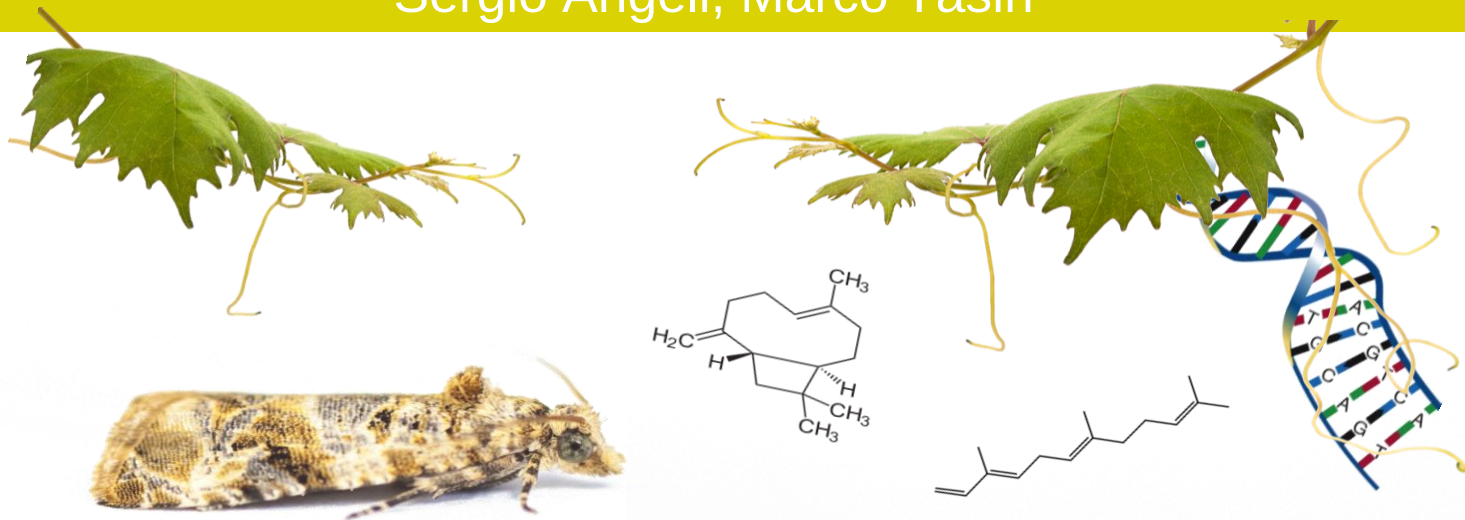


Control of the grapevine moth *Lobesia botrana* through the genetic engineering manipulation of the host plant's volatiles

Mickael Malnoy, Umberto Salvagnin, Stefan Martens, Gianfranco Anfora, Sergio Angeli, Marco Tasin

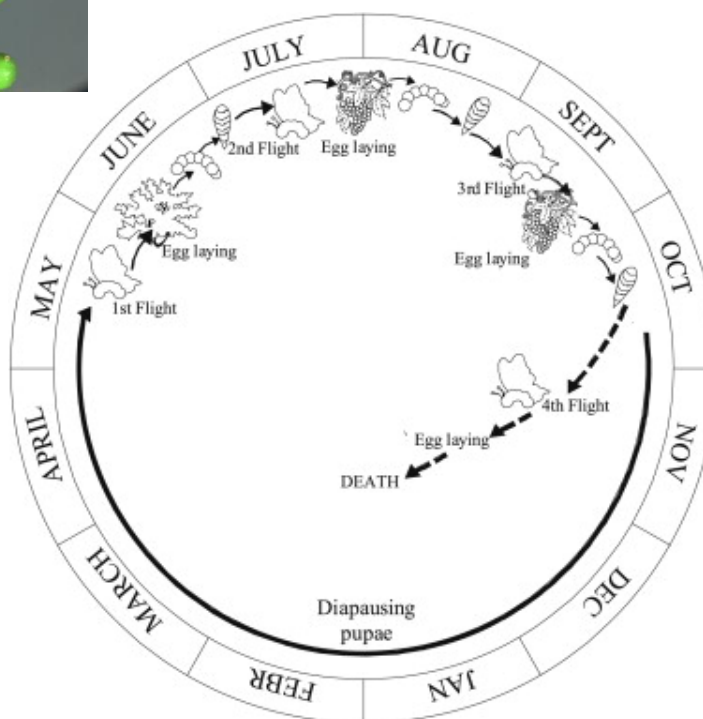
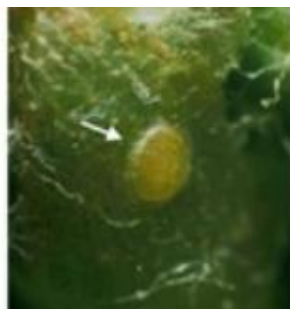


