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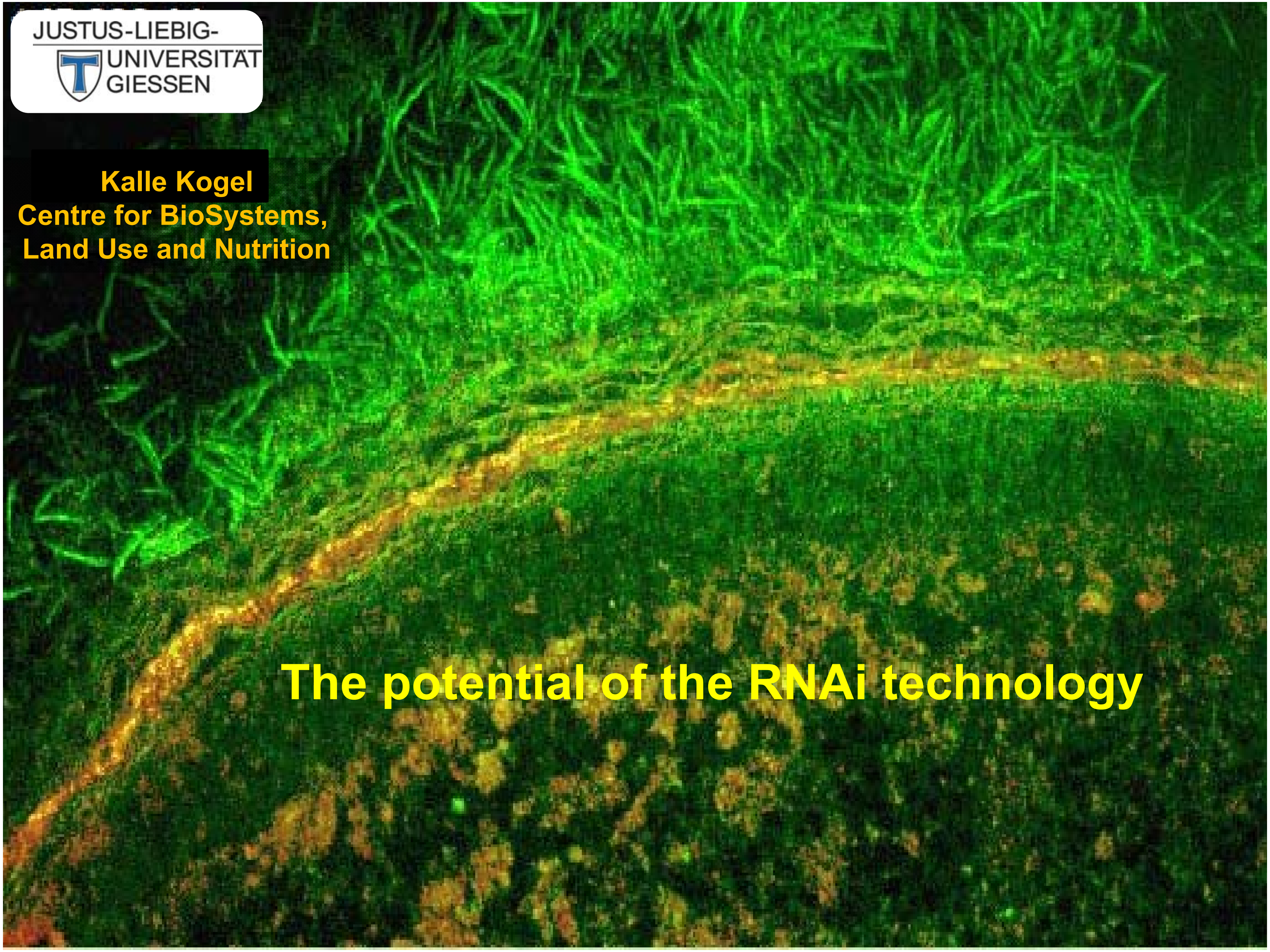
Kalle Kogel
Centre for BioSystems,
Land Use and Nutrition



The potential of the RNAi technology

RNA-based Plant Protection

Fighting against pest and diseases
using non-coding RNAs

outline

- ***Promising data for insect control …***
- ***What about fungal pathogens …?***
- ***Many question when it comes to practical application…***

Agricultural application of double-stranded (ds)RNA

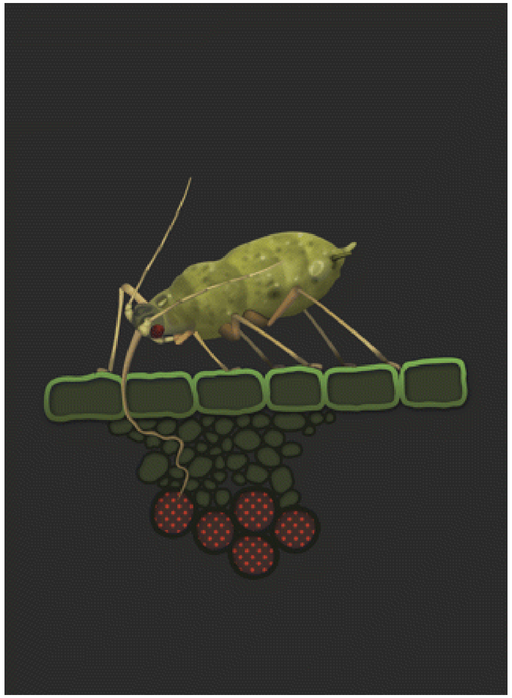
Proof-of-Concept

viruses √
insects √
nematodes √
fungi √
oomycets √

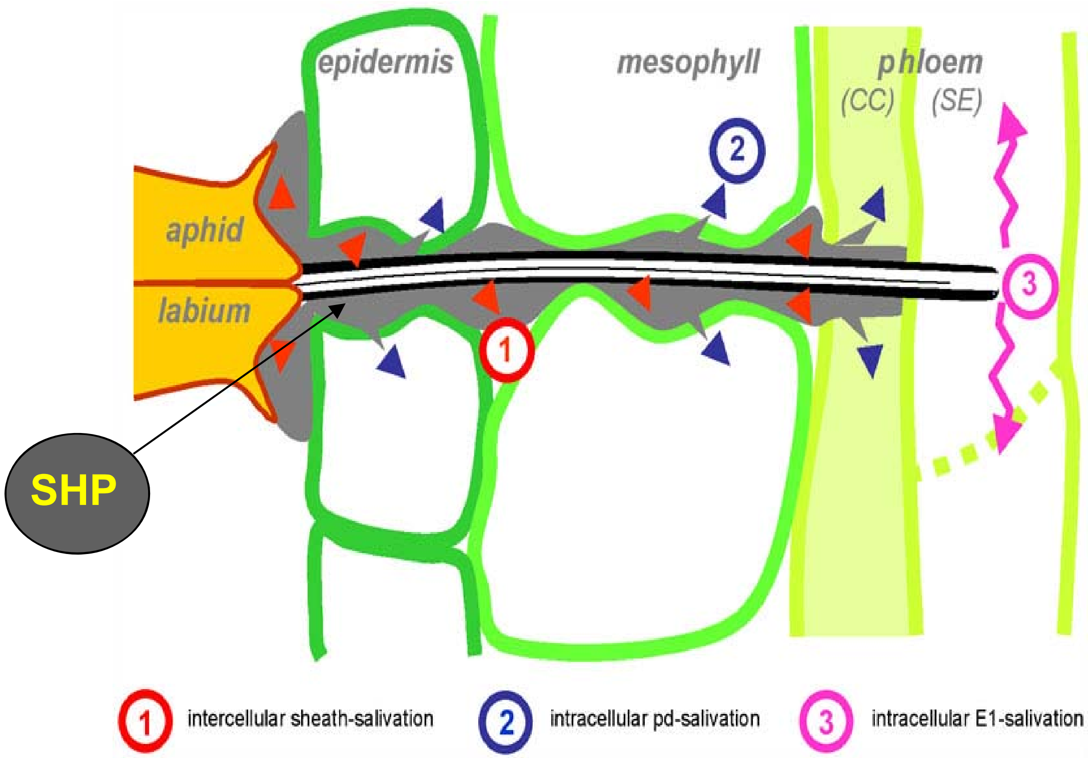
Example from the lab

Aphid target „SHEATH PROTEIN“ (SHP)

Target: *Sitobion avenae*



from Plant Physiology, USA (2012)

