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…

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Host-plant resistance to insects

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

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Host-plant resistance to insects

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

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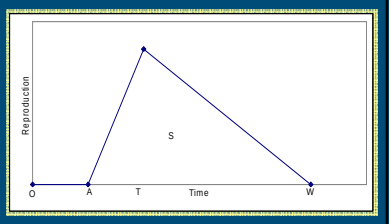
Host-plant resistance to insects

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



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Host-plant resistance to insects

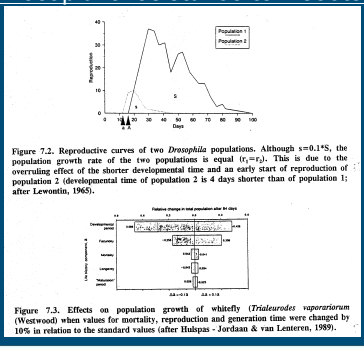

Figure 7.2. Reproductive curves of two *Drosophila* populations. Although s=0.1*S, the population growth rate of the two populations is equal ($r_1=r_2$). This is due to the overruling effect of the shorter developmental time and an early start of reproduction of population 2 (developmental time of population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

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Host-plant resistance to insects

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

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- How to measure approaching and landing?

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Approaching and landing

Y

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

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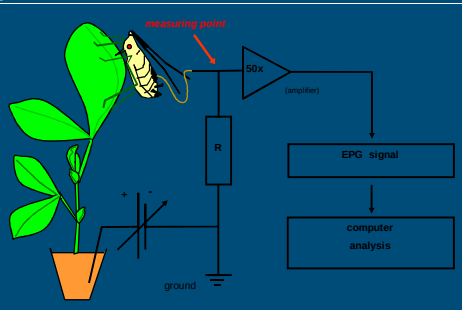
EPG



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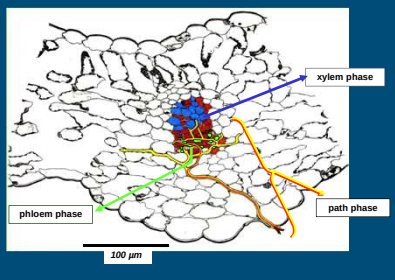
Aphid feeding



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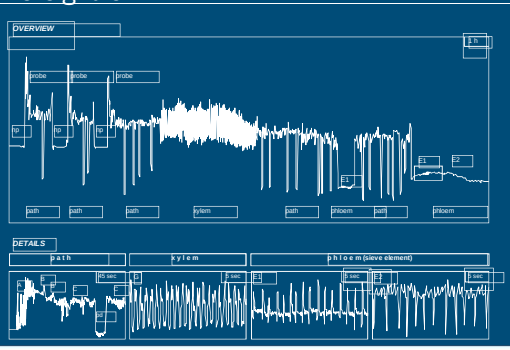
EPG signals



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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

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- (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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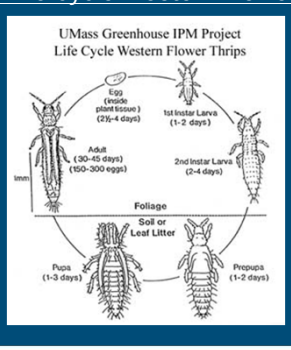
Life cycle Western Flower Thrips



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Host plants: chrysanthemum, cucumber

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Thrips damage

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TSWV damage

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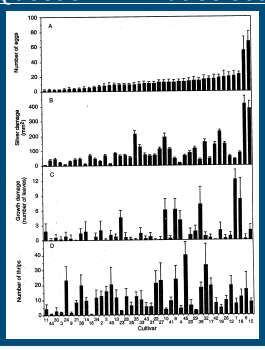
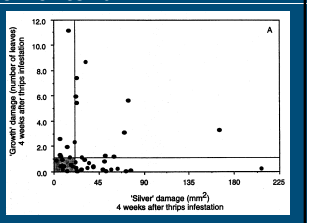
Question 1: what selection criteria?