The european grapevine moth *Lobesia Botrana*

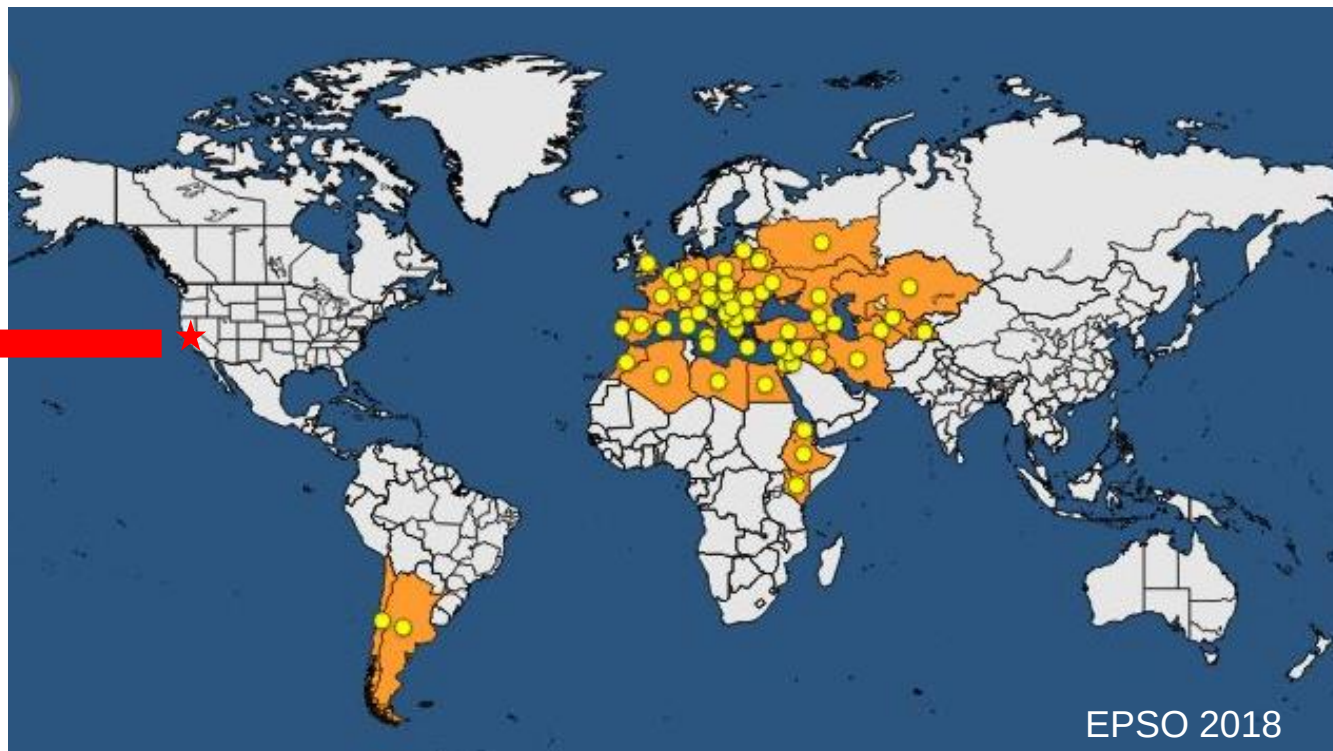


Bruno Bagnoli

Zangheri



The european grapevine moth *Lobesia Botrana*



Eradication :

- ✓ Cost >\$100M
- ✓ 6 years (2009-2015)

- Insecticides 100-150 euro/ha
- Pheromone trap 150-200 euro/ha

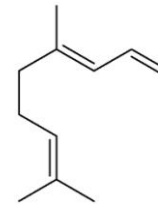
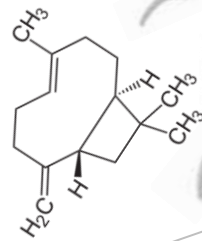
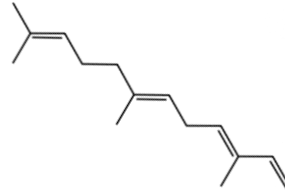


Semiochemicals in the vineyards

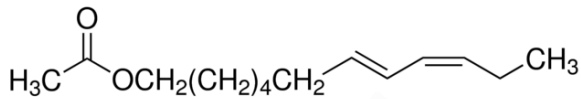
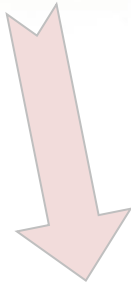
♂



Kairomones



Mating



Pheromones

♀

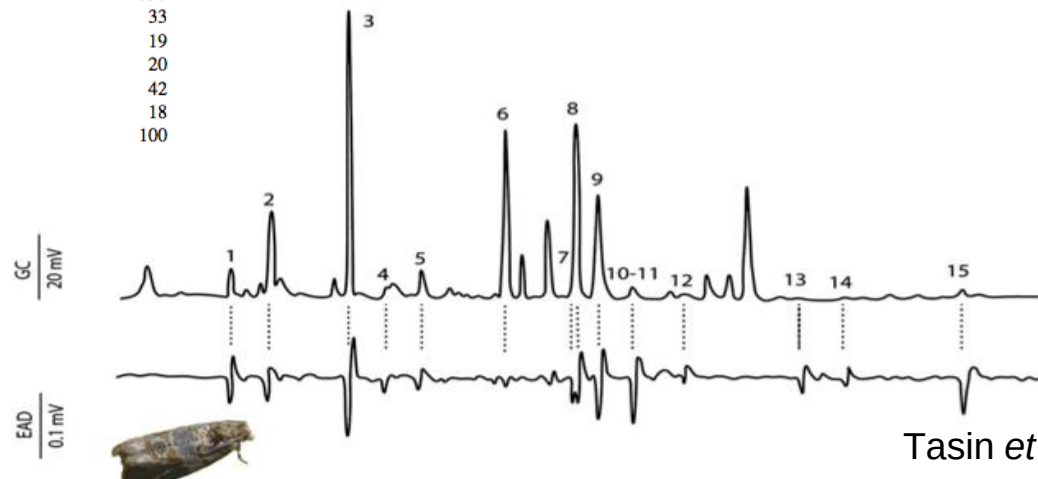
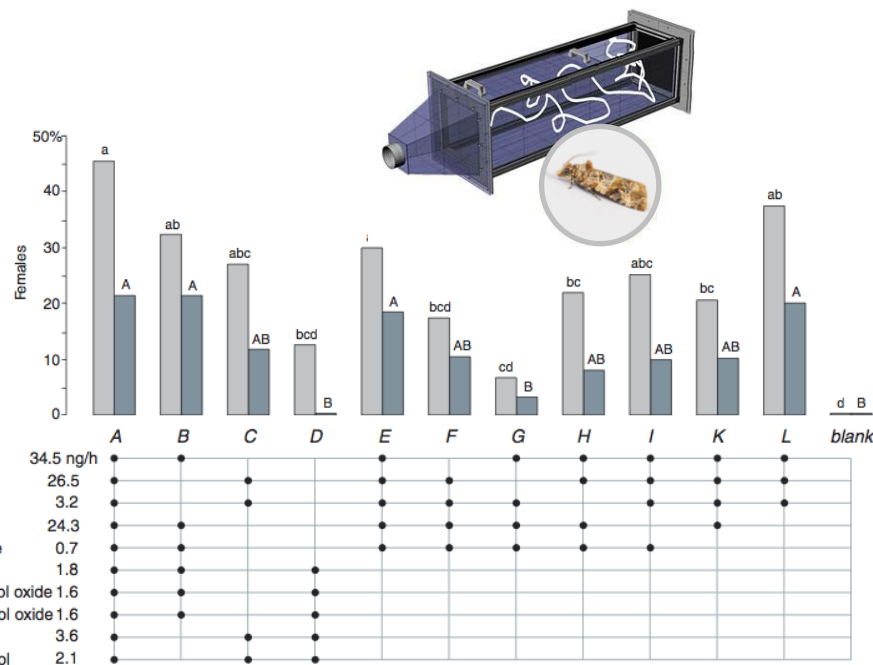