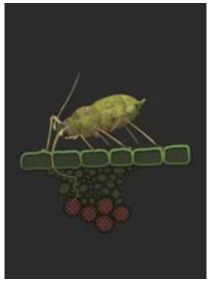


Tjallingii W F J. Exp. Bot. 2006;57:739-745

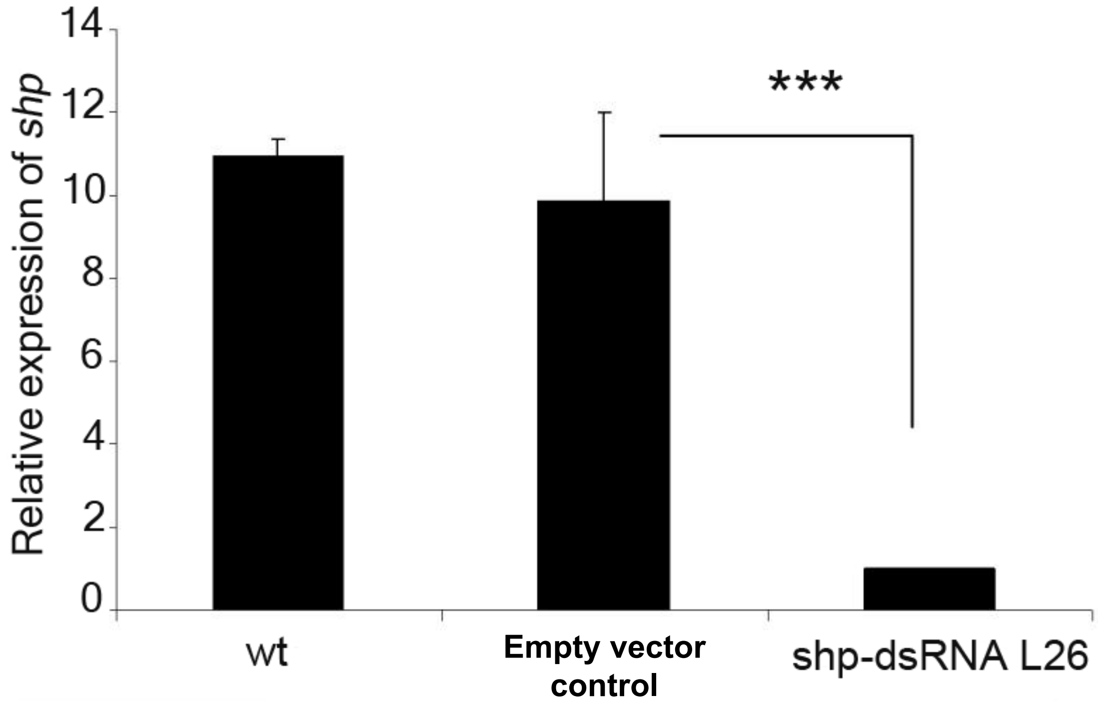
Abdellatef et al. 2015, Plant Biotech. J. 13:849

Aphid target „SHEATH PROTEIN“ (SHP)

HIGS



Sitobion avenae

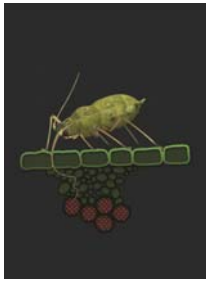


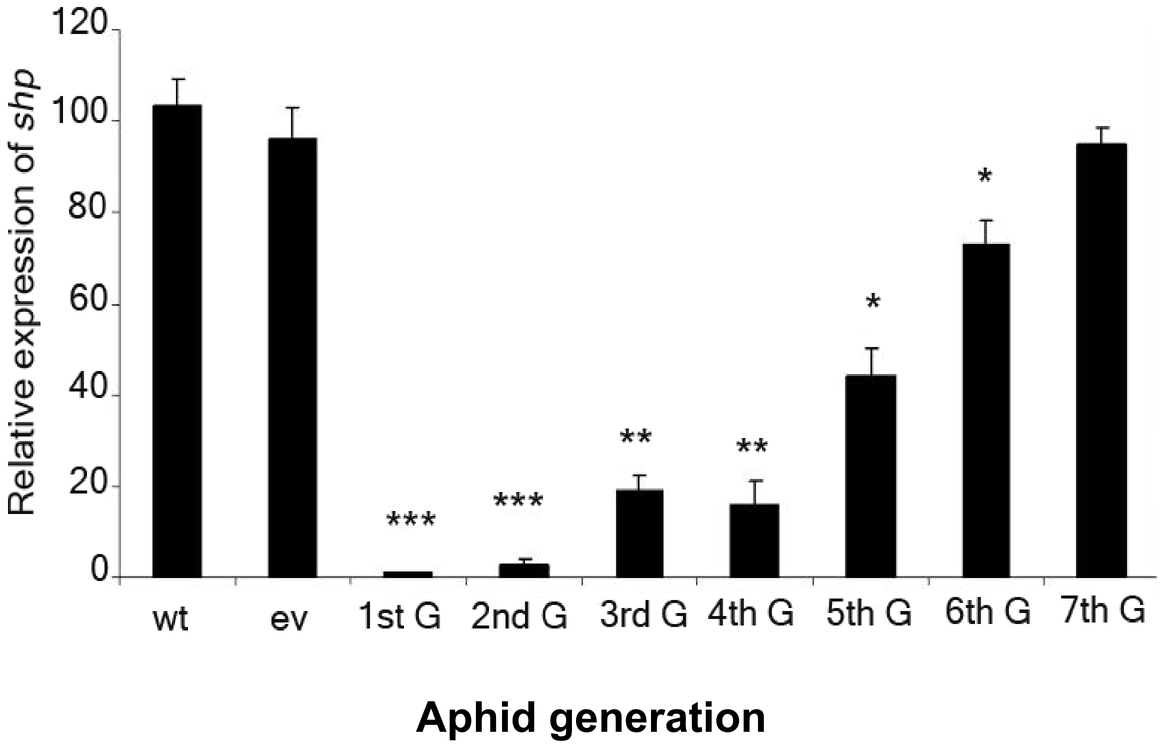
2 weeks on shp-dsRNA plants

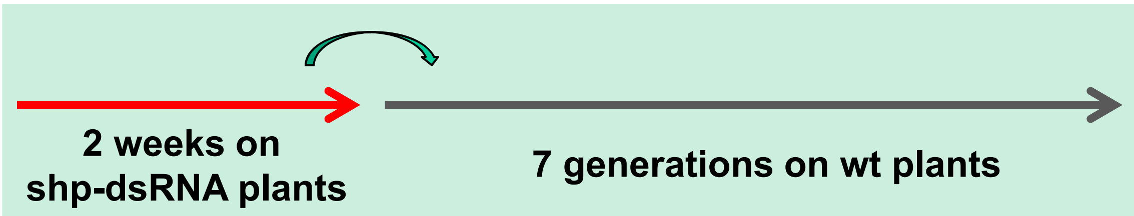
Abdellatef et al. 2015, Plant Biotech. J. 13:849

Transgenerational silencing = parental RNAi

HIGS







Abdellatef et al. 2014, Plant Biotech. J. 13:849

RNAi-based approach is realistic

SmartStax PRO corn
Monsanto/Bayer

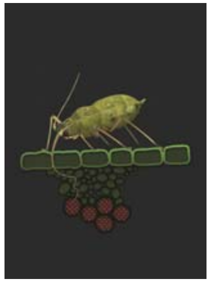
- **contains a *Snf7*-dsRNA construct**
- **together with three *crystal (Cry)* genes constructs**
  (from *Bacillus thuringiensis* (*Bt*) against Western corn rootworm, WCR)

Approved by CFIA in 2016 and EPA in 2017

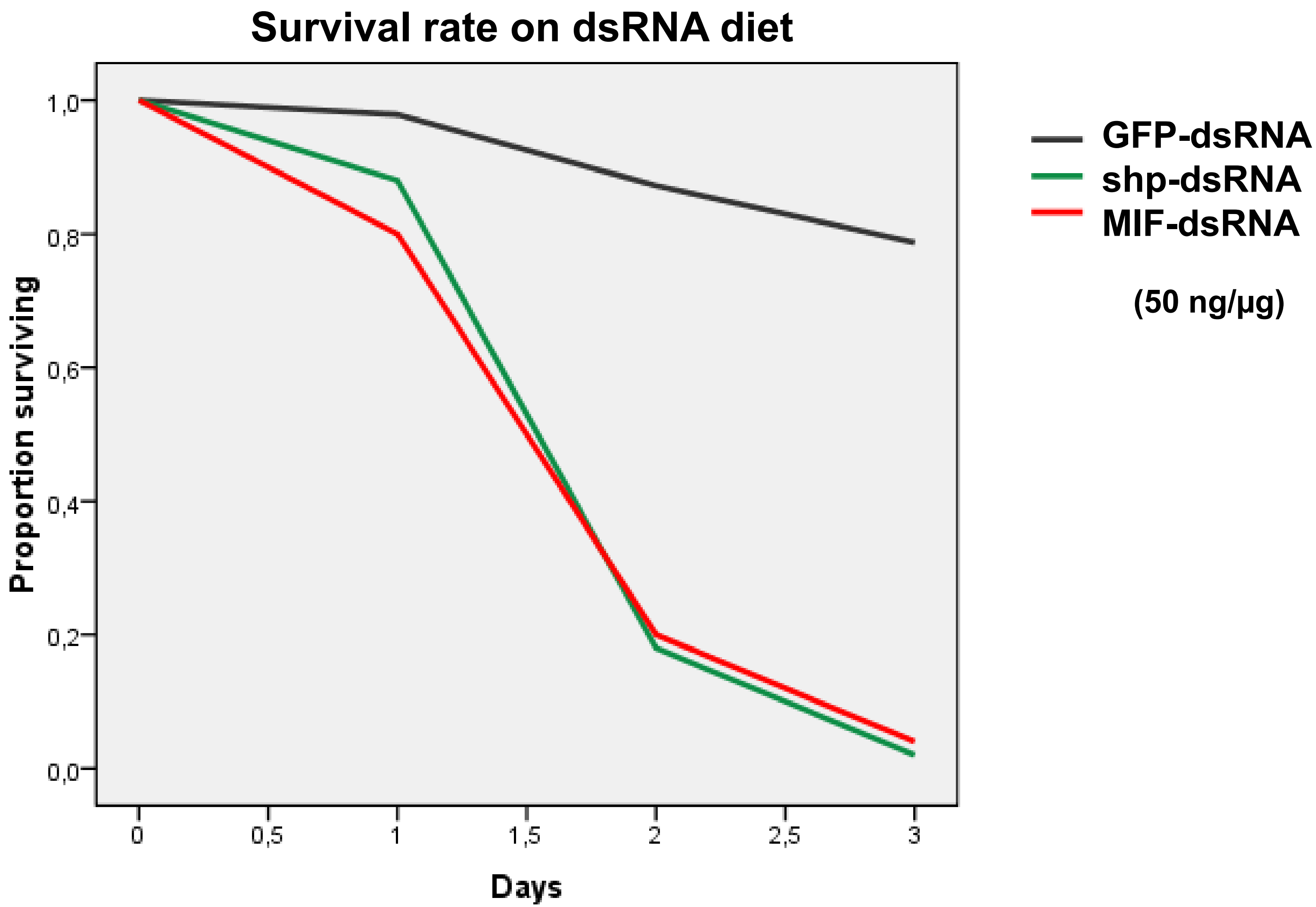
Head et al. 2017

Snf7 functions as a part of the vesicle transport system

dsRNA Feeding (environmental RNAi)