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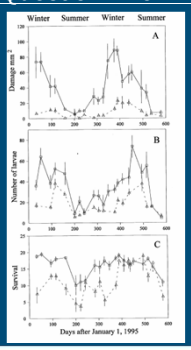
Question 2: environmental variation?



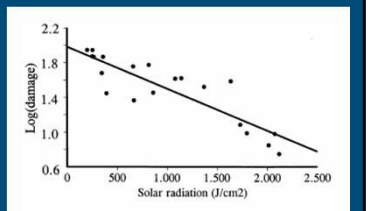


Figure 2. Correlation between log (thrips damage) as determined on leaves under uniform conditions, and mean daily solar radiation (J/cm²) during plant growth on susceptible chrysanthemum cultivar 'Pink Pompon', r = - 0.874, P < 0.0001, n = 20.

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Effect fertilization?

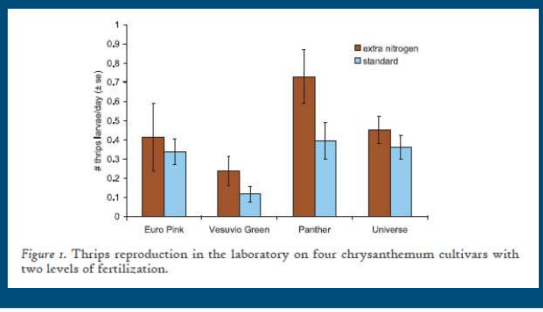

Figure 1. Thrips reproduction in the laboratory on four chrysanthemum cultivars with two levels of fertilization.

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

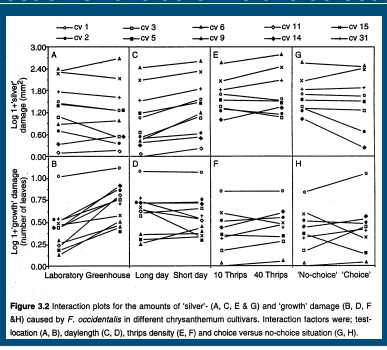

Figure 3.2 Interaction plots for the amounts of 'silver'- (A, C, E & G) and 'growth' damage (B, D, F &H) caused by *F. occidentalis* in different chrysanthemum cultivars. Interaction factors were; test-location (A, B), daylength (C, D), thrips density (E, F) and choice versus no-choice situation (G, 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 mm^2 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 (mm^2)	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?

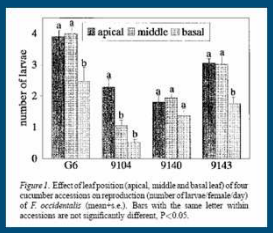

Figure 1. Effect of leaf position (apical, middle and basal leaf) of four cucumber accessions on reproduction (number of larvae female/day) of *F. occidentalis* (mean±s.e.). Bars with the same letter within accessions are not significantly different, $P<0.05$.

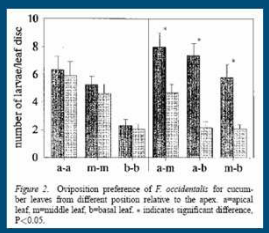

Figure 2. Oviposition preference of *F. occidentalis* for cucumber leaves from different position relative to the apex. a=apical leaf, m=middle leaf, b=basal leaf. * indicates significant difference, $P<0.05$.

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Question 7: effect of insect sex?

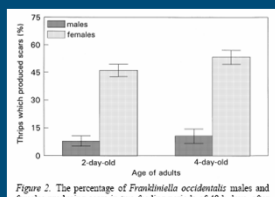

Figure 2. The percentage of *Frankliniella occidentalis* males and females producing scars in two feeding periods of 48 h days, after adult emergence. Vertical bars indicate s.e.'s.

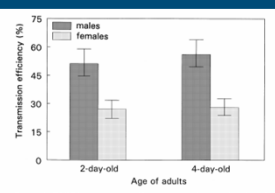

Figure 3. TSWV transmission efficiency by males and females of *Frankliniella occidentalis* during two feeding periods of 48 h days after adult emergence. Vertical bars indicate s.e.'s

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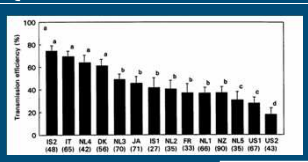
Question 8: variation within insect species (biotypes)?



