G. (2015). *Sources of essential oils. Handbook of Essential Oils: Science, Technology, and Applications, Second Edition*, p.52 . <https://doi.org/10.1201/b19393>

Selection under greenhouse conditions

Preselection of **22 EOs for herbicidal action** and test on :

-Monocotyledons : grass (Festuca 70%, Lolium 30%)



- Dicotyledons : *Urtica dioica*, *Chenopodium*, *Papaver*, *Trifolium incarnatum*



Selection under greenhouse conditions

Preselection of **22 EOs for herbicidal action** and test on :

- Bryophyte

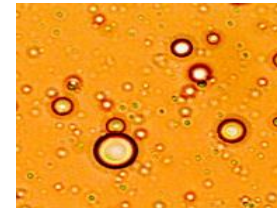


- Pteridophyte



Basic Formulation

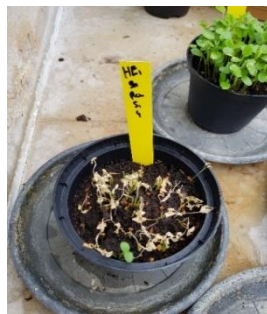
- active substance
- chemical adjuvants



Selection under greenhouse conditions

Activity against

	Type of major peak	Monocotyledon	Dicotyledon	Mosses	Horsetail
EO1	Aromatic	+	++++	++++	++++
EO2	Aromatic	++++	++++	undertermined	undertermined
EO3	Terpenic	++++	+	undertermined	undertermined



