# High-throughput microscopic phenotyping of traits related to plant susceptibility to two-spotted spider mite



**5. NUMBER OF EGGS PER LEAF** 

**FROM ONE PLANT** 

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#### SUMMARY

The two-spotted spider mite (TSSM), Tetranychus urticae Koch, represents one of the most destructive generalist mite herbivores. TSSM feeds on hundreds of plant species belonging to various botanical families. The ability of TSSM to flexibly adapt to multiple host plants results from innate tolerance to different plant xenobiotics. Since the effective resistance loci are unknown novel approaches are required to identify quantitative phenotypes related to TSSM susceptibility and to discover their genetic background.

We configured the efficient stereomicroscope-based workstation for high-resolution imaging of mite-infested leaves. The scans allowed to identify and measure several parameters related to plant susceptibility including: (1) mite performance - no of eggs laid by one female after 72h on one rosette, (2) damaged leaf area, (3) feces area, and (4) the distribution of these parameters on consecutive leaves.

The new software – MITESPOTTER, was developed to automatically analyze the defined traits on high-resolution scans. The trait detection was based on neural networks which were trained with manually marked objects. The mite damaged area detection was using the Feature Pyramid Network (FPN). For the detection of mite eggs and feces, the Detectron 2 library was used and the model combined the architectures of Faster RCNN, ResNet, and FPN.

The rosettes of three-week-old seedlings of 130 Arabidopsis thaliana accessions deriving from remote world locations were infested with synchronized young TSSM females and after 72 hours the abaxial and adaxial leaf surface was scanned producing 1700 high-resolution images. The scans were computer analyzed and showed remarkable differences in the damaged leaf area, laid eggs, and feces area. Our method allowed also to count eggs on the consecutive leaves and analyze ab/adaxial egg distribution.



## 3. IMAGE OF A. THALIANA LEAF DISSECTED FROM FULL SCAN DIGITAL CLOSE-UPs ON EGGS, FECES AND DAMAGED AREAS





Guanine feces (white/yellow) - the development of a new TSSM susceptibility-related trait. Building the training set based on visible and fluorescence scans. Image courtesly by A. Jezierska



TSSM young females were placed uniformly on young rosette leaves (3-5 consecutive leafs) – females showed sometimes unexpected preference to n+3 or n+4 leaf in terms of laying eggs.

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The recorded quantitative traits are: female fertility, i.e. the number of eggs per plant per female, the amount of feces and the damaged area. Female fertility is counted per plant in proportion to the abaxial and adaxial side of the leaf. The amount and area of feces is calculated per plant per 1 female. In the total area of damage, we examine the percentage of the total area of the plant.

#### SCANNING DETAILS

The infested leaves are immobilized on the dish by the double-sided and transparent tape, which allows us to scan the leaves from both sides. The imaging was performed using Leica Thunder Imaging System and a stereoscopic microscope MF205 with a motorized table and a RGB camera LEICA dfc 7000 T. The image acquisition was performed using reflected light in 25x magnification. Using a navigator from the LasX program an area of image acquisition was marked and when the imaging starts the program controls the camera and the table and image acquisition is made automatically for one side of all the leaves placed on one dish. The whole imaging of one side of a dish takes up to 6 minutes and around 450 single images are taken and merged in one 3-4 GB image in TIFF format. The obtained images are analyzed in the MITESPOTTER software and the results are exported to CSV file for GWAS analysis.

### 2. MERGED SCAN











## **6. LITERATURE**

Kiełkiewicz M., Barczak-Brzyżek A., Karpińska B., Filipecki M., 2019. Unravelling the Complexity of Plant Defense Induced by a Simultaneous and Sequential Mite and Aphid Infestation. International Jurnal of Molekular Science

Agut B., Pastor V., Jaques J.A., Flors V., 2018. Can Plant Defence Mechanisms Provide New Approaches for the Sustainable Control of the Two-Spotted Spider Mite Tetranychus urticae? Int. J. Mol. Sci.

## 7. CONCLUSION

The proposed analytical pipeline facilitates the screening of large segregating populations of different plant species leading to the identification of loci for breeding of TSSM tolerant plants. One of the most promising applications is using the described phenotyping data in a genome-wide association study. The MITESPOTTER software can be adapted for traits related to other pests and pathogens.



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