



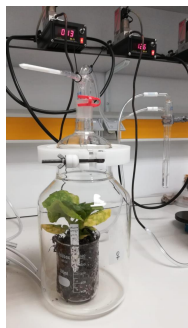
SUSPHIRE

Sustainable Bioproduction of Pheromones
for Insect Pest Control in Agriculture

WP2 Lepidoptera Pheromone

Task 2.4: Investigate the pheromone content and the electrophysiological and behavioural response of target insects to extracts of plants and fungi expressing Lepidoptera Pheromones (P1 [Lead] & P5) Duration: Month 18 – Month 24

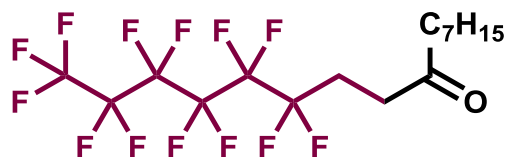
Ismael Navarro
Ecología y Protección Agrícola SL
Sandra Vacas
CEQA-UPV



Quantification

Volatile and Plant extraction

Internal standard selection: Esterification of fluorinated alkyl





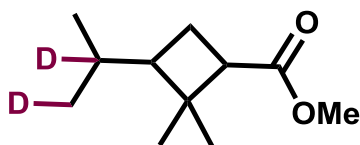
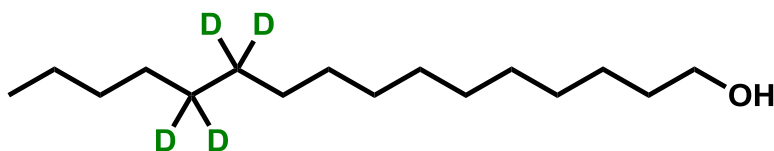
Quantification

Volatile and Plant extraction

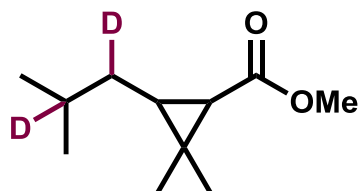


MATRIX EFFECTS!!

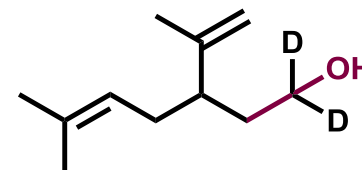
Internal standard selection: Deuterated samples



d₂-labelled Planoccociol



d₂-labelled Crisantemol

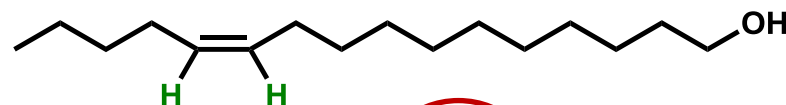


d₂-labelled Lavandulol

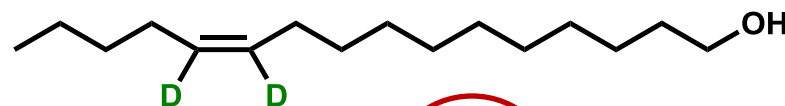


Plant extraction

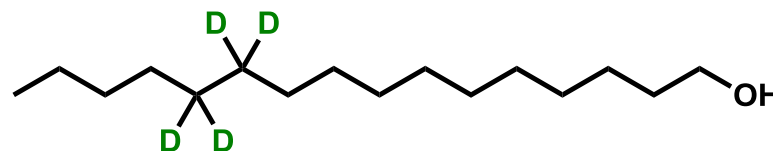
We can compare the intensity of the deuterated mass peaks with a spiked sample.