Efficacy of EO1



EO2



EO3



Untreated



Untreated



Efficacy of EO1

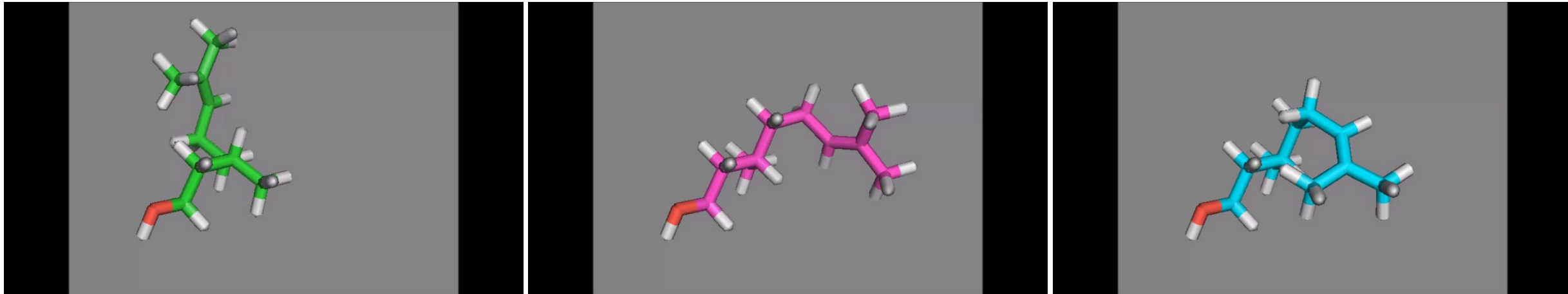
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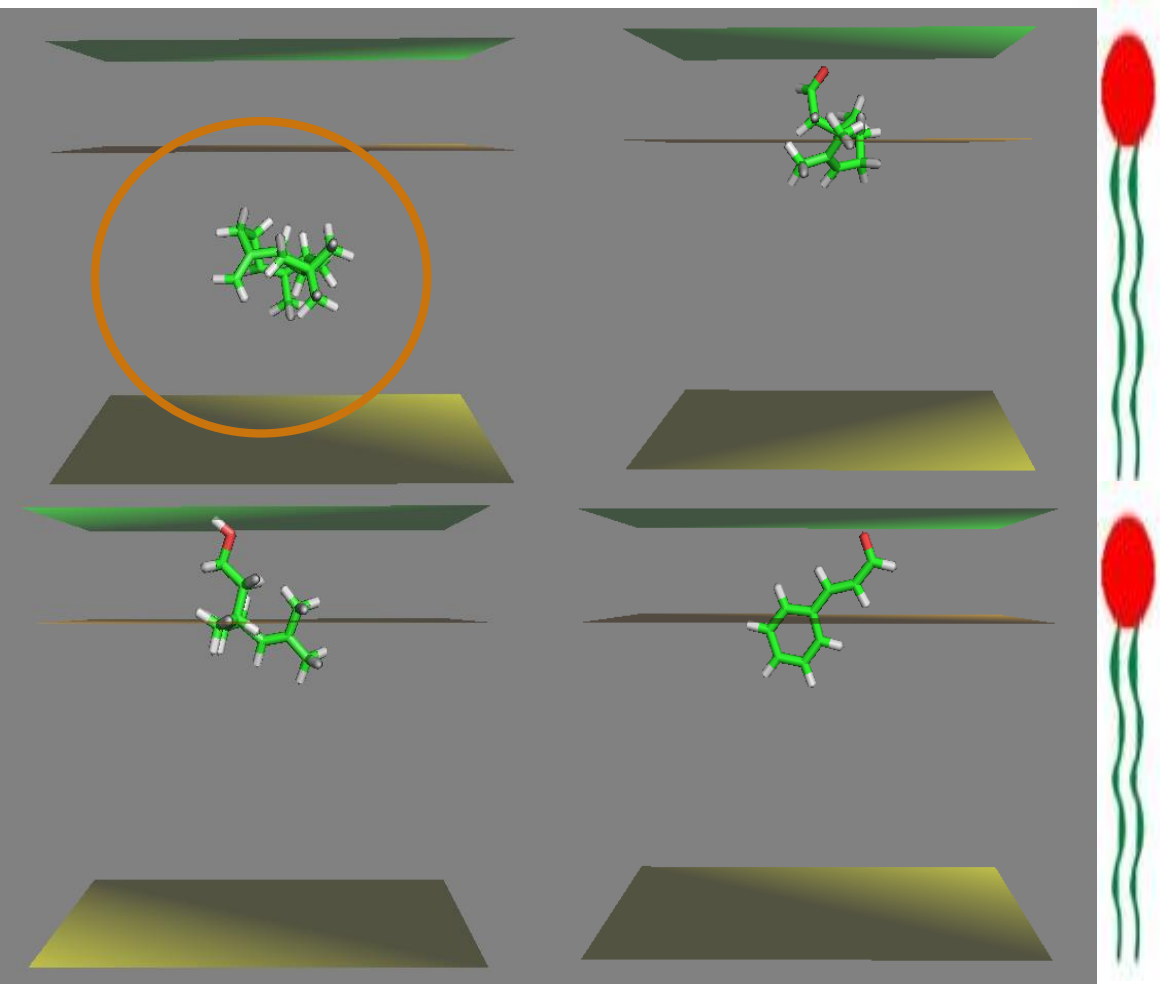
in silico analysis – 3D Modelling

Terpenic compound with 3 different conformations naturally present in Essential oils

