Breeding for resistance to insects

Willem Jan de Kogel

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Breeding for resistance to insects

- General introduction
- Terpenoids

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Host-plant resistance to insects

- Plants can adapt to stress
- Prerequisite: genetic variation

Organism	**% polymorphic loci/ population**	**% heterozygous loci/ individual**
Invertebrates	47	13
Vertebrates	25	6
Man	28	7
Plants	46	17

Dobzhansky et al. 1977

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- How can plants defend themselves against herbivores?

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Host-plant resistance to insects

- Plant defence against herbivores:
  - Association with other species (unapparent, masking)
  - Escape in space and/or time
  - Tolerance
  - Resistance (morphological, chemical, nutritional)
  - Attraction of natural enemies
- But: herbivores can adapt…

Host-plant resistance to insects

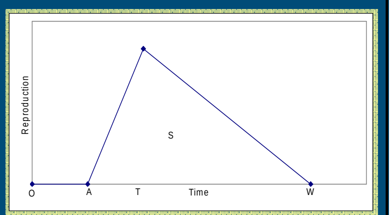
- Host-plant resistance (HPR): reduction in population growth rate of the pest
- HPR:
  - Antixenosis (disturbing behaviour)
  - Antibiosis (disturbing physiology)

Host-plant resistance to insects

- Host-plant resistance
  - Heritable
  - Relative
  - Measurable
  - Variable

Host-plant resistance to insects

- Population growth rate of insects:
  - Survival
  - Reproduction
  - Generation time
  - O = birth
  - A = start reproduction
  - T = peak reproduction
  - W = end reproduction
  - S = total reproduction



Host-plant resistance to insects

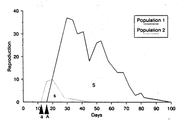


Figure 7.2. Reproductive curves of two *Drosophila* populations. Although s=0.1*S, the population growth rate of the two populations is equal ($r_1=r_2$). This is due to the overruling effect of the shorter developmental time and an early start of reproduction of population 2 (developmental time of population 2 is 4 days shorter than of population 1; after Lewontin, 1965).

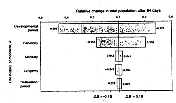


Figure 7.3. Effects on population growth of whitefly (*Trialeurodes vaporariorum* (Westwood) when values for mortality, reproduction and generation time were changed by 10% in relation to the standard values (after Hulspas - Jordaan & van Lenteren, 1989).

Host-plant resistance to insects

- Insect:
  - Approaching
  - Landing
  - Probing
  - Feeding
  - Oviposition
- Antixenosis: repellent, antifeedant (early stage of attack)
- Antibiosis: toxic compounds or deficient nutrition

- How to measure approaching and landing?

Approaching and landing

Y

- How to measure probing and feeding?
- Feeding:
  - Chewing
  - Piercing/sucking
  - Phloem feeding

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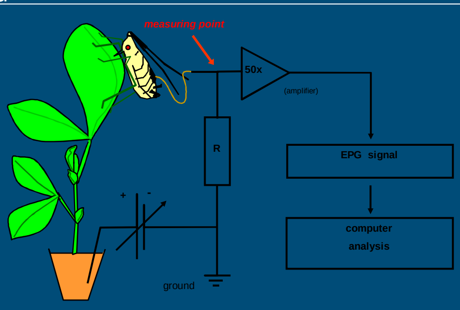
EPG



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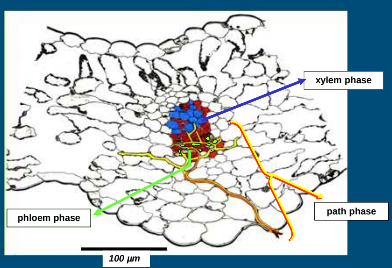
Aphid feeding