Host-finding



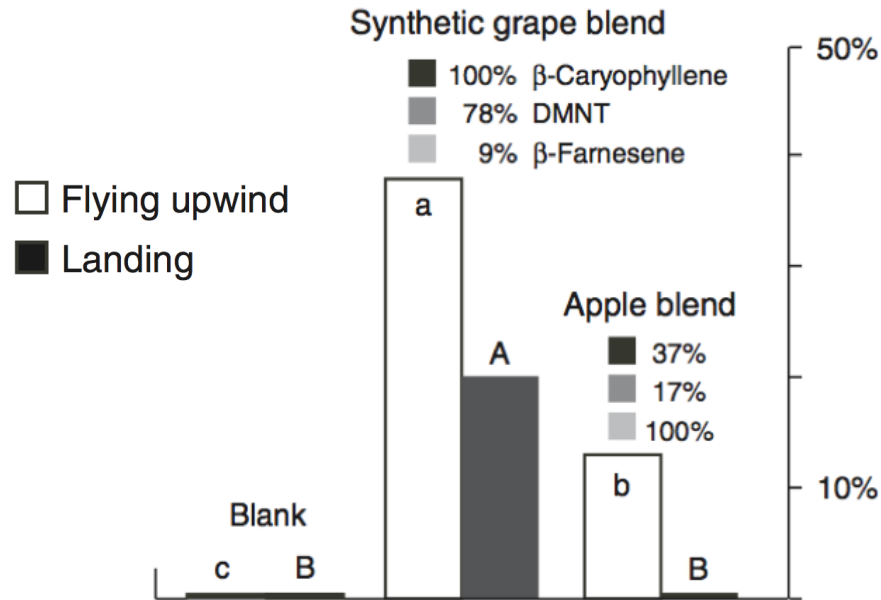
Background

Compound	Antennal response $\pm$ SD (mV $\times 10^4$ ng) ^a	Relative response (%) vs. (<i>E,E</i>)- α -farnesene
Hydrocarbons		
pentadecane ^{b,c,d,e,f}	2.03 $\pm$ 0.91	12
1-pentadecene ^{b,d,e}	0.39 $\pm$ 0.04	2
heptadecane ^{b,d,f}	5.48 $\pm$ 0.93	33
1-heptadecene ^{d,f}	1.08 $\pm$ 0.12	6
octadecane ^{b,d}	1.99 $\pm$ 0.61	12
nonadecane ^{b,d}	6.51 $\pm$ 1.52	39
1-nonadecene ^d	0.95 $\pm$ 0.28	6
heneicosane ^{d,e}	7.16 $\pm$ 1.71	43
Alcohols		
(<i>Z</i>)-3-hexenol ^f	2.22 $\pm$ 1.16	13
1-hexadecanol ^{d,e}	2.23 $\pm$ 1.91	13
1-heptadecanol ^{d,e}	4.76 $\pm$ 2.21	28
1-octadecanol ^{d,e}	6.59 $\pm$ 2.14	39
Aldehydes		
(<i>Z</i>)-3-hexenal ^{b,e}	4.76 $\pm$ 0.32	28
nonanal ^{b,c,d,e,f}	4.98 $\pm$ 4.18	30
Ketones		
2-undecanone ^{d,e}	1.86 $\pm$ 1.04	11
2-dodecanone ^{d,e}	6.30 $\pm$ 5.03	38
2-tridecanone ^{d,e}	1.03 $\pm$ 0.67	6
Esters		
(<i>Z</i>)-3-hexenyl acetate ^{e,f}	2.50 $\pm$ 1.27	15
Aromatic compounds		
methyl salicylate ^{b,d,e}	5.37 $\pm$ 3.12	32
Terpenoids		
(<i>E</i>)-4,8-dimethyl-1,3,7-nonatriene ^{b,e}	66.02 $\pm$ 39.14	393
limonene ^{c,d,e}	5.61 $\pm$ 4.55	33
(<i>E</i>)- β -ocimene ^{b,c,e}	3.25 $\pm$ 2.55	19
linalool ^f	3.33 $\pm$ 1.68	20
β -caryophyllene ^{c,d,e}	7.03 $\pm$ 4.50	42
(<i>E</i>)- β -farnesene ^{d,e}	2.95 $\pm$ 1.07	18
(<i>E,E</i>)- α -farnesene ^{b,c,d,e,f}	16.79 $\pm$ 3.53	100



Tasin et al. (2005; 2007; 2009)

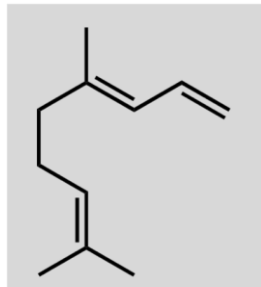
Background



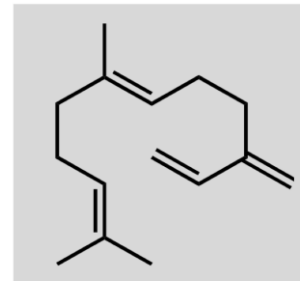
Tasin *et al.* (2005; 2007; 2009)

The ratio between the compounds seems to be crucial for the behavioral activity.

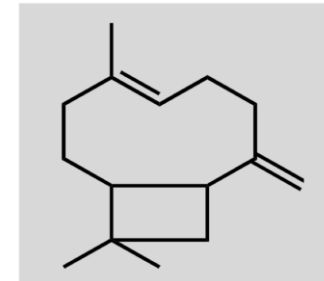
Could we test these compounds in the vineyards for pest control?



(*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT)