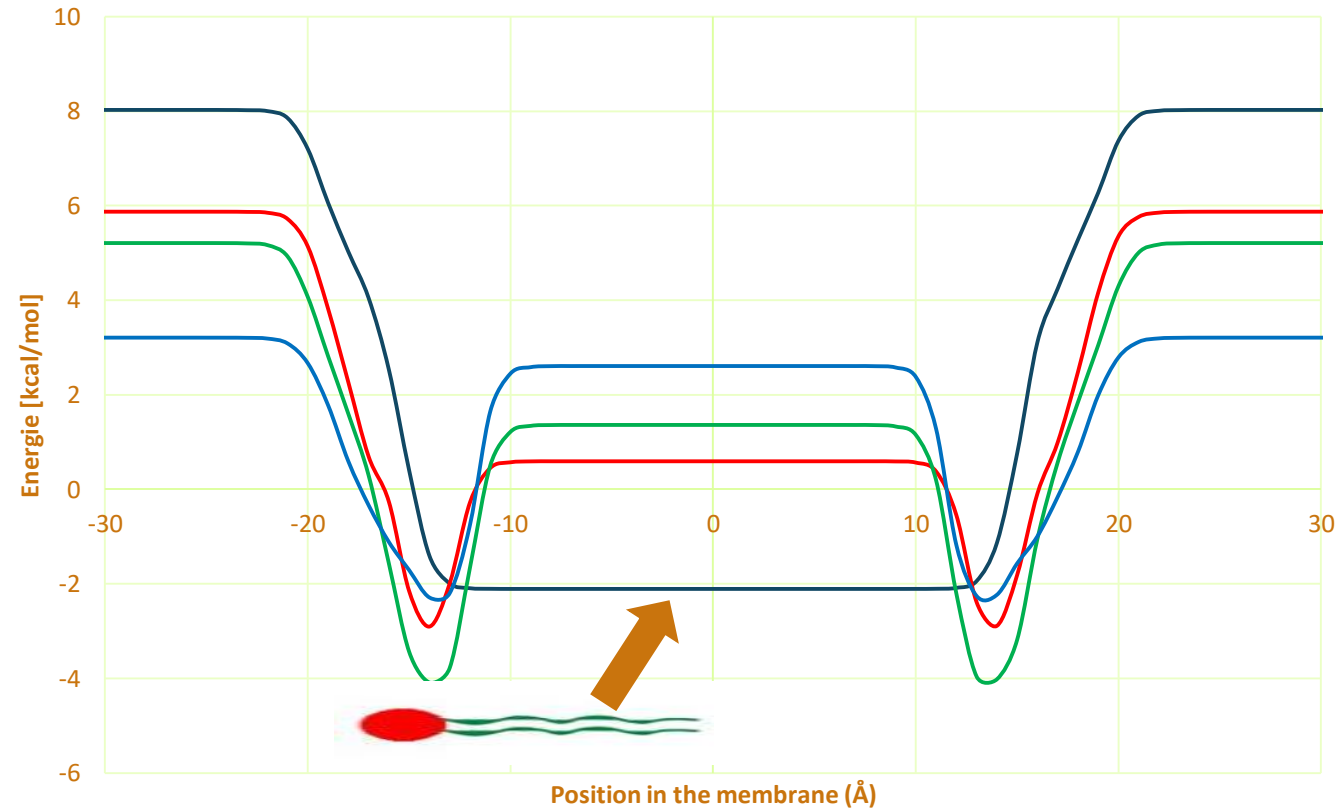


in silico analysis – Membrane insertion

Representative compounds in Essential oils

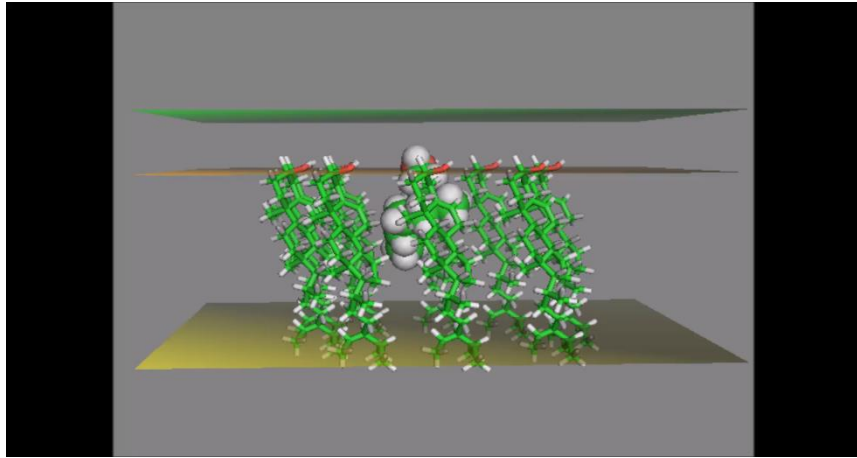


Low energy = most probable position in the membrane (IMPALA method)

