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A GENETIC ENGINEERING APPROACH TO STUDY THE ADAPTATION OF THE GRAPEVINE MOTH *LOBESIA BOTRANA* FROM ITS WILD HOSTS TO *VITIS*

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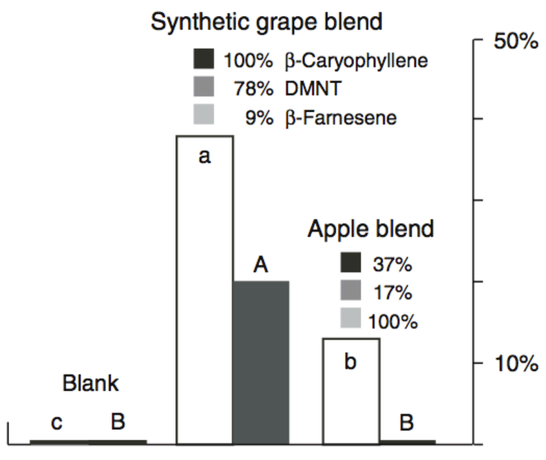
Introduction: The grapevine moth *Lobesia botrana* is an extremely polyphagous insects, endemic of the Palearctic Region and known across all the Mediterranean area as an economically important pest in the vineyards. Since the chemical ecology of the moth has been a major topic of research for decades, much is known about its behavior. Larvae of the moth can feed up to 40 plant species belonging to 27 different families and the major targets of feeding, if available, are always the reproductive tissues (flowers, fruits). A crucial role in host finding and egg-laying behaviors is played by specific volatile compounds, released by the plant and perceived by the insect olfactory system.

Wind Tunnel Studies

Tasin et al., *Naturwissenschaften* (2006) 93: 141–144



Upwind attraction of gravid grapevine moth females L. botrana to blends of the synthetic plant volatiles (E)-β-caryophyllene, (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and (E)-β-farnesene.

Since the adaptation to *Vitis* is recent (the first intense damages were recorded only from the early 20th century), experiments were carried out comparing the emission of volatiles between grapevine and *Daphne gnidium*, considered the first host of *L. botrana* in the wild: as expected, the volatiles profile partially overlapped.

Wind-tunnel studies have shown that a blend of the 3 grape terpenoids elicits attraction comparable to that of the complete fruit headspace collection. It was shown also that the specific ratio among compounds is crucial, since both the subtraction and the percentage variation of any of the three chemicals resulted into an almost complete loss of activity.

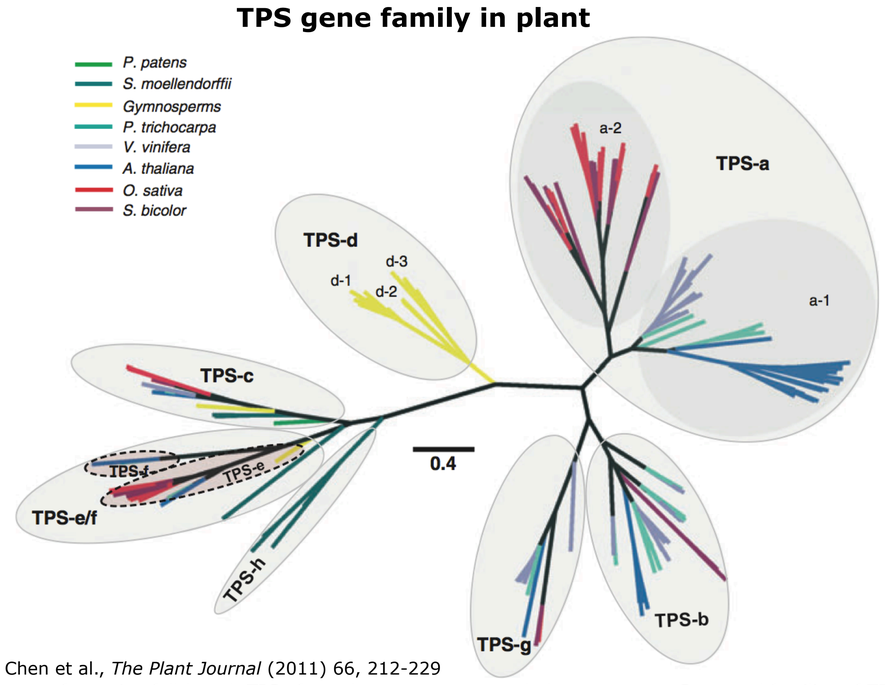
Of these terpenoids, only one (β-caryophyllene) is common between the two plants, while the others are present only in *Vitis* and were thus the target of the recent adaptation.

