JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

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OUR CHALLENGE

Current Situation (EU)

The number of approved **active substances** in pesticides has been cut down by 50% during the past years…

Nine of the ***ten*** best-selling cereal fungicides are out…

Vytenis Andriukaitis, Commissioner for Health and Food Safety (2017)

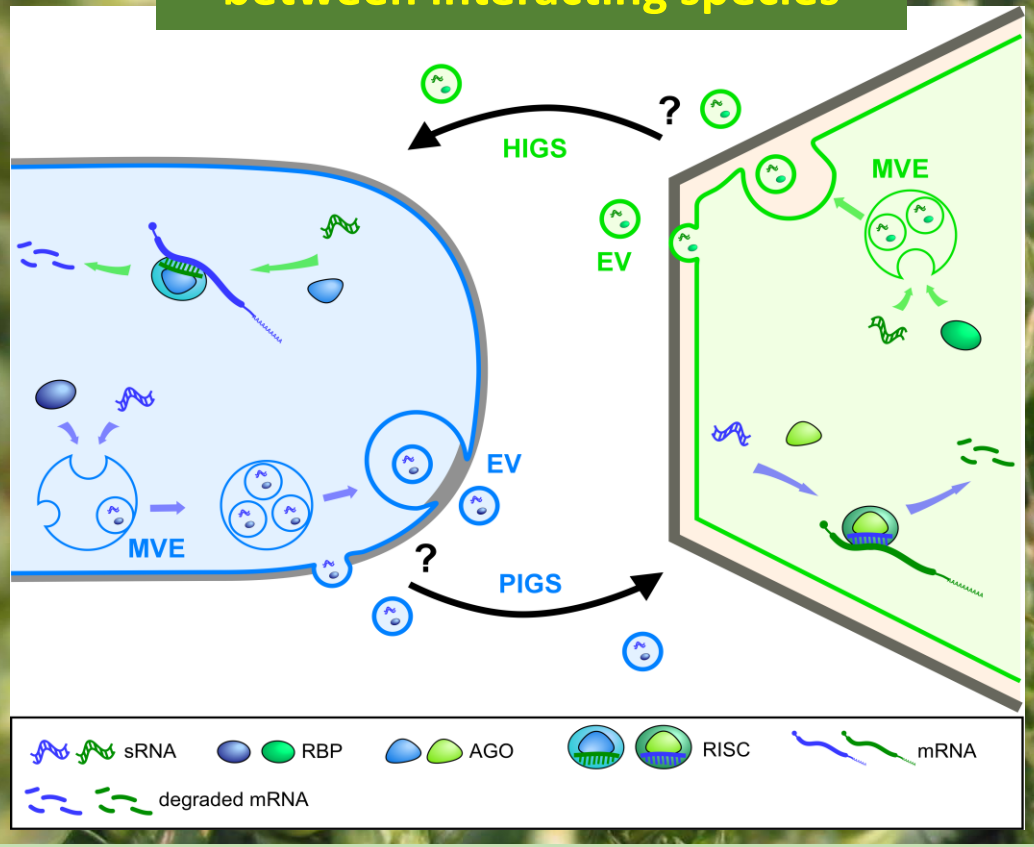
Volker Koch-Achelpöhler, Perspektiven des chem. Pflanzenschutzes, Industrieverband Agrar (2015)

OUR SOLUTION

Plant Protection by Non-coding RNAs

LEARNING FROM NATURE

Exchange of small RNA
between interacting species



Modulation of defence and virulence

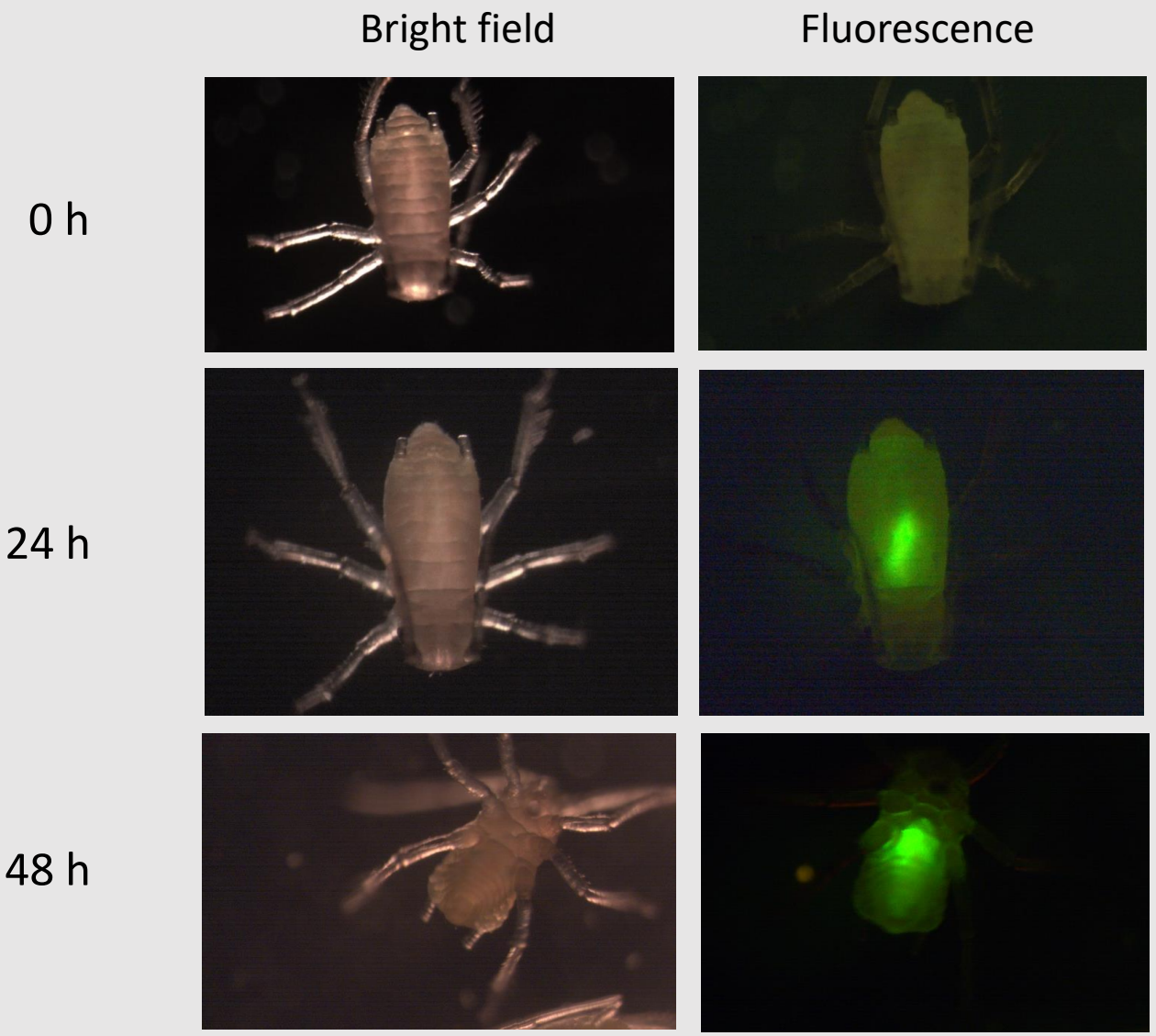
RNA input
(dsRNA, sRNA, circRNA, mRNA)

Fight against pest and diseases

- viruses
- insects
- nematodes
- fungi
- oomycetes

Example:
insect control by RNA

Uptake of fluorescent dsRNA$_{AF488}$ from artificial diet



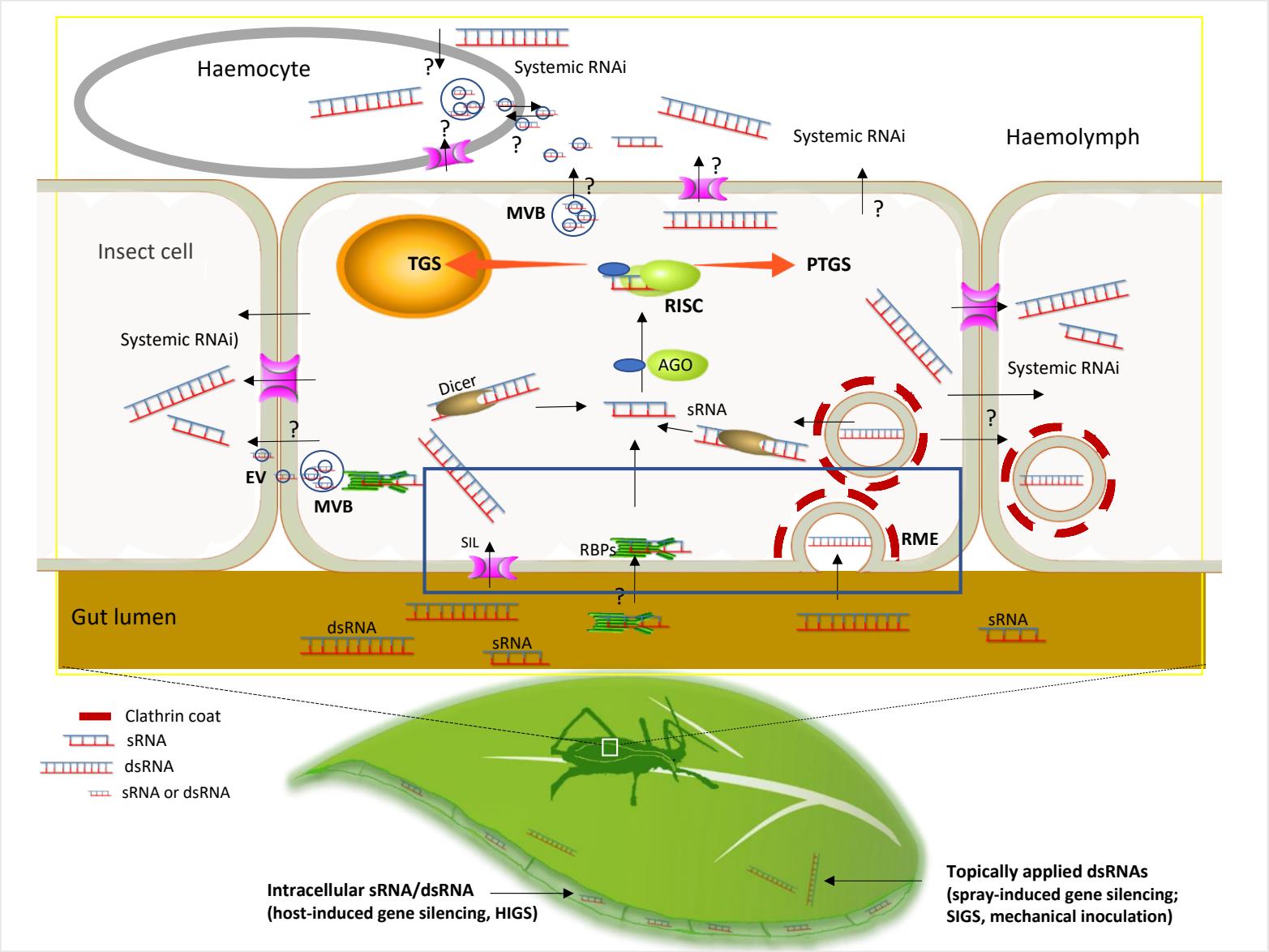
ecitation/emission wavelength (480 nm/510 nm)

***Efficiency -**
reduced aphid survival:

- RNA via HIGS
  very high (>80%)
- RNA via artificial diet
  high (>70%)
- RNA via SIGS
  very low (<10 %)

**SaMIF1*-dsRNA:
MIF: macrophage-migration inhibitor factor

Molecular components of dsRNA uptake in insects



Efficiency aspects:

- RNA length

> 60 nt for some insects

- RNA transport

chemical formulations

- RNA stability

inactivation by nucleases

Liu et al 2020, Advanced Biotechnology

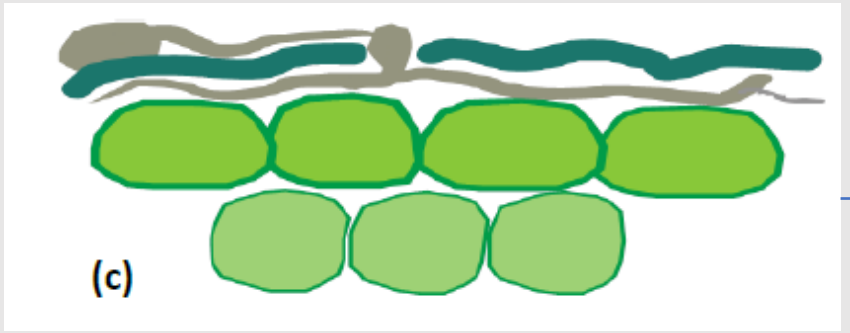
Example:
fungus control by dsRNA

Uptake of fluorescent dsRNA from liquid culture

Fungus		L-culture	Leaf/root SIGS	Ref.
Rhynchosporium secalis	hemibiotrophic	efficient	n.d	not publ.
Fusarium graminearum	necrotrophic	weak	yes	Koch et al. 2016 Plos Path
Fusarium culmorum	necrotrophic	weak	yes	Koch et al. 2018 Eur J Plant Path
Verticillium longisporum	necrotrophic	efficient	yes	Galli et al. 2020 Meth Mol Biol 2166
Piriformospora indica	biotrophic/ saprophytic	efficient	n.d.	not publ.
Zymoseptoria tritici	hemibiotrophic	no	no	Kettles et al. 2019