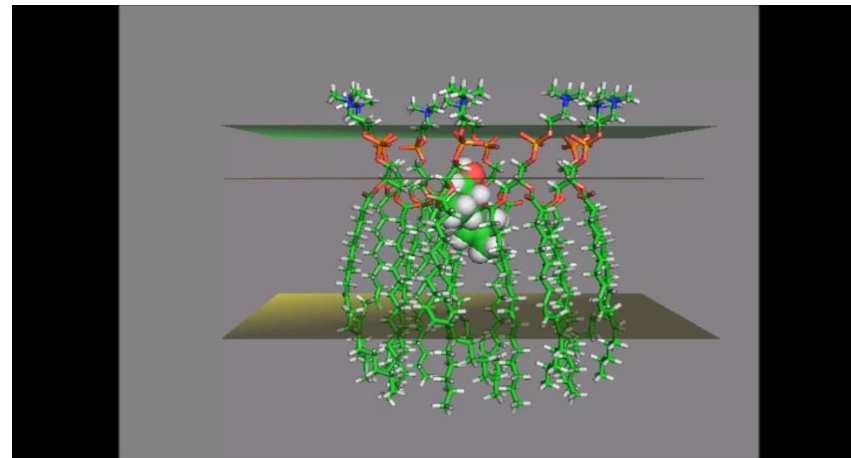


in silico analysis – Lipid-EO molecules interactions

SITOSTEROL



PLPC



INSERTION AND INTERACTION STUDIES COULD LEAD TO

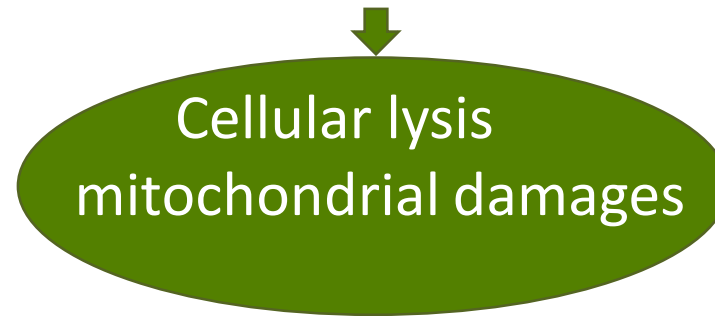
- RIGIDIFIED CELL MEMBRANE
- PERTURBATED CELL MEMBRANE
- CASCADE REACTIONS INSIDE THE CELLS

Modes of action after membrane damage

- Cytotoxicity

- No specific targets

- Lipophiles → membrane damage
cytoplasm coagulation
lipids and proteins damages



- Importance of phenols aldehydes and alcohols

- Risk of weed resistance reduced

- Several targets affected by EOs

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Importance of the formulation

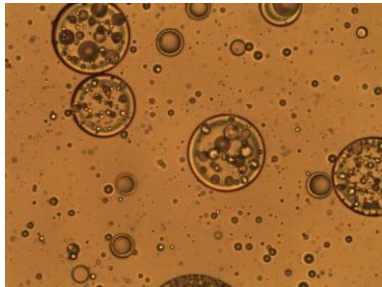


- Influences the modes of action
- Protects the EOs against the environment
 - Volatilization
 - UV radiation
- Influences the stability

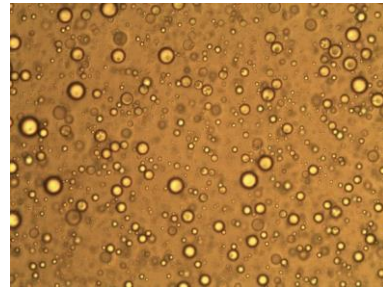
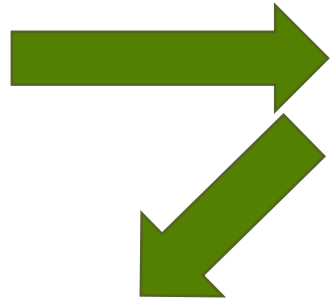
It affects the efficacy

