CONTROL OF THE GRAPEVINE MOTH *L. BOTRANA* THROUGH THE MANIPULATION OF THE PLANT TERPENOID PROFILE

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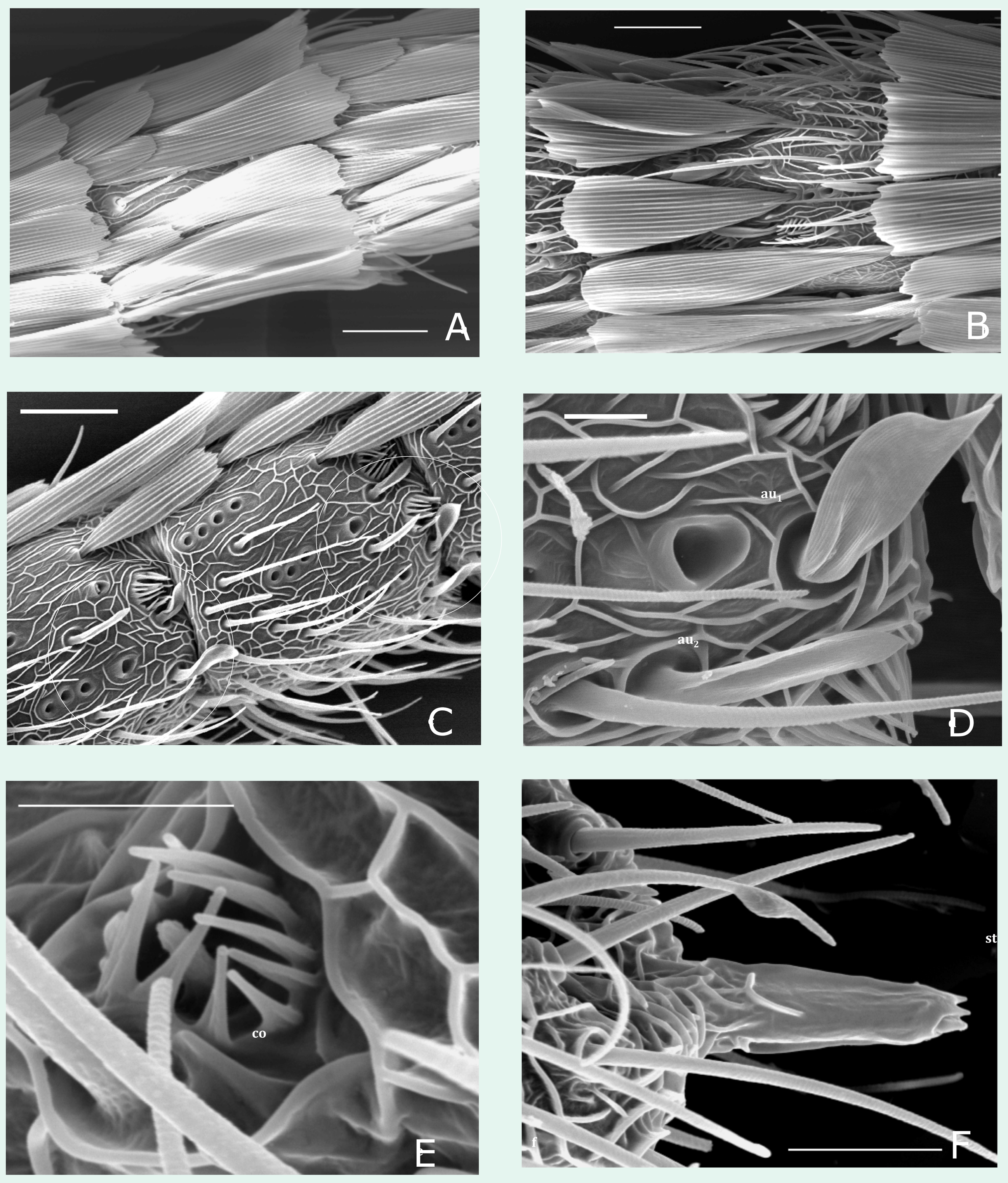
Introduction: The grapevine moth *Lobesia botrana* is one of the key pests of grape. Damages of the vineyard are achieved both by direct larval feeding on reproductive tissue of the plant (flowers, berries) and by secondary infections of microorganisms. Current control systems are either based on pesticides (many of which are currently being phased out) and mating disruption, that does not work well in non-delimited areas, or areas where pest population is high. We therefore suggest a method that instead works on the female by modifying the host-finding and the egg-laying behaviors, which in herbivore insects are mostly mediated by host plant volatiles (kairomones).
Recent wind-tunnel studies have shown that a blend of the 3 grape terpenoids (*E*)-b-caryophyllene, (*E*)-b-farnesene and (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) elicits attraction comparable to that of the complete fruit headspace collection in laboratory essays. The same blend gave also promising result when tested in field conditions. 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.