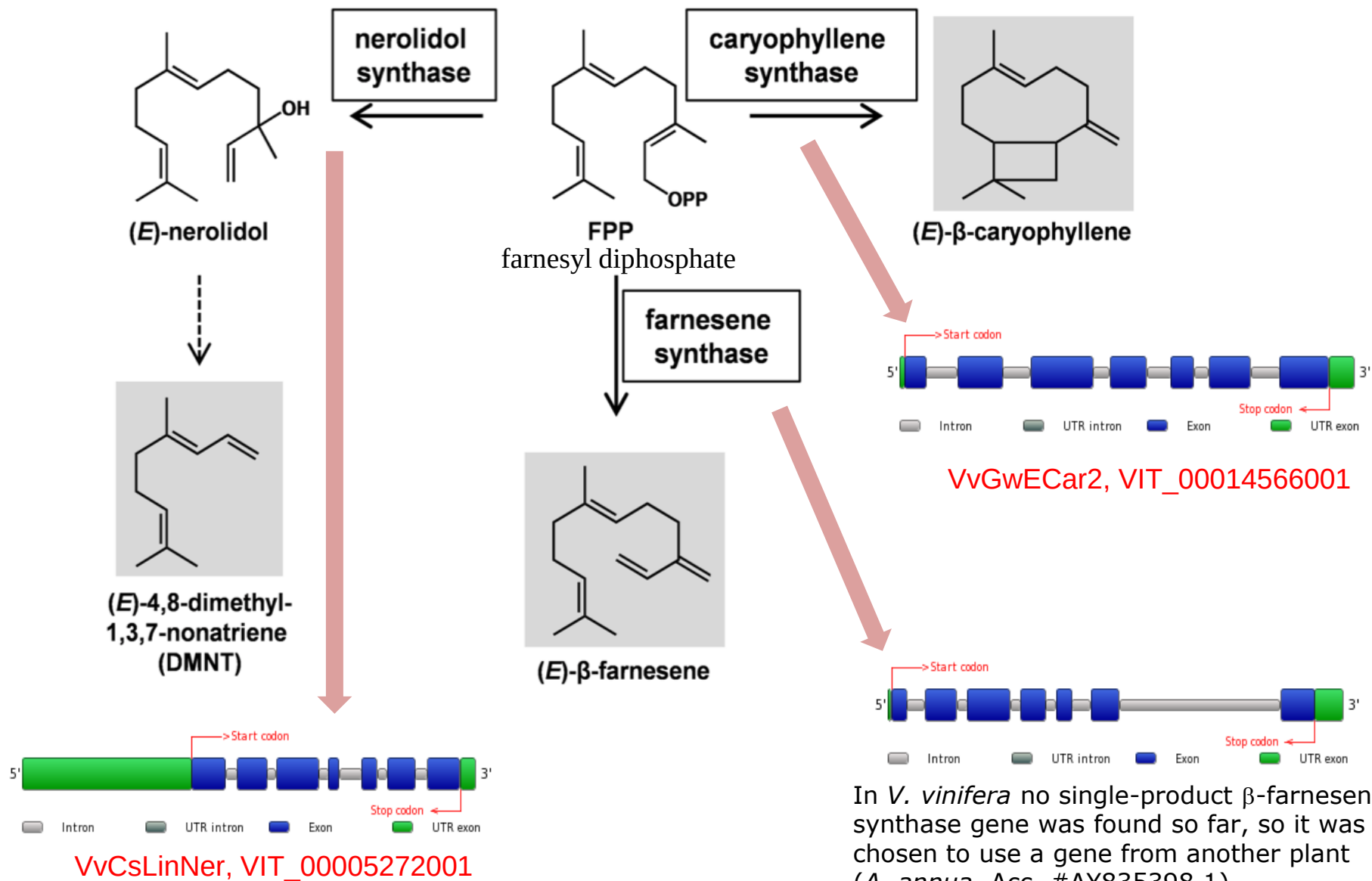


(*E*)- β -farnesene
VIT_00005272001



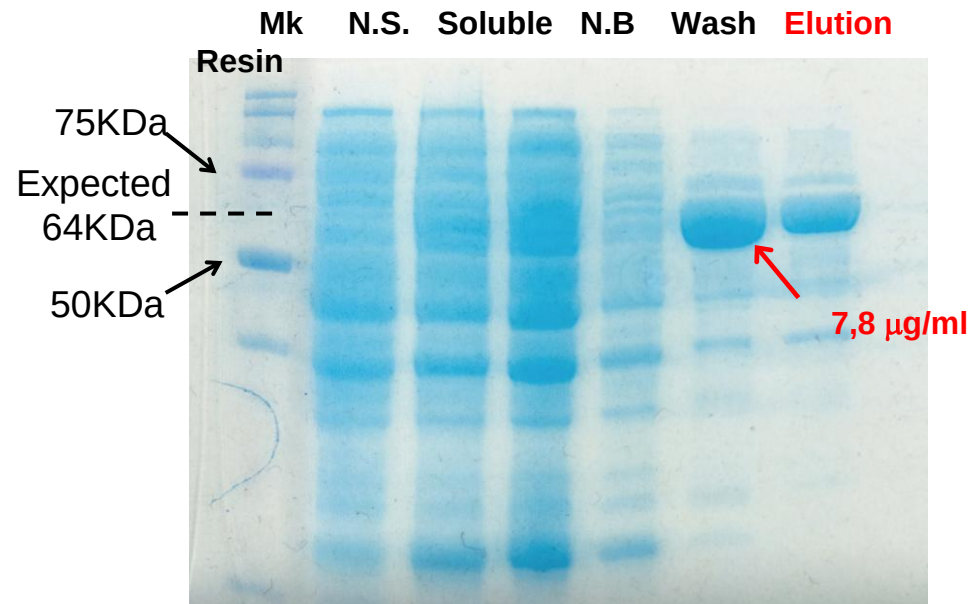
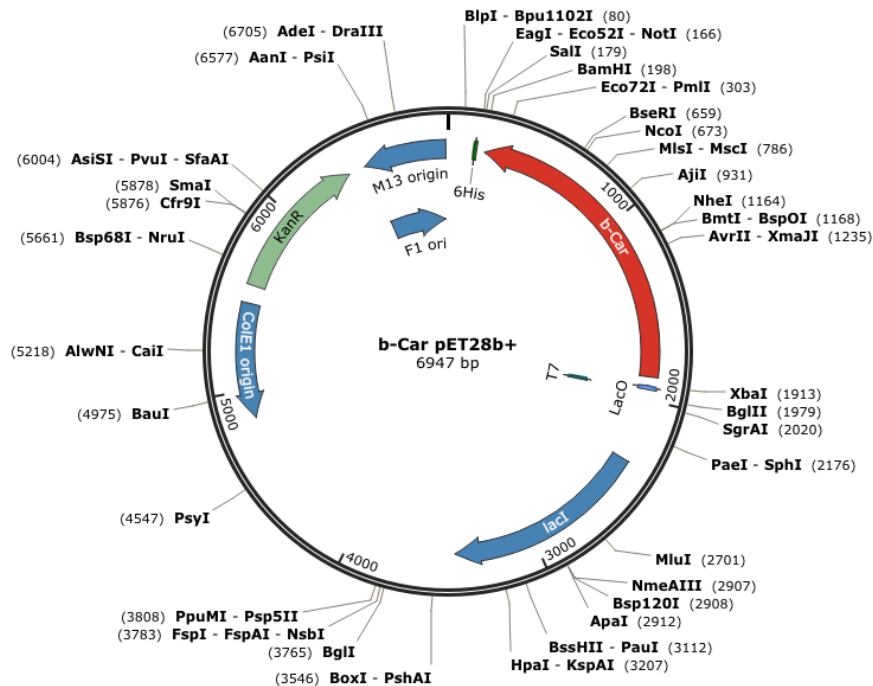
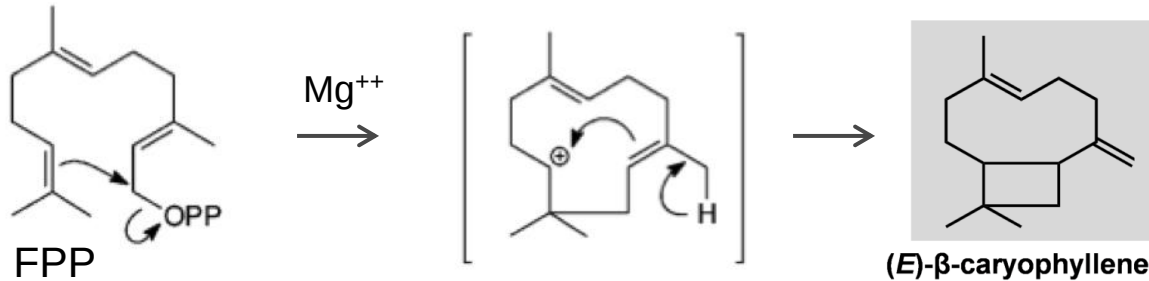
(*E*)- β -caryophyllene