Rhynchosporium commune

Rhynchospores



hemibiotrophic growth

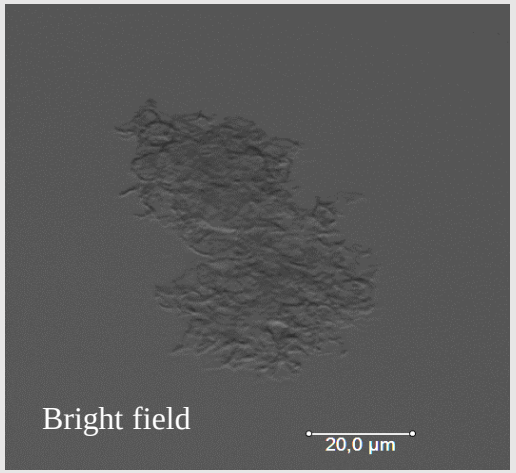
Leaf blotch symptomes on barley

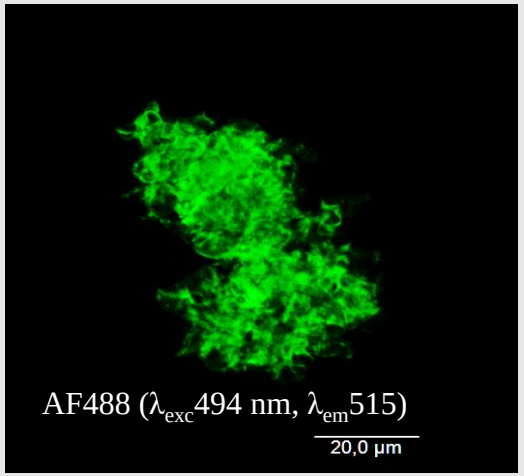
+ dsRNA$_{AF488}$

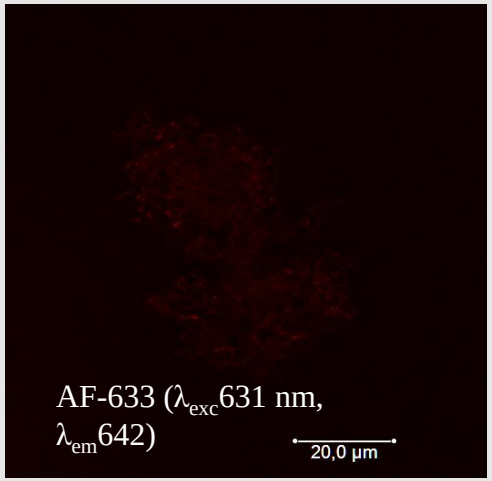
Experiment:

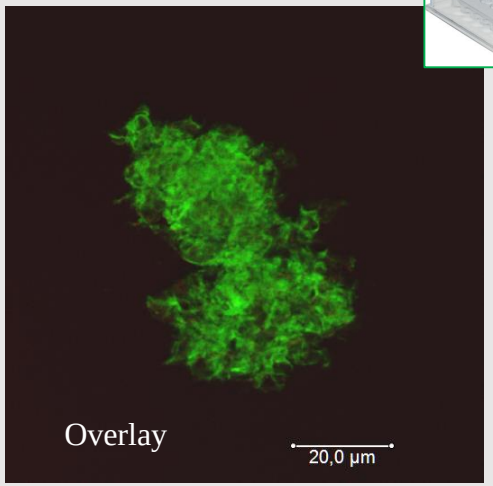
Treatment of 96-well-plate liquid cultures with fluorescent 420 nt dsRNA$_{AF488}$

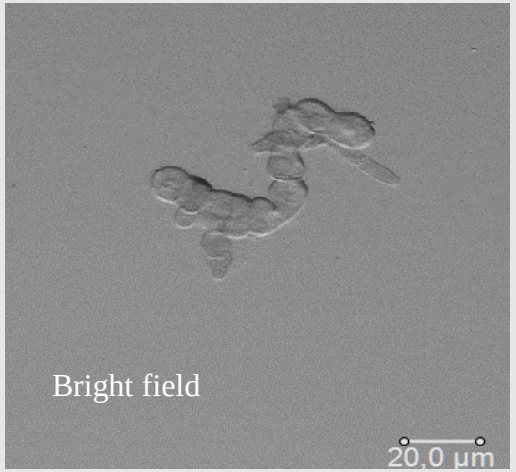
Uptake of 420 bp dsRNA$_{AF488}$ by *R. commune*

