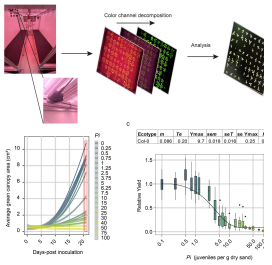
Jun 23, 2023 Version 1

High-throughput phenotyping of green canopy area during nematode infection V.1



DOI
https://dx.doi.org/10.17504/protocols.io.kqdg39167g25/v1

Jaap-Jan Willig[1], Devon Sonneveld[1], Joris J.M. van Steenbrugge[1], Laurens Deurhof[2], Casper C. van Schaik[1], Misghina G. Teklu[3], Aska Goverse[1], Jose L. Lozano Torres[1], Geert Smant[1], Mark G. Sterken[1]

[1]Laboratory of Nematology, Wageningen University & Research, 6708PB Wageningen, The Netherlands;
[2]Laboratory of Phytopathology, Wageningen University & Research, 6708PB Wageningen, The Netherlands;
[3]Agrosystems Research, Wageningen University & Research, 6708PB Wageningen, The Netherlands

Jaap-Jan Willig

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.kqdg39167g25/v1

Protocol Citation: Jaap-Jan Willig, Devon Sonneveld, Joris J.M. van Steenbrugge, Laurens Deurhof, Casper C. van Schaik, Misghina G. Teklu, Aska Goverse, Jose L. Lozano Torres, Geert Smant, Mark G. Sterken 2023. High-throughput phenotyping of green canopy area during nematode infection. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kqdg39167g25/v1Version created by **Jaap-Jan Willig**

Protocol status: Working
We use this protocol and it's working

Created: June 14, 2023

Last Modified: June 23, 2023

Protocol Integer ID: 83399

Keywords: High-throughput phenotyping, Arabidopsis, green canopy area, nematodes, plant defence response, activation of plant defence response, plant interaction, throughput phenotyping of green canopy area, nematode infection nematode migration, plant assimilate, feeding nematode, significant impact on plant growth, model plant arabidopsis thaliana, plant growth, withdrawal of plant assimilate, biotic interaction, intraspecific variation in tolerance limit, cyst nematode infection, green canopy area growth, nematode infection, distinct trait in biotic interaction, plant, arabidopsi, throughput phenotyping, phenotyping platform, feeding site formation, cellular mechanism, crop, imaging of tolerance

Funders Acknowledgements:
Dutch Top Sector Horticulture & Starting Materials
Grant ID: TU18152
NWO domain Applied and Engineering Sciences VENI
Grant ID: 14250
NWO domain Applied and Engineering Sciences VENI
Grant ID: 17282
NWO domain Applied and Engineering Sciences VIDI
Grant ID: 18389

Abstract

Nematode migration, feeding site formation, withdrawal of plant assimilates and activation of plant defence responses have a significant impact on plant growth and development. Plants display intraspecific variation in tolerance limits for root-feeding nematodes. Although disease tolerance has been recognised as a distinct trait in biotic interactions of mainly crops, we lack mechanistic insights. Progress is hampered by difficulties in quantification and laborious screening methods. We turned to the model plant *Arabidopsis thaliana*, since it offers extensive resources to study the molecular and cellular mechanisms underlying nematode-plant interactions. We established through imaging of tolerance-related parameters that green canopy area was an accessible and robust measure for assessing damage as the consequence of cyst nematode infection. Subsequently, we developed a high-throughput phenotyping platform to non-destructively measure the green canopy area growth of 960 *A. thaliana* plants simultaneously.

Attachments

Supplementary File 1... 11KB

Supplementary File 2... 3KB

Materials

- 200mL pots
- Silversand
- 3 mm thick black nonreflective foamed PVC coversheet
- Hyponex: (1.7 mM/L $NH4^{+}$, 4.1 mM/L K^{+}, 2 mM/L Ca_2^{+}, 1.2 mM/L Mg_2^{+}, 4.3 mM/L NO_3^{-}, 3.3 mM/L SO_4^{2-}, 1.3 mM/L $H2PO_4^{-}$, 3.4 µm/L Mn, 4.7 µm/L Zn, B 14 µm/L, 6.9 µm/L Cu, 0.5 µm/L Mo, 21 µm/L Fe, pH 5.8)
- UI-1490LE-C-HQ cameras (IDS Imaging) mounted with 12mm lenses (Cat. No. B5M12056, IDS Imaging)

1 Fill 200mL pots with silversand.

2 Place the pots in the steel frames.

2.1 Once all the pots are filled with silversand, cover them with the black nonreflective foamed PVC coversheet.

3 Prior to sowing, silver sand was watered with Hyponex for five minutes.

4 Sow 2 to 5 Arabidopsis seeds per pot using a toothpick.

5 Seven days after sowing, remove redundant seedlings were using a tweezer.

5.1 At eight days after sowing, water the plants with Hyponex for five minutes.

6 Inoculate the seedlings with nematode at nine days after sowing

7 After inoculation, start the camera system to take pictures of the Arabidopsis seedlings (15 pictures per day with an hour interval for a period 23 days).

8 Finally, the surface area of the rosette was determined using a custom-written ImageJ macro (ImageJ 1.51f; Java 1.8.0_321 [32-bit]) and Java was used to make GIFs.