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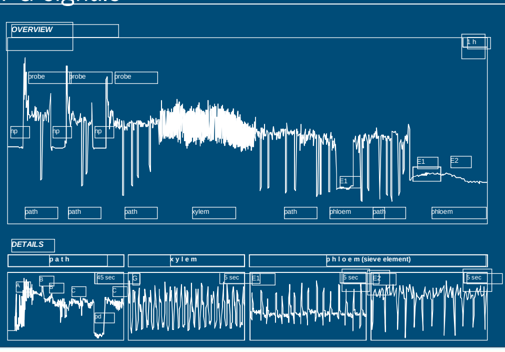
EPG signals



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Testing of HPR: important to standardize tests

- Plant variables:
  - Age, tissue
  - Induced responses
  - Whole plant/plant parts
- Insect variables:
  - Stage, age
  - Sex
  - Pre-test conditioning
  - Biotypes
  - Selection/adaptation

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Testing of HPR: important to standardize tests

- Environmental variables
  - Light
  - Temperature
  - Humidity
  - Nutrition
  - Etc.
- No-choice vs. Choice-experiments

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- What do we need when we want to select a resistant plant?

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Evaluation of resistance

- Collection of plant material
- Mass-rearing of insects
- Bio-assay
- Selection criteria

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Breeding for resistance

- Screening
- Genetic analysis (dominant/recessive, # genes, linkage)
- Commercial cultivar
- Estimation of durability (variability of pest)
- Management of HPR

- (Dis)advantages of HPR?

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Advantages of HPR

- Easy to apply
- Relatively inexpensive
- Usually no negative effect on environment
- Can be used in IPM (combined with biological control)
- Generally accepted by public (except for transgenic plants)

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Disadvantages of HPR

- Long developmental time
- Crop/cultivar specific
- Break-down of resistance

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Case study: Western Flower Thrips

- *Frankliniella occidentalis* (Thysanoptera, Thripidae)
- Polyphagous pest world-wide
- Transmits virus (TSWV)

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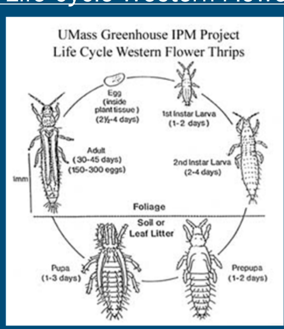
Life cycle Western Flower Thrips



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Host plants: chrysanthemum, cucumber

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Thrips damage

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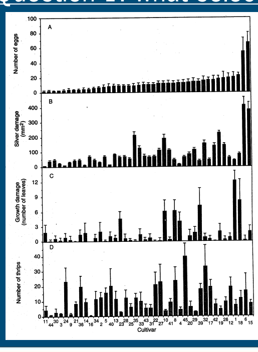
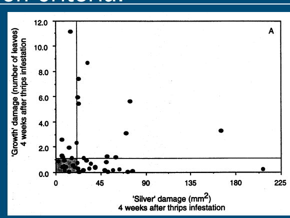
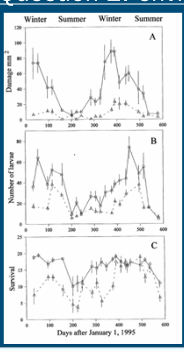
TSWV damage

Question 1: what selection criteria?





Question 2: environmental variation?



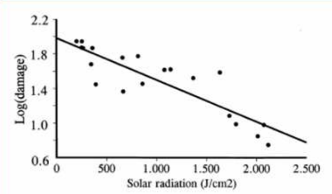


Figure 2. Correlation between log (thrips damage) as determined on leaves under uniform conditions, and mean daily solar radiation (J/cm²) during plant growth on susceptible chrysanthemum cultivar 'Pink Pompon', r = - 0.874, P < 0.0001, n = 20.

Effect fertilization?