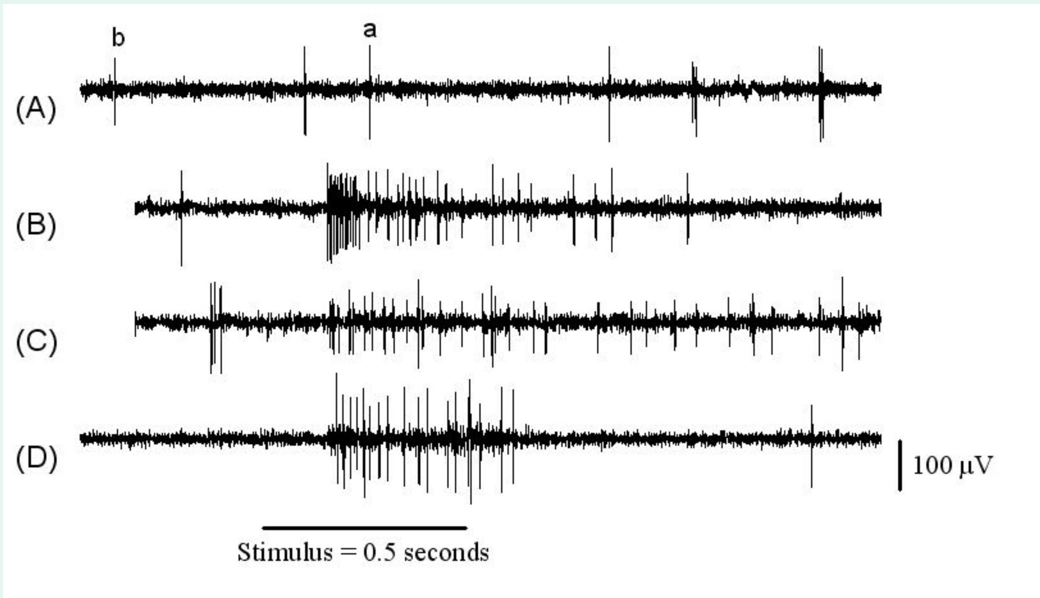
Scanning Electron Microscopy (SEM)



Dorsal side (A) covered with scale few sensilla project from the surface. In the ventral side (B), only partially covered by scales, several types of sensilla are placed. The most densely distribuited are the sensilla trichodea. Sensilla chaetica, easily distiguishable for a raised socket and a sculptured cuticle, are set, both in the ventral and dorsal part of the antenna, one in middle and two in the lateral sides. Two morphological different types of sensilla auricillica, the rabbit eared shoehorn characterized by an enlarged shape and the regular shoehorn, are set in small groups, partially covered by scales, in the distal edge of each segment (C-D) where few sensilla coeloconica (C-E) and one sensillum styloconicum (F) are also present.

Single-Cell Recording

Recordings were made from the ventral surface of medial segments of the antenna, which lodges most of the sensilla trichodea and sensilla auricillica. An electrolytically sharpened tungsten wire was used to penetrate the insect head cuticle to serve as ground electrode, while another electrolytically sharpened tungsten electrode was inserted at the base of the sensilla to establish contact with the receptor neurons.