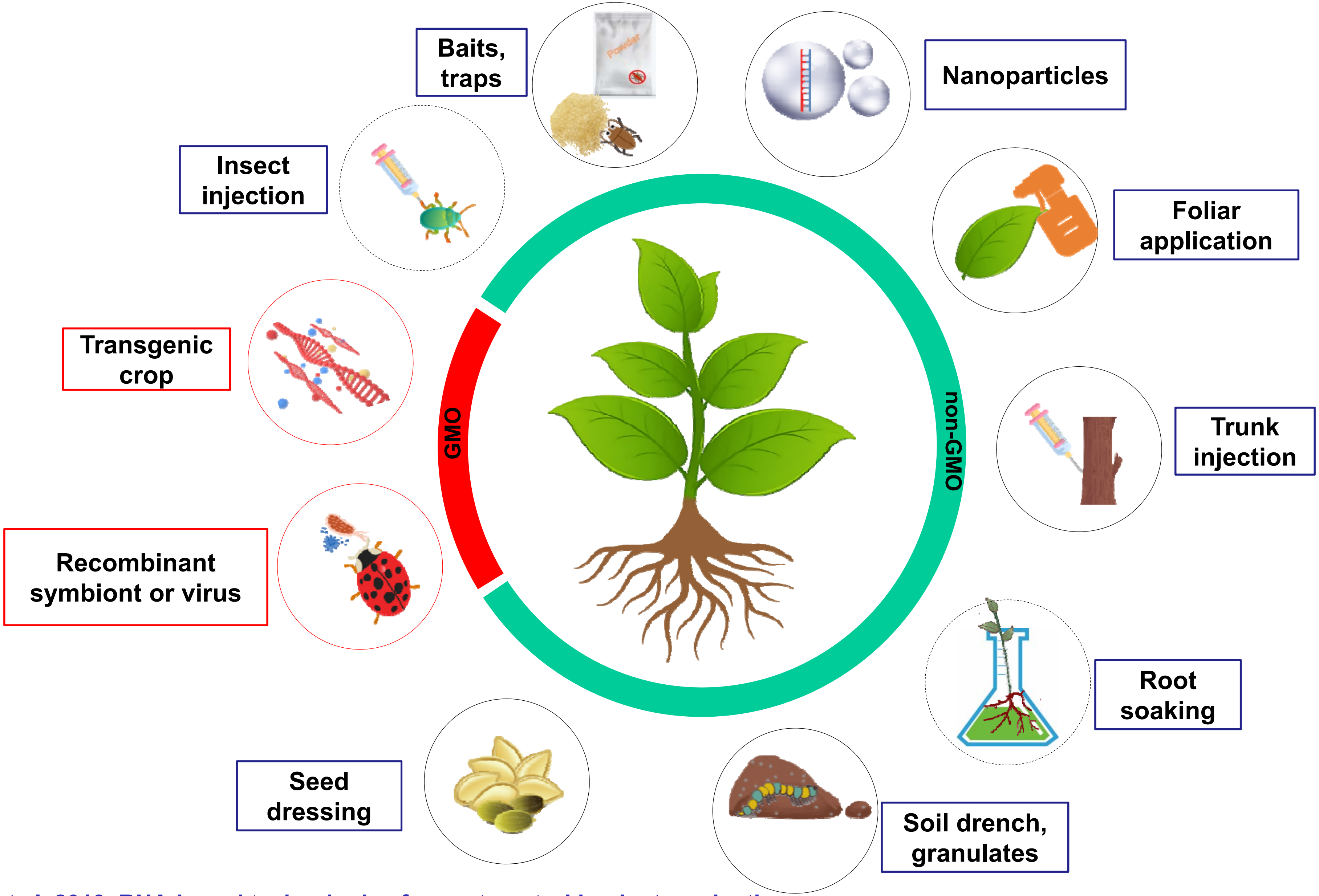


eRNAi



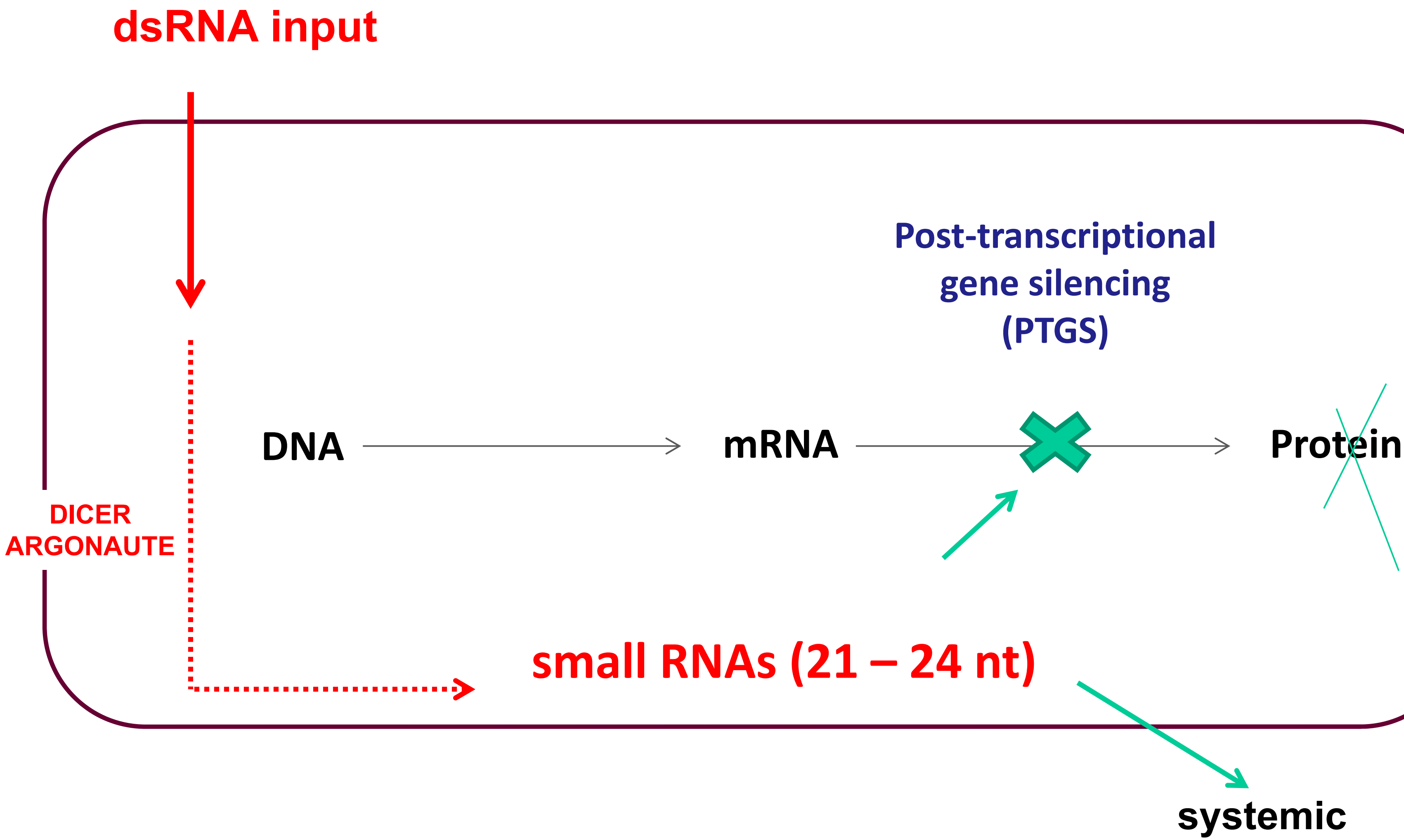
MIF: Macrophage migration inhibitor factor