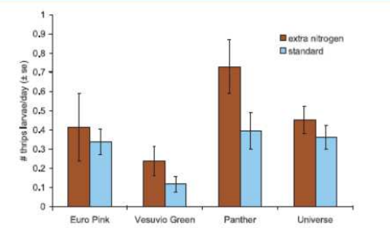


Figure 1. Thrips reproduction in the laboratory on four chrysanthemum cultivars with two levels of fertilization.

Question 3: choice or no-choice set-up?

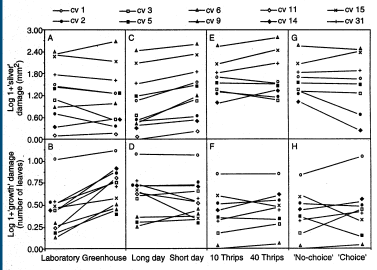


Figure 3.2 Interaction plots for the amounts of 'silver'- (A, C, E & G) and 'growth' damage (B, D, F &H) caused by *F. occidentalis* in different chrysanthemum cultivars. Interaction factors were; test-location (A, B), daylength (C, D), thrips density (E, F) and choice versus no-choice situation (G, H).

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Question 5: whole plant or plant part?

Table 1. Damage (mean ± SEM) caused by *F. occidentalis* to four cucumber accessions (n = 3). Damage in mm² on first leaf measured two weeks after inoculation and damage index to leaves (0 = no damage, 5 = maximum damage) determined 7.5 weeks after inoculation

Accession	Damage ± SEM 1st leaf (mm²)	Damage index ± SEM
G6	623 ± 78[a]	3.25 ± 0.42[a]
9104	335 ± 25[b]	0.27 ± 0.12[b]
9140	265 ± 33[b]	0.43 ± 0.10[b]
9143	224 ± 14[b]	0.61 ± 0.14[b]

Values followed by the same letter (columns) are not significantly different (P < 0.05).

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Question 6: what plant age?

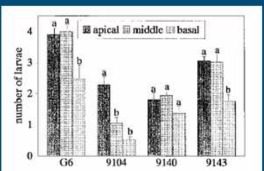

Figure 1. Effect of leaf position (apical, middle and basal leaf) of four cucumber accessions on reproduction (number of larvae/female/day) of *F. occidentalis* (mean+s.e.). Bars with the same letter within accessions are not significantly different, P<0.05.

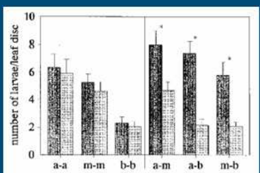

Figure 2. Oviposition preference of *F. occidentalis* for cucumber leaves from different position relative to the apex. a=apical leaf, m=middle leaf, b=basal leaf. * indicates significant difference, P<0.05.

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Question 7: effect of insect sex?

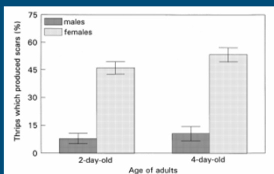

Figure 2. The percentage of *Frankliniella occidentalis* males and females producing scars in two feeding periods of 48 h days, after adult emergence. Vertical bars indicate s.e.'s.

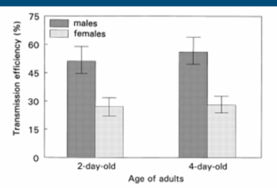

Figure 3. TSWV transmission efficiency by males and females of *Frankliniella occidentalis* during two feeding periods of 48 h days after adult emergence. Vertical bars indicate s.e.'s

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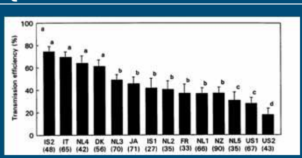
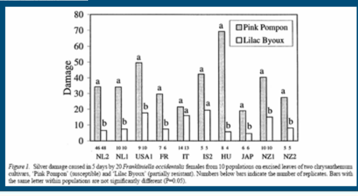
Question 8: variation within insect species (biotypes)?