Tasin et al., Chem. Senses (2010) 35: 57–64

compound	release rate (ng/h)	%	Grape mimic (GS + C)	Grape specific (GS)	Common blend (C)	Daphne specific (DS)	Daphne blend (DS + C)
(E)-DMNT	26.9	76.9	●	●	○	○	○
(E)-linalool oxide (furanoid)	2.7	7.7	●	○	●	○	●
1-octen-3-ol	6.0	17.1	●	●	○	○	○
(Z)-linalool oxide (furanoid)	2.7	7.7	●	○	●	○	●
2-ethyl-hexan-1-ol	2.9	8.3	●	●	○	○	○
linalool	2.6	7.4	●	○	●	○	●
(E)-β-caryophyllene	35.0	100.0	●	○	●	○	●
(E)-β-farnesene	3.6	10.3	●	●	○	○	○
(E,E)-α-farnesene	24.0	68.6	●	○	●	○	●
methyl salicylate	1.6	4.6	●	○	●	○	●
ethyl benzoate	12.7	36.3	○	○	○	●	●
(E)-linalool oxide (pyranoid)	19.7	56.3	○	○	○	●	●
(Z)-linalool-oxide (pyranoid)	6.3	18.0	○	○	○	●	●
benzothiazole	0.1	0.3	○	○	○	●	●
(Z)-3-hexenyl benzoate	0.5	1.4	○	○	○	●	●

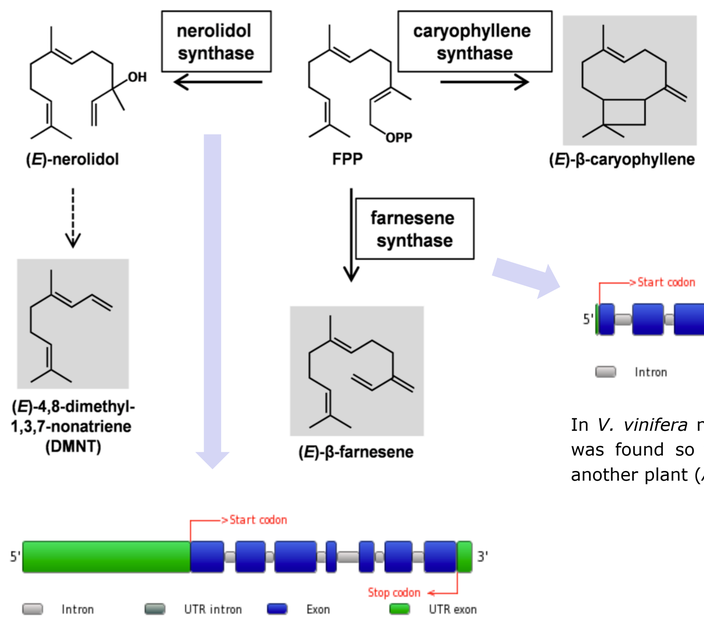
Scheme of the main volatile compounds identified in the headspace of the two plants, *V. vinifera* (recent adaptation) and *D. gnidium* (old host).

TPS gene family in plant



From Chen et al., *The Plant Journal* (2011) 66, 212-229
Phylogeny of putative full-length TPSs from the seven sequenced plant genomes. Seven subfamilies of TPSs are recognized. These include subfamily TPS-c (most conserved among land plants), subfamily TPS-e/f (conserved among vascular plants), subfamily TPS-h (Selaginella moellendorffii specific), subfamily TPS-d (gymnosperm specific), and three angiosperm-specific subfamilies TPS-b, TPS-g and TPS-a.