RNA application for insect control



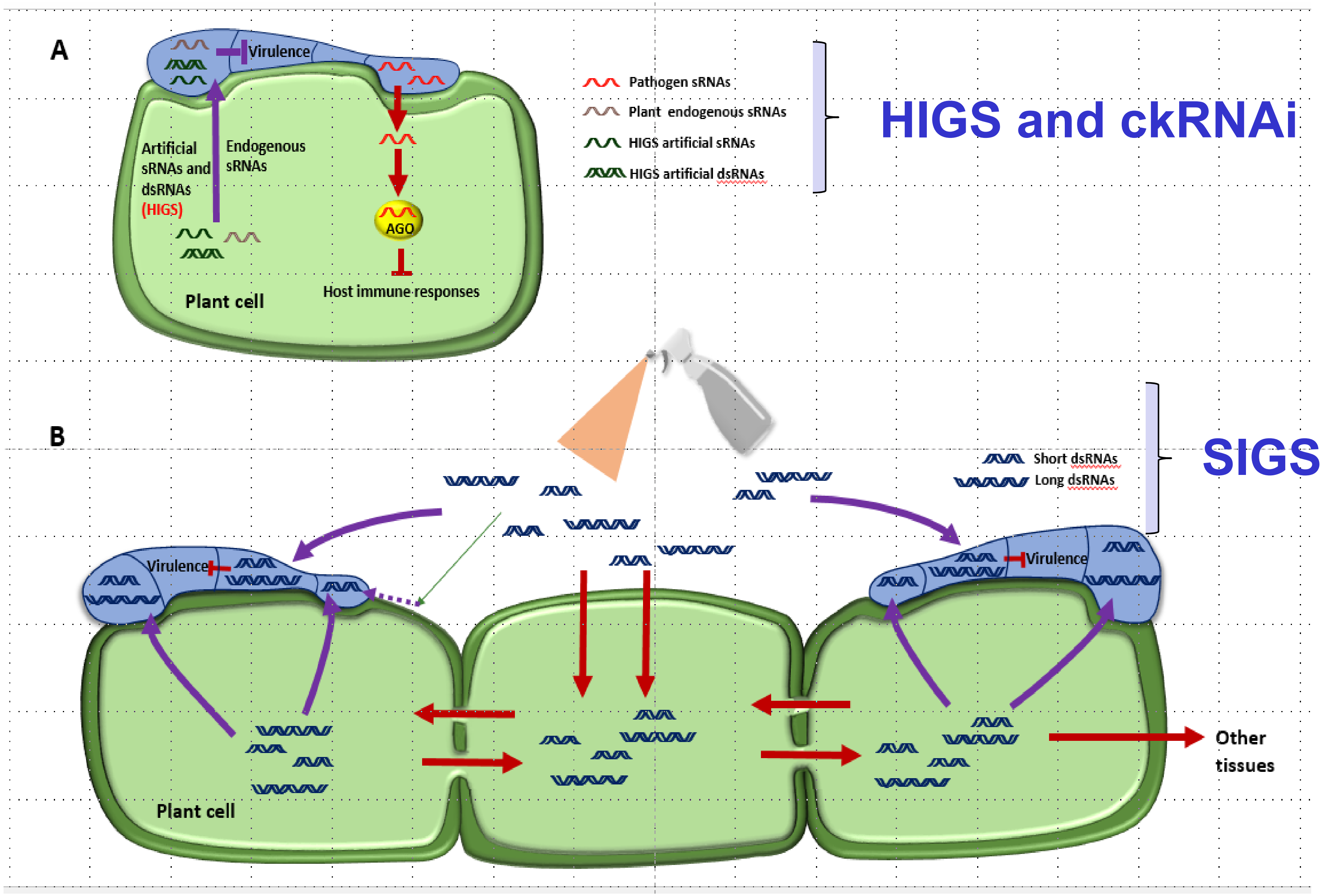
Liu et al. 2019. RNA-based technologies for pest control in plant production. Biotech. Adv.doi.org

Mechanism of target gene silencing with artificial RNA



Andrew Z. *Fire* and Craig C. Mello (Noble Prize 2006)

Mechanism of gene silencing
HIGS, SIGS and natural cross-kingdom (ck)RNAi

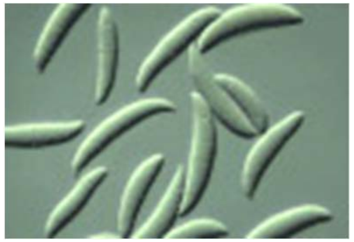


Parameters affecting RNAi efficiency	Putative underlying factors in insects and environment	Examples of insect species/order investigated
Copy number of core RNAi machinery genes	Genome evolution of the RNAi pathway: -Gene duplication -Gene loss	*Drosophila melanogaster;* Red flour beetle (*Tribolium castaneum*); Pea aphid (*Acyrthosiphon pisum*)
Presence of systemic RNAi	Existence of cell-to-cell movement of dsRNA (e.g. dsRNA channels; receptor-mediated endocytosis; dsRNA uptake and escape from vesicle)	*Drosophila melanogaster*; Red flour beetle (*Tribolium castaneum*); Western corn rootworm (*Diabrotica virgifera virgifera*)
Presence of pRNAi	-dsRNA uptake in oocytes -Secondary amplification -Epigenetic modifications directed by sRNA -Time and exposure of dsRNA treatment correlates with pRNAi strength	Red flour beetle (*Tribolium castaneum*); Green peach aphid (*Myzus persicae*); Grain aphid (*Sitobion avenae*); Western Corn Rootworm (*Diabrotica virgifera virgifera*)
Insect status	-Physiological status -Life stage -Tissues	Armyworm (*Spodoptera frugiperda*); Triatomine bug (*Rhodnius prolixus*); Colorado potato beetle (*Leptinotarsa decemlineata*); Lepidoptera; Desert locust (*Schistocerca gregaria*); Migratory locust (*Locusta migratoria*)
dsRNA stability/degradation	-Abundance of dsRNases/nucleases in insect fluids (saliva, gut lumen, haemolymph) -Insect gut pH -Soil bacteria/microbiome	Brown planthopper (*Nilaparvata lugens*); Fall armyworm (*Spodoptera frugiperda*); Tarnished plant bug (*Lygus lineolaris*); Pea aphid (*Acyrthosiphon pisum*); Tobacco cutworm (*Spodoptera litura*); Migratory locust (*Locusta migratoria*); American cockroach (*Periplaneta americana*); Giant mealworm beetle (*Zophobas atratus*); Asian corn borer (*Ostrinia furnacalis*); Brown marmorated stinkbug (*Halyomorpha halys*); Domestic silkmoth (*Bombyx mori*)

Liu et al. 2019. RNA-based technologies for pest control in plant production.
Biotech. Adv.doi.org

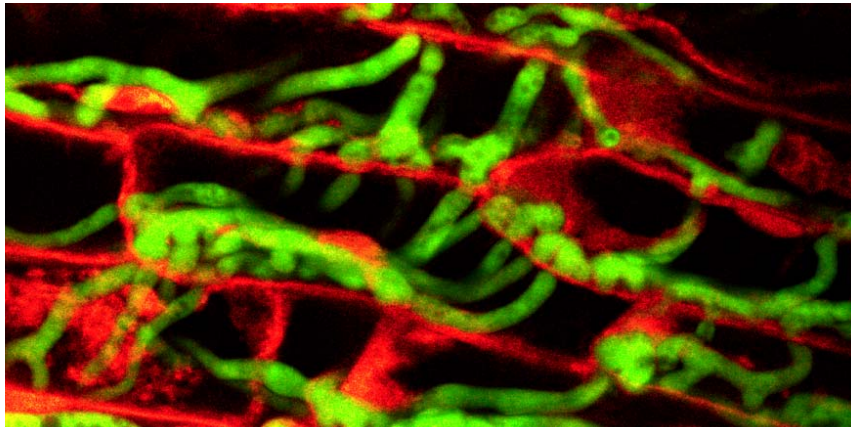
Fungal control by RNAi

Targeting the mycotoxin-producing fungus *Fusarium graminearum* by RNA



Macroconidia

Axenic culture



Necrotrophic growth

Head blight disease

Jansen et al. 2005 PNAS 102:16892

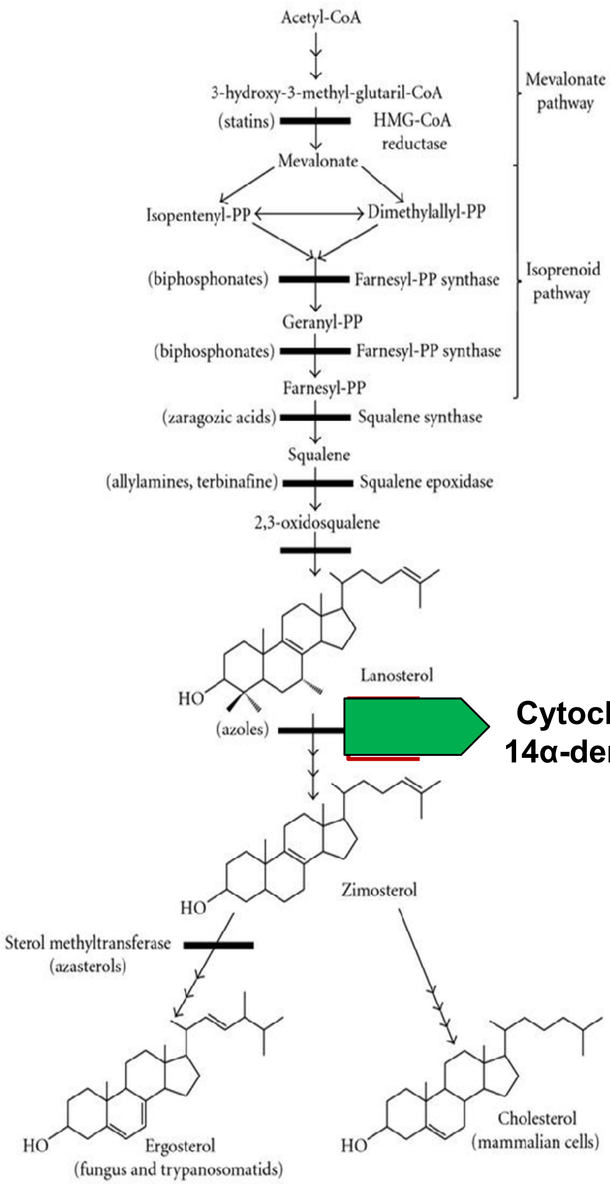
An „old“ fungicide target is a good target for dsRNA

Fusarium graminearum
has three *CYP51* genes
(Fan et al. 2013)