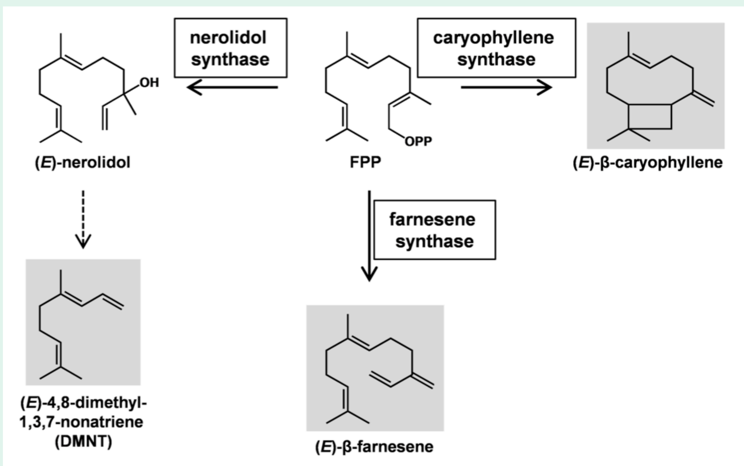
Samples of spikes recorded on stimulation of receptor neurons housed in a sensillum auricillicum on the antennae of female *L. botrana* with host plant volatiles: solvent (A:10 µl hexane), methyl salicylate (B: 10 µg), nonatriene (C: 10 µg) and (+)-linalool (D: 10 µg).
The horizontal bar below the recordings indicates the stimulus duration (0.5 sec), the vertical bar on the right side the scale amplitude (40 µV).

Responses elicited in the ORNs were assigned to three different categories, according to different levels of increase in spike frequency.

compound	olfactory neuron																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Responding neurons (%)
(S)-octen-3-ol	0	0	1	0	0	2	3	1	2	2	0	0	2	2	1	0	2	1	1	1	65
(R)-octen-3-ol	0	0	2	0	0	2	3	2	3	3	0	0	3	3	2	1	2	1	2	2	70
(-)-limonene	0	0	0	0	0	0	1	1	2	1	0	0	0	0	3	0	0	0	1	1	35
(+)-limonene	-	-	-	-	-	-	-	-	-	-	0	0	0	0	3	0	0	0	0	1	20
(E)-α-farnesene	0	0	0	2	0	0	2	3	1	1	0	0	0	0	2	0	0	2	2	1	45
(E)-β-farnesene	0	0	0	2	0	0	2	1	1	1	0	0	0	0	1	0	0	1	1	2	45
methyl salicylate	0	0	0	0	0	3	3	3	3	3	0	3	2	3	2	0	2	2	2	1	65
(E)-β-caryophyllene	0	0	0	0	0	0	1	2	0	1	0	0	0	0	2	0	0	1	1	0	30
(E)-3,7-dimethyl nonatriene	0	0	2	0	0	0	0	3	1	1	0	0	0	0	2	0	0	2	1	3	40
E7,Z9-12:Ac	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
(+)-linalool	0	0	0	0	0	0	2	2	2	2	0	0	0	0	3	0	2	3	3	3	45
(-)-linalool	0	0	2	0	0	0	3	2	2	1	0	0	0	0	2	0	2	3	3	1	50
(-)-E- linalool oxide pyranoid	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	2	2	2	0	16
(+)-E- linalool oxide pyranoid	0	0	0	0	0	0	2	0	1	0	0	-	0	0	1	0	1	2	2	0	32
(+)-Z- linalool oxide pyranoid	0	0	0	0	0	0	1	0	0	0	0	-	0	0	0	0	2	3	2	1	26
(-)-Z- linalool oxide pyranoid	0	0	0	0	0	0	0	0	0	0	0	-	0	1	0	0	2	2	2	1	26
(+)-E- linalool oxide furanoid	0	0	0	0	0	0	0	0	0	0	0	-	0	0	1	0	2	3	2	0	21
(+)-Z-linalool oxide furanoid	0	0	0	0	0	0	0	0	0	0	0	-	0	0	1	0	2	1	2	0	21
(-)-Z-linalool oxide furanoid	0	0	0	0	0	0	0	0	0	0	0	-	0	0	1	0	2	3	1	0	21
% perceived compounds	0	0	22	11	0	17	61	56	56	56	0	8	16	21	79	5	63	84	90	63	