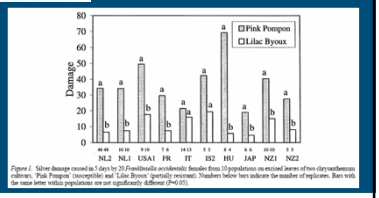


Figure 1. Silver damage caused in 5 days by 20 *Frankliniella occidentalis* females from 10 populations on excised leaves of two chrysanthemum cultivars, 'Pink Pompon' (susceptible) and 'Lilac Byoux' (partially resistant). Numbers below bars indicate the number of replicates. Bars with the same letter within populations are not significantly different (P>0.05).

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Conclusions

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

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Questions?

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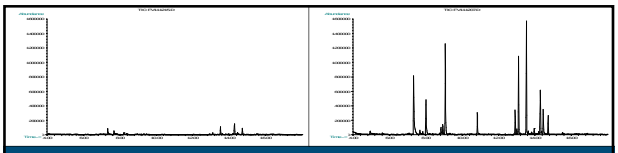
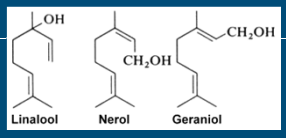


Terpenes for insect resistance

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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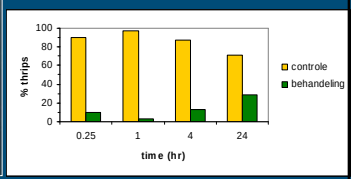
Repellent effect of plant extract applied on leaf discs



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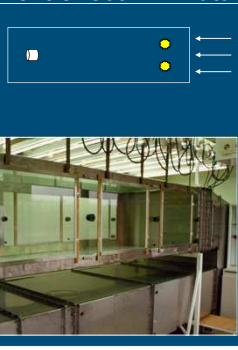
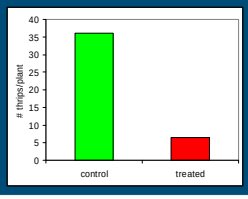
Repellent effect in windtunnel





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Direct toxicity: example Gladiolus thrips

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

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Gladiolusthrips, *Thrips simplex,* pest in gladiolus

- In the field
  foliage and flower damage

- During storage
  corm damage

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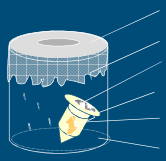
Fumigation, lab-assay

- Experimental set up



parafilm sandwich with 10% sucrose solution
thrips
lid with 80 µm gauze
microcentrifuge tube
filterpaper with test compound
glass beaker, 100 ml

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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
    - GN010 5 ml/m3 on filterpaper
    - GN010 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)

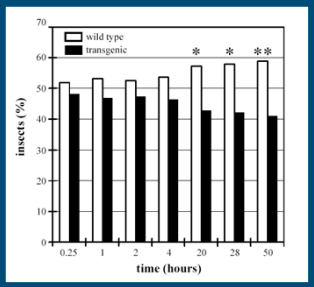
Treatment	Corms with thrips	Undamaged corms
Untreated	83%	17%
Treatment 5x	0%	93%
Chemical standard	0%	92%