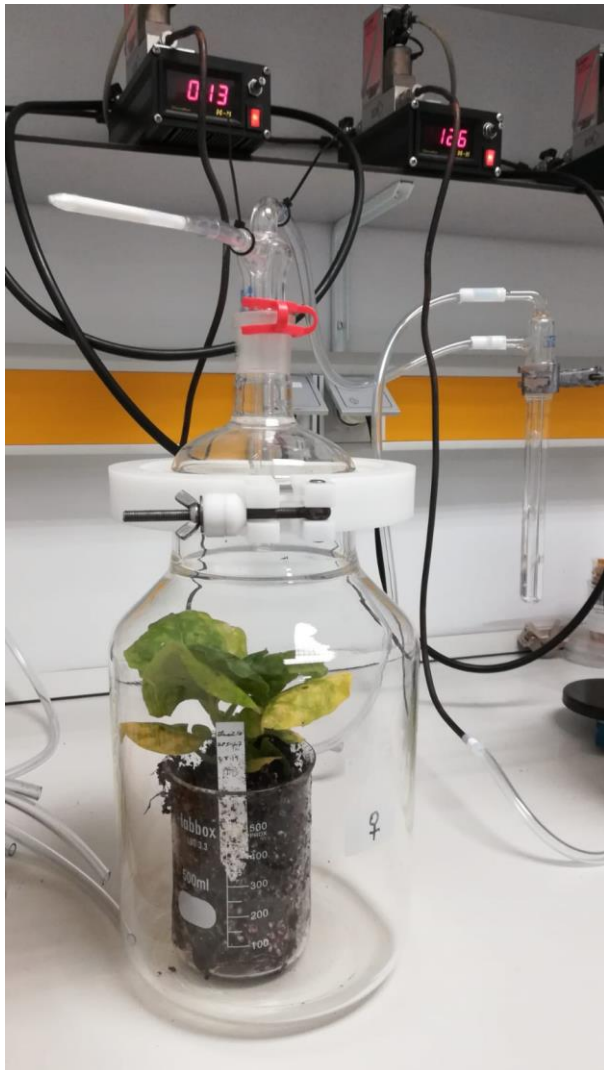
Exact Mass: 240,2453



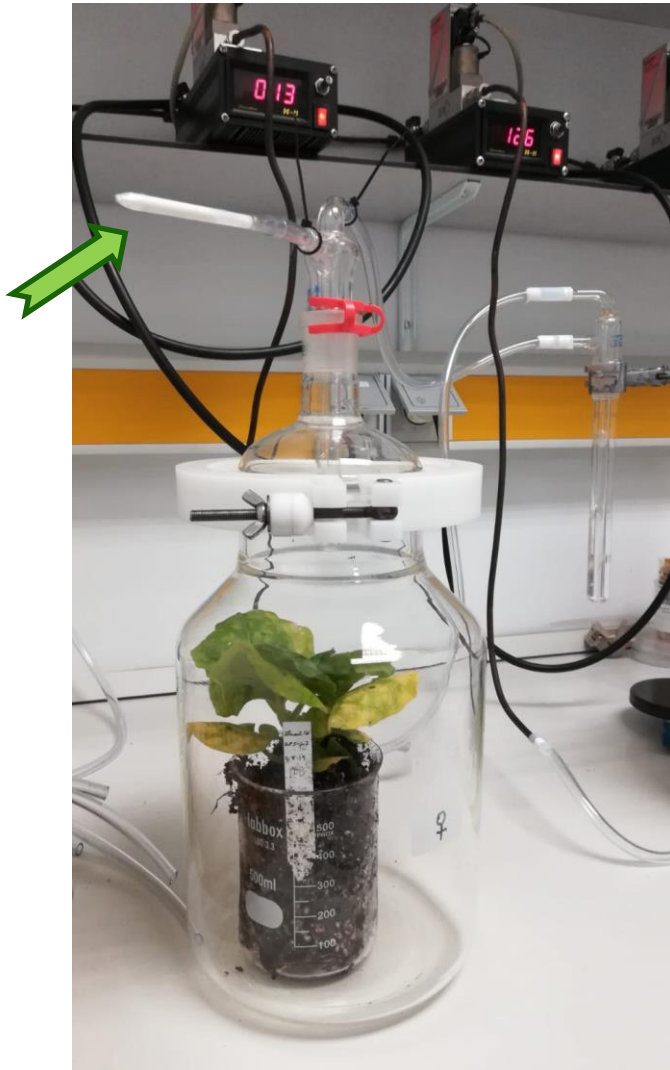
Exact Mass: 242,2579



Exact Mass: 246,2861



Volatile Collection



Aeration conditions

- 5 L glass reactor
- Ultrapurified air stream: 150 mL/min
- 300 mg Porapak Q
- 72 h collection
- Extraction: 5 mL pentane
- GC/MS/MS analysis