***FgCYP51B*:**
sterol 14α-demethylation

***FgCYP51A*:**
sterol 14α demethylase induced on ergosterol depletion

***FgCYP51C*:**
virulence on wheat ears



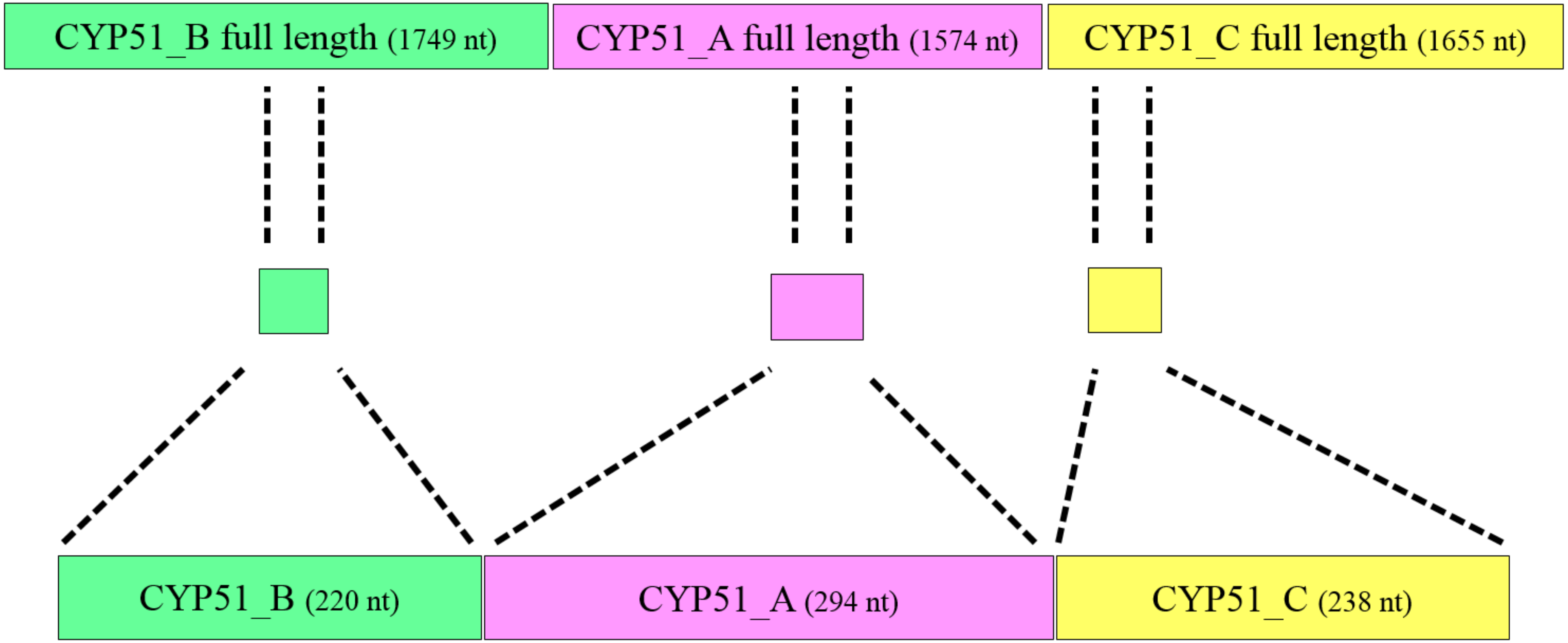
Ergosterol biosynthesis

Cytochrome P450 sterol 14α-demethylase (CYP51)

= Target of AZOLE fungicides:
sterol demethylation inhibitors (DMI)

De Souza et al. 2009

CYP51-dsRNA targets all three fungal *CYP51* genes



= 791 nt long

Control of *Fusarium graminearum* on *CYP51-dsRNA* expressing Arabidopsis

HIGS

Fusarium

Fusarium + dsRNA

Koch et al. 2013, PNAS 110:19324

dsRNA as fungicide?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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

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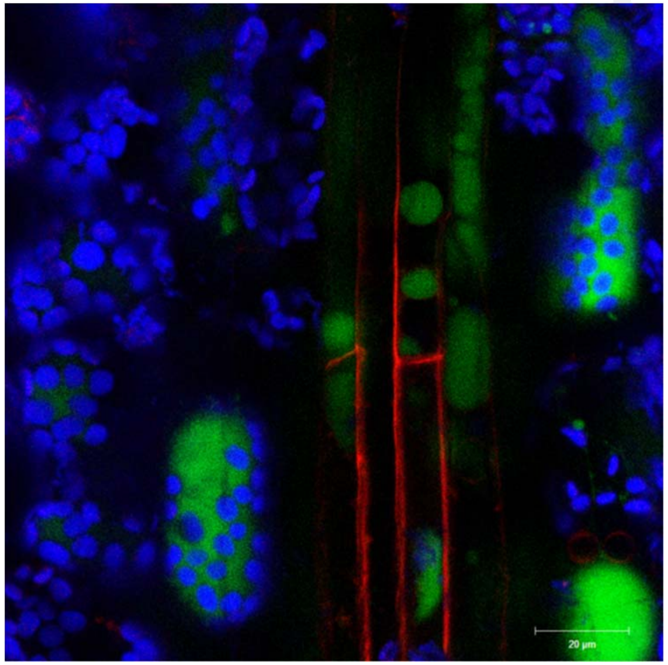
Abstract
Author Summary
Introduction
Results
Discussion
Materials and Methods
Supporting Information
Acknowledgments