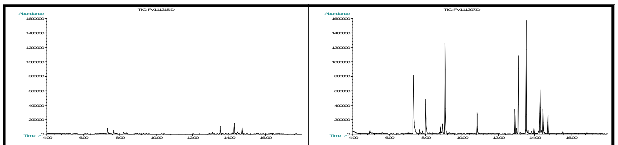




Conclusions

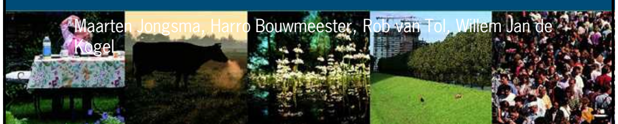
- Beware of variation in insect, plant and environment
- Important to have insight in insect-plant-(virus)-interaction: know your enemies!

Questions?

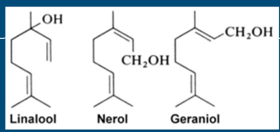


Terpenes for insect resistance

Maarten Jongsma, Harro Bouwmeester, Rob van Tol, Willem Jan de Kogel



Terpenes



- Volatile
- Components of essential oils
- More than 10.000 terpenes have been described
- Thyme – thymol; Geranium – geraniol; Peppermint – menthol; Citrus - linalool

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Terpenes: breeding for resistance

- Direct effects of terpenes on pest insects?
- Can we use terpenes as marker of resistance?
- Effects of terpenes on third trophic level?

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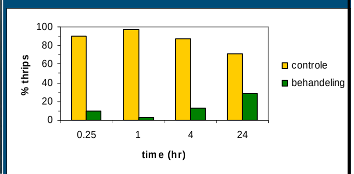
Repellent effect of plant extract applied on leaf discs



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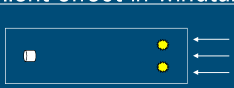
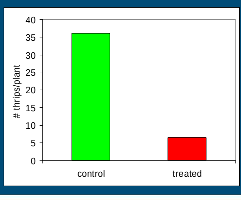
Repellent effect in windtunnel





Direct toxicity: example Gladiolus thrips

- Plant extract to control gladiolus thrips
  - From lab-assay to practical conditions

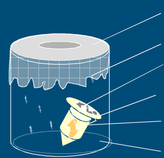
Gladiolusthrips, *Thrips simplex*, pest in gladiolus

- In the field
  foliage and flower damage

- During storage
  corm damage

Fumigation, lab-assay

- Experimental set up



Toxicity of essential oils; *Thrips simplex*

Thrips simplex, mortality (24 h)			
Essential oil	0.1µl	1 µl	10 µl
a	-	0	2.2
b	-	79.6	100
c	-	6.1	12.2
d	-	0	2
e	-	0	0
f	10.2	100	100
g	-	0	16.3
h	10.4	100	100
i	-	19.2	97.9
j	-	41.	100
k	-	66.1	100
l	31	100	100
m	-	0	71.4

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Fumigation, 0.25 m^3 containers

- *Materials and methods*
  - Air-tight containers with air circulation
  - Thrips
    - infested corms together with uninfested corms in the same container
  - 2 or 4 fumigation treatments
  - Treatments
    - untreated
    - GNO10 5 ml/m3 on filterpaper
    - GNO10 10 ml/m3 on filterpaper

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Fumigation, 0.25 m^3 containers

Treatment	# thrips/ 10 infested corms	# thrips/ 20 uninfested corms
2x control	512	923
2x 5 ml/m^3	17	137
2x 10 ml/m^3	0	4
4x control	243	782
4x 5 ml/m^3	2	29
4x 10 ml/m^3	0	0

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Fumigation, palletcontainer storage (practical conditions)

- Storage rooms (138 m^3)
- Corms cv VeraLyn with natural infestation
- Storage period:
  - 5 weeks at 20-23°C
- Treatment:
  - weekly 10 ml/m^3 (5 times)

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Fumigation, palletcontainer storage (practical conditions)

