Control of the grapevine moth *Lobesia botrana* through the genetic engineering manipulation of the host plant's volatiles

Mickael Malnoy, Umberto Salvagnin, Stefan Martens, Gianfranco Anfora, Sergio Angeli, Marco Tasin
The European grapevine moth *Lobesia Botrana*
The European grapevine moth *Lobesia Botrana*

- **Eradication:**
  - Cost >$100M
  - 6 years (2009-2015)

- Insecticides: 100-150 euro/ha
- Pheromone trap: 150-200 euro/ha
Semiochemicals in the vineyards

Kairomones

Mating

Host-finding

Pheromones

H₃C

O

CH₂(CH₂)₄CH₂

CH₃

CH₃
Background

Tasin et al. (2005; 2007; 2009)
The ratio between the compounds seems to be crucial for the behavioral activity.

Could we test these compounds in the vineyards for pest control?
In *V. vinifera* no single-product β-farnesene synthase gene was found so far, so it was chosen to use a gene from another plant (*A. annua*, Acc. #AY835398.1).
(E)-β-caryophyllene synthase enzyme characterization

VvGwECar2, VIT_00014566001
(E)-β-caryophyllene synthase enzyme characterization

- $K_m = 33.8 \mu M$
- $K_{cat} = 0.204 \text{ sec}^{-1}$
- $V_{max} = 14.1 \text{ nM/sec}$

- β-caryophyllene: 70%
- α-humulene: 23.5%
- germacrene D: 6.5%

- pH optimum
- Substrate Saturation
Kairomone ratio change in *V. vinifera*

- VvGwECar2, VIT_00014566001
- *A. annua*, *Beta farnesene* Acc. #AY835398.1

2 days co-culture *Agrobacterium*

Embryogenic callus

2 to 6 months

Selection of transgenic embryos

1 to 2 months

Plant regeneration
Brachetto over-expressing b-Caryophyllene

![Image of Brachetto plant]

Graph showing relative fold change with controls and VvGwEcar2-overexpression lines.
VOCs quantification

CLSA (Closed-Loop Stripping Analysis)

Abundance

RT (min)

Abundance

b-Caryophyllene / b-Farnesene RATIO

ng / h * plant

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Wind Tunnel
**Wind Tunnel**

- Wind
- Plant extract to testor plant
- **β-Caryophyllene / β-Farnesene RATIO**

![Wind Tunnel Diagram](image)

- Upwind flights
- landing

![Graphs](image)

- Proportion of tested females
- **A** - **I**

**CENTRO RICERCA E INNOVAZIONE**
Future

✧ Validation in (semi) field conditions
✧ Collateral effects in the agroecosystem
✧ Effects on yield and wine quality
✧ Insect adaptation to the new kairomonal blend (IPM?)


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Adjusting the scent ratio: using genetically modified *Vitis vinifera* plants to manipulate European grapevine moth behaviour
THANK YOU FOR YOUR ATTENTION