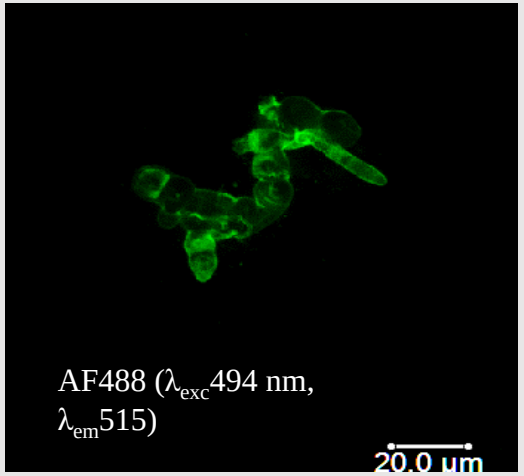


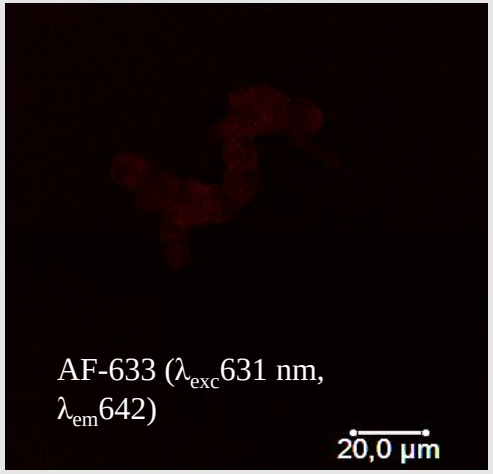


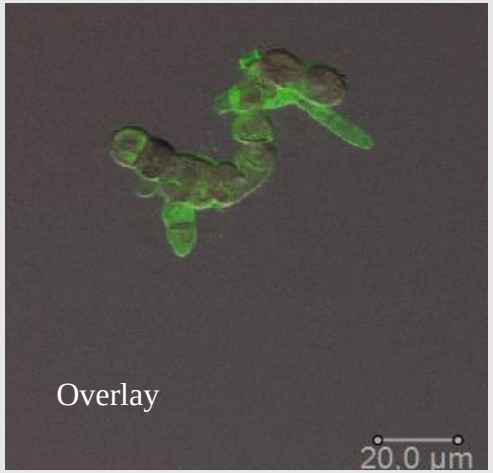






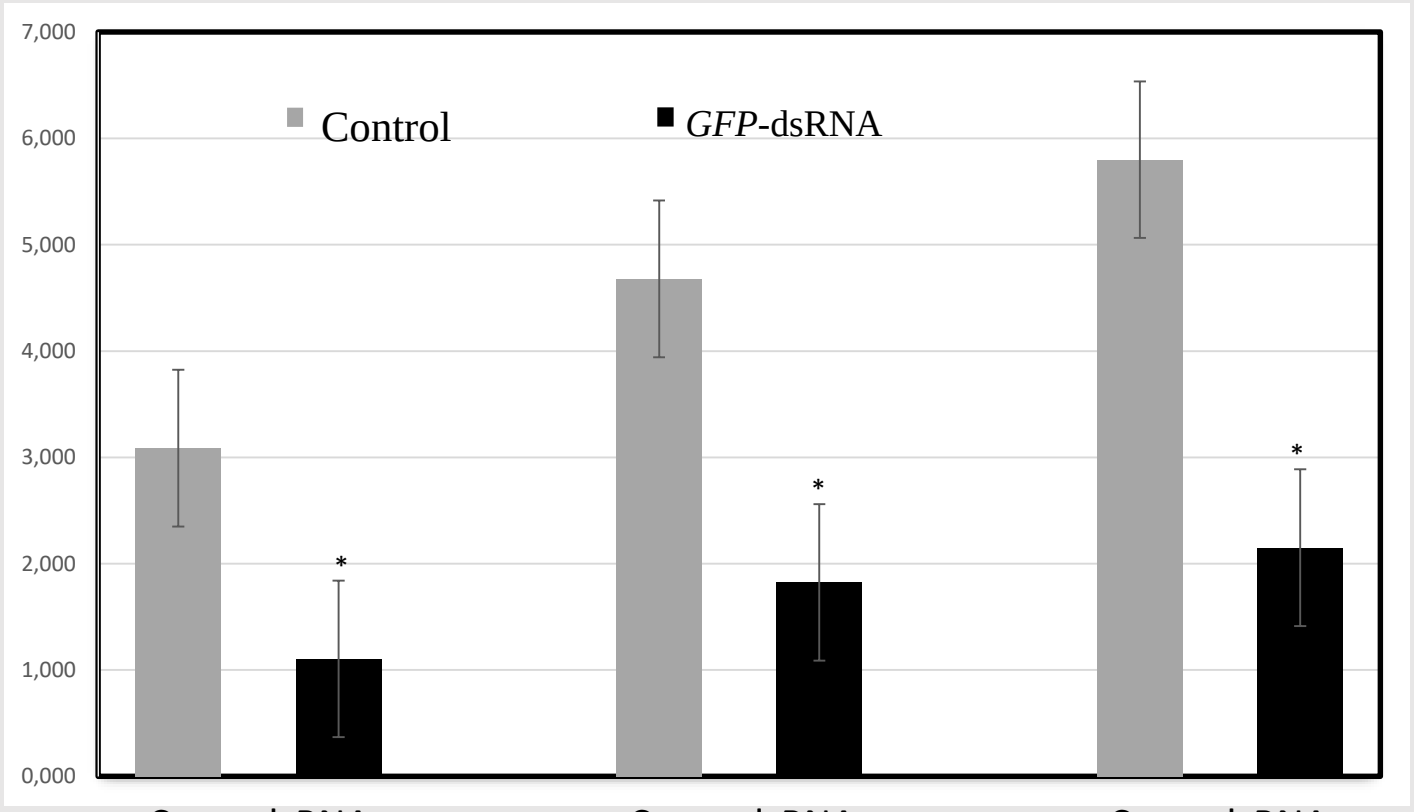


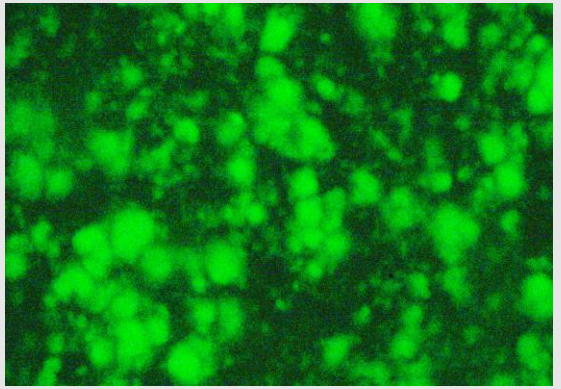




Confocal laser microscopy (CLM) - 4 dpt of conidia

Silencing of *GFP* in *R. commune*$_{GFP}$ by 481 bp dsRNA$_{GFP}$

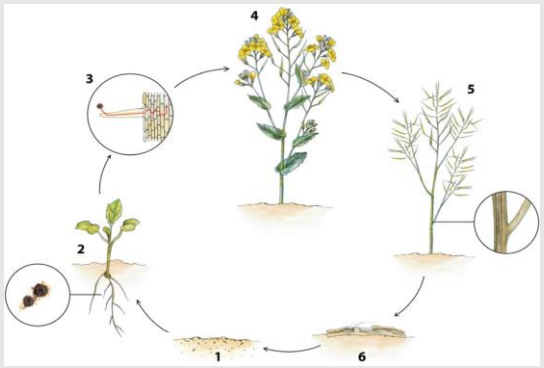




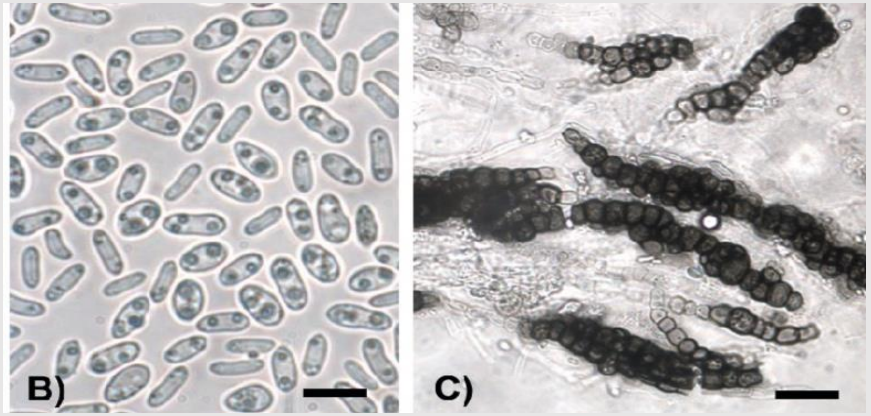
Rc-UK7-GFP
Czapek Dox Agar

***GFP* expression in *Rc*-UK7:** Bars, SD of three replicates; * $p<0.05$ (student's t-test)

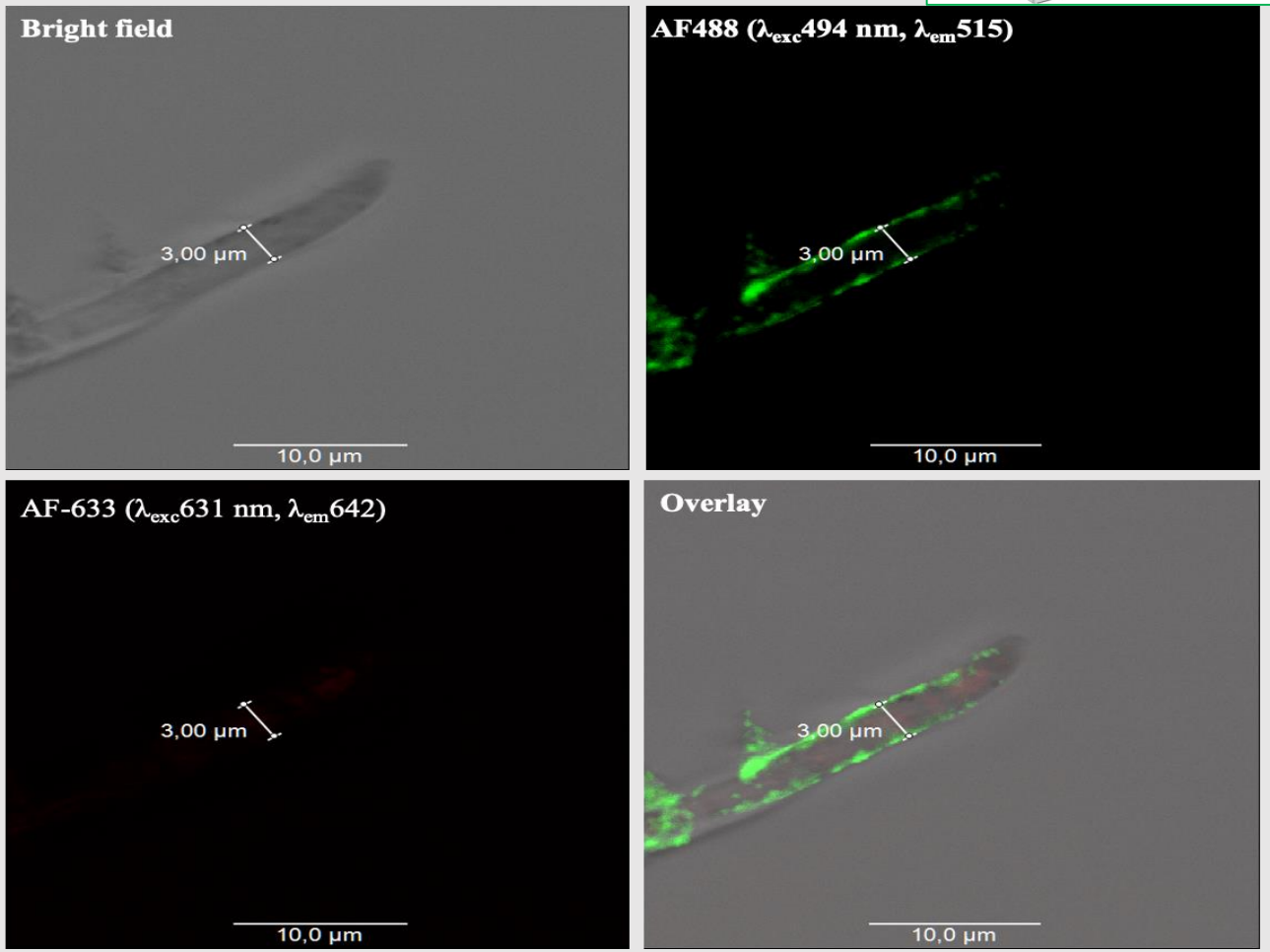
Uptake of 420 bp dsRNA$_{AF488}$ by *Verticillium longisporum*



Verticillium wilt

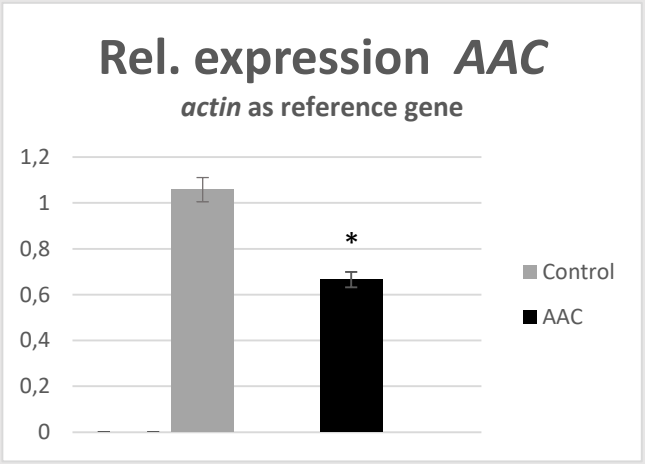


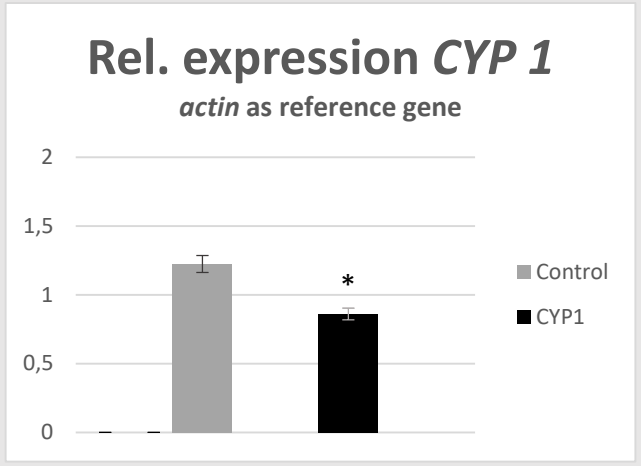
conidia microsclerotia

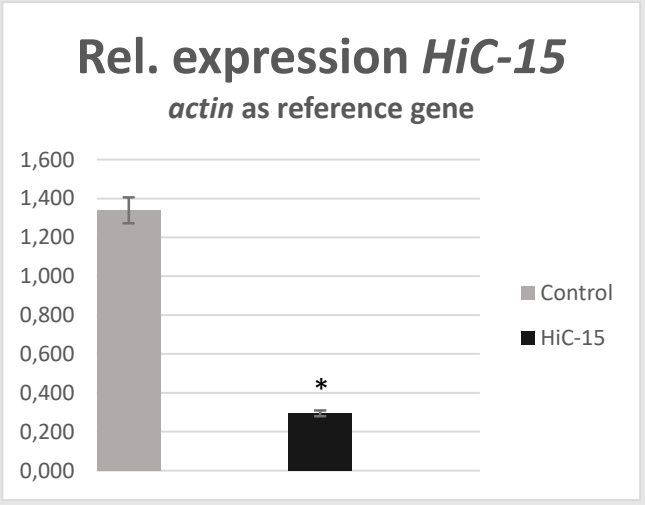


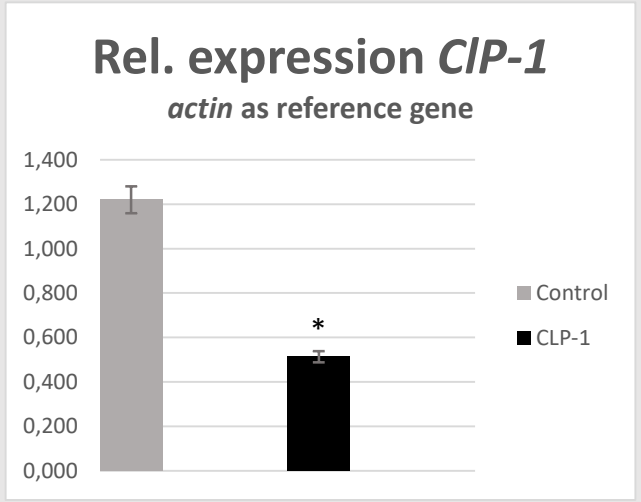
CLM 4 dpt of conidia

Gene silencing in *V. longisporum* by dsRNA









AAC: ADP/ATP carrier
CYP1: Cytochrome P450 monooxygenase

HiC-15: Isotrichodermin C-15 hydroxylase
ClP-1: Cysteine protease1

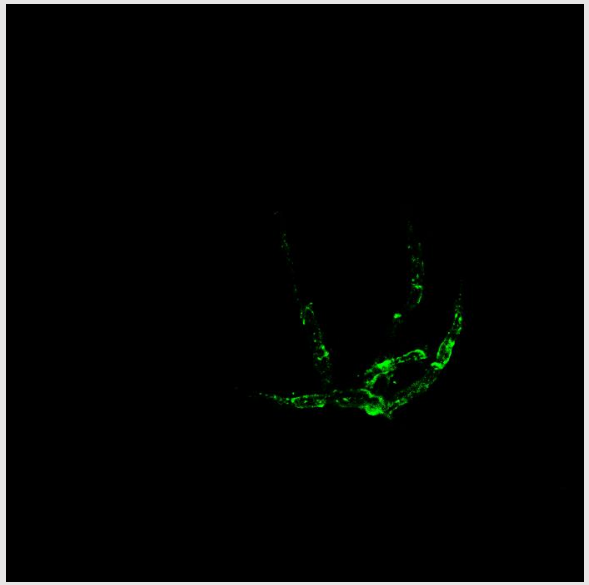
Uptake of fluorescent dsRNA by *V. longisporum*

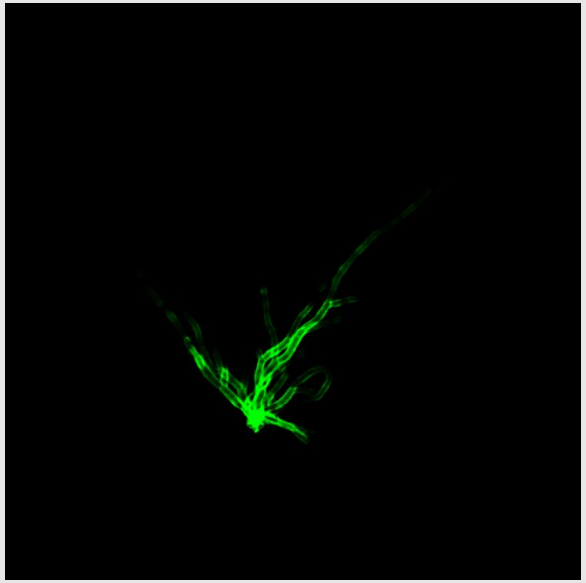
24 h

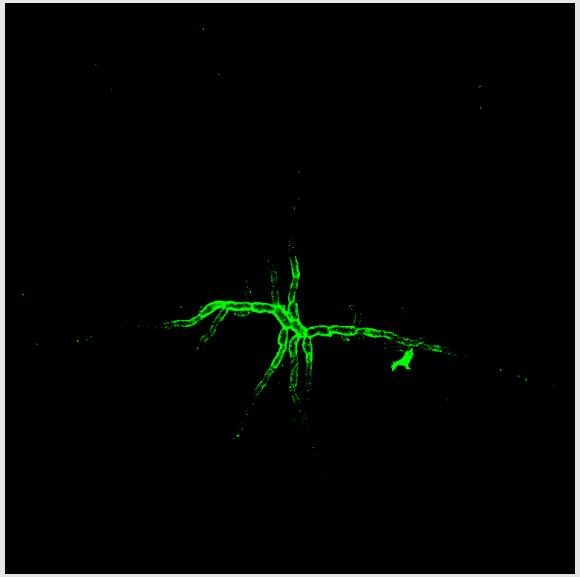
21 bp dsRNA$_{FAM}$	**420 bp** **dsRNA$_{AF488}$**	**1451 bp** **dsRNA$_{AF488}$**