Targeted sesquiTPS genes



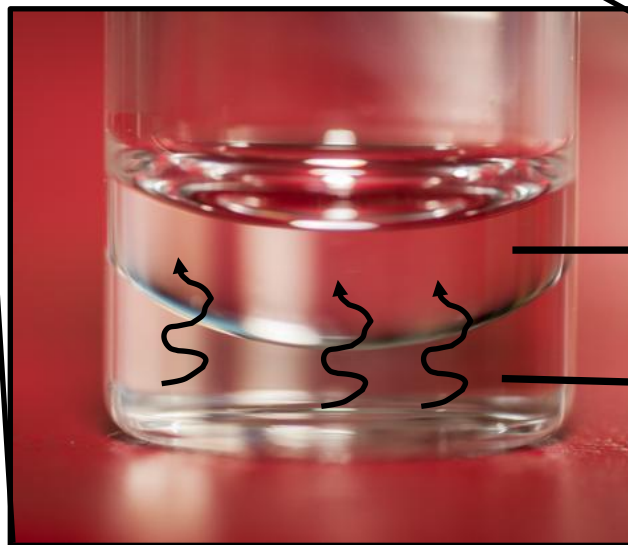
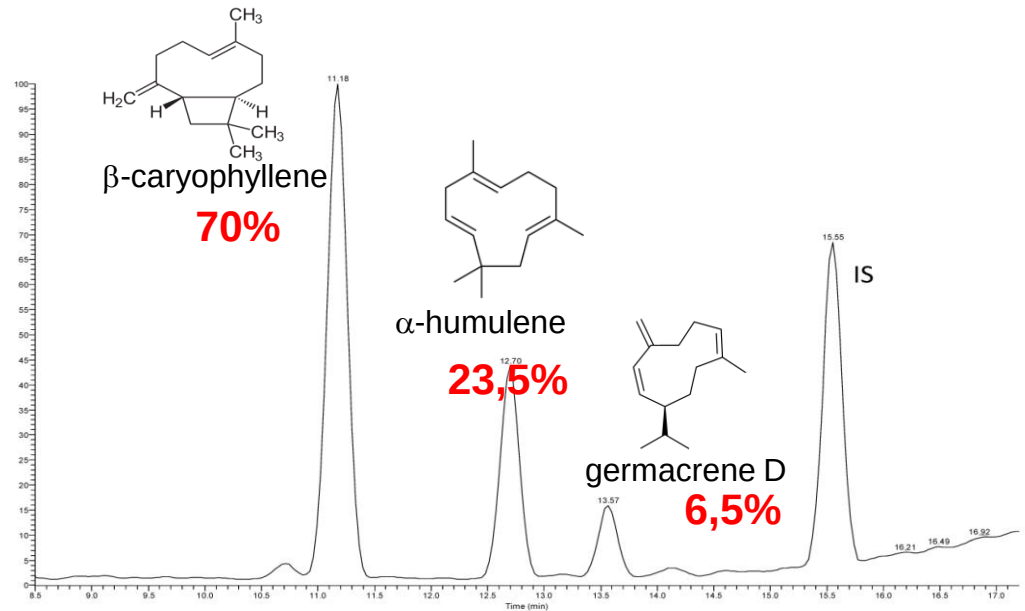
(E)-β-caryophyllene synthase enzyme characterization

VvGwECar2, VIT_00014566001

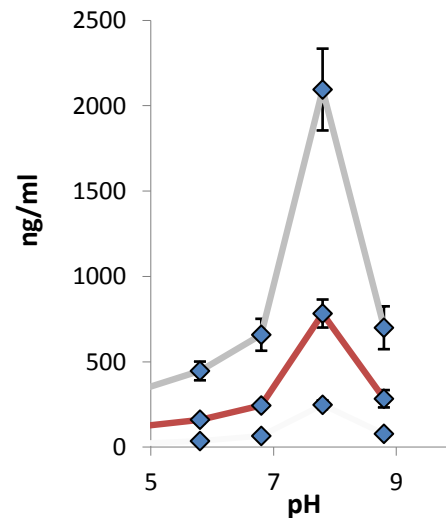


(E)- β -caryophyllene synthase enzyme characterization

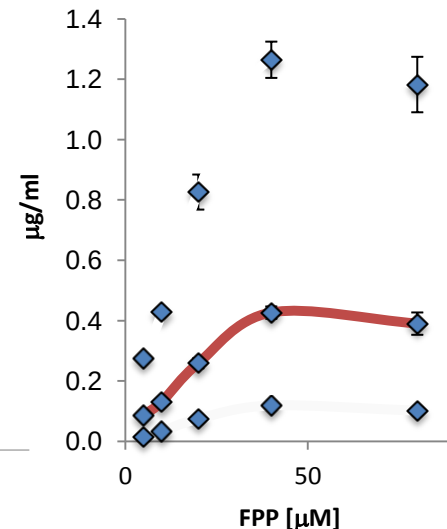
$K_m = 33,8 \mu\text{M}$
 $K_{cat} = 0,204 \text{ sec}^{-1}$
 $V_{max} = 14,1 \text{ nM/sec}$



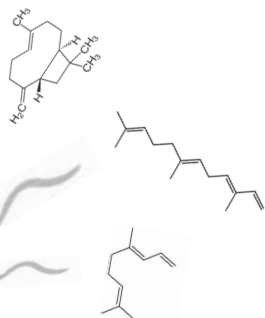
pH optimum

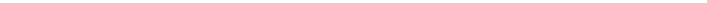


Substrate Saturation



The image is a composite. On the left is a photograph of a grapevine with a thickened stem, characteristic of a corky disease. On the right, there is a chemical structure of a terpenoid, specifically a bicyclic compound with a methyl group (CH₃) and a vinyl group (CH=CH₂). Below the chemical structure are three wavy lines, which are likely representing chemical structures or spectra.



