



## Breeding for insect resistance @WUR



WAGENINGENUR  
For quality of life

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# Aim

- Insects cause damage through feeding or through transmission of viruses
- Controlled using pesticides
- Aim to reduce use of pesticides
- Resistance in (wild relatives of) crops



# Pest insects

- Aphids: *Myzus persicae*, *Aulacorthum solani*, *Aphis gossypii*
- Whiteflies: *Bemisia tabaci*, *Trialeurodes vaporariorum*, *Aleyrodes proletella*
- Thrips: *Frankliniella occidentalis*, *Thrips tabaci*
- Colorado Potato Beetle: *Leptinotarsa decemlineata*

Tomato, Potato, Pepper, Pumpkin, Squash,  
Chrysant, Poinsettia



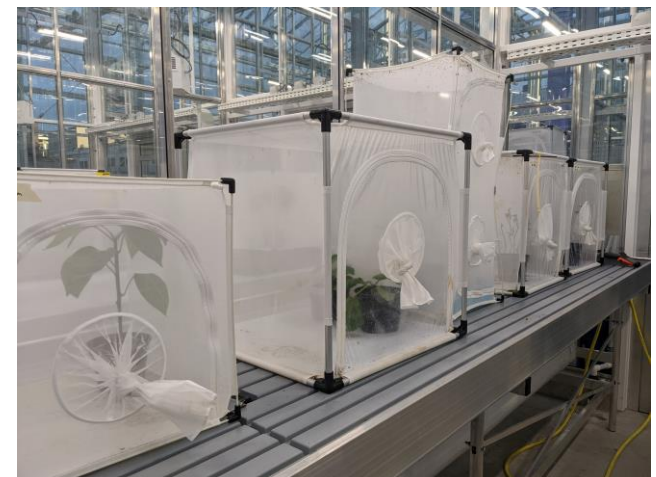
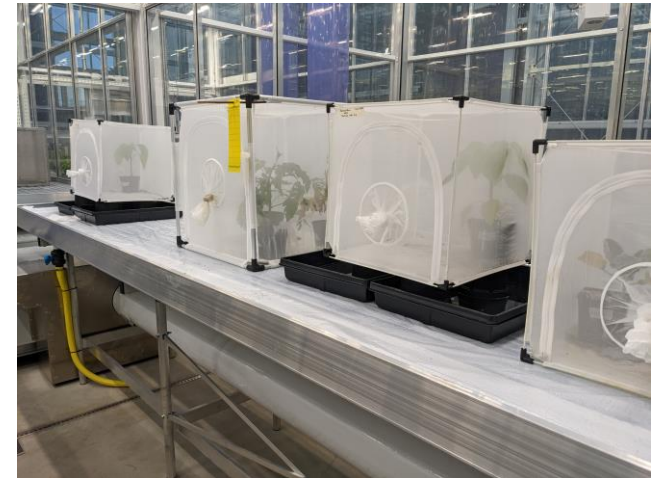
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Rearing on hostplant

Different biotypes

## Insect rearing



# Projects: Public-Private Partnerships

- Most research done in projects
- Funded by Dutch government and companies



Whitefly resistant Poinsettia to reduce insecticide use



Host plants resistance against aphids in Capsicum



Aphid and whitefly resistance in Pumpkin and Squash



Resistance mechanisms against thrips in Chrysanthemum and its relatives



Broad spectrum resistance against insects in potato

Resistance against Colorado Potato beetle for organic market

# Projects: Public-Private Partnerships

- Most research done in projects
- Funded by Dutch government and companies
  
- Screening germplasm for resistance against insects
- Developed phenotyping assays for different insects
- Characterization of resistance mechanism
- Genetic analysis (mapping)

# Insect resistance screening

Look for antibiosis type of resistance

Affecting survival and fecundity of the insect



# Insect resistance screening



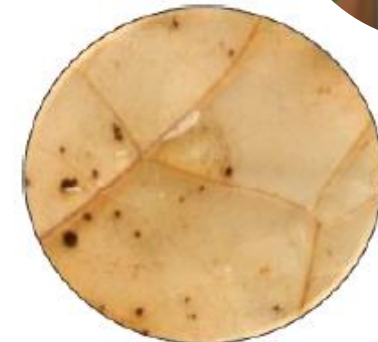
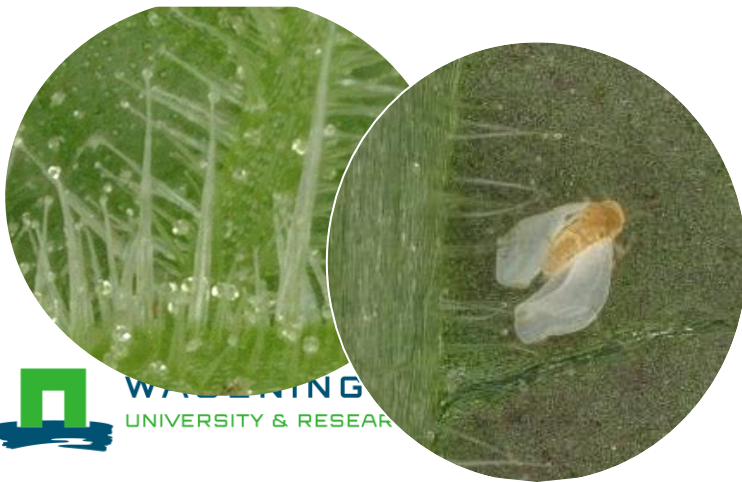