Volatile Collection

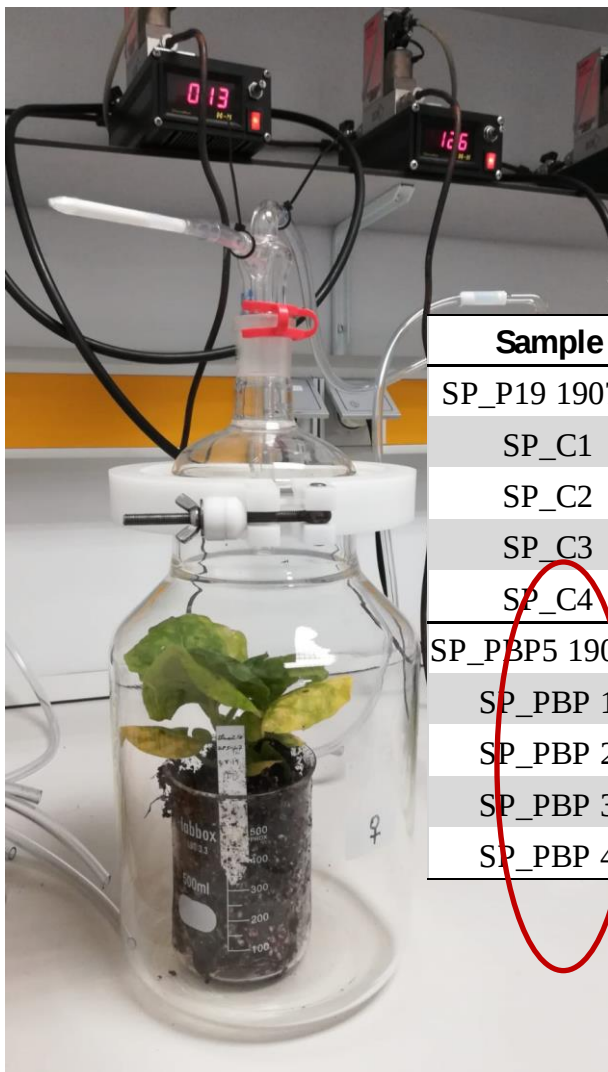
Quantification

- Internal standard (TFN)
- Triple quadrupole mass detector
- MS/MS parameters

	Transition	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)
TFN	1	375	263	10
	2	393	373	5
Z11C16ol	1	82	67	5
	2	96	81	5
	3	95	67	10
	4	96	54	10



Volatile Collection SxP 1.0 Sexy plant



**Only Z-11 hexadecenol was quantified.
No acetate was detected**



Sample	TFN area	OL area	ratio	ng/ul calc	ng collected	ng/day	
SP_P19 190711	141800	21597	0,1523	1,73	345,2	115,1	
SP_C1	255100	31310	0,1227	1,46	219,3	73,1	
SP_C2	208300	18680	0,0897	1,17	175,0	58,3	
SP_C3	222300	-					
SP_C4	236600	9973	0,0422	0,74	111,4	37,1	70,9
SP_PBP5 190711	200800	47970	0,2389	2,50	437,3	145,8	
SP_PBP 1	244100	8025	0,0329	0,66	98,9	33,0	
SP_PBP 2		-					
SP_PBP 3	269300	23050	0,0856	1,13	169,5	56,5	
SP_PBP 4	249300	-					78,4

