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…
OUR SOLUTION

Plant Protection by Non-coding RNAs
LEARNING FROM NATURE

Exchange of small RNA between interacting species

RNA input (dsRNA, sRNA, circRNA, mRNA)

Modulation of defence and virulence

Fight against pest and diseases

viruses
insects
nematodes
fungi
oomycetes
Example:
insect control by RNA
Uptake of fluorescent dsRNA$_{AF488}$ from artificial diet

**Brightness field**

**Fluorescence**

<table>
<thead>
<tr>
<th></th>
<th>0 h</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bright field</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
</tbody>
</table>

*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

excitation/emission wavelength (480 nm/510 nm)
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

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Culture Type</th>
<th>Uptake L-culture</th>
<th>Leaf/root SIGS</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rhynchosporium secalis</em></td>
<td>hemibiotrophic</td>
<td>efficient</td>
<td>n.d</td>
<td>not publ.</td>
</tr>
<tr>
<td><em>Fusarium graminearum</em></td>
<td>necrotrophic</td>
<td>weak</td>
<td>yes</td>
<td>Koch et al. 2016 Plos Path</td>
</tr>
<tr>
<td><em>Fusarium culmorum</em></td>
<td>necrotrophic</td>
<td>weak</td>
<td>yes</td>
<td>Koch et al. 2018 Eur J Plant Path</td>
</tr>
<tr>
<td><em>Verticillium longisporum</em></td>
<td>necrotrophic</td>
<td>efficient</td>
<td>yes</td>
<td>Galli et al. 2020 Meth Mol Biol 2166</td>
</tr>
<tr>
<td><em>Piriformospora indica</em></td>
<td>biotrophic/saprophytic</td>
<td>efficient</td>
<td>n.d.</td>
<td>not publ.</td>
</tr>
<tr>
<td><em>Zymoseptoria tritici</em></td>
<td>hemibiotrophic</td>
<td>no</td>
<td>no</td>
<td>Kettles et al. 2019</td>
</tr>
</tbody>
</table>
Treatment of 96-well-plate liquid cultures with fluorescent 420 nt dsRNA\textsubscript{AF488}

Experiment:

Rhynchosporium commune

- Rhynchosporium rhyncospores
- hemibiotrophic growth
- + dsRNA\textsubscript{AF488}

Leaf blotch symptoms on barley
Uptake of 420 bp dsRNA_{AF488} by *R. commune*

Confocal laser microscopy (CLM) - 4 dpt of conidia
Silencing of GFP in *R. commune*<sub>GFP</sub> by 481 bp dsRNA<sub>GFP</sub>

**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* 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

CIP-1: Cysteine protease1
Uptake of fluorescent dsRNA by *V. longisporum*

24 h

**21 bp**

**420 bp**

**1451 bp**

Bright field

AF488 laser
Uptake of fluorescent dsRNA by *Piriformospora indica*

**WGA Alexa Fluor 488**

8 days after inoculation

**Yield increase in barley**

**Growth promotion**

**Chlamydospores**

**Protection of roots**
Uptake of dsRNA_{FAM} by *P. indica*

**Bright field**

- 24 h
- 48 h

**AF488 laser**
Uptake of dsRNA_{AF488} by *P. indica*

24 h

**Bright field**

**AF488 laser**

420 bp dsRNA_{AF488}
Uptake of dsRNA_{AF488} by \textit{P. indica}
Uptake of dsRNA_{AF488} by *P. indica*

**48 h**

- 751 bp
- 1451 bp
- 1451 bp
24 h

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

1775 bp

Bright field  
AF488
dsRNA as fungicide

**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**

**INOCULATION**

Systemic leaf area

- dsRNA<sub>CYP51</sub>
- GFP-dsRNA control

Koch et al. 2016, Plos Path
Uptake of dsRNA$_{AF488}$ from semi-systemic leaf sites

Systemic leaf area

CYP51-dsRNA$_{AF488}$
Alternative Screening designs
Screening design: Uptake of dsRNA_{AF488} by cut barley leaves

420 nt dsRNA_{AF488}

Bright field

- xylem
- phloem
- Bundle sheath
- bs
- ph

Cross-section

- 5 cm

Base

- 2 cm
- 5 cm
Screening design: uptake of 21 nt siRNA<sub>Cy3</sub> by Arabidopsis petioles

Bright field    SUC2::YPF    siRNA<sub>Cy3</sub>    Merge

base

middle
Screening design: 
uptake of 21 bp siRNA<sub>Cy3</sub> in leaves

Drop: foliar uptake – 120 h
Uptake of 21 nt siRNA_{Cy3} by Arabidopsis leaves
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
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