Treatment	Corms with thrips	Undamaged corms
Untreated	83%	17%
Treatment 5x	0%	93%
Chemical standard	0%	92%

Conclusion

- (Plantextracts containing) terpenes can have repellent and toxic effects on insects

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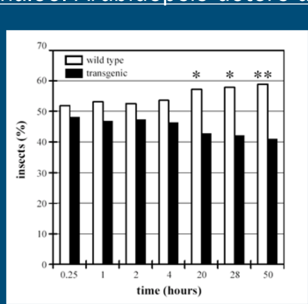
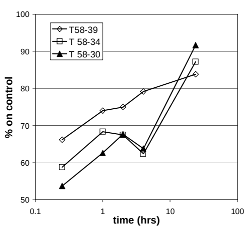
- Role of terpenes in insect resistance
  - Transgenic approach

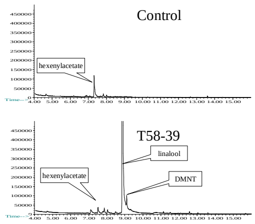
Linalool Arabidopsis deters aphids



Aharoni et al
Plant Cell 2003

Choice assay for thrips on linalool chrysanthemum





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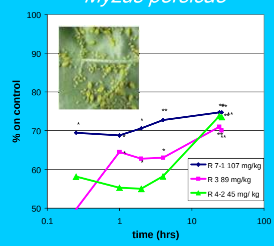
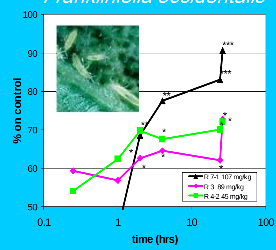
Choice assays for aphids and thrips on linalool potato





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Conclusion

- Specific terpenes can have negative impact on insects (repellent or toxic)
- Question: can we find correlations between HPR and certain terpenes in plants?

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- Non targeted GC metabolomics for insect resistance
  - Case of chrysanthemum and thrips

Example of chrysanthemum - thrips

- Available: a collection of chrysanthemum cultivars with strongly different thrips resistance traits

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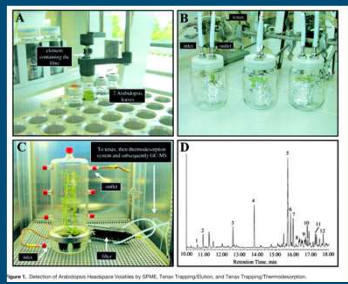
GC-Metabolomics to find markers for resistance

- non destructive sampling of volatiles from headspace
- correlation of peaks with resistance traits