Quantification SxP1.2 Sexy plant

[illegible]

WT 1

Z-11-OL

Z-11-AC

SxP 1.2 T1-2



Volatile Collection SxP1.2 Sexy plant

Sample	TFN area	Z -11-hexadecen-1-ol				Z -11-hexadecenyl acetate			
		area	ratio OL/TFN	µg collected	ng/day	area	ratio AC/TFN	µg collected	ng/day
vol_SxP 1.2 T1-1	358133,3	30260,0	0,084	0,209	69,8	149366,7	0,418	0,193	64,4
vol_SxP 1.2 T1-2	400833,3	39573,3	0,099	0,226	75,2	312533,3	0,780	0,359	119,8
vol_SxP 1.2 T1-3	233133,3	22506,7	0,096	0,223	74,2	126666,7	0,543	0,251	83,6
vol_SxP 1.2 T1-4	238600,0	37870,0	0,159	0,294	98,0	132600,0	0,556	0,257	85,6
				0,238	79,3			0,265	88,4



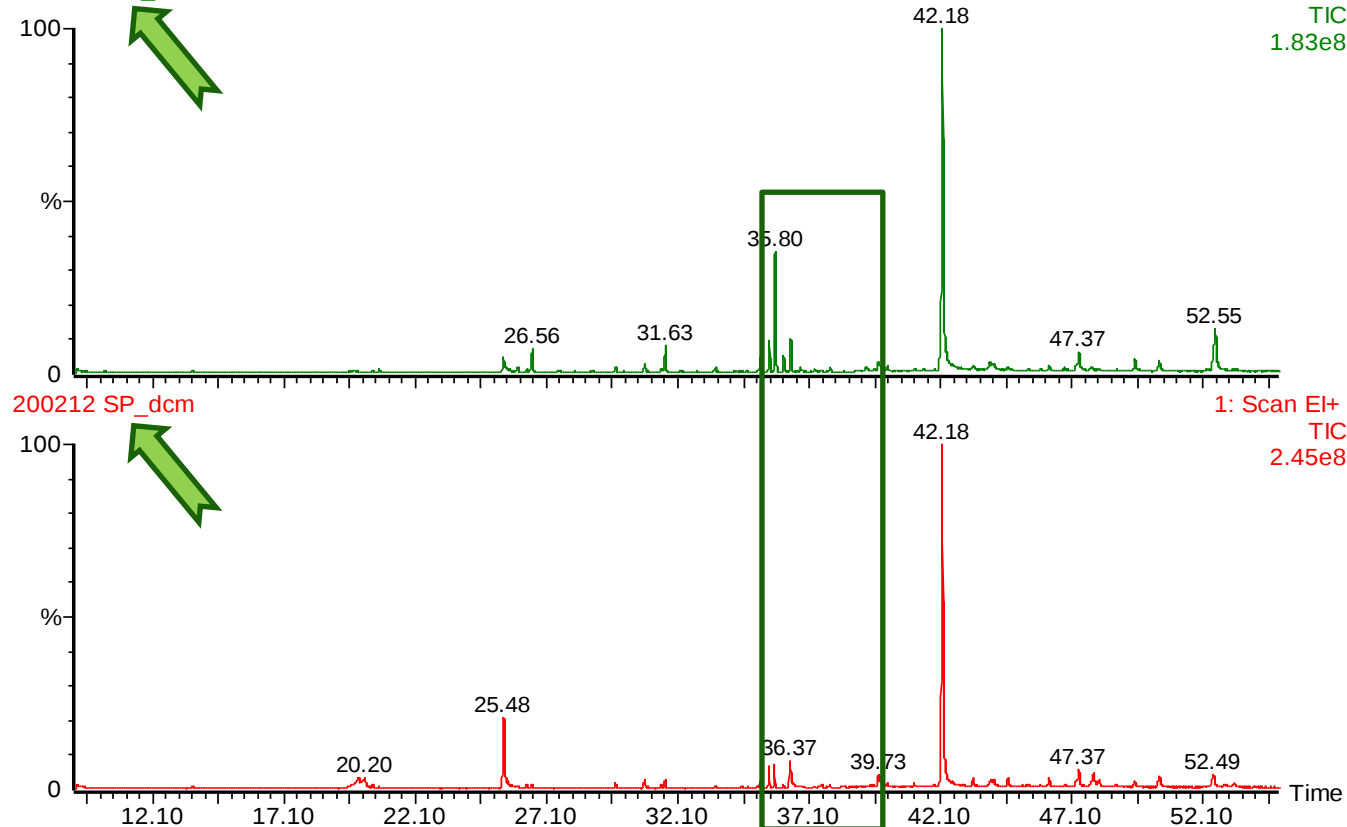
Plant extraction



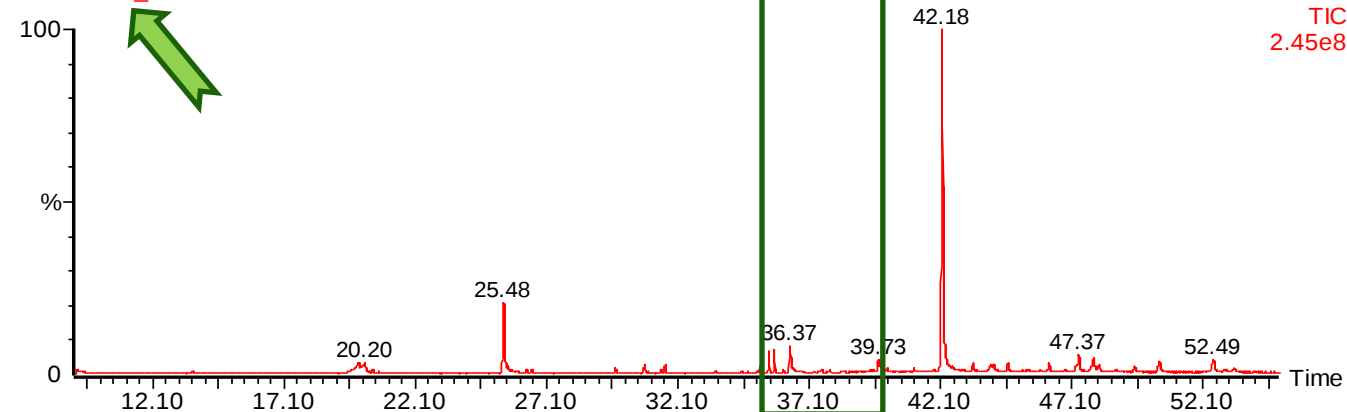
Plant extraction Conditions

- 5 mL TLN/g plant
- Sonication
- Filtration + Concentration
- GC/MS/MS analysis (internal standard TFN)

200212 WT_dcm



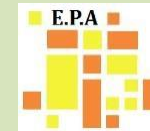
200212 SP_dcm



20.20 alifatic acid
25.48 nicotine
26.56 tetradecane
31.63 hexadecane
35.57 isopropyl myristate
35.78 phytol type
36.13 phytol type
36.37 Z-11-c16ol
36.39 phytol type
42.18 diisooctyl adipate
47.37 adipate...
52.55 octacosane