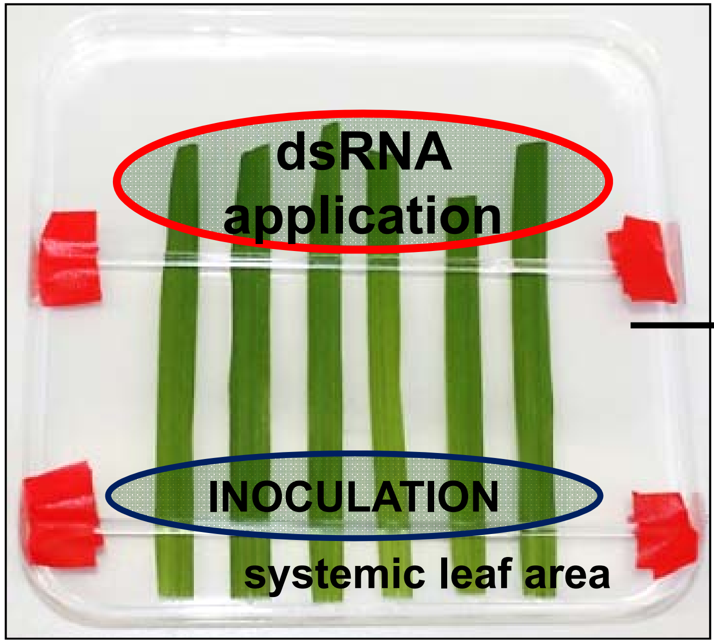
Abstract

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

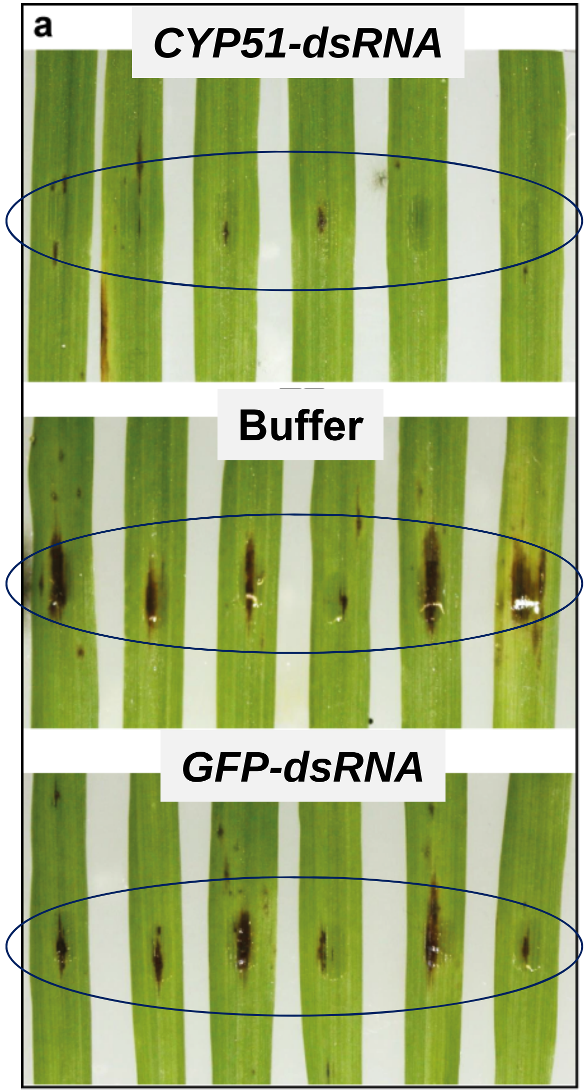


Koch et al. 2016, Plos Pathogens ppat.1005901

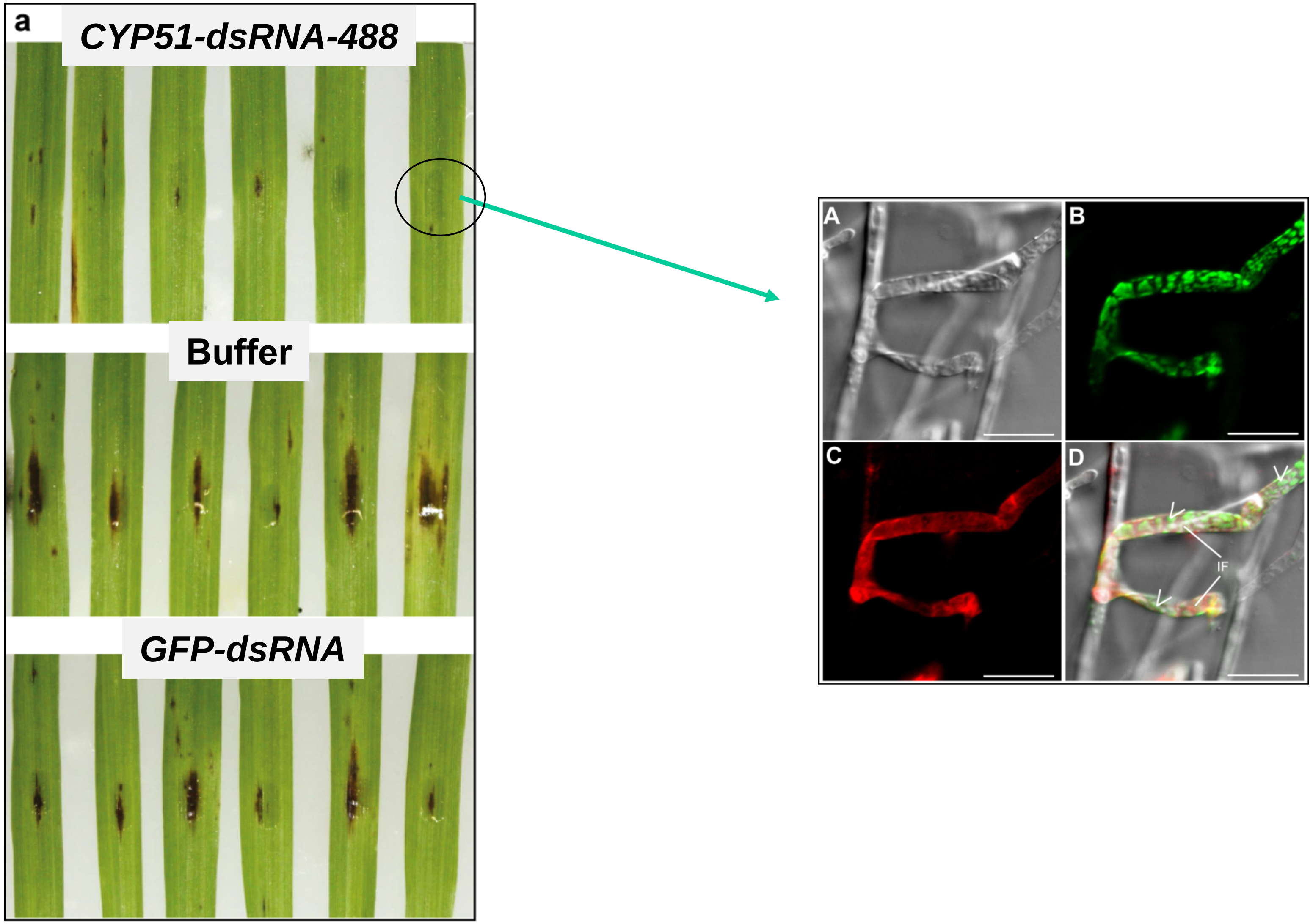
Spray-mediated systemic control of *Fusarium graminearum*



systemic leaf area



Uptake of *CYP51*-dsRNA$_{atto488}$ from the systemic leaf area



Fungi responsive to environmental RNAi

Fungus	Natural ckRNAi	HIGS	RNA spray	Treat	Activity	Ref.
Botrytis cinerea	Ath tomato	target: DICER	target: DICER	dsRNA siRNA foliar	reduced virulence	Weiberg et al. 2013 Wang et al. 2016
Verticillium dahliae	tomato	cotton target: *Vd*RGS1	n.d	-	reduced virulence	Wang et al. 2016 Xu et al. 2018
Fusarium graminearum	n.d. (hints)	targets: -CYP51 -Chitin synthase	target: CYP51	dsRNA siRNA foliar, liquid culture	reduced virulence	Koch et al. 2016 Cheng et al. 2015
Fusarium culmorum	n.d.	target glucan synthase	target: CYP51	dsRNA liquid culture	reduced growth	Koch et al. 2018 Chen et al. 2015

Fungi responsive to environmental RNAi

Fungus	Natural ckRNAi	HIGS	RNA spray	Treat	Activity	Ref.
Sclerotinia sclerotiorum	n.d.	n.d.		dsRNA foliar	reduced virulence	McLoughlin et al. 2018
Puccinia striiformis	 wheat	 targets: -MAPKK -*CPK1* -CP	n.d.	-	reduced virulence	Wang et al. 2017 Zhu et al. 2017 Qi et al. 2017 Li et al. 2018
Puccinia triticina	n.d.	 Targets: MAPK, cyclophilin	n.d.	-	reduced virulence	Panwar et al. 2017
Zymoseptoria tritici	n.d. (no hints)			dsRNA axenic culture	none	Kettles et al. 2018

Fungi responsive to environmental RNAi

Fungus	Natural ckRNAi	HIGS	RNA spray	Treat	Activity	Ref.
Blumeria graminis	n.d.	☺ Targets: effectors	n.d.	-	reduced virulence	Nowara et al. 2010 Pliego et al. 2013
Magnaporthe oryzae	☺ Brachypodium many hints	☺	n.d.		reduced virulence	Zanini et al. BioRxiv Zhu et al. 2017
Aspergillus flavus		☺ aflatoxin biosynthesis				Sharma et al. 2018

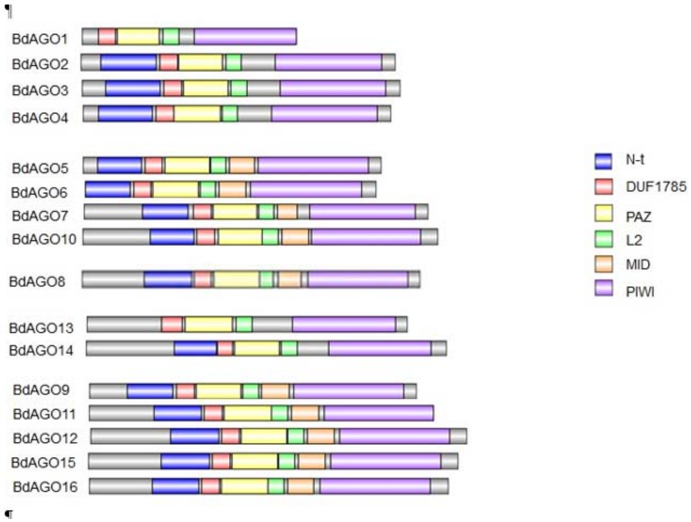
Fundamental questions to be addressed in view of future applications

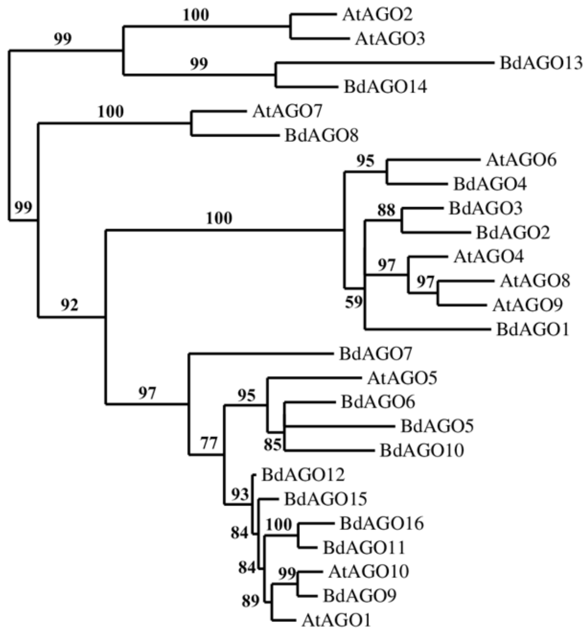
- **Factors involved in RNA processing (CROP PLANTS) ?**

- **RNA release / transport / uptake**

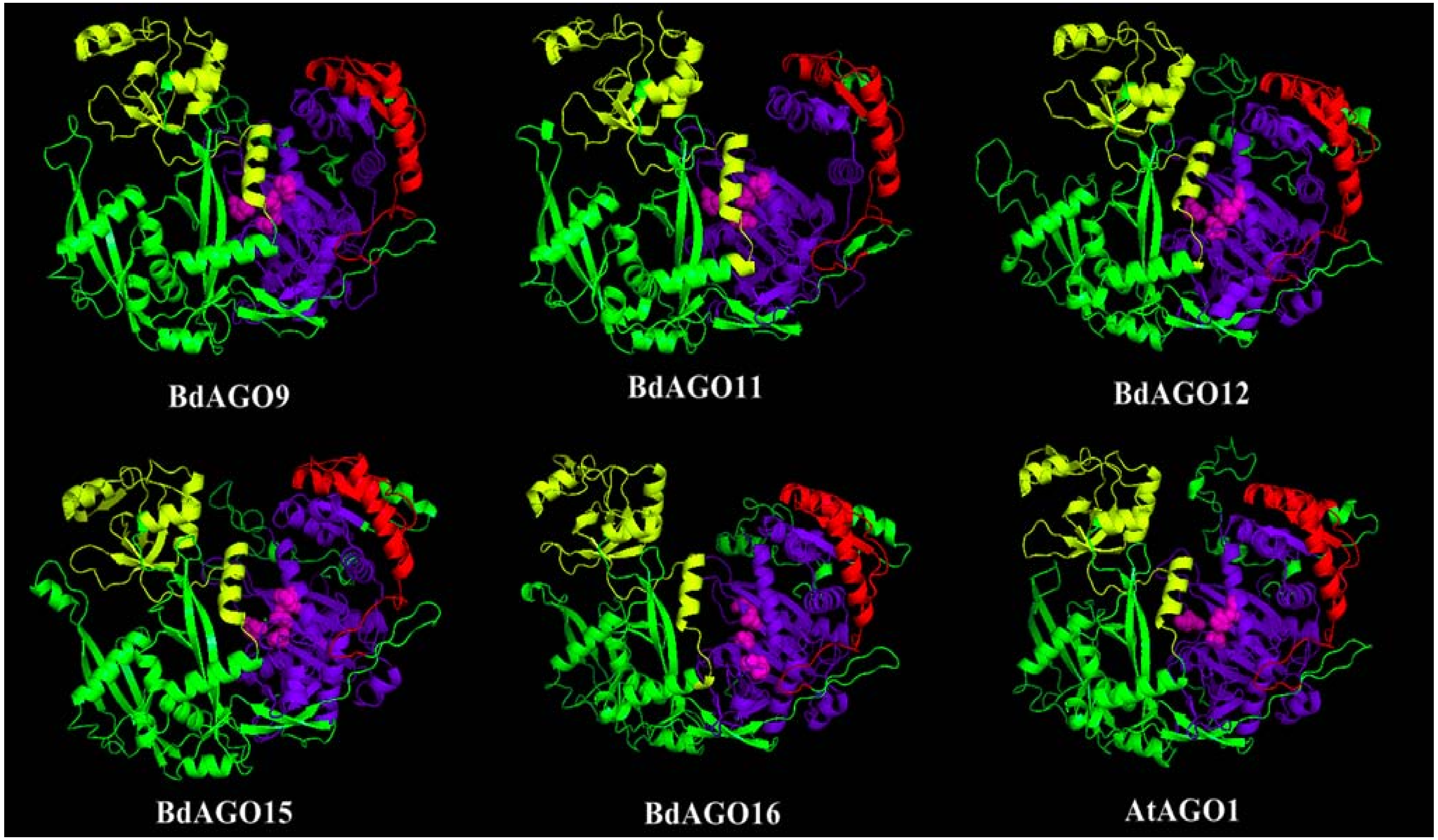
RNAi genes in the grass model *Brachypodium distachyon*