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Thrips damage index

117

81

77

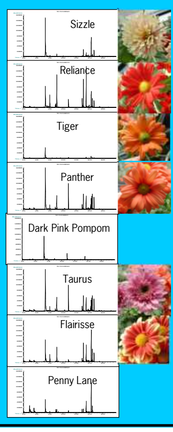
34

29

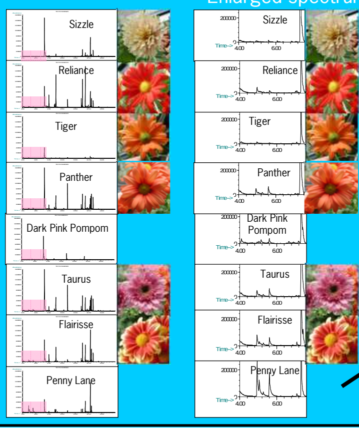
28

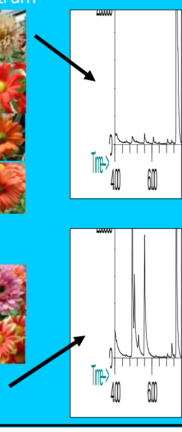
11

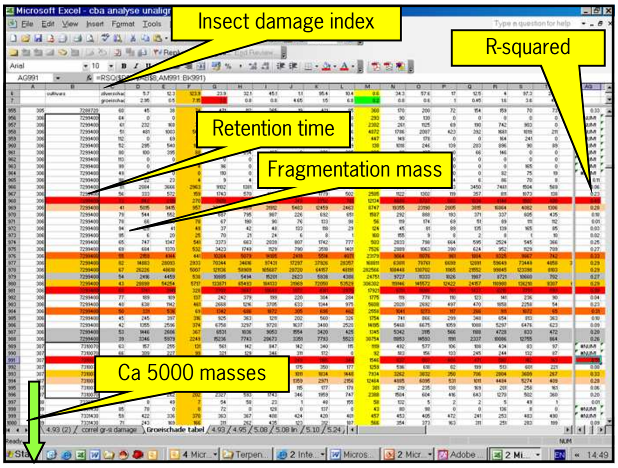
6

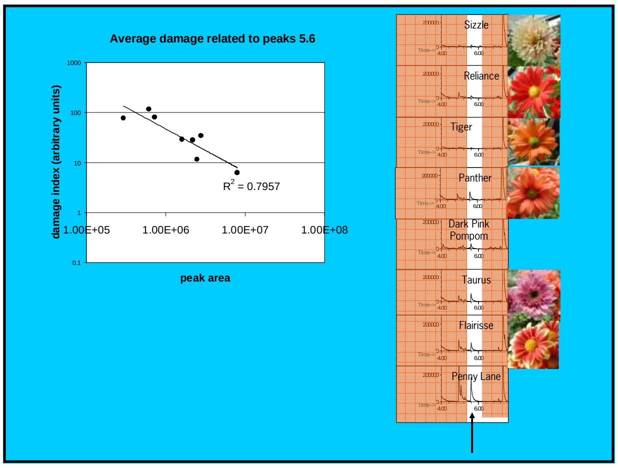


Enlarged spectrum

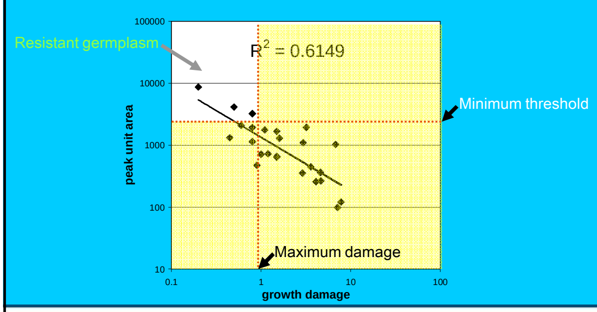








Correlation of specific VOCs from leaves with insect damage



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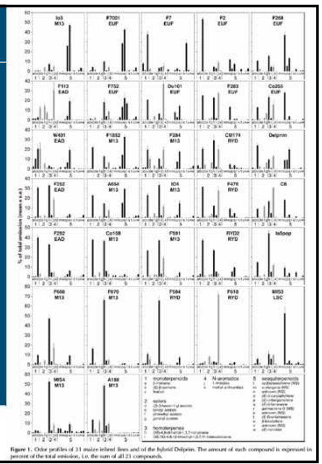
- Genetic variation for 23 VOC's in 31 maize inbred lines
  - Degen et al Plant Phys 135: 1928, 2004



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- Induced variation for VOC's in rice
  - Obara et al. BBB 66: 2549, 2002

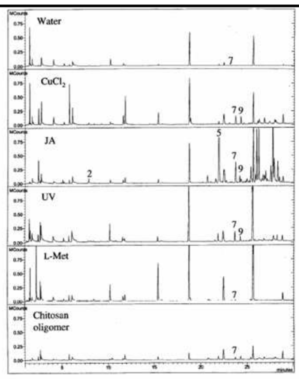

Fig. 4. GC Profiles of the Volatiles Induced in Rice Leaves with Water, $CuCl_2$ (0.5 mM), JA (0.5 mM), L-Met (5 mM) and Chitosan Oligomer (10%, v/v) after a 48-h Incubation and UV Irradiation for 20 min, before Being Incubated for 48 h.
Compound names: α-copaene (5), β-caryophyllene (7), and (Z)-α-bergamotene (9).