# Insect resistance screening



# Resistance mechanisms

- Knowledge on mechanism of resistance and genes involved
- Feeding behavior analysis by EPG, video tracking
- Plant staining cellular processes
- Metabolomics
- Trichomes

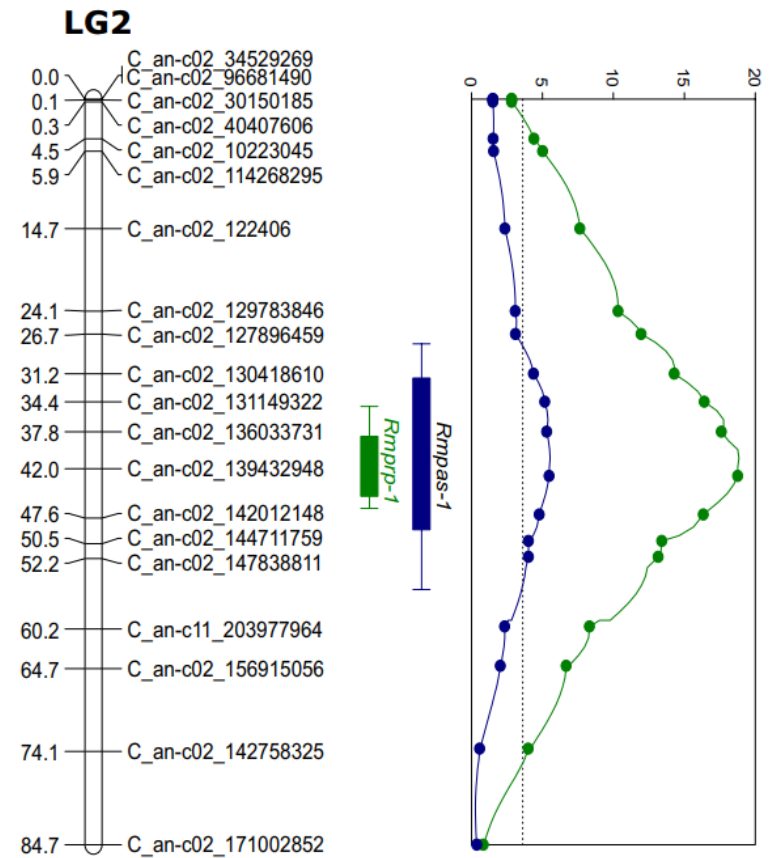


# Mapping of resistance locus: example

## Aphid resistance in *Capsicum* maps to a locus containing LRR-RLK gene analogues

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# Prospects

- Resistance against multiple insects
- Interaction with biological control
- Automatization of phenotyping




## Eggsplore: A workflow to rapidly evaluate insect resistance based on oviposition

**Micha Gracianna Devi<sup>1</sup> & Dan Jeric Arcega Rustia<sup>2</sup>**  
<sup>1</sup> Insect Resistance Breeding, Wageningen University and Research, The Netherlands  
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**Abstract**

Up to date, conventional method is still used to quantify insect egg for enhancing plant resistance. Commonly, this method involves the use of a bioassay and manually separating the forms over a leaf segment, followed by counting with a video microscope as egg is spotted. This procedure is laborious, and require a good eye skill to distinguish the eggs. In susceptible tomato and potato plants, whiteflies lay more than a hundred eggs inside a clip cage (2 x 2 cm). These eggs are vulnerable to predation in the soil and images from microscope gives to know depending on the egg. Moreover, whitefly eggs that are covered in whitefly honey dew and hidden among trichomes are difficult to detect. Furthermore, there is a limitation to continuously document samples using images from a bioassay. Eggsplore is a workflow especially developed to rapidly evaluate whitefly oviposition. This includes the use of a digital microscope that can quickly capture high resolution Z-stacked images of the whole leaf surface, then importing the images to the Eggsplore program for egg quantification. A deep learning model, based on YOLOv3 <sup>3</sup>, was trained to detect the eggs automatically in the images and deployed using an executable graphical user interface. An evaluated using a strong image dataset, the algorithm has an average accuracy of 0.93 with an error of 2.7 eggs per image and average  $r^2$  of 0.91, relative to the manual count.






**Challenges in conducting whitefly assays**

1. High quantity of samples for phenotyping population.
2. Manual quantification using bioassay requires constant focus adjustment, a lot of time is required.
3. Leaf samples after counting are then discarded, no documentation.
4. Variation in human eye capacity, human error.


### 1. Image acquisition

Things to consider:


1. How low can it go?  
Magnification, resolution, cost
2. How efficient in terms of time & human resource?


Tomato



Potato



Arabidopsis




### 2. Image processing


**Main advantages:**

1. Documented images of leaves.
2. High throughput screening.
3. Handles eggs between houseplant.
4. Website-based (internet).
5. Exported data (date, of egg count and processed image with level of confidence).

**Things to be optimized:**

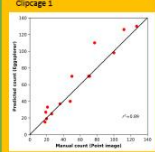
- Under/overestimation of egg count.
- The more variation in images for training, the smarter the program becomes.



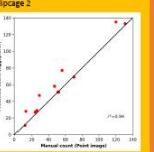


**Model evaluation on tomato samples showed high fitted values between manual and predicted count**

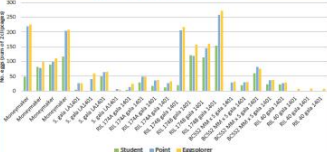
Chicago 1



Chicago 2



**Eggsplore performs similarly to manual point count and better than student count**



**Outlook**

1. Eggsplore optimized for thrips eggs
2. Yachone quantification tool with similar workflow.

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