Formulation from chemical to biological adjuvants

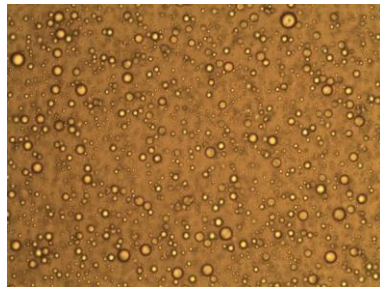
Microscopy



Basic Formulation



chemical Formulation

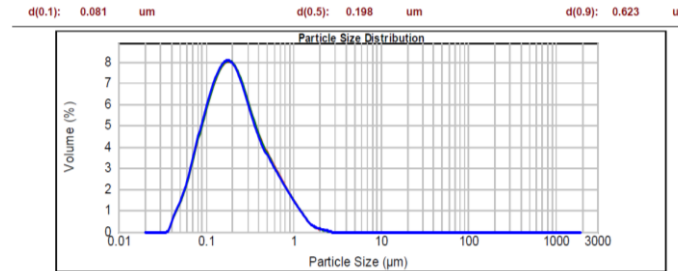


Biological Formulation

Type	Droplet size	appearance	Stability
Marco emulsion	2-20 μ m	Milky white	Instable
Mini emulsion	0.1-0.3 μ m	Bluish white	Several week
Micro emulsion	<0.1 μ m	translucent	Several months