Bright field

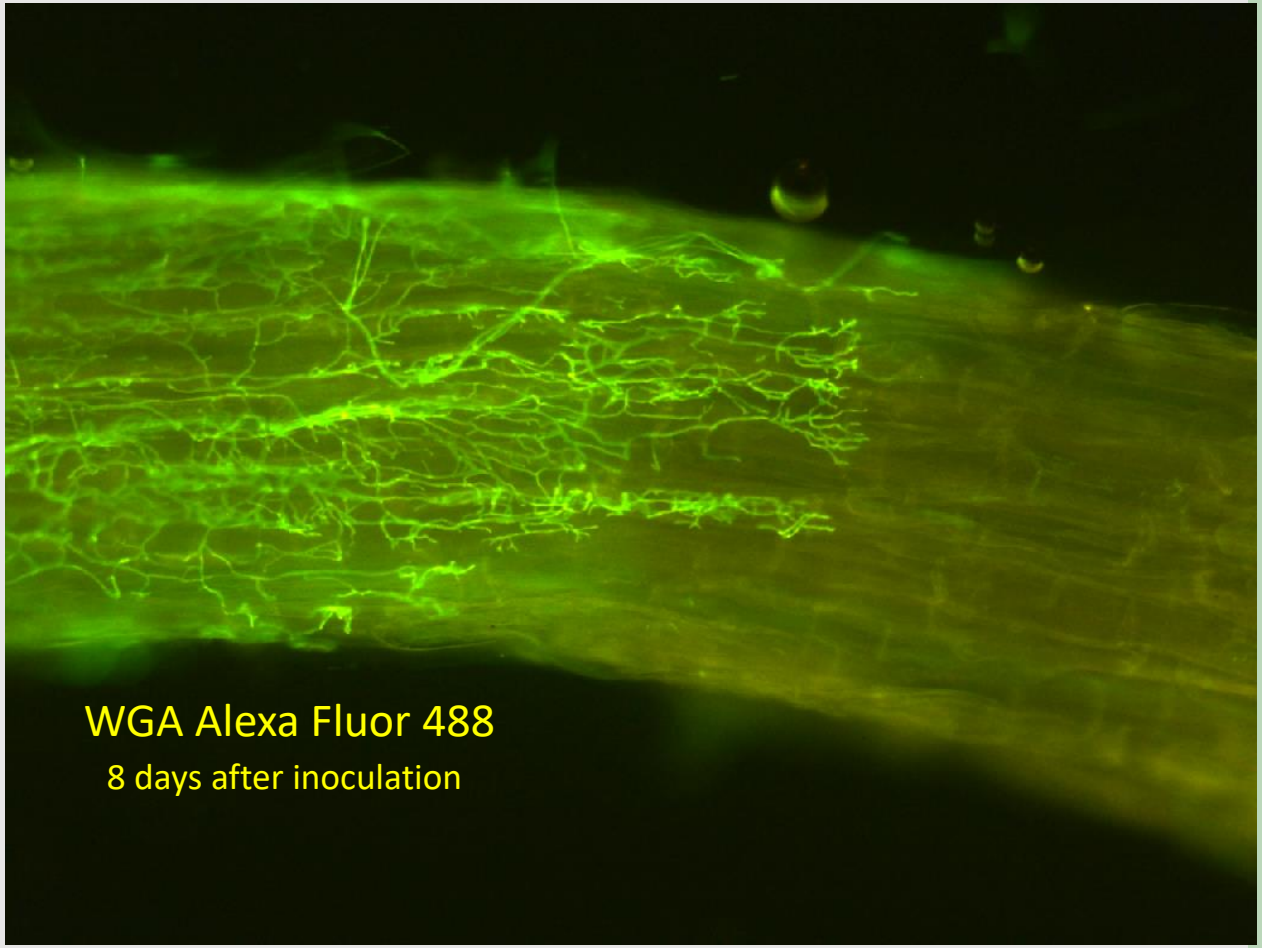
AF488 laser







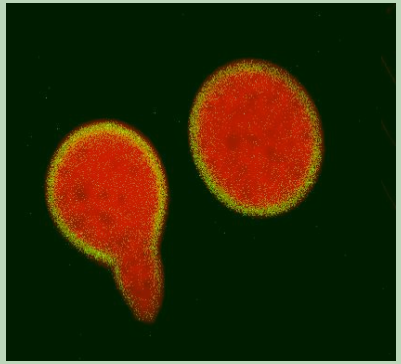
Uptake of fluorescent dsRNA by *Piriformospora indica*



Yield increase in barley

Growth promotion

Protection of roots



Chlamydospores

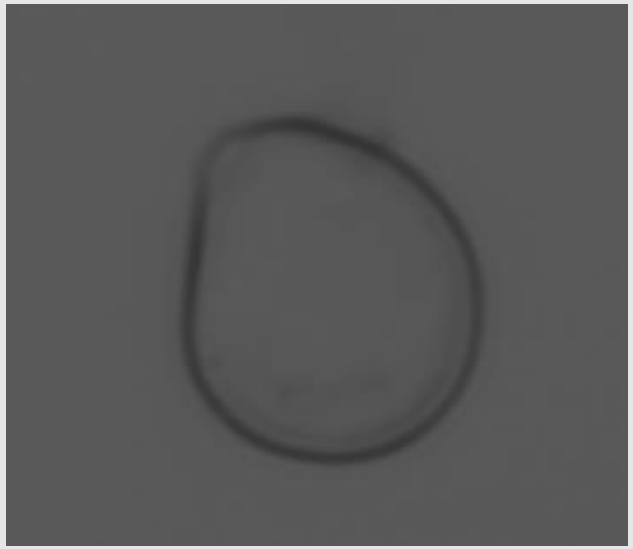
Uptake of dsRNA$_{FAM}$ by *P. indica*

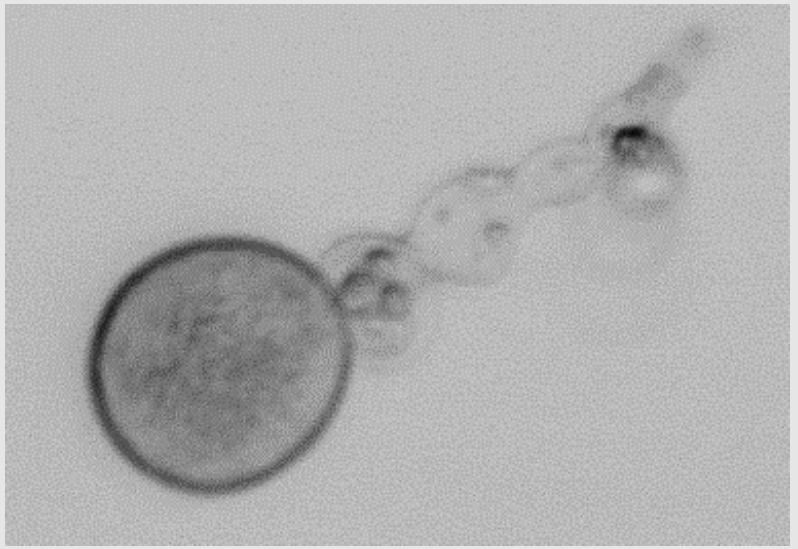
21 bp
dsRNAFAM

24 h

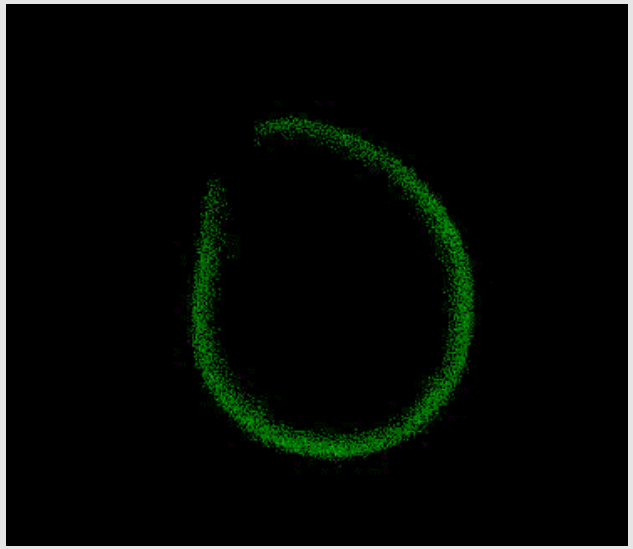
48 h

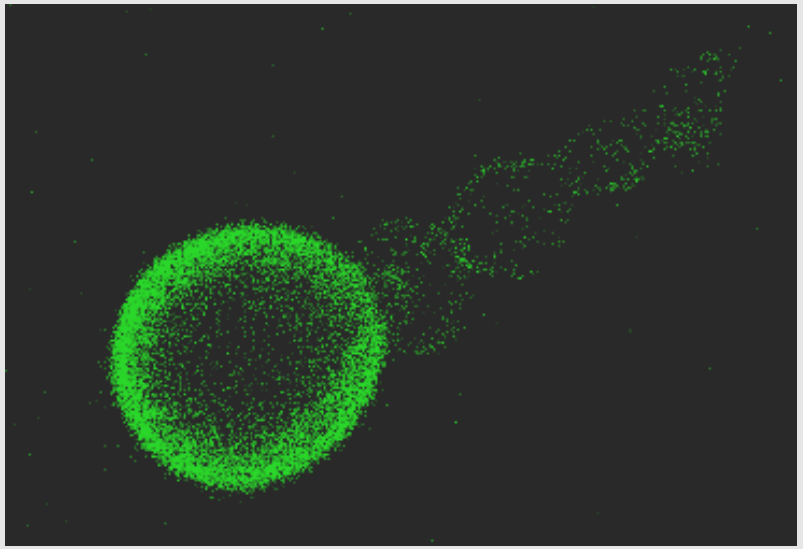
Bright field





AF488
laser



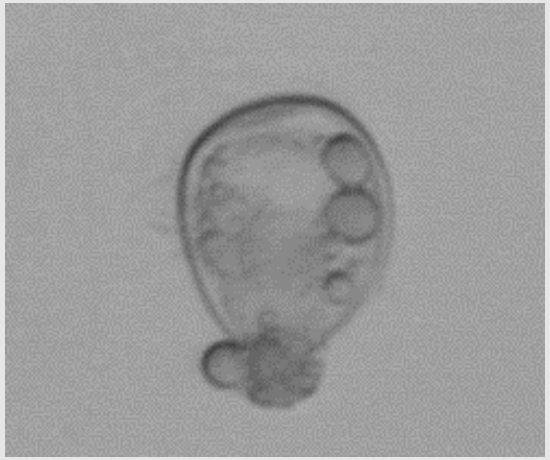
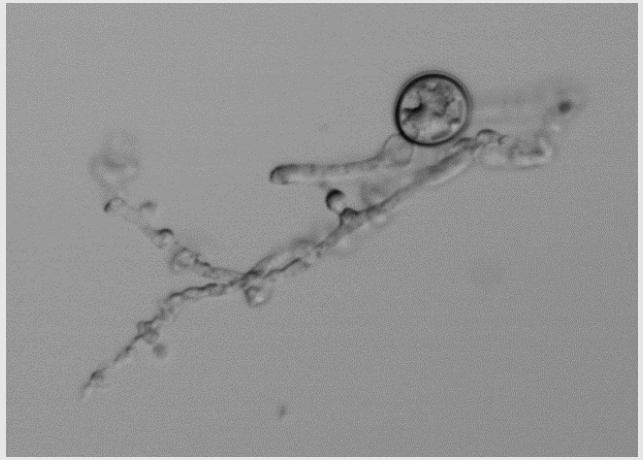
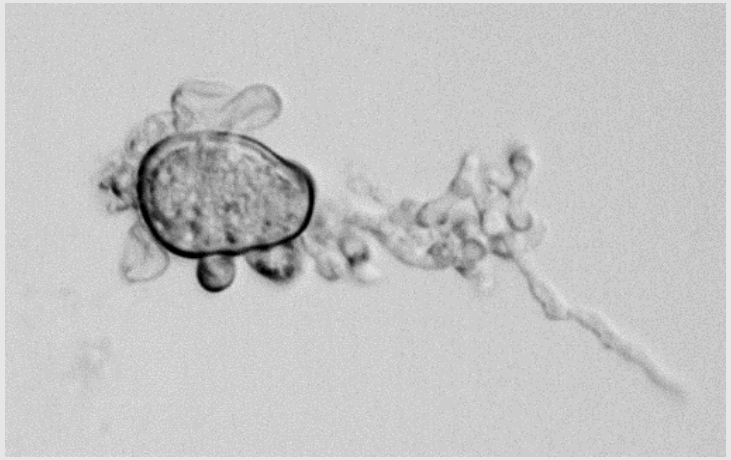