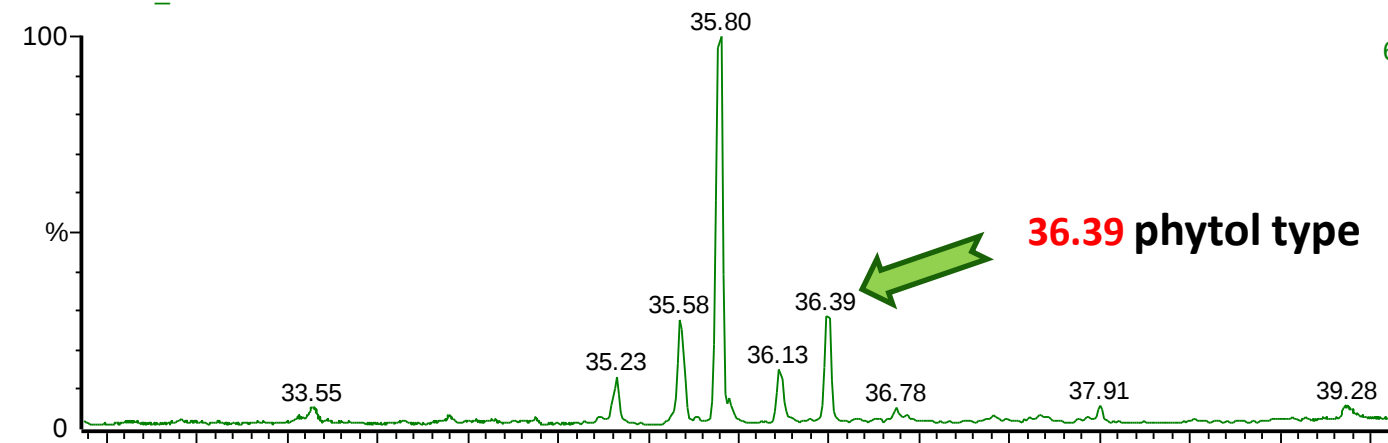


WP2 Lepidoptera Pheromone

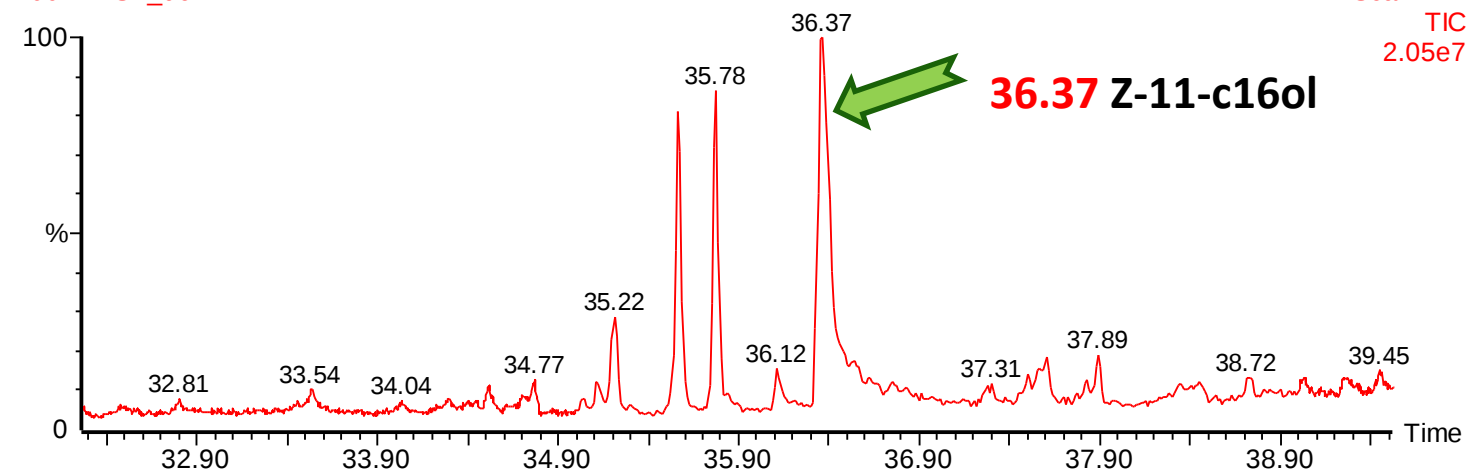


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200212 WT_dcm



200212 SP_dcm





Plant extraction SxP 1.0 Sexy plant



Z-11-hexadecen-1-ol

Sample	TFN area	OL area	ratio	ng/ul calc	ng collected	ug/plant	ug/g plant
P19_B TLN dil	82730	9357	0,1131	1,38	688,0	206,4	73,7
P19_A TLN dil	86840	4928	0,0567	0,87	436,4	174,6	43,6
PBP 1 TLN dil	84720	12950	0,1529	1,73	865,4	259,6	92,7