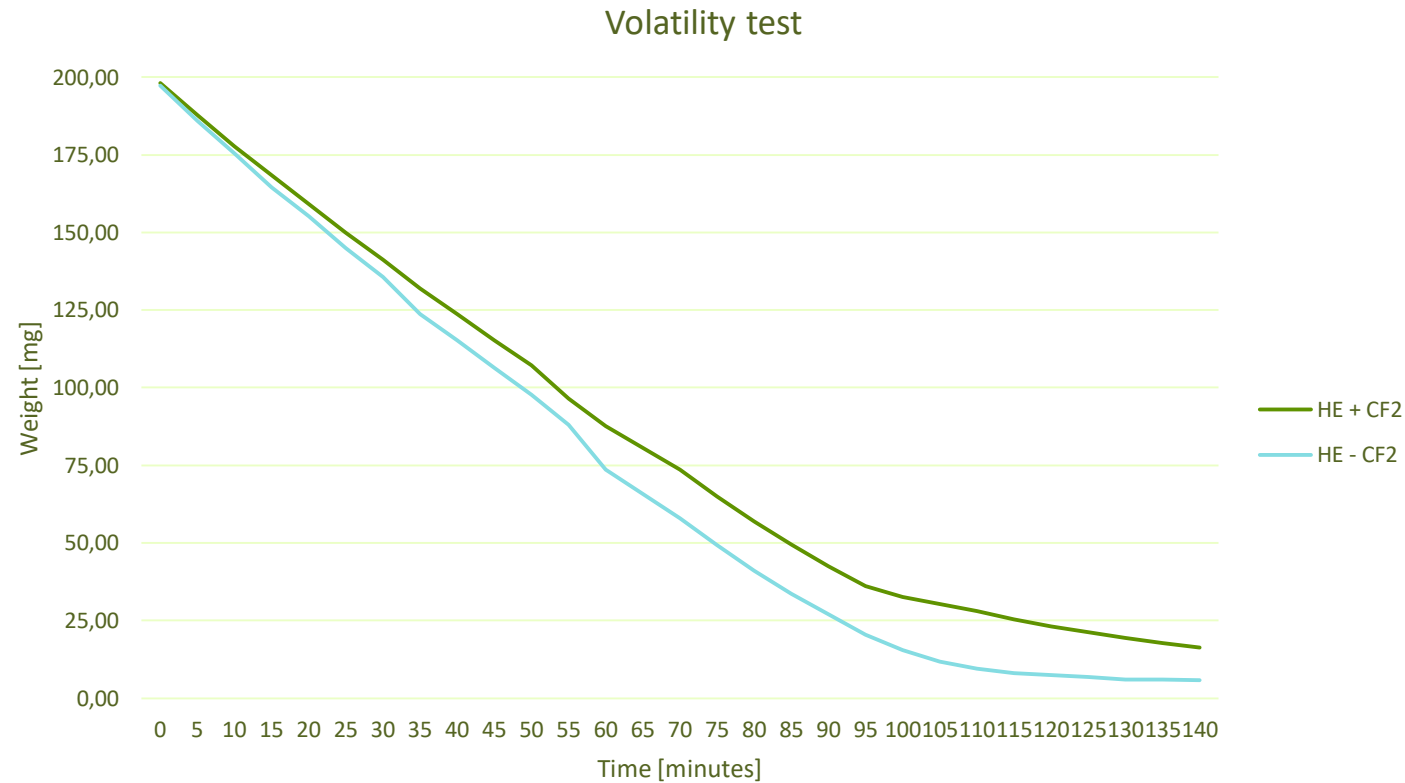


Laser granulometry



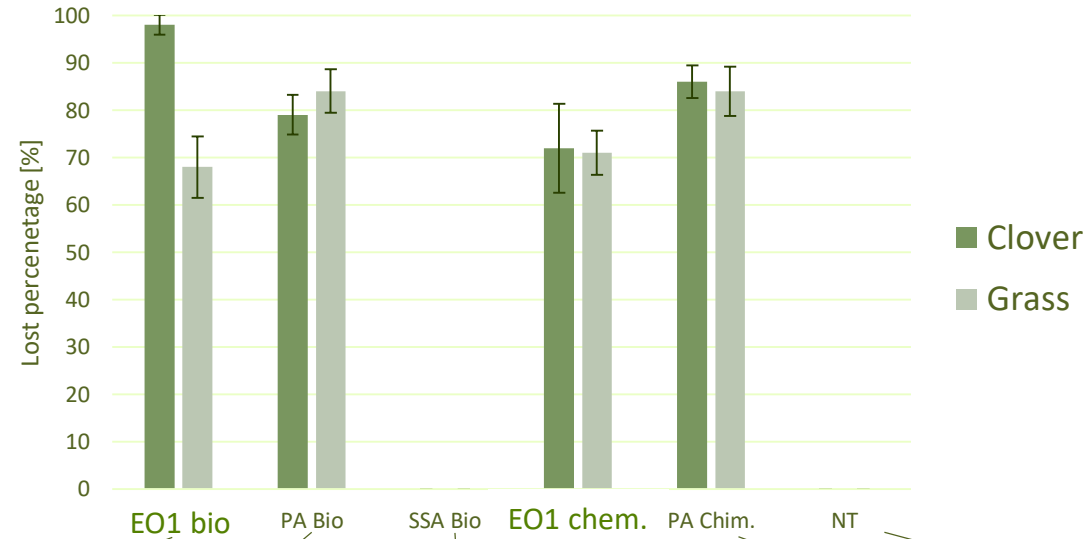
Formulation from chemical to biological adjuvants

Formulation reducing volatility



Formulation from chemical to biological adjuvants

Efficacy on
clover and grass



SSA: Without active substance
PA: Self formulated Pelargonic Acid
NT: Untreated
Bio: Biological formulation
Chim.: Chemical formulation
*7 DAA



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Efficacy trials under field conditions 2019

Monocotyledons : 4

Poa annua (POAAN)



Lolium perren (LOLPE)



Festuca rubra (FESRU)



Echinochloa crus-galli (ECHCG)



Efficacy trials under field conditions 2019

Dicotyledons : 12

Veronica persica (VERPE)



Trifolium repens (TRFRE)



Taraxacum officinale (TAROF)



Plantago lanceolata (PLALA)



Matricaria chamomilla (MATCH)



Geranium dissectum (GERDI)



Lamium purpureum (LAMPU)



Daucus carota (DAUCA)



Sonchus arvensis (SONAR)



Chenopodium album (CHEAL)



Fumaria officinalis (FUMOF)