- 



embryogenic callus
↑ 2 days co-culture *Agrobacterium* ↑

2 to 6 months

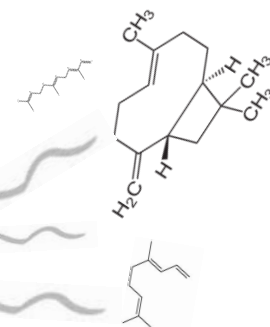
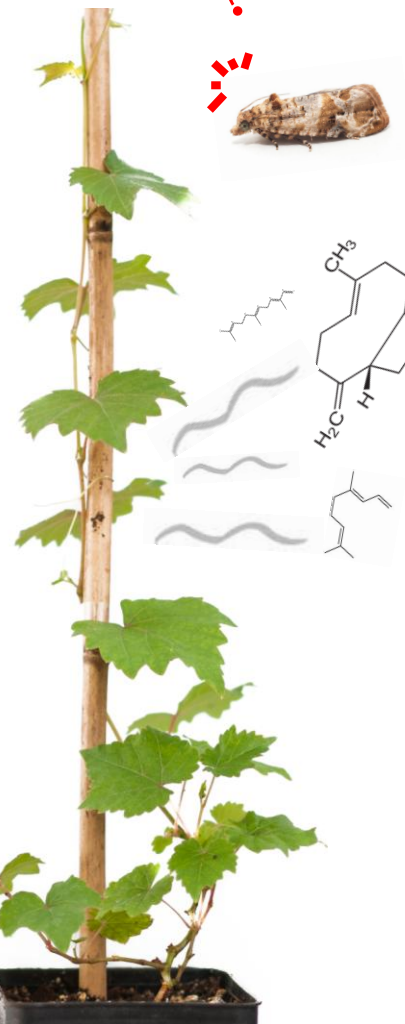


Selection of transgenic embryos

1 to 2 months



Plant regeneration

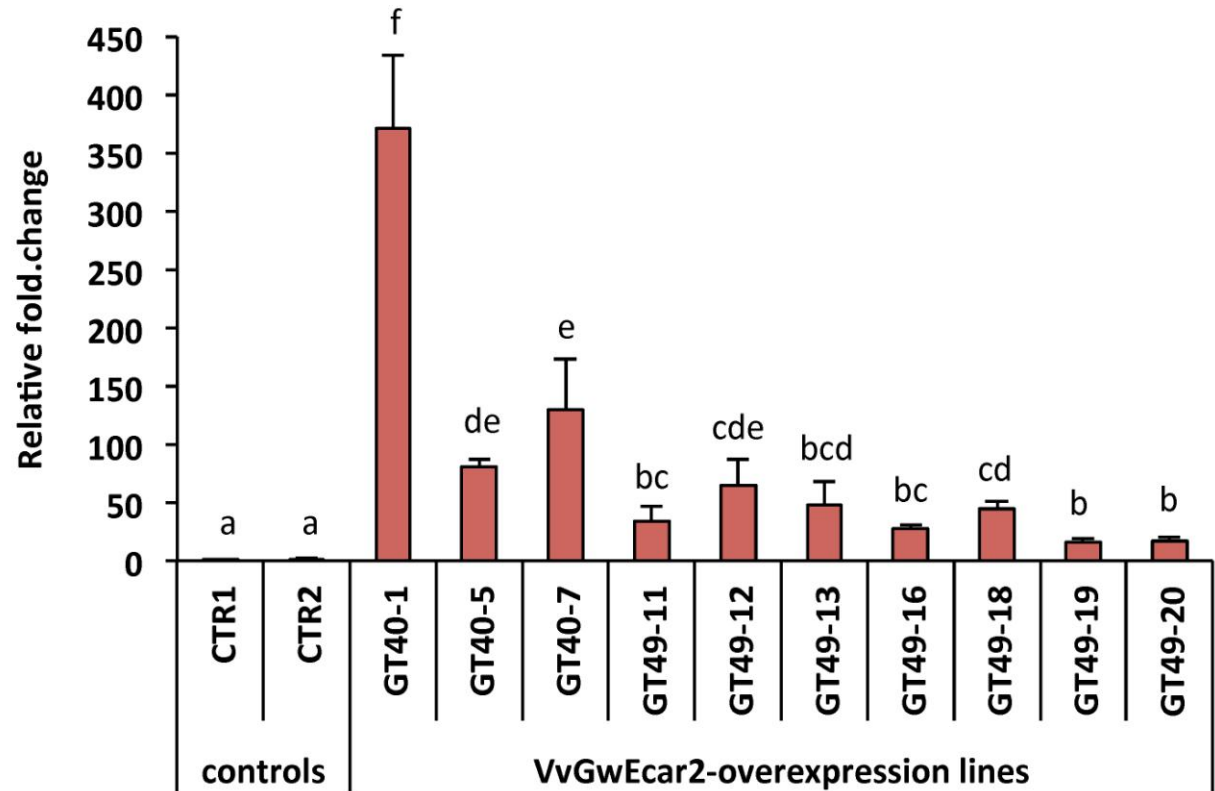


GM

Brachetto over-expressing b-Caryophyllene



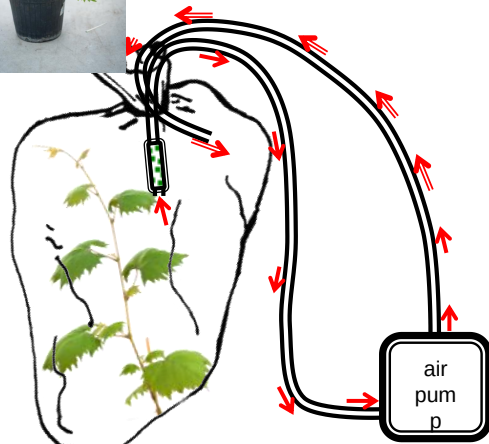
Brachetto over-expressing
b-Caryophyllene