- **Arabidopsis has 10 AGOs and 4 DCLs**
- **Brachypodium has <u>16</u> AGOs and <u>6</u> DCLs**



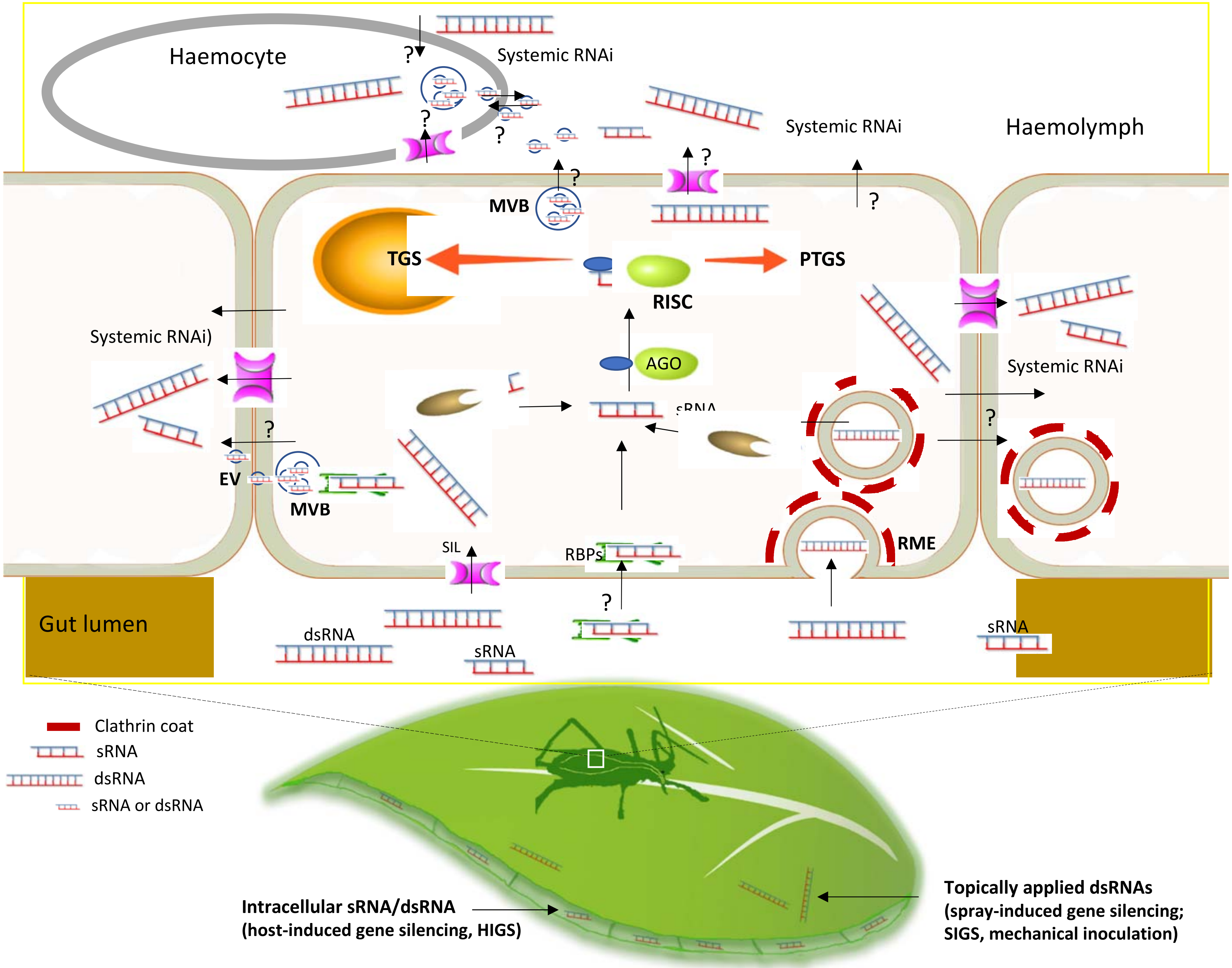


Brachypodium AGOs in the *AtAGO1* clade



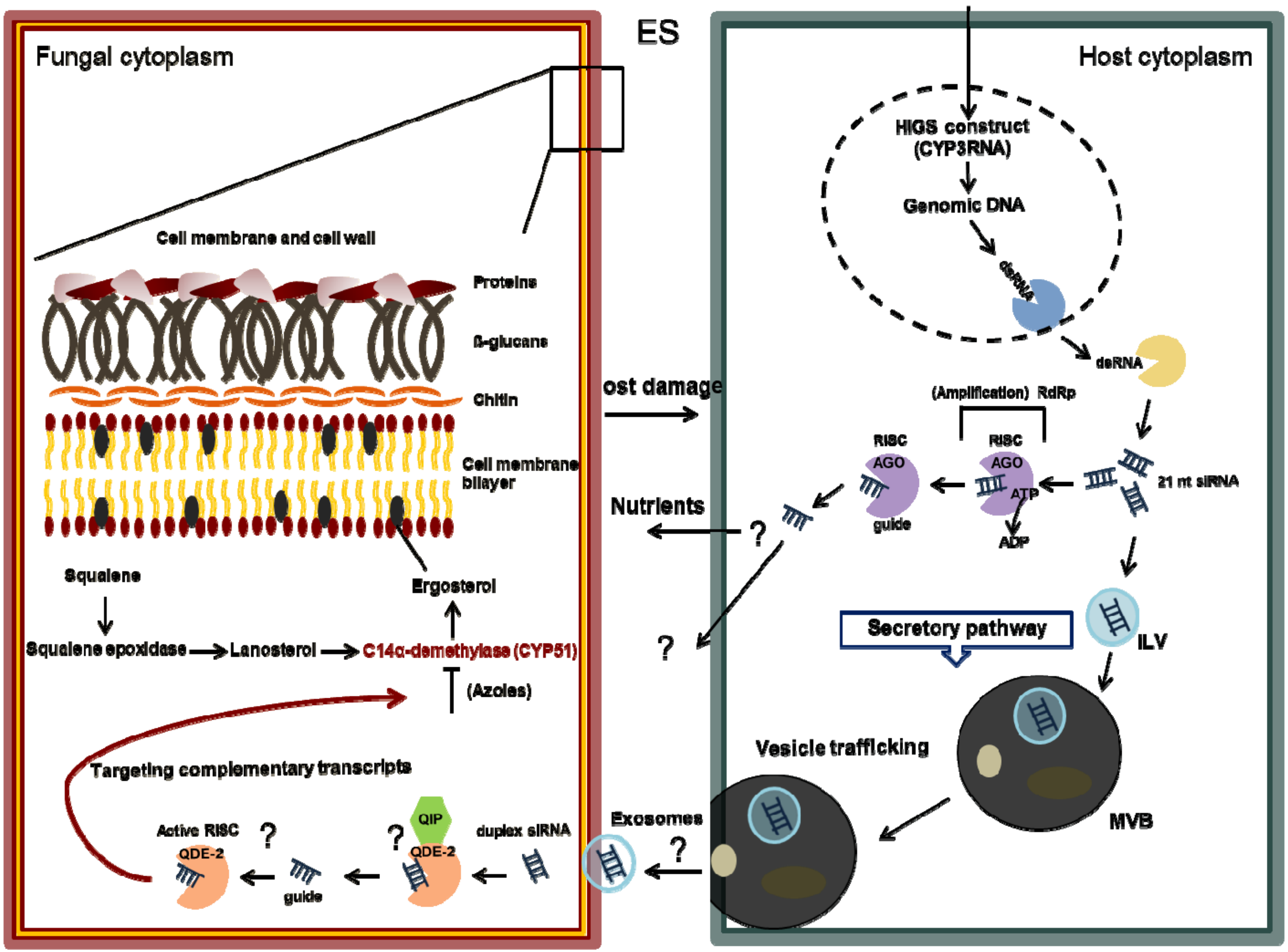
3D structure prediction for BdAGO9, BdAGO11, BdAGO12, BdAGO15 and BdAGO16 as modelled by Swiss Prot. AtAGO1 3D structure prediction is displayed as the closest homolog in Arabidopsis. PAZ (yellow), MID (red) and PIWI (blue) domains as predicted by SMART and PFAM displayed. The catalytic tetrad within the PIWI domain (DEDH) marked by magenta spheres. Visualization by PyMOL.