Leucanthemum vulgare (CHYLE)



Efficacy trials under field conditions 2019



June 2019 in Belgium

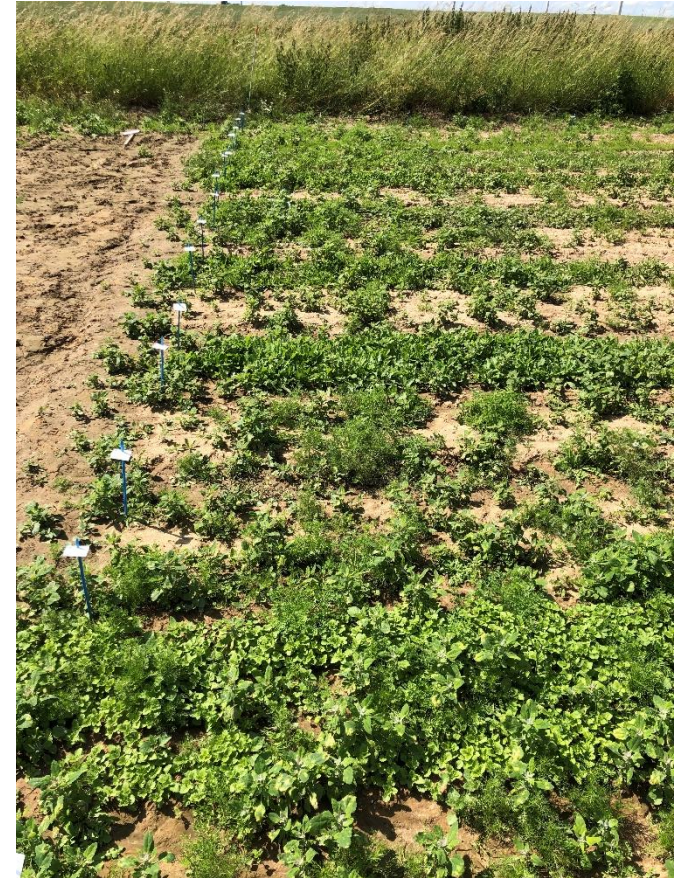
Sown weeds in fields

Application of HE1 with final formulation

Application of HE2 or HE3 with suboptimal formulation

2 applications (10 days between applications)

