JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

PHYTO Phytopathologie JLU Gießen

RESISTENZTAGUNG FULDA 2021 XII

Neue Pflanzenschutzkonzepte: RNAi-Technologie

Wie realistisch ist der zukünftige Einsatz von RNA im Pflanzenschutz – eine datenbasierte Einschätzung

Ena Šečić, Dr. rer. nat.

Contact: Ena.Secic@agrar.uni-giessen.de

Host - Induced Gene Silencing (HIGS)

Spray - Induced Gene Silencing (SIGS)

Examples (and requirements) for RNAi-based plant protection against:

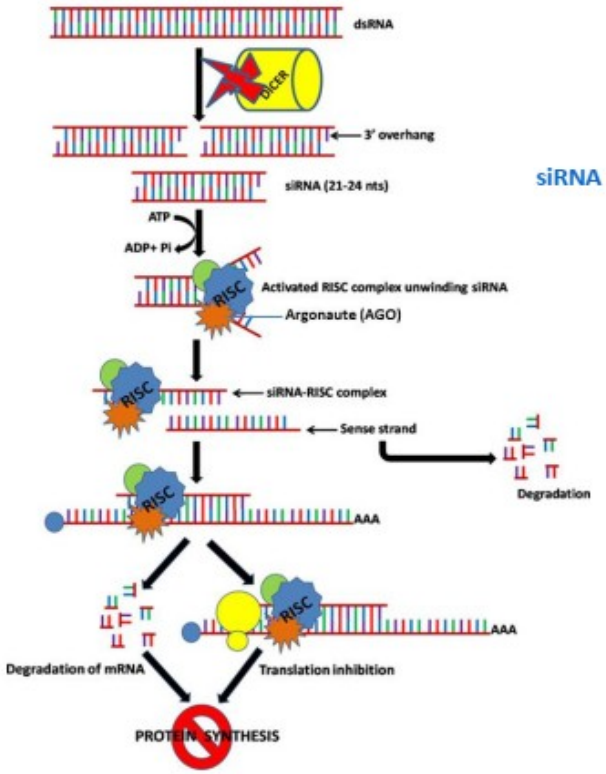
- Insects
- Fungi

- RNAi-based plant protection - outlook

A quick reminder...

siRNA = small interfering RNA

RISC = RNA-induced silencing comlex

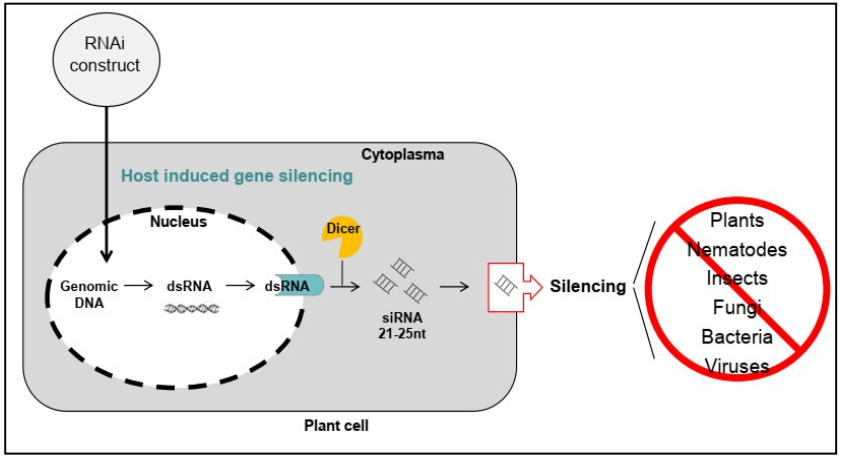


Jagtap et al., 2011

Host – Induced Gene Silencing (HIGS)
Example: *Fusarium graminearum*

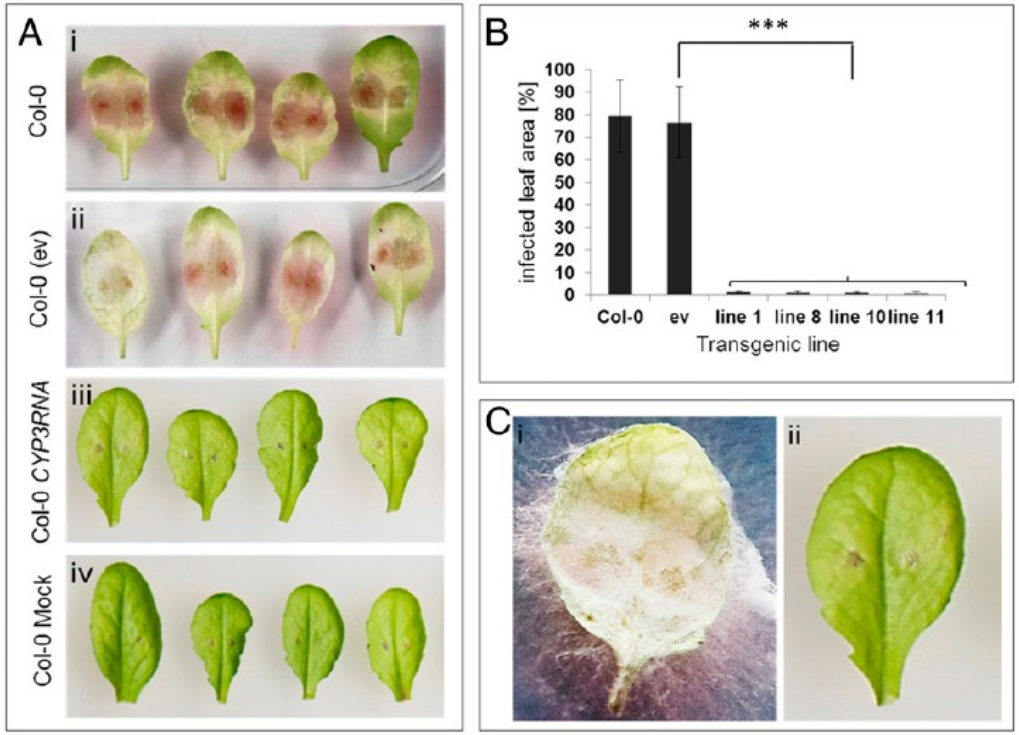
Transgenic approach (**Nowara et al. (2010) Plant Cell 22:3130-3141**)