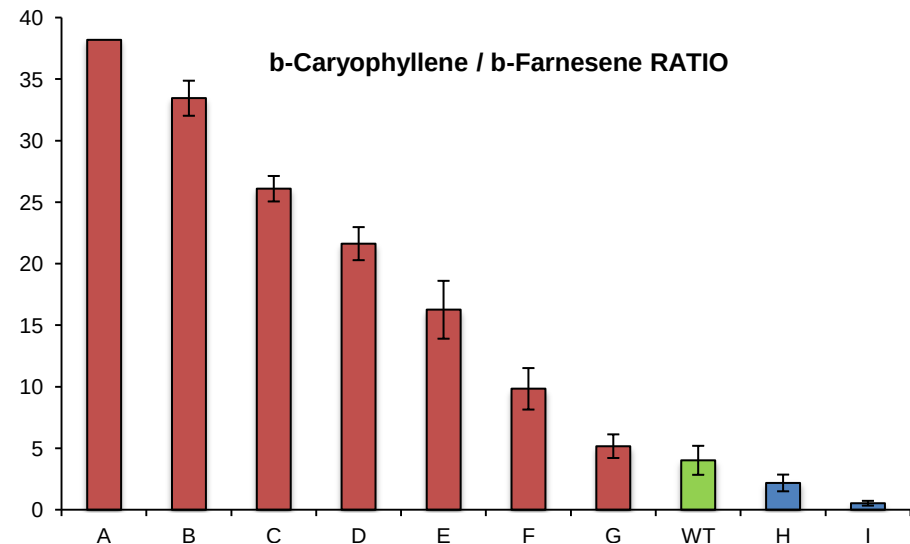
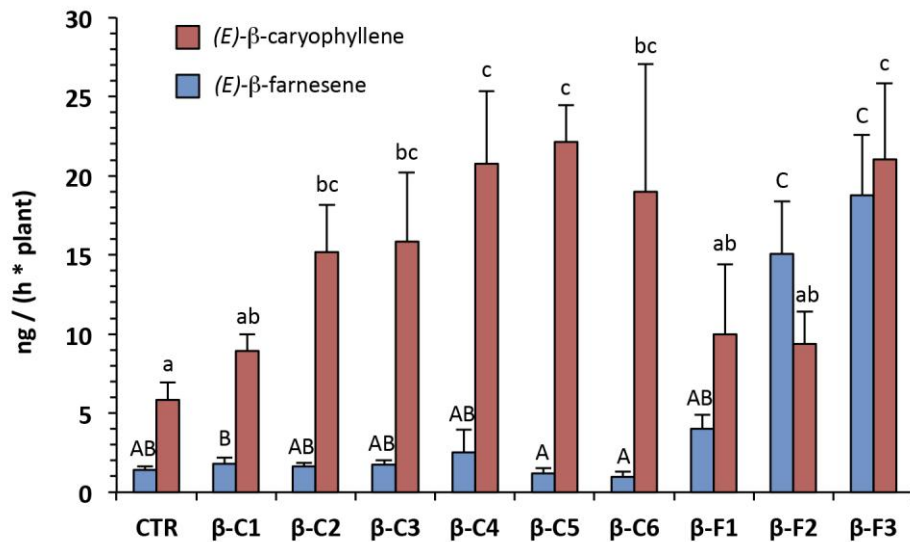
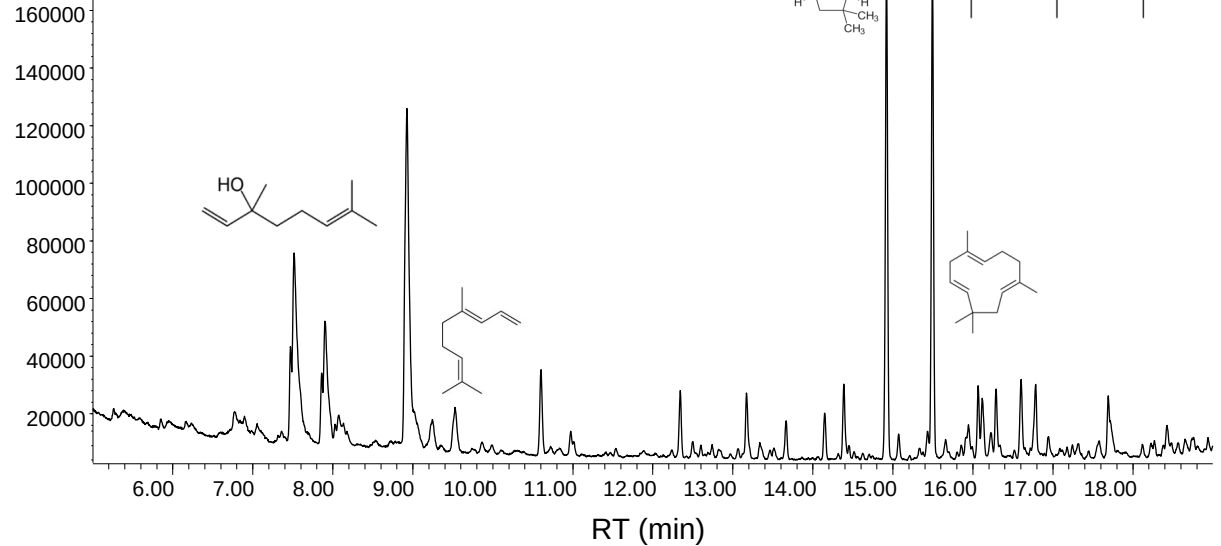
VOCs quantification



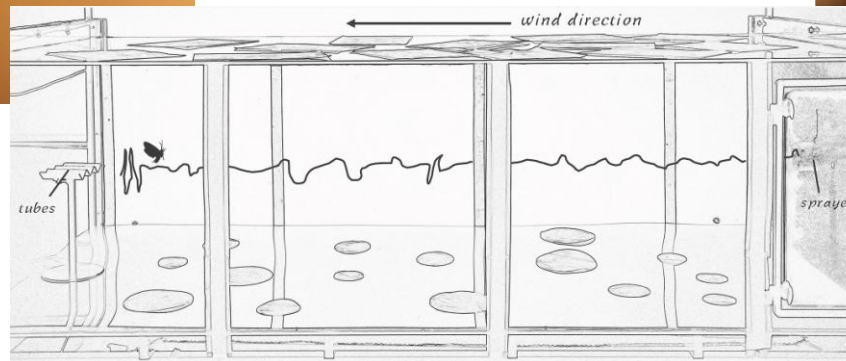
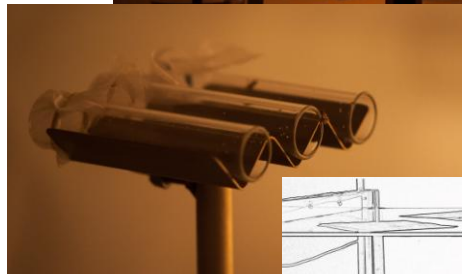
CLSA (Closed-Loop Stripping Analysis)