Uptake of dsRNA$_{AF488}$ by *P. indica*

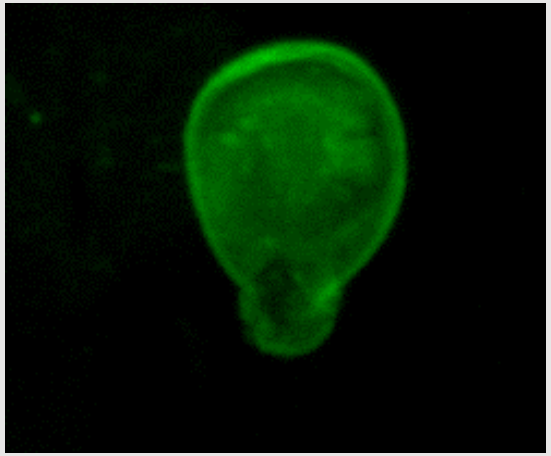
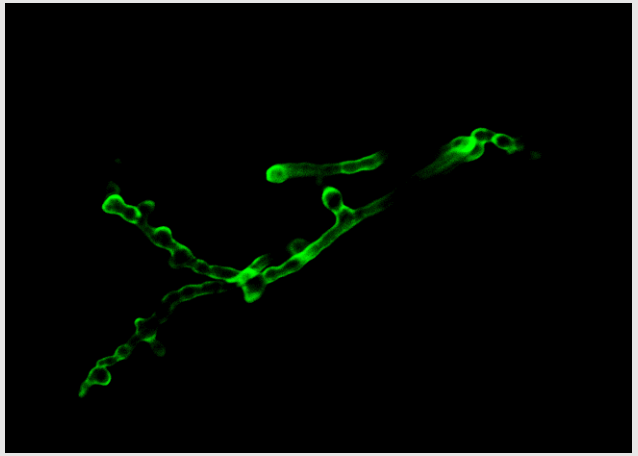
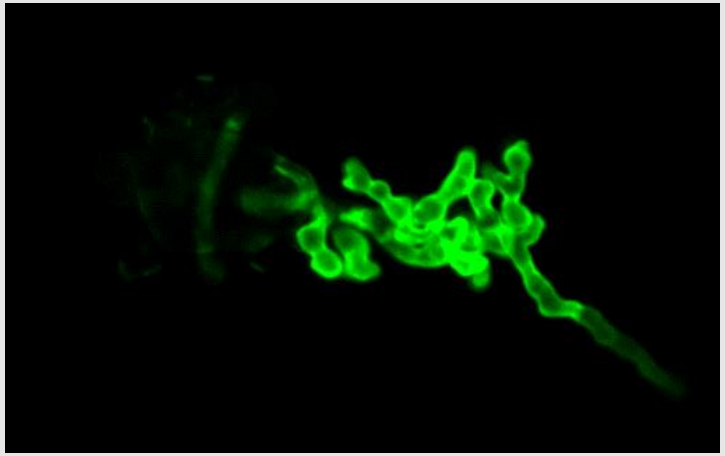
24 h

420 bp
dsRNAAF488

Bright field

AF488 laser

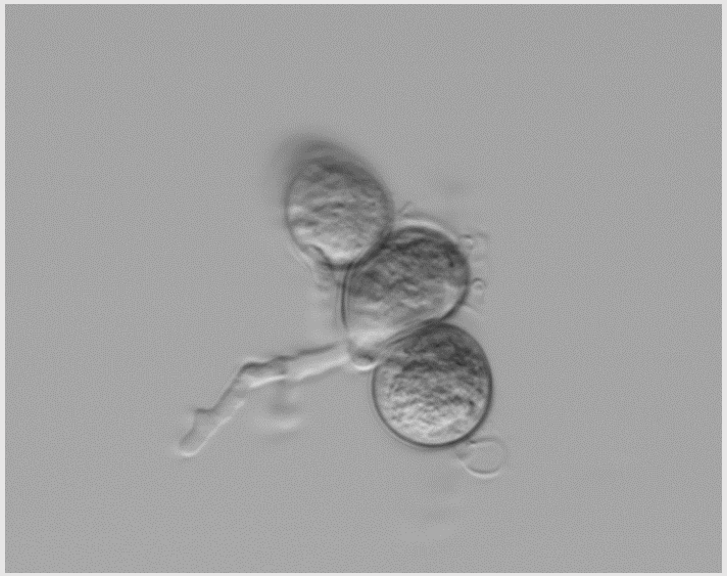




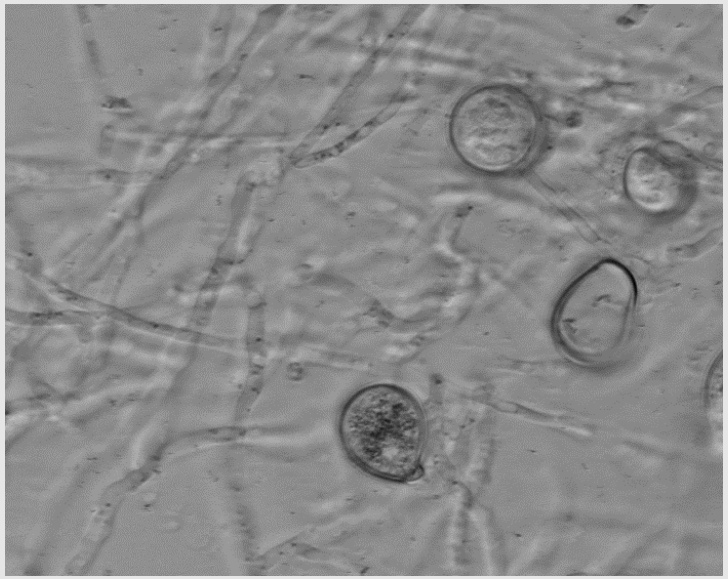
Uptake of dsRNA$_{AF488}$ by *P. indica*

48 h

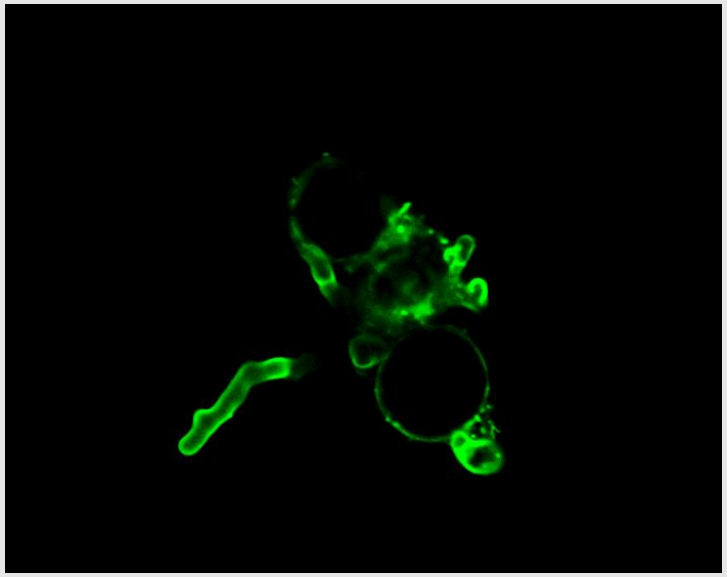
420 bp
dsRNAAF488

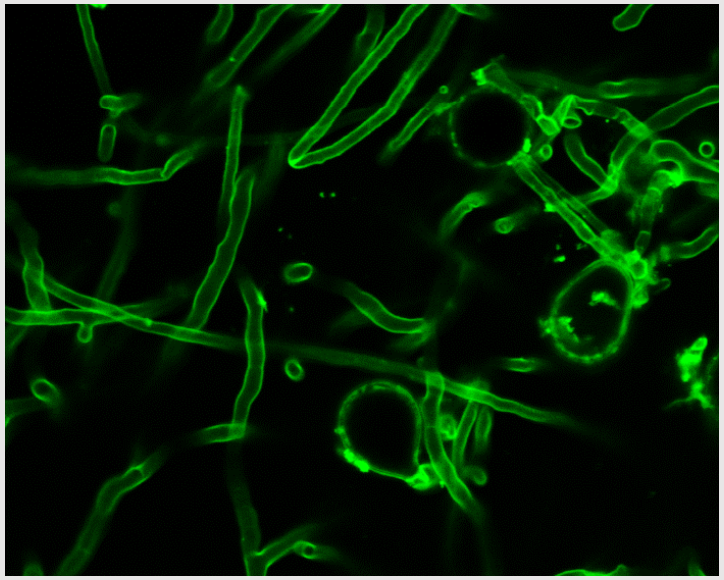
Bright field





AF488
laser



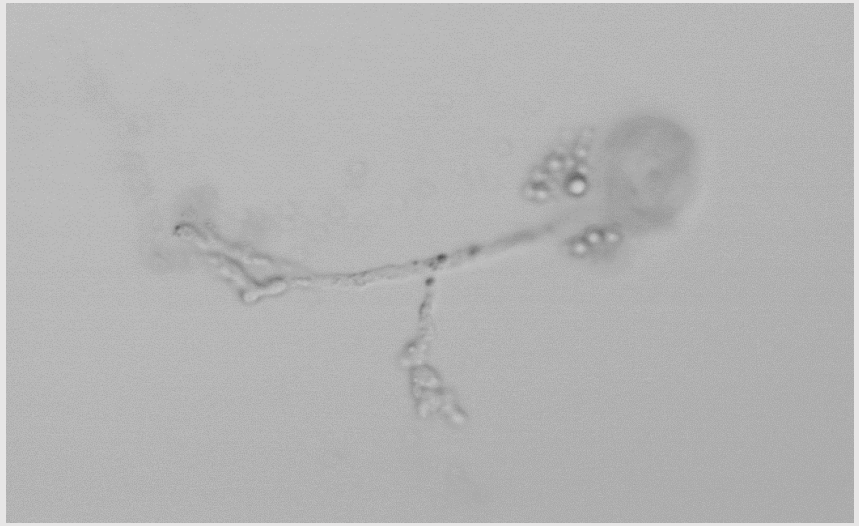
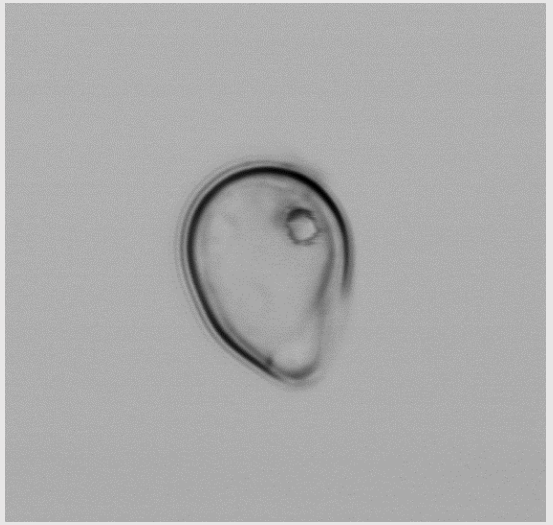