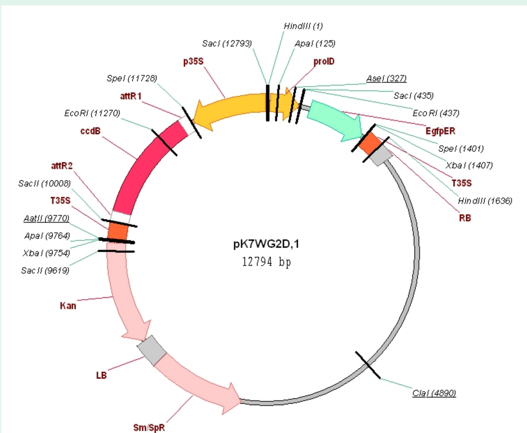
SCR responses to kairomones were strongly female-specific and sensilla auricillica-specific, and in the same range of neuron activation as those elicited in males by E7,Z9-12:Ac. Sensilla auricillica play hence a key role in the perception of host plant compounds.

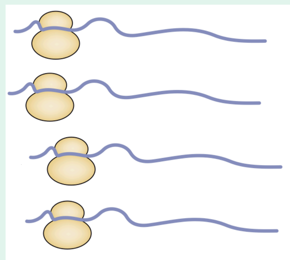
Targeted Terpens



The three specific terpenes β-caryophyllene, β-farnesene and DMNT are evolved by different Terpene Synthase Enzymes (TPS) from the same substrate, Farnesyl Pyrophosphate (FPP). Many TPS gene families were characterized in recent years in various species, also in Grapevine. It is now possible a genetic engineering approach for the manipulation of the terpenoid profile emitted by the plant.

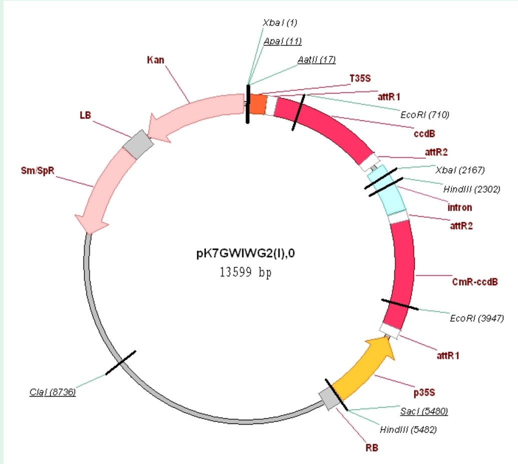
First Strategy: TPS genes overexpression

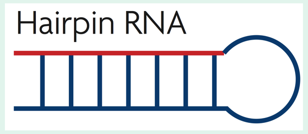




Candidate TPS genes expression is driven by strong constitutive promoter

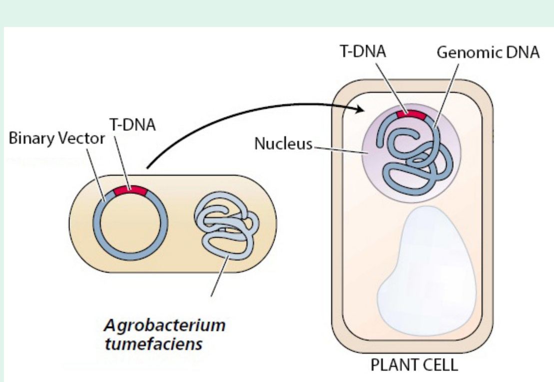
Second Strategy: RNAi on TPS genes





The formation of a hpRNA targeting the TPS candidate genes lead to RNAi.

Agrobacteria-mediated transformation of grapevine embryogenic calli



Regenerants will give plants with altered terpenoid profile.

Conclusion: The plants obtained will be a potential useful tool to investigate further the plant-insect interactions, and are a likely starting point of new insect control strategies based on kairomones manipulation *in planta*.