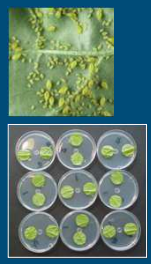
Conclusion

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

- Role of terpenes in insect resistance
  - Transgenic approach

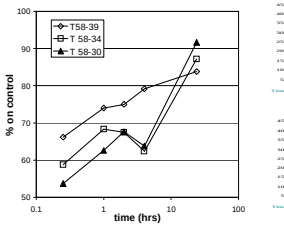
Linalool Arabidopsis deters aphids

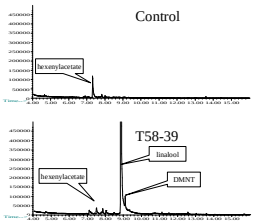




Aharoni et al
Plant Cell 2003

Choice assay for thrips on linalool chrysanthemum





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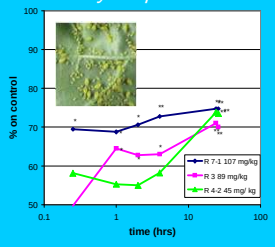
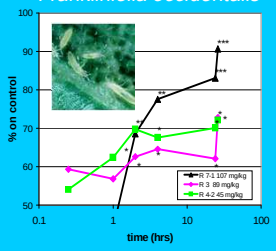
Choice assays for aphids and thrips on linalool potato

Myzus persicae



Frankliniella occidentalis



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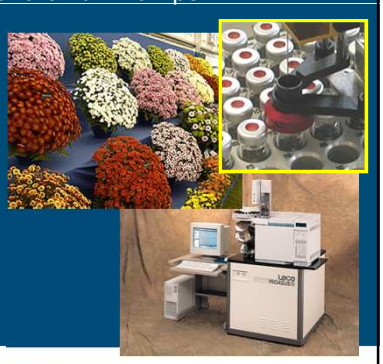
- Non targeted GC metabolomics for insect resistance
  - Case of chrysanthemum and thrips

Example of chrysanthemum - thrips

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



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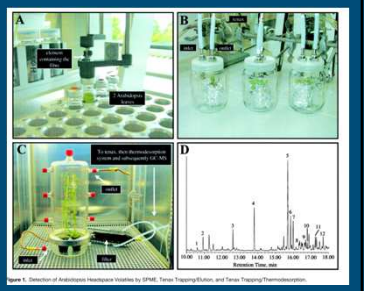
GC-Metabolomics to find markers for resistance

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



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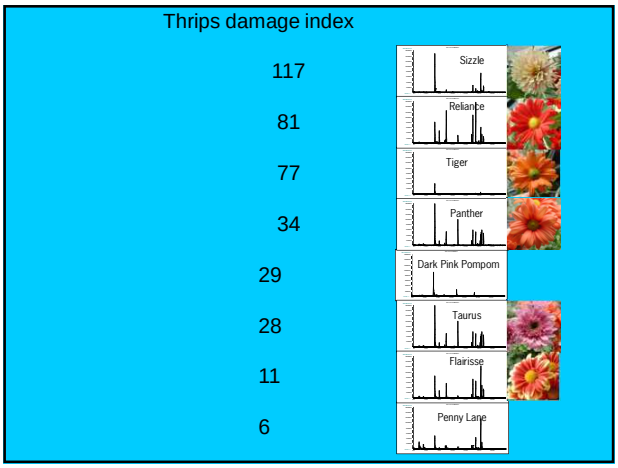
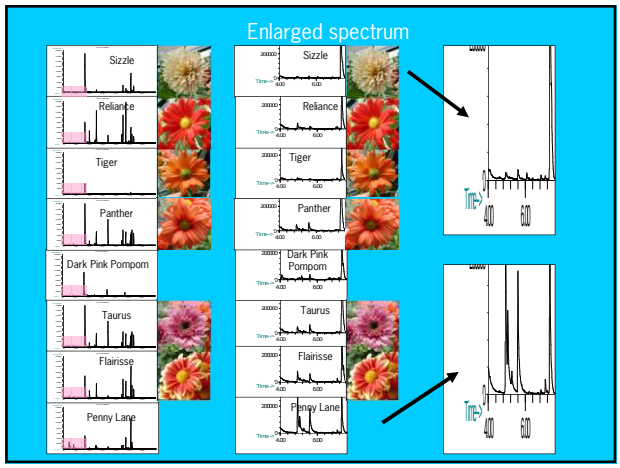
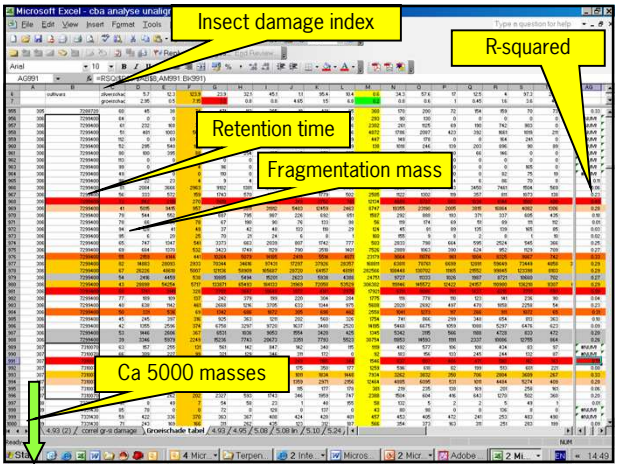
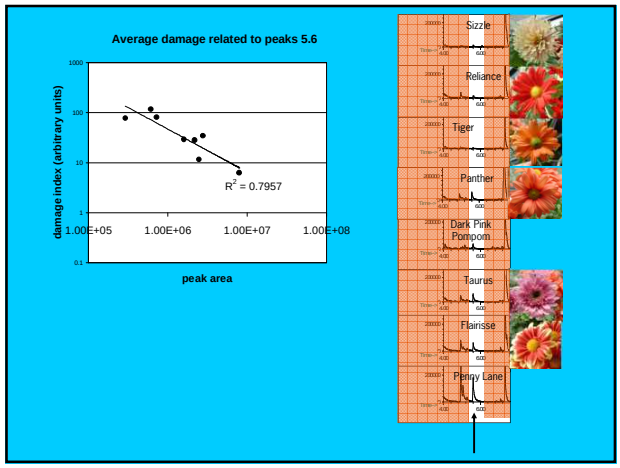
Thrips damage index