Uptake of dsRNA$_{AF488}$ by *P. indica*

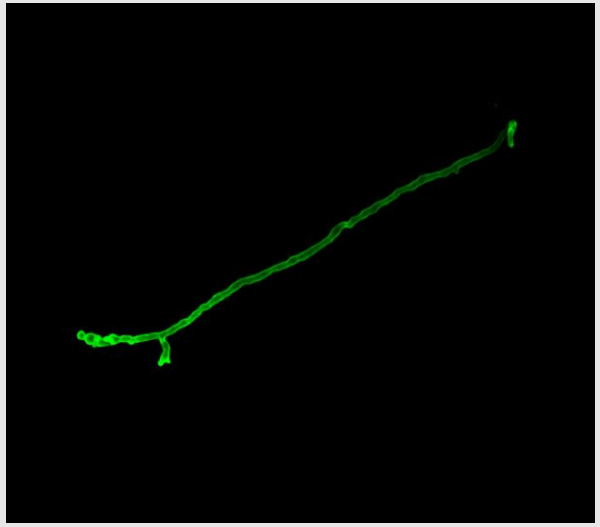
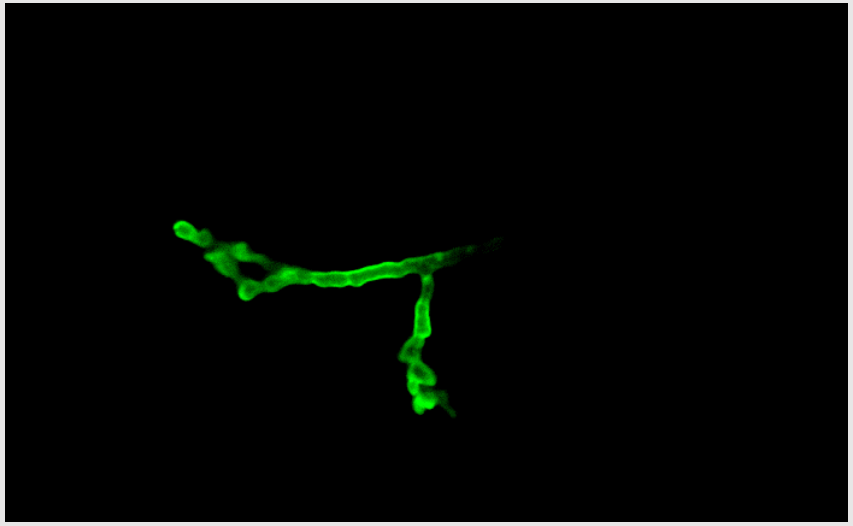
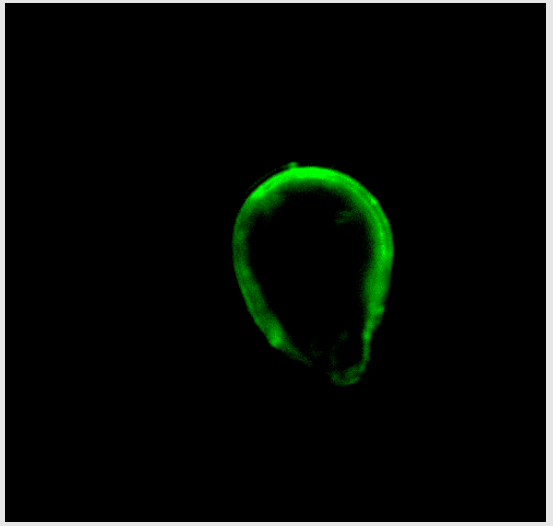
48 h

751 bp | **1451 bp** | **1451 bp**

Bright field

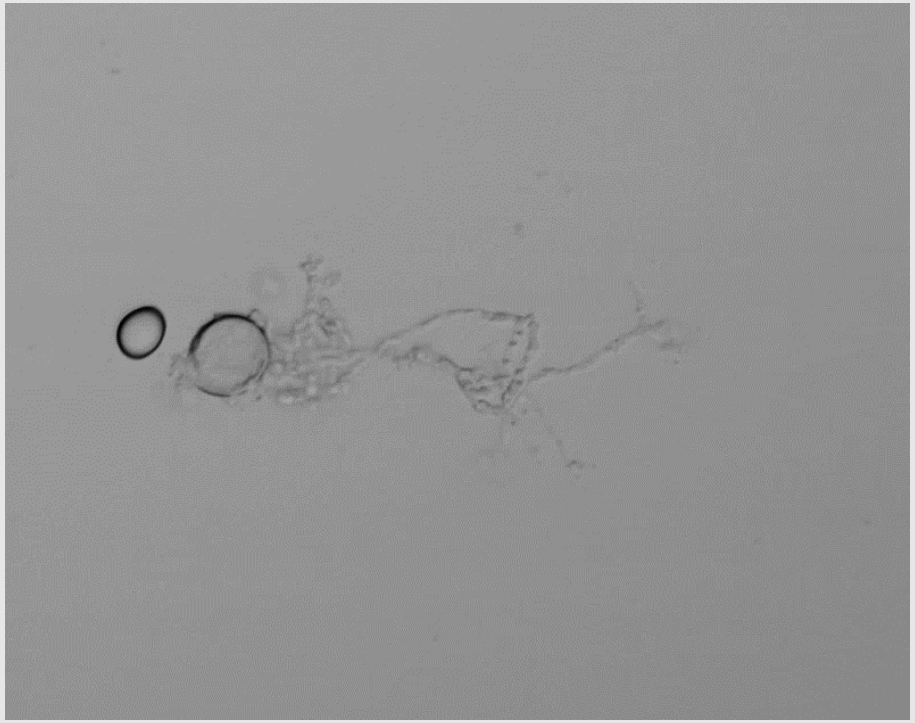
AF488 laser

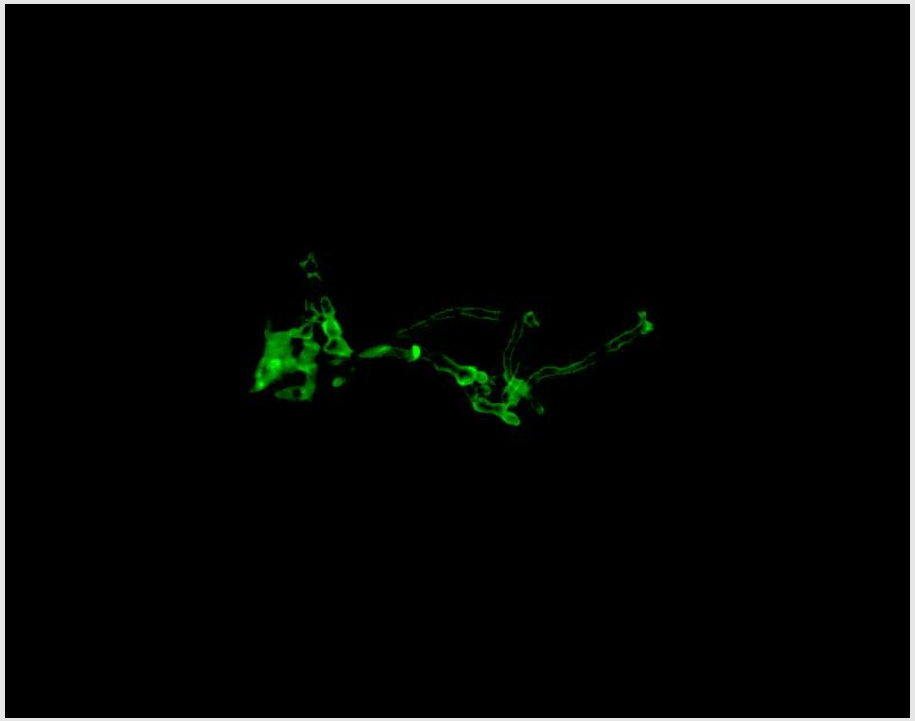
24 h

Uptake of dsRNA$_{AF488}$ by *P. indica*

1775 bp



Bright field



AF488

dsRNA as fungicide

Koch et al. 2016, Plos Pathogens ppat.1005901

OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

An RNAi-Based Control of *Fusarium graminearum* Infections Through Spraying of Long dsRNAs Involves a Plant Passage and Is Controlled by the Fungal Silencing Machinery

Aline Koch, Dagmar Biedenkopf, Alexandra Furch, Lennart Weber, Oliver Rossbach, Eltayb Abdellatef, Lukas Linicus, Jan Johannsmeier, Lukas Jelonek, Alexander Goesmann, Vinitha Cardoza, John McMillan, Tobias Mentzel, Karl-Heinz Kogel

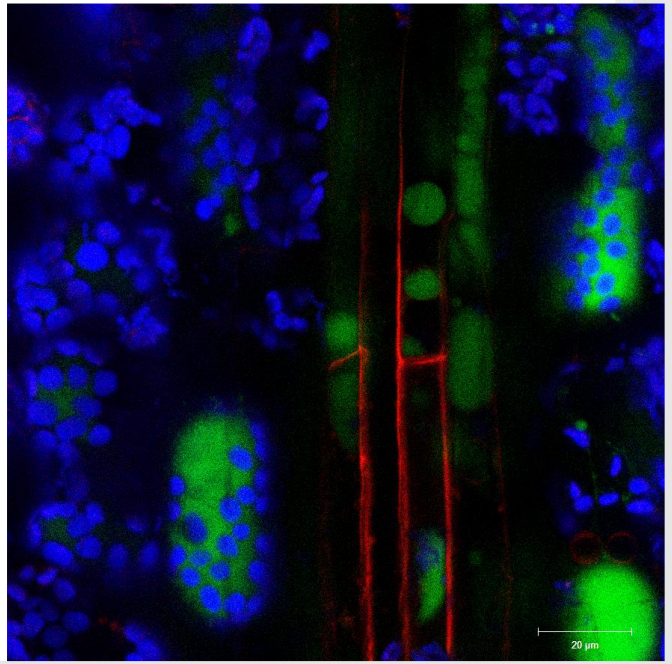
Published: October 13, 2016 • http://dx.doi.org/10.1371/journal.ppat.1005901

Article Authors Metrics Comments Related Content

Abstract
Author Summary
Introduction
Results
Discussion
Materials and Methods
Supporting Information
Acknowledgments

Abstract

Meeting the increasing food and energy demands of a growing population will require the development of ground-breaking strategies that promote sustainable plant production. Host-induced gene silencing has shown great potential for controlling pest and diseases in crop plants. However, while delivery of inhibitory noncoding double-stranded (ds)RNA by transgenic expression is a promising concept, it requires the generation of transgenic crop plants which may cause substantial delay for application strategies depending on the transformability and genetic stability of the crop plant species. Using the agronomically important barley—*Fusarium graminearum* pathosystem, we alternatively demonstrate that a spray application of a long noncoding dsRNA (791 nt *CYP3*-dsRNA), which targets the three fungal cytochrome P450



Efficiency aspects:

- RNA uptake

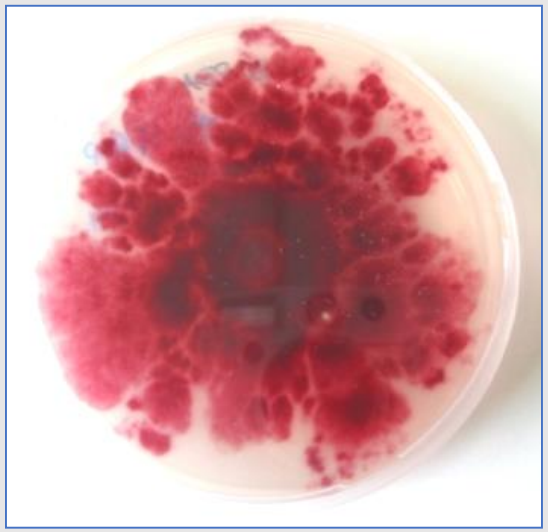
chemical formulations

- RNA stability

inactivitation of nucleases ?

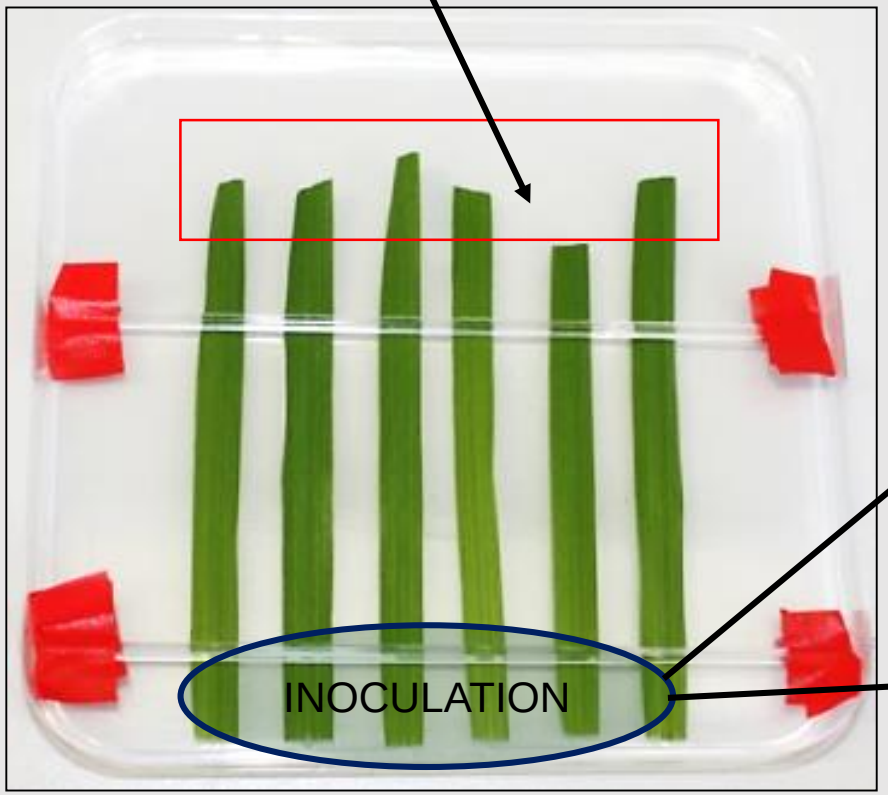
Spray-mediated systemic control of *Fusarium graminearum*