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New tools for insect resistance breeding?

- Current insect assays are expensive and inheritance can be too complex for marker assisted breeding
- Measuring headspace components is at least 10-20 fold less expensive
- Results can be delivered in one week using only a small piece of leaf
- Breeder will receive a subpopulation with a high probability of insect resistance
- **Correlations are indicators of resistance mechanism, which can lead to genes**

Conclusion

- There may be correlations between the amount of certain terpenoids and HPR
- Question also effect on natural enemies of pest insects?

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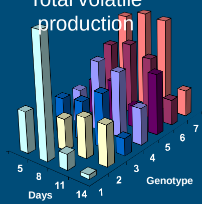
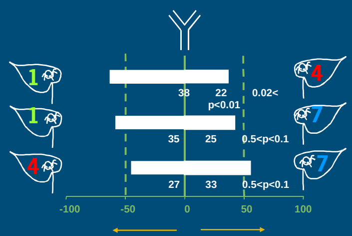
Cucumber genotypes vary in volatile production

Total volatile production





Variation also in individual components

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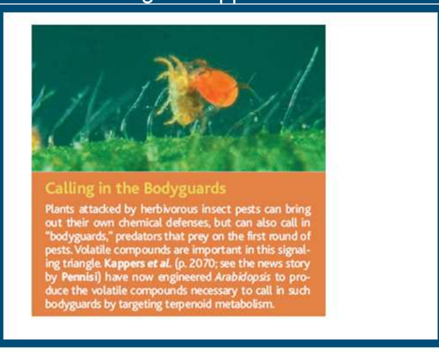
Transgenic approach



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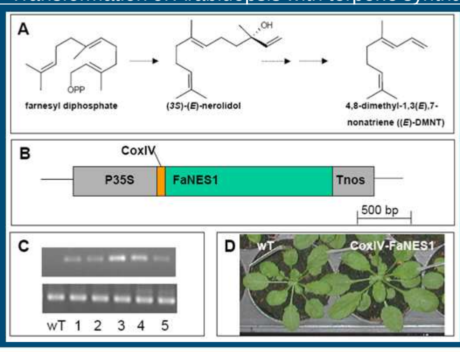
Transformation of Arabidopsis with terpene synthase



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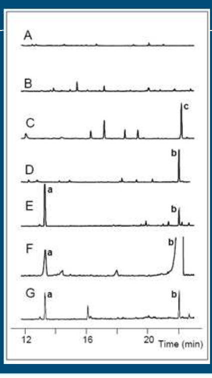

A: wildtype

B: wildtype with spidermite

D & E: transgenic plants emitting SOS-compounds nerolidol and DMNT

a: DMNT

b: nerolidol

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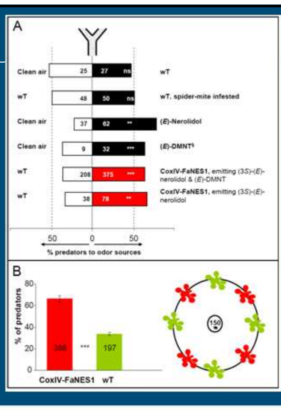

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- In the future plants may be improved by (molecular) breeding for efficient attraction of natural enemies of herbivores

Conclusions

- Direct effects of terpenes on insect behaviour and mortality
- Indirect effect of terpenes on third trophic level
- Terpenes may serve as marker of resistance

- We can select for high levels of terpene production
- We can transform plants to manipulate terpene production
- We can induce terpene production

- Question: possible disadvantage…?

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Possible disadvantage

- Different taste/odour
- Toxicity to non-target organisms
- “Cost” of resistance for plant
- Pleiotropy (one gene effects several phenotypic traits)

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