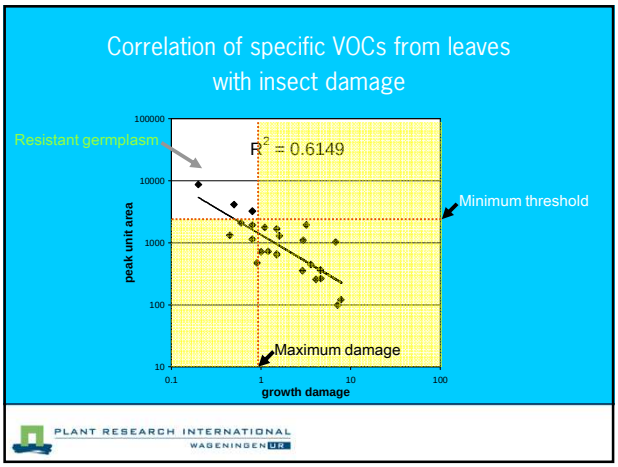


Enlarged spectrum




Insect damage index
R-squared
Retention time
Fragmentation mass
Ca 5000 masses


Average damage related to peaks 5.6
damage index (arbitrary units)
peak area
R2 = 0.7957
1.00E+05
1.00E+06
1.00E+07
1.00E+08
Sizzle
Reliance
Tiger
Panther
Dark Pink Pompom
Taurus
Flairisse
Penny Lane


Correlation of specific VOCs from leaves with insect damage
Resistant germplasm
R2 = 0.6149
Minimum threshold
Maximum damage
peak unit area
growth damage
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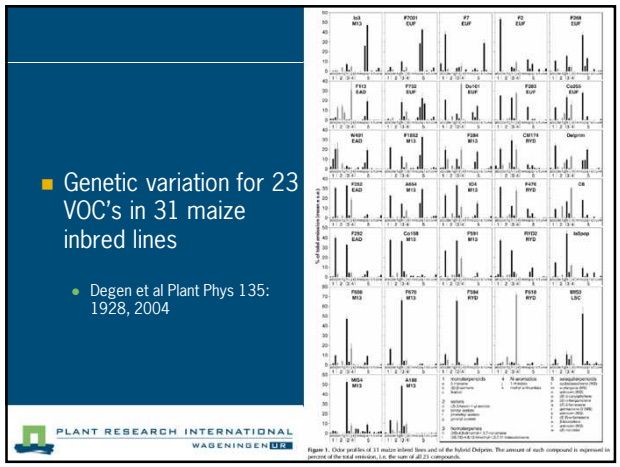
Genetic variation for 23 VOC's in 31 maize inbred lines
Degen et al Plant Phys 135: 1928, 2004
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- Induced variation for VOC's in rice
  - Obara et al. BBB 66: 2549, 2002