Macroconidia



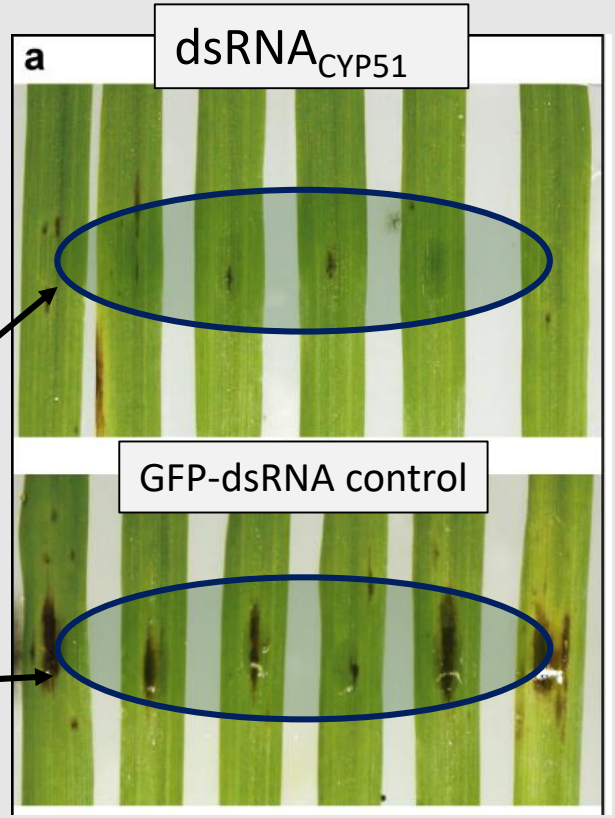
Axenic culture

dsRNA application



Fusarium graminearum

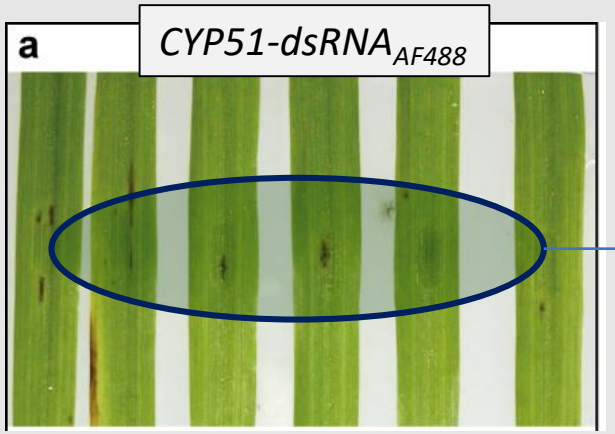
Systemic leaf area

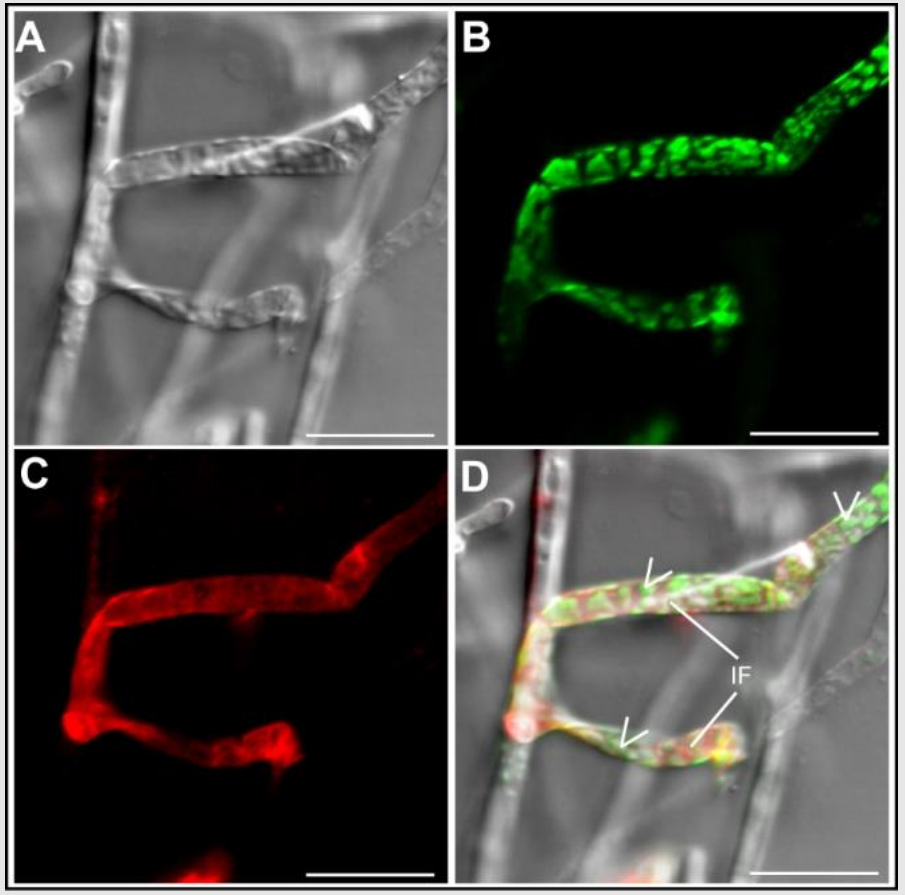


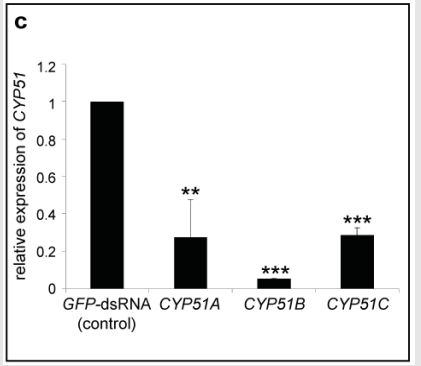
Koch et al. 2016, Plos Path

Uptake of dsRNA$_{AF488}$ from semi-systemic leaf sites

Systemic leaf area



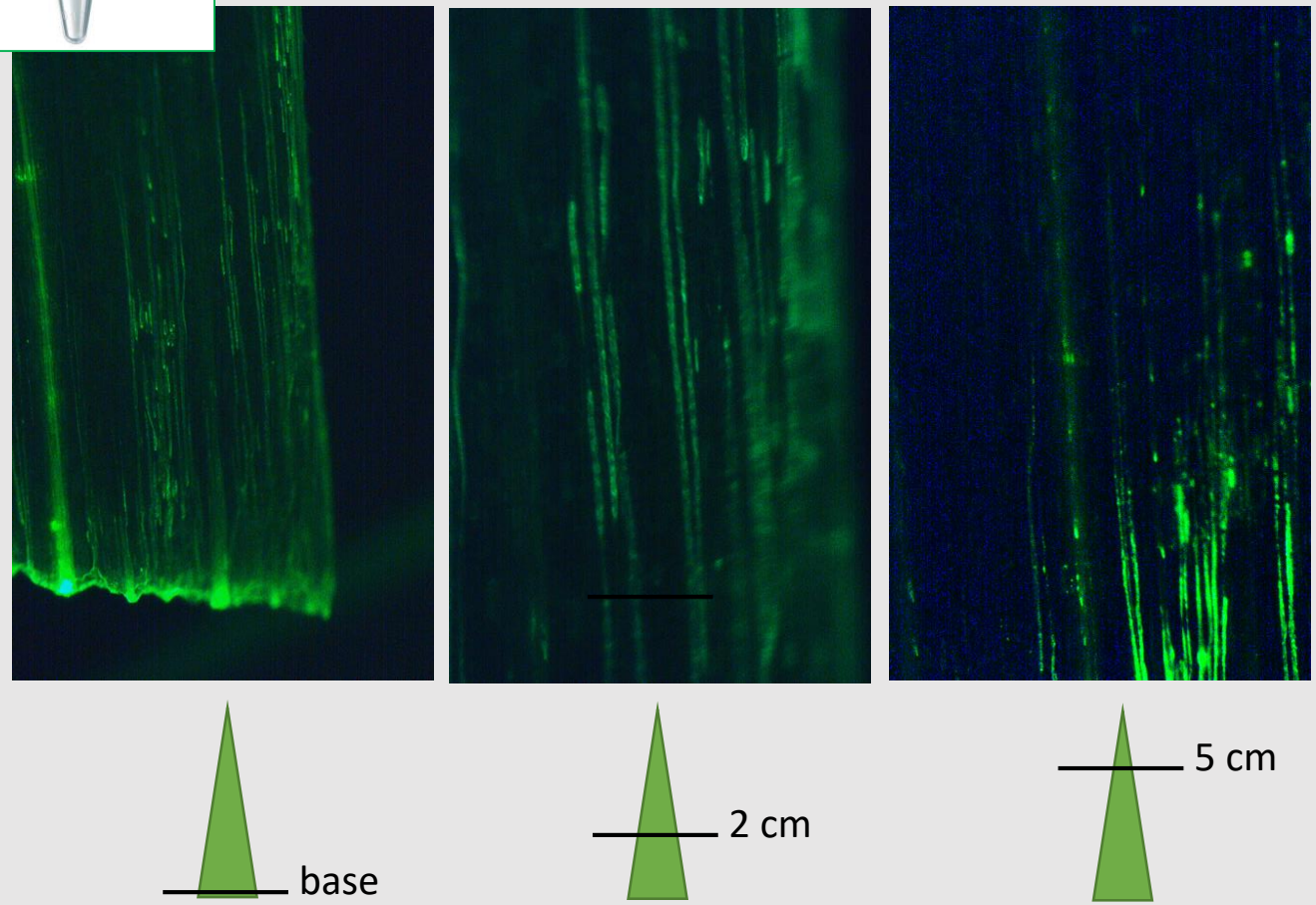




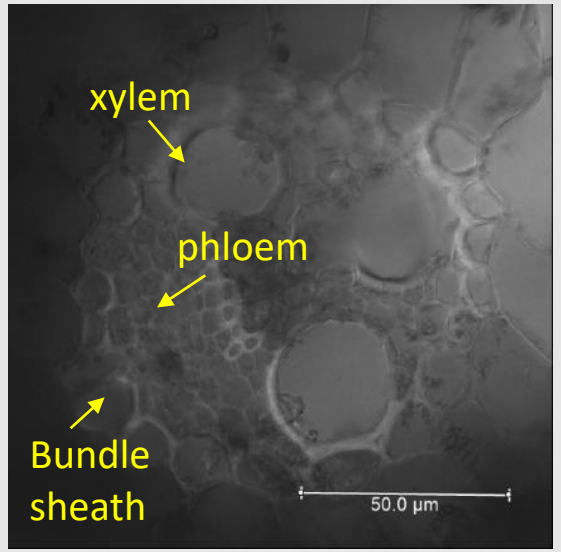
Alternative Screening designs

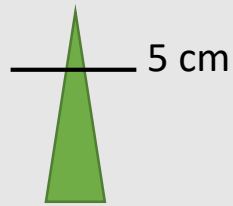
Screening design: Uptake of dsRNA$_{AF488}$ by cut **barley** leaves