4 replicates



Efficacy trials under field conditions 2019

E01 – Belgium

M
o
n
o

D
I
C
O



E01 N/2



E01 N



E01 2N



Positive control 1



Positive control 2



Untreated control

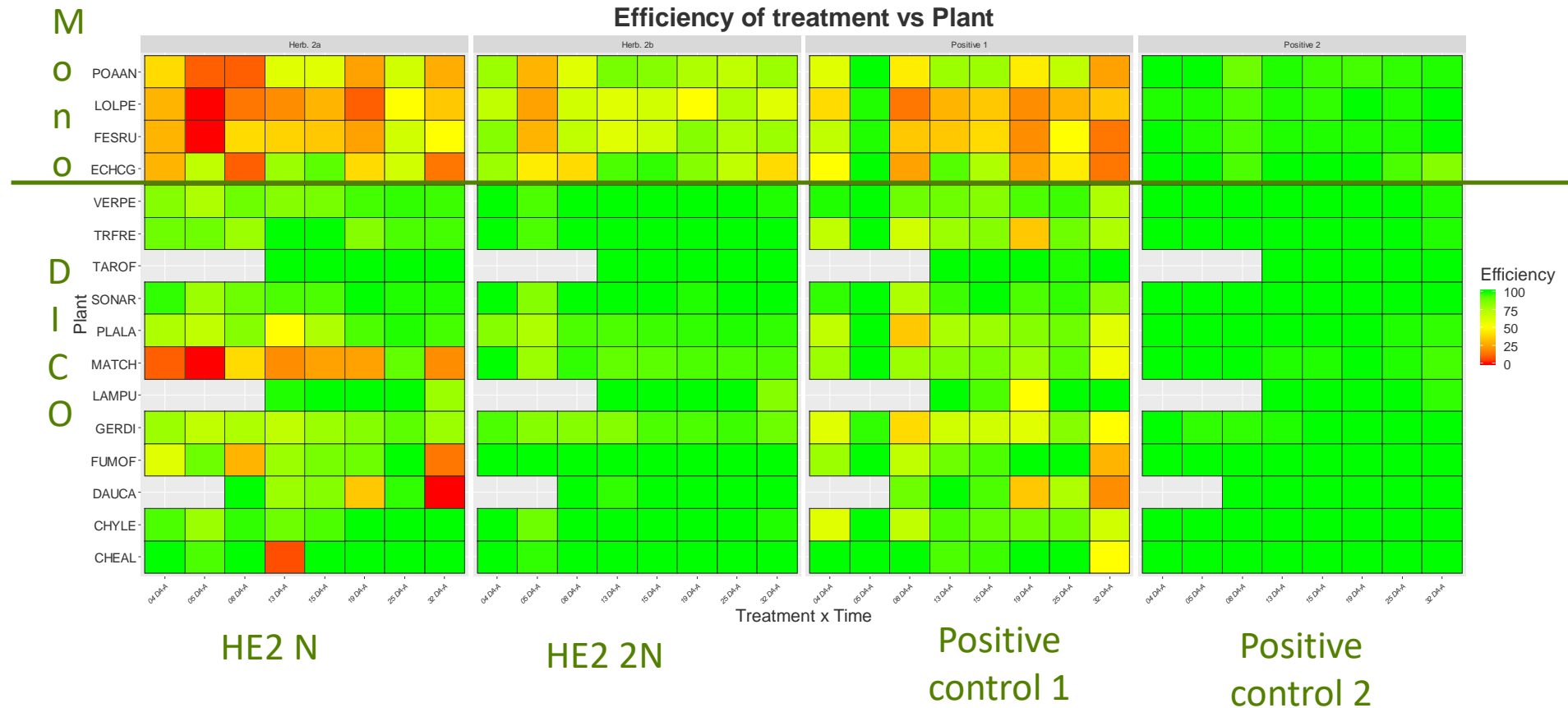
Efficacy trials under field conditions 2019



- Similar results with EO1 in South of France and UK
- Home & garden market to treat grass against Dicot and mosses

Efficacy trials under field conditions 2019

EO2 – Belgium



Efficacy trials under field conditions 2019

EO2 – Belgium

M
o
n
o

D
I
C
O



EO2 N



EO2 2N



Positive
control 1



Positive
control 2



Untreated
control

Efficacy trials under field conditions 2019



- Similar results with EO2 in South of France and UK
- Home & garden and professional markets as non-selective herbicide



Agronomical Plant Extracts & Essential Oils

- APEO is a spin off of Gembloux Agro-Bio Tech (Uliege)
- A **team** of 2 seniors (scientist in plant protection & business developer) and one junior researcher specialist in organic chemistry
- **Mission** is to create/find, develop, formulate, register and “go-to-market” (directly or indirectly) of innovative products taking care of environment & people
- **Business model** is based on “organic growth” built on several solutions (3 EOs & 1 PE, others to come) being developed, registered & sold by either APEO or 3rd parties partners
- APEO is among the 7 nominated to the Blum Award

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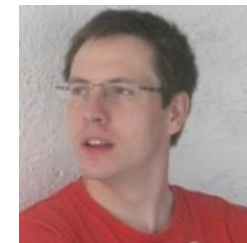


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Conclusions



- Screening methods are crucial to start with the good candidates. We must applied in parallel 3 criteria (cost and quality production, efficacy and tox)
- Mode of action and formulation are essential to overcome some drawbacks of EOs and insure stability of the product and easy application
- 3 essential oils are developed as biological herbicides :
 - ✓ EO1 : Home & Garden (semi-selective)
 - ✓ EO2 : Home & Garden and professional (non-selective)
 - ✓ EO3 : to be determined



Simon Dal Maso



Agronomical Plant Extracts & Essential Oils

Thank you