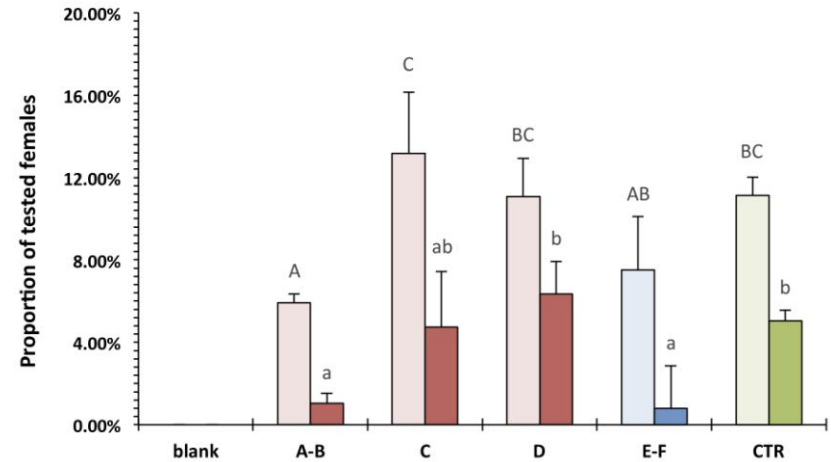
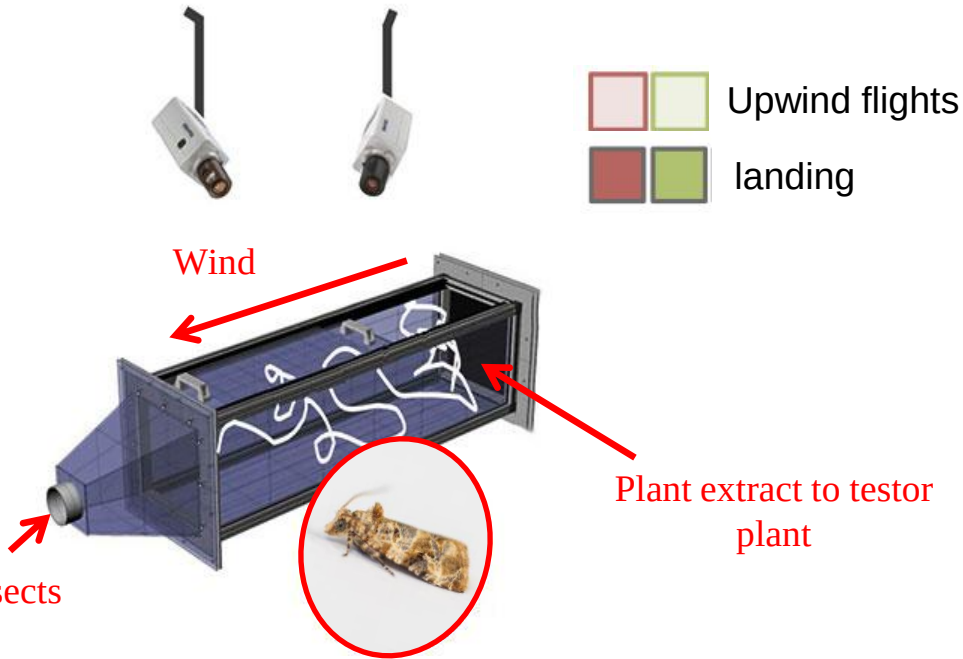
Abundance



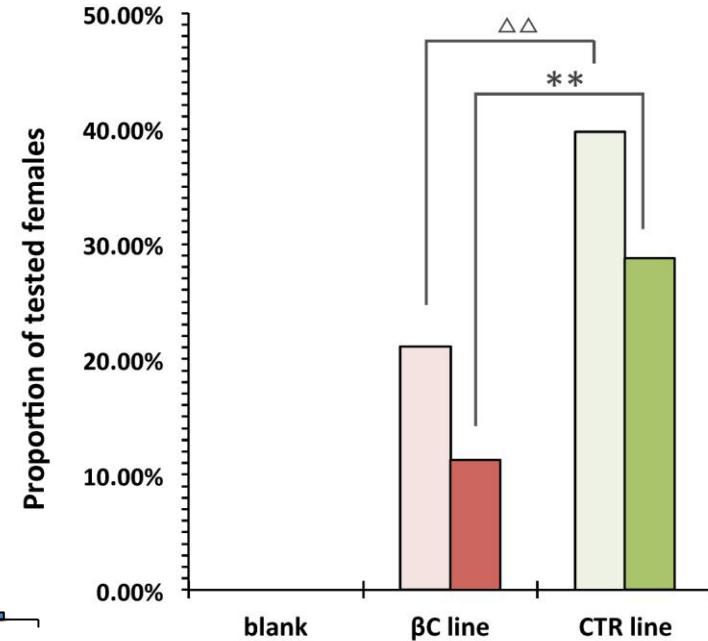
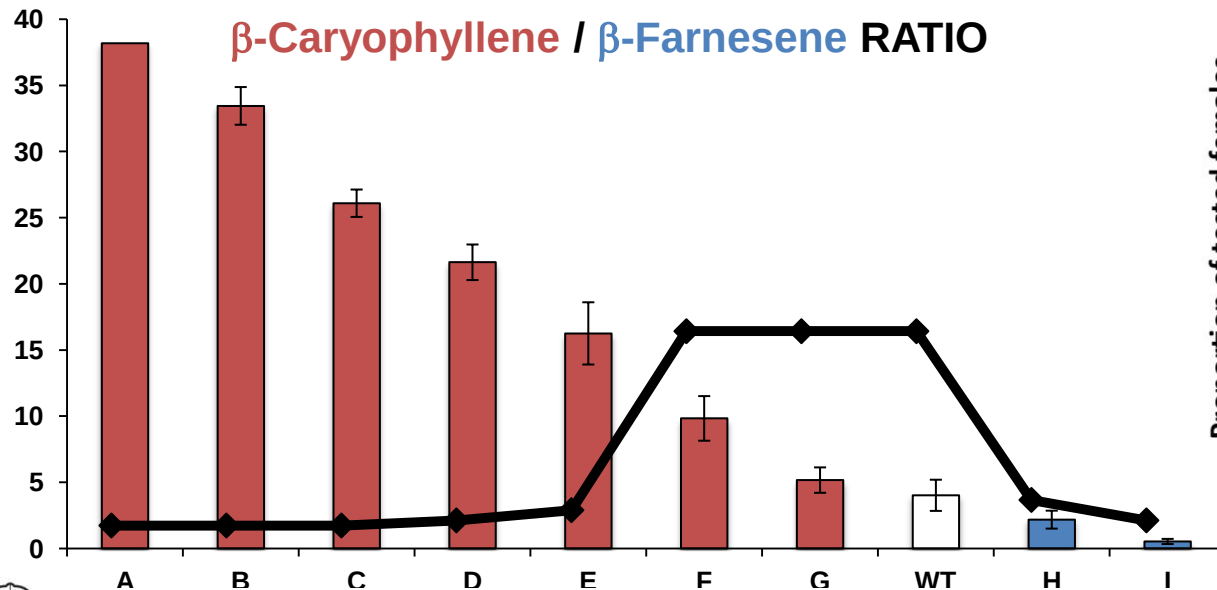
Wind Tunnel



Wind Tunnel



β -Caryophyllene / β -Farnesene RATIO



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



Dr Umberto Salvagnin



Dr Lorenza Dalla Costa



Dr Stefan Martens



Pr Sergio Angeli



Pr Gianfranco Anfora



Dr Gunda Thöming



Dr Marco Tasin

**THANK YOU
FOR YOUR
ATTENTION**