420 nt dsRNA$_{AF488}$

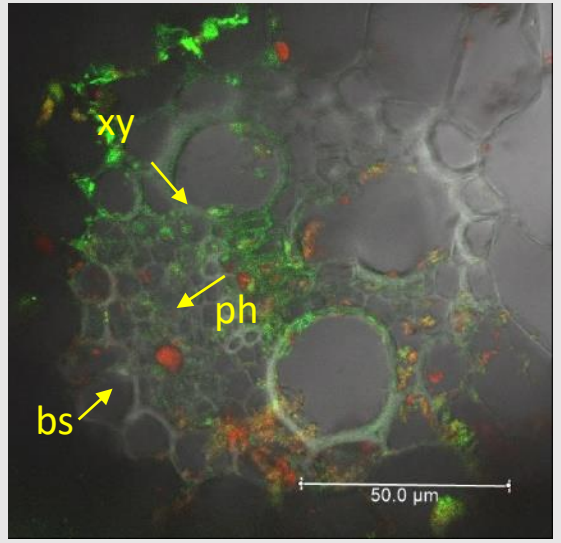


Bright field

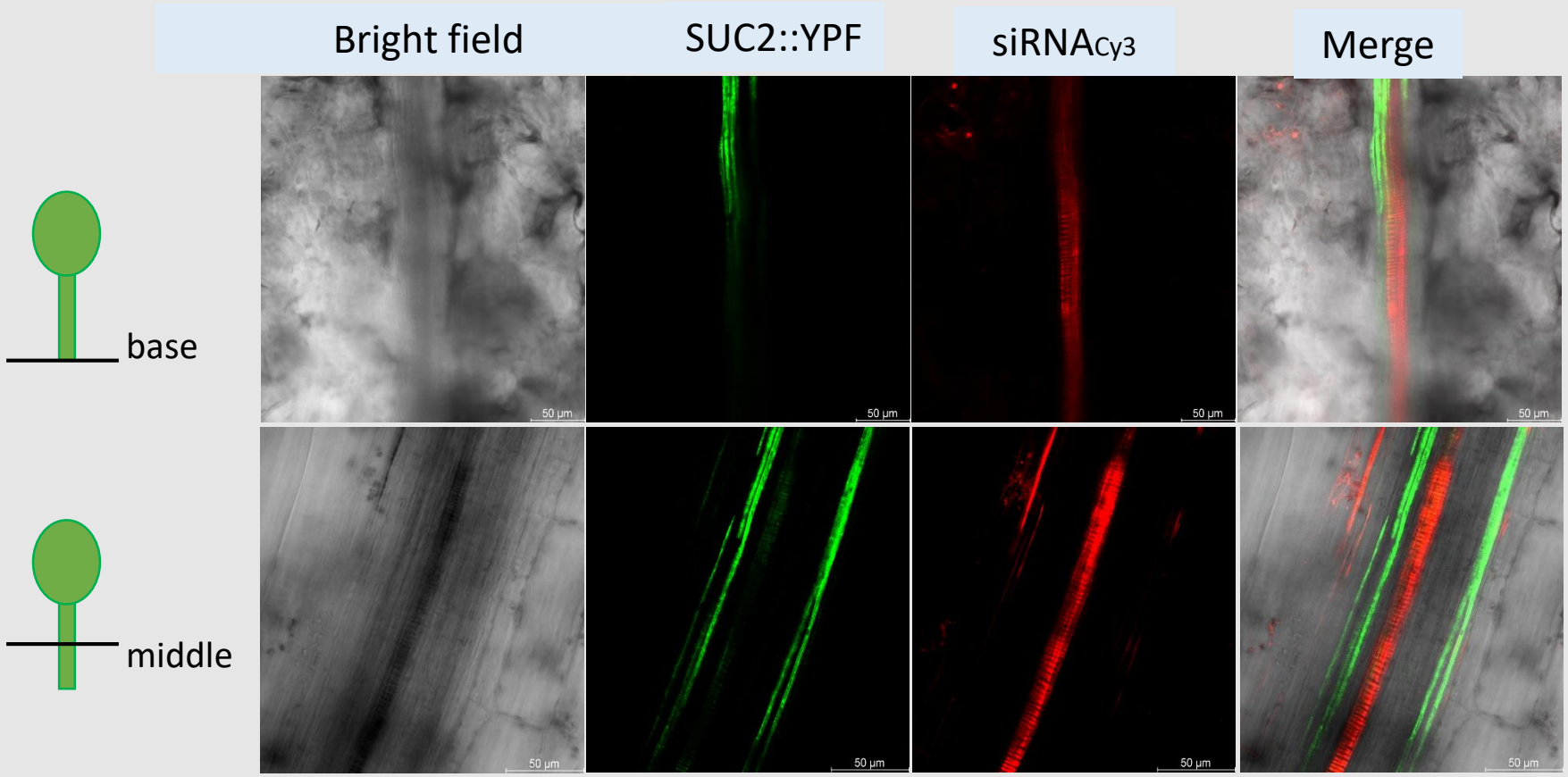




Cross-section



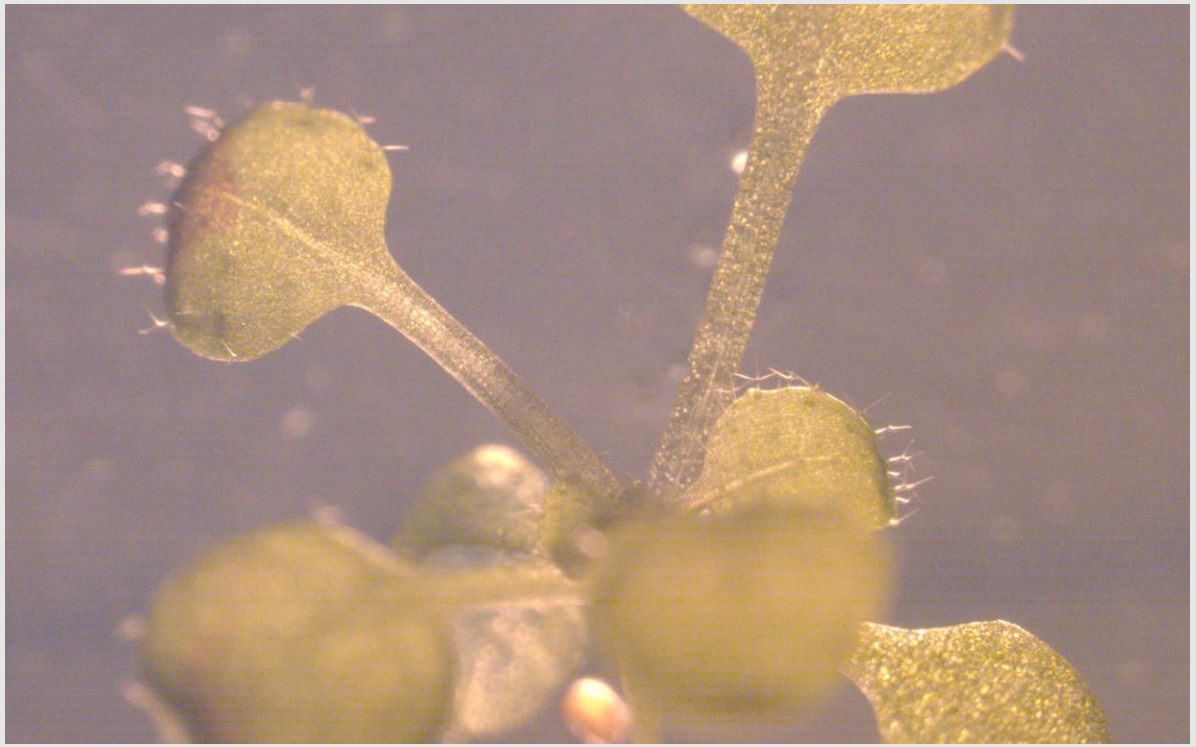
Screening design: uptake of 21 nt siRNA$_{Cy3}$ by Arabidopsis petioles



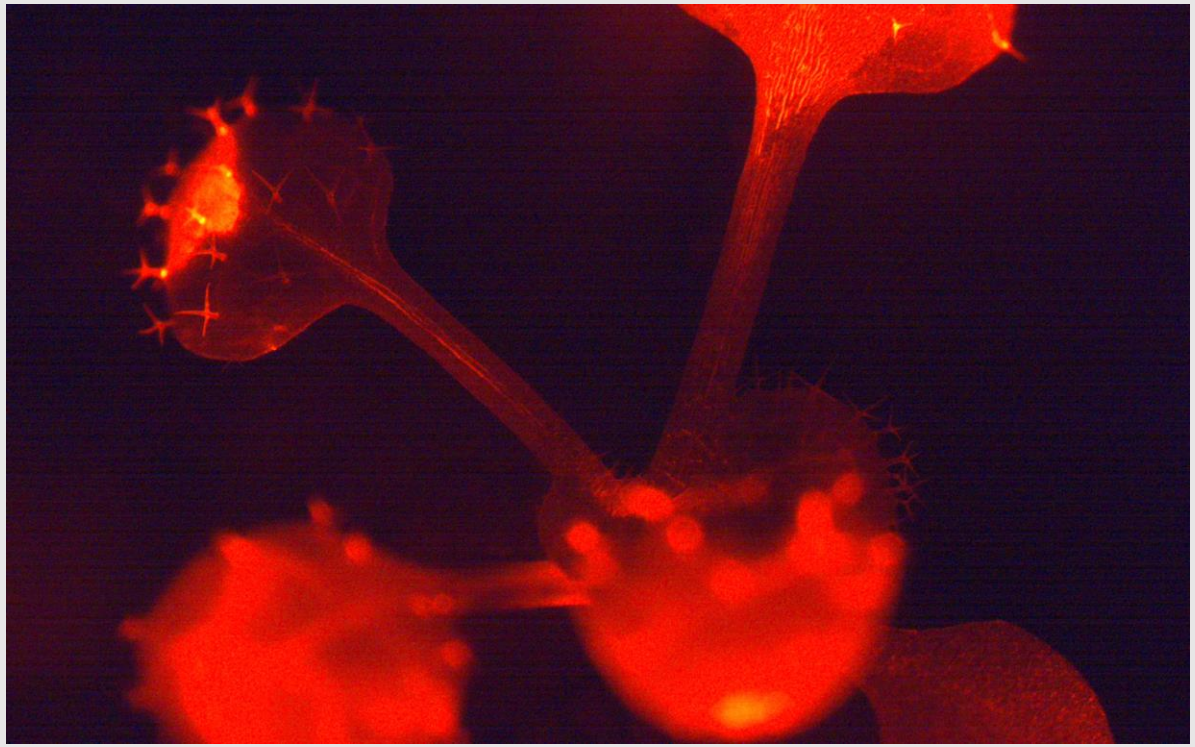
Screening design: uptake of 21 bp $siRNA_{Cy3}$ in leaves

Drop: foliar uptake – 120 h

Bright field



DSR

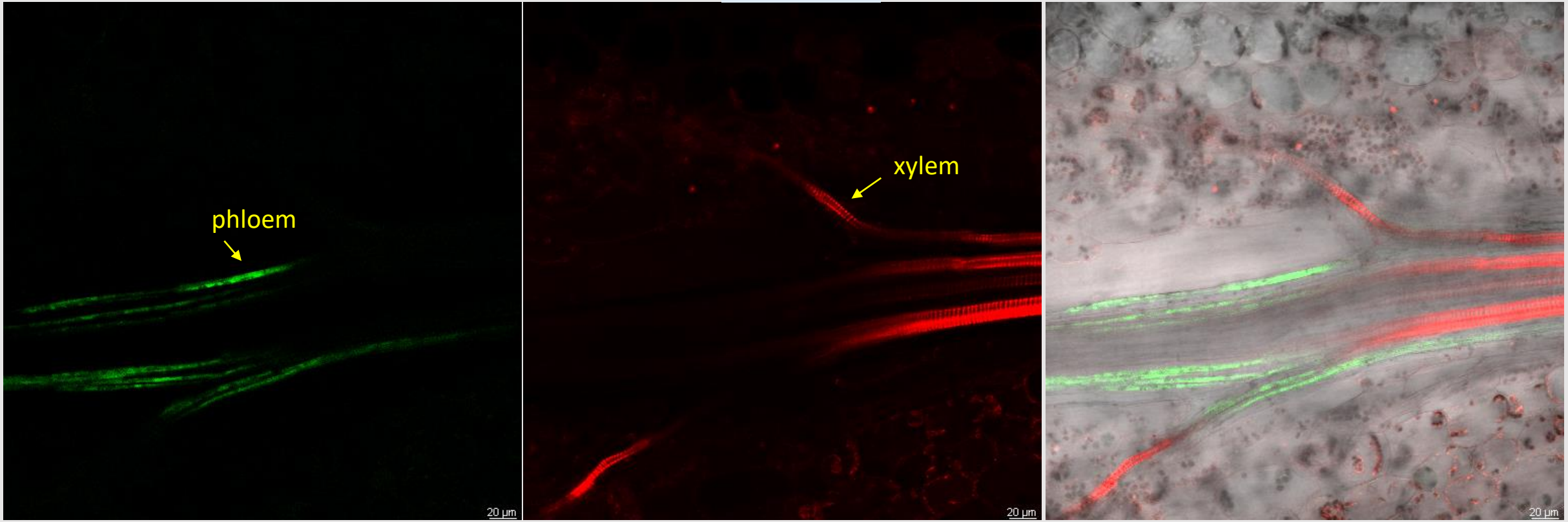


Uptake of 21 nt siRNA$_{Cy3}$ by Arabidopsis leaves

SUC2::YPF

siRNA$_{Cy3}$

Merge



Summary

- Pests: exogenous dsRNA is promising and very efficient against selected pests
- Fungi: exogenous dsRNA treatments not yet established; open questions !
- Innovative dsRNA compositions with formulations
- dsRNA can move inside the plant
- Apoplastic cell-to-cell or xylematic route

ACKNOWLEDGEMENT

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

Cooperation

Wolfgang Knogge, Halle, Germany
Minna Poranen, Helsinki
Manfred Heinlein, Straßbourg
Christine Coustau, Nice

ptj Projektträger Jülich Forschungszentrum Jülich

Federal Ministry of Education and Research

Jafar Imani **Maria Ladera Carmona**

Ena Šečić

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