How is sRNA and dsRNA transferred from plants to insects and microbial pathogens ?

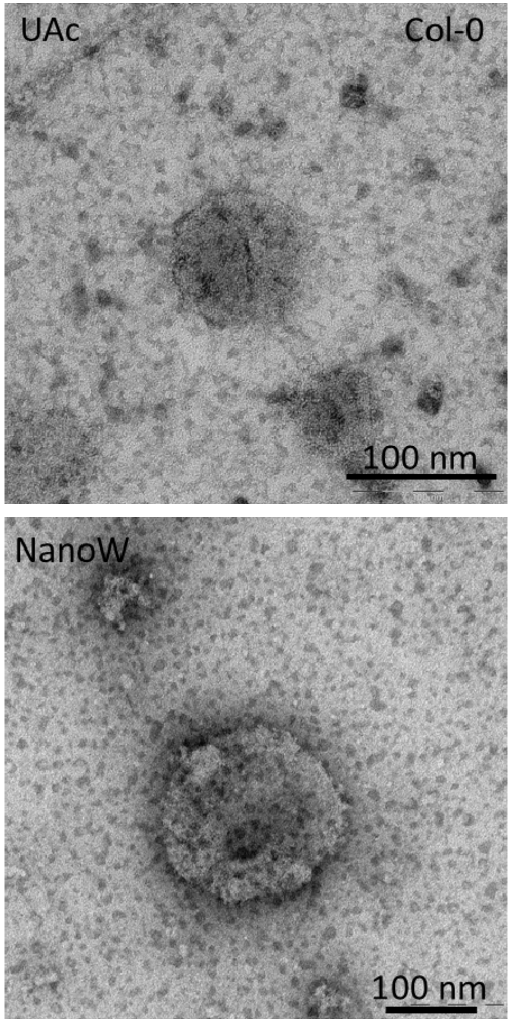


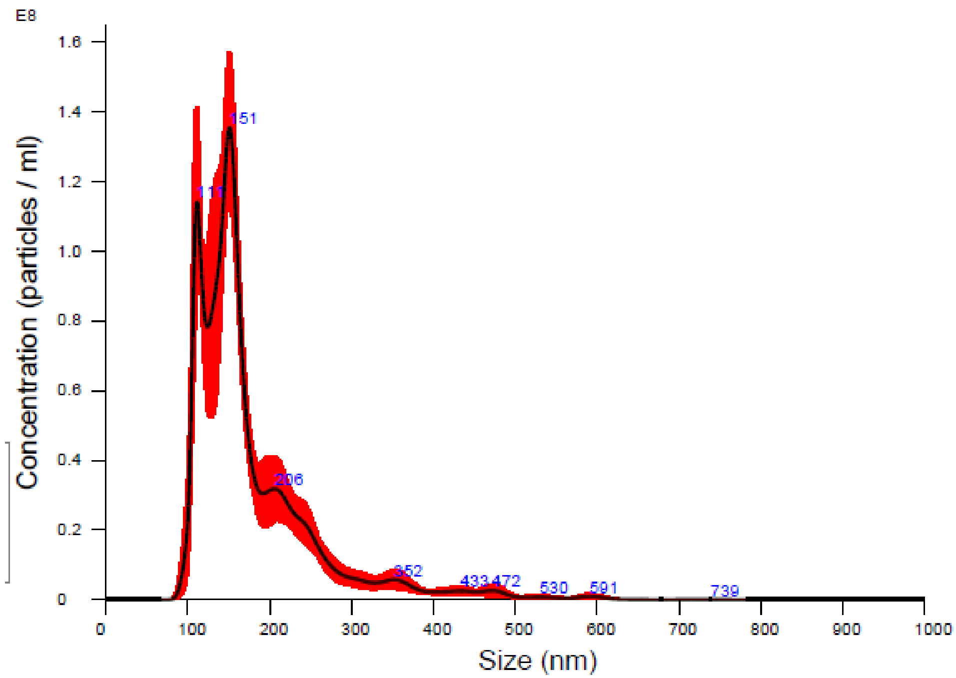
Haemocyte
Systemic RNAi
Systemic RNAi
Haemolymph
MVB
TGS
PTGS
RISC
Systemic RNAi)
AGO
Systemic RNAi
sRNA
EV
MVB
RME
SIL
RBPs
Gut lumen
dsRNA
sRNA
sRNA
Clathrin coat
sRNA
dsRNA
sRNA or dsRNA
Intracellular sRNA/dsRNA (host-induced gene silencing, HIGS)
Topically applied dsRNAs (spray-induced gene silencing; SIGS, mechanical inoculation)

Transfer of RNA during HIGS (?)

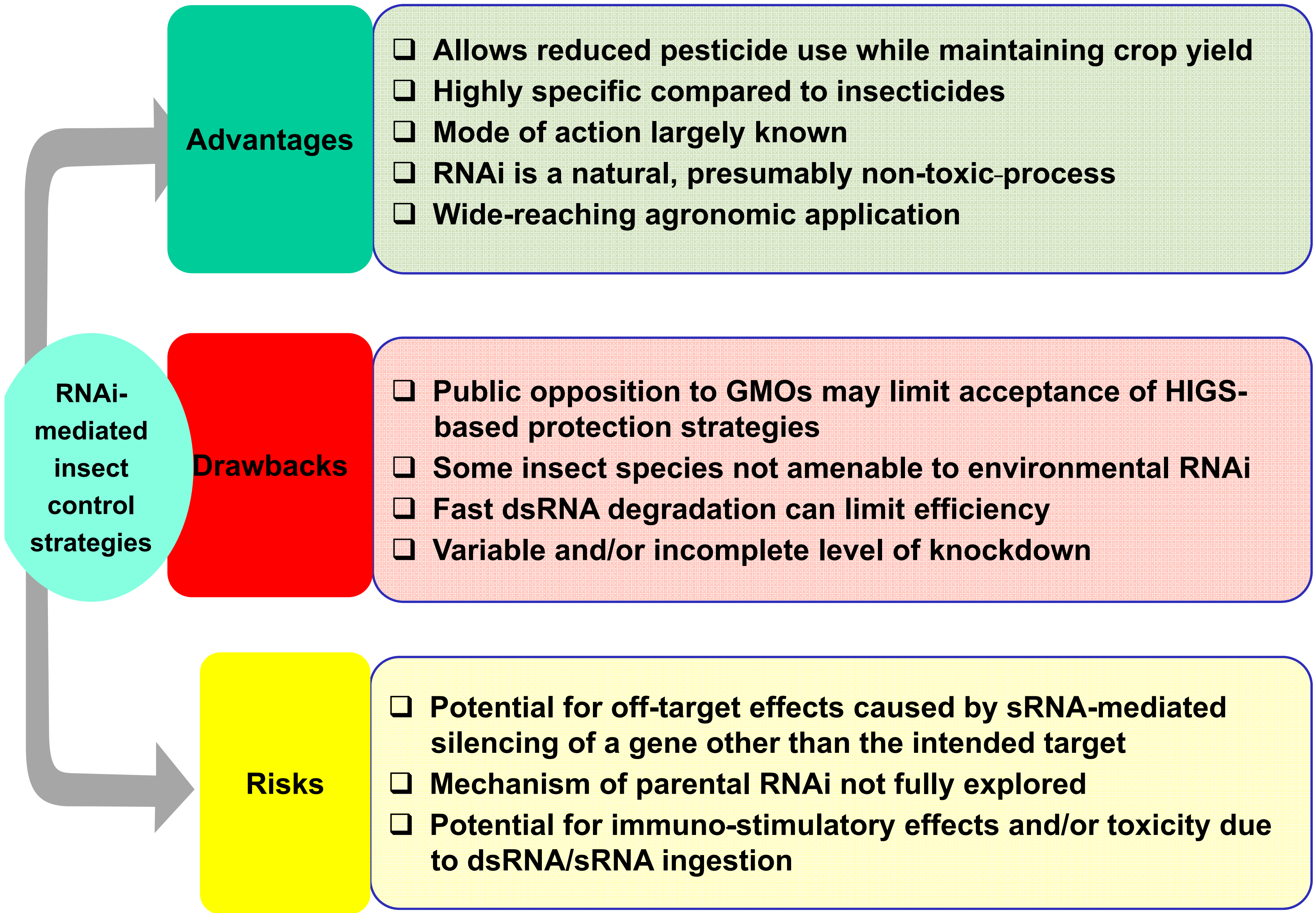


Purification of exosomes from Arabidopsis expressing CYP3RNA





Averaged particle concentration per particle size purified from *Arabidopsis thaliana* HIGS plants (black line) and +/- 1 standard error (red). Purified extracellular vesicles have an average size of 139 +/- 7.7 nm the sample concentration was $1.0x10^{10}$ +/- $5.2x10^{8}$ particles/mL.


RNAi-mediated insect control strategies
Advantages
Allows reduced pesticide use while maintaining crop yield
Highly specific compared to insecticides
Mode of action largely known
RNAi is a natural, presumably non-toxic-process
Wide-reaching agronomic application
Drawbacks
Public opposition to GMOs may limit acceptance of HIGS-based protection strategies
Some insect species not amenable to environmental RNAi
Fast dsRNA degradation can limit efficiency
Variable and/or incomplete level of knockdown
Risks
Potential for off-target effects caused by sRNA-mediated silencing of a gene other than the intended target
Mechanism of parental RNAi not fully explored
Potential for immuno-stimulatory effects and/or toxicity due to dsRNA/sRNA ingestion

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Shaoshuai Liu

BASF The Chemical Company

PLANT 2030

Deutsche Forschungsgemeinschaft DFG

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