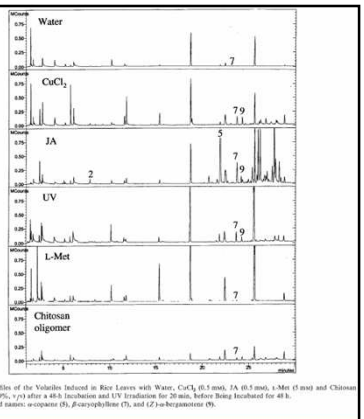

Fig. 4. GC Profiles of the Volatiles Induced in Rice Leaves with Water, $CuCl_2$ (0.5 mM), JA (0.5 mM), L-Met (5 mM) and Chitosan Oligomer (10%, v/v) after a 48-h Incubation and UV Irradiation for 20 min, before Being Incubated for 48 h. Compound names: α-copaene (3), β-caryophyllene (7), and (Z)-α-bergamotene (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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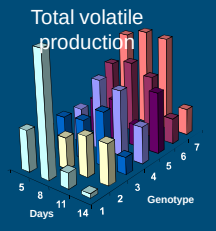
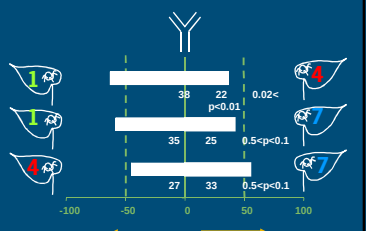
Cucumber genotypes vary in volatile production

Total volatile production





Variation also in individual components

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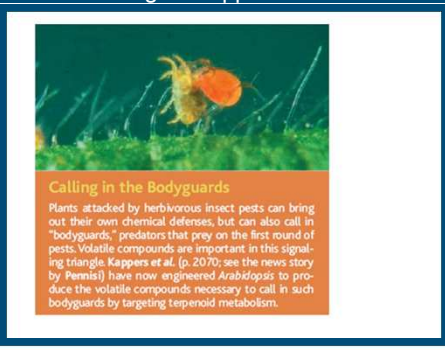
Transgenic approach



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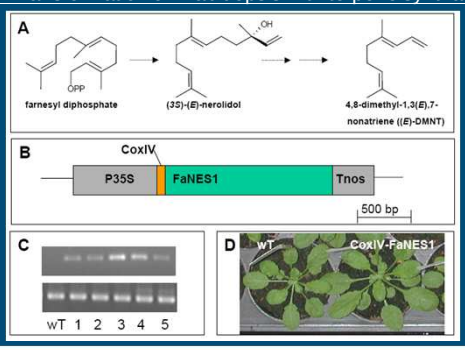
Transformation of Arabidopsis with terpene synthase



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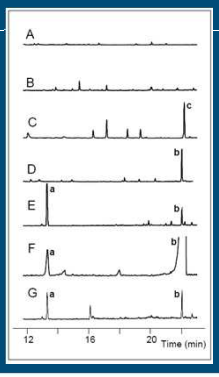

A: wildtype

B: wildtype with spidermite

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

a: DMNT

b: nerolidol

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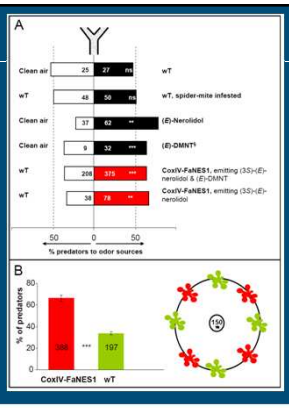

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- In the future plants may be improved by (molecular) breeding for efficient attraction of natural enemies of herbivores

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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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