Targeted SesquiTerpene Synthase genes



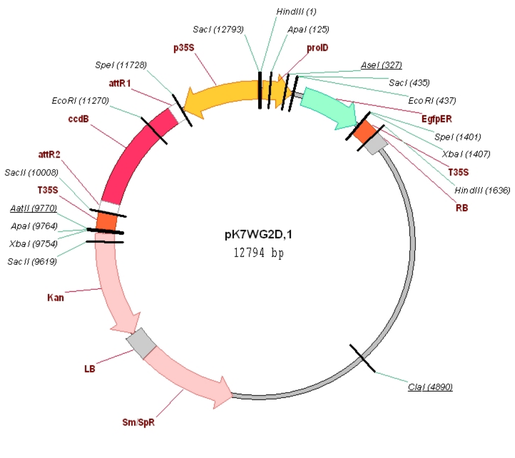
It was used the sequence of the gene from the Cabernet Sauvignon variety (VvCsLinNer, Acc. # HM807393).
The gene belong to the VvTPS-g subfamily.

It was used the sequence of the gene from the Gewürztraminer variety (VvGwECar2, Acc. # HM807374). The gene belong to the VvTPS-a subfamily.

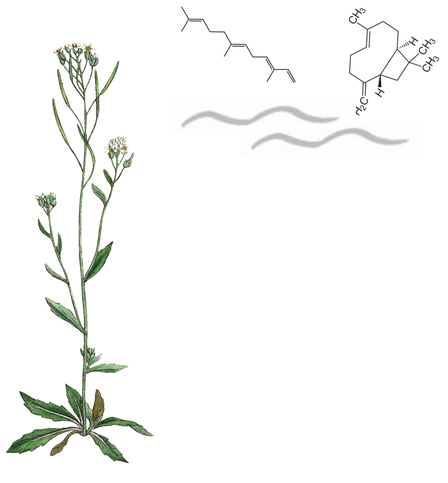
In *V. vinifera* no single-product β-farnesene synthase gene was found so far, so it was chosen to use a gene from another plant (*A. annua,* Acc. #AY835398.1).

TPS genes overexpression in *Arabidopsis thaliana*

Wild Type Col-0 Arabidopsis thaliana plant (low basal terpenes emission).

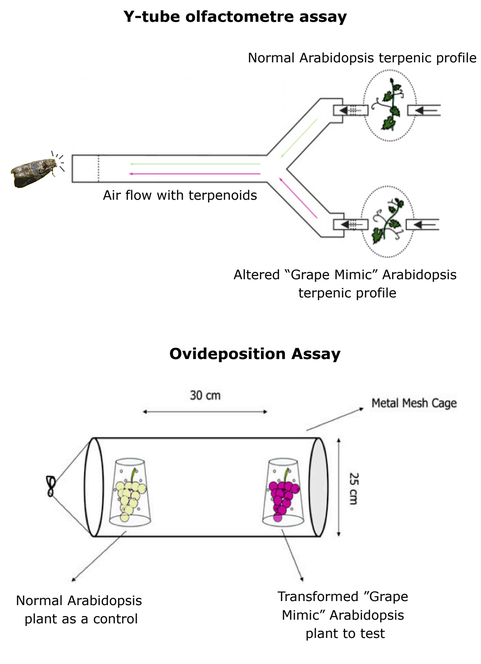


Candidate TPS genes expression is driven by strong constitutive promoter and inserted into the plant via Agrobacterium-mediated transformation.



New Transgenic Arabidopsis Plant, with a higher terpens emission rate, and a terpenic profile more simnilar to that one of grapevine.

Behavioral assays with *Lobesia botrana*



Conclusion: The plants obtained will be a potential useful tool to investigate plant-insect interactions and to better understand the role of terpenoids in the adaptation of *Lobesia botrana* to Grapevine. Moreover, the manipulation of the three terpenoids biosynthesis in grapevine, which has already started in our lab, could provide a novel way to set up a sustainable pest control method, based on the volatile compounds directly released by the plants.