Quantification SxP1.2 Sexy plant

[illegible]

phytol type

WT 1



Z-11-OL

 Z-11-AC

SxP 1.2 T1-2



Volatile Collection

Quantification SxP1.2 Sexy plant



Sample	TFN area	Z -11-hexadecen-1-ol					Z -11-hexadecenyl acetate				
		area	ratio OL/TFN	µg collected	ug/plant	ug/g plant	area	ratio AC/TFN	µg collected	µg/plant	µg/g plant
TLN_SxP 1.2 T1-1	190533,3	327666,7	1,718	4,7	298,7	28,5	35833,3	0,188	0,2	11,4	1,1
TLN_SxP 1.2 T1-2	200200,0	530600,0	2,651	8,8	561,4	56,1	74410	0,372	0,5	32,1	3,2
TLN_SxP 1.2 T1-3	underway										
TLN_SxP 1.2 T1-4	underway										
					430,05	42,3				21,75	2,15



Volatile vs. plant extract SxP1.0 Sexy plant

Sample	TFN area	OL area	ratio	ng/ul calc	ng collected	ug/plant	ug/g plant
P19_B TLN dil	82730	9357	0,1131	1,38	688,0	206,4	73,7
P19_A TLN dil	86840	4928	0,0567	0,87	436,4	174,6	43,6
PBP 1 TLN dil	84720	12950	0,1529	1,73	865,4	259,6	92,7

Sample	TFN area	OL area	ratio	ng/ul calc	ng collected	ng/day	
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SP_C1	255100	31310	0,1227	1,46	219,3	73,1	
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SP_C3	222300	-					
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SP_PBP 2		-					
SP_PBP 3	269300	23050	0,0856	1,13	169,5	56,5	
SP_PBP 4	249300	-					78,4



Plant extraction

VS

Volatile Collection



**Seems that only 0,1 %
of the produced Z-11-
hexadecen-1-ol is
emitted**





Volatile vs. plant extract SxP1.2 Sexy plant

Sample	TFN area	Z -11-hexadecen-1-ol				Z -11-hexadecenyl acetate			
		area	ratio OL/TFN	µg collected	ng/day	area	ratio AC/TFN	µg collected	ng/day
vol_SxP 1.2 T1-1	358133,3	30260,0	0,084	0,209	69,8	149366,7	0,418	0,193	64,4
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vol_SxP 1.2 T1-4	238600,0	37870,0	0,159	0,294	98,0	132600,0	0,556	0,257	85,6
				0,238	79,3			0,265	88,4

Sample	TFN area	Z -11-hexadecen-1-ol					Z -11-hexadecenyl acetate				
		area	ratio OL/TFN	µg collected	ug/plant	ug/g plant	area	ratio AC/TFN	µg collected	µg/plant	µg/g plant
TLN_SxP 1.2 T1-1	190533,3	327666,7	1,718	4,7	298,7	28,5	35833,3	0,188	0,2	11,4	1,1
TLN_SxP 1.2 T1-2	200200,0	530600,0	2,651	8,8	561,4	56,1	74410	0,372	0,5	32,1	3,2
TLN_SxP 1.2 T1-3	underway										
TLN_SxP 1.2 T1-4	underway										
					430,05	42,3				21,75	2,15



Plant extraction

VS

Volatile Collection



Seems that only 0,05 %
of the produced
Z-11-hexadecen-1-ol
but 1,21 % of the
**Z-11-hexadecenyl
acetate** is emitted





Behavioral response

*Sesamia
nonagrioides*

New tests will be
performed by
electroantennography