Application: GMO Maize vs WCR (Head et al., 2017)



Host-induced gene silencing of cytochrome P450 lanosterol C14α-demethylase–encoding genes confers strong resistance to *Fusarium* species

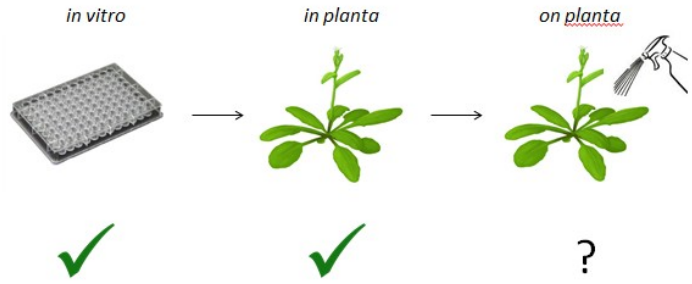
Aline Koch[a], Neelendra Kumar[a], Lennart Weber[b], Harald Keller[c], Jafargholi Imani[a], and Karl-Heinz Kogel[a,1]

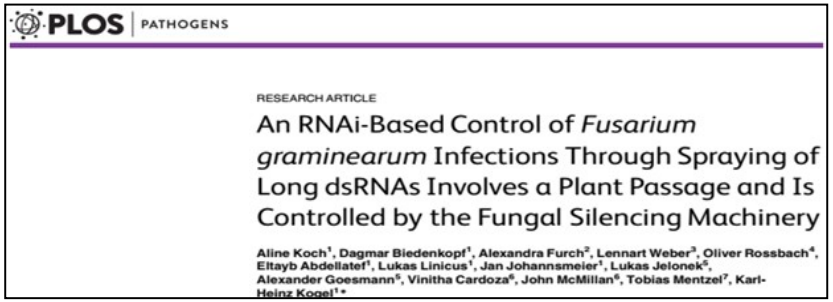


Spray-Induced Gene Silencing (SIGS)
Topical application of RNA – a GMO-free approach

Non-GMO approach **(Koch et al., 2016)**

Patent: Bayer (Monsanto): patent WO 2011/112570 (Sammons et al., 2011)

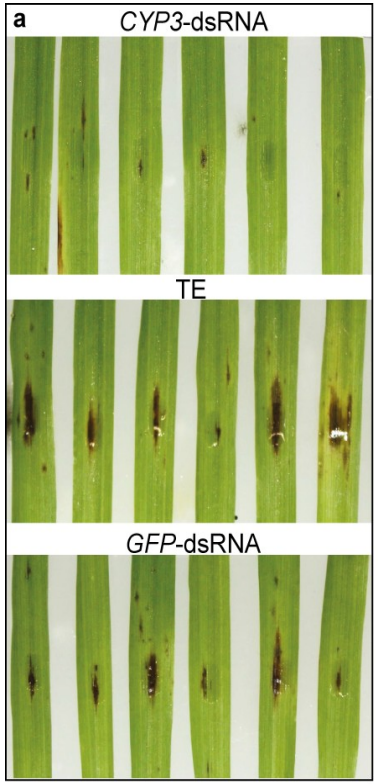


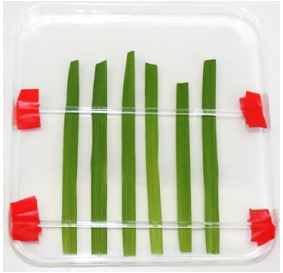

PLOS | PATHOGENS

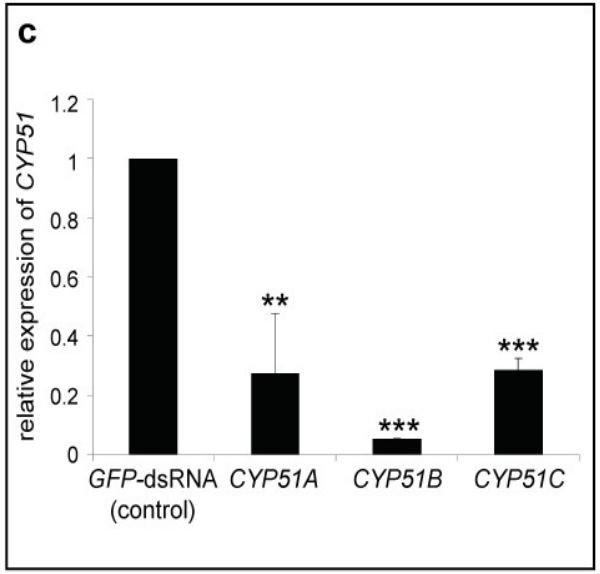
RESEARCH ARTICLE

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[1], Dagmar Biedenkopf[1], Alexandra Furch[2], Lennart Weber[3], Oliver Rossbach[4], Eltayb Abdellatef[1], Lukas Linicus[1], Jan Johannsmeier[1], Lukas Jelonek[5], Alexander Goesmann[5], Vinitha Cardoza[6], John McMillan[6], Tobias Mentzel[7], Karl-Heinz Kogel[1]*







Non-GMO approach **(Wang et al., 2016)**

Patent: Bayer (Monsanto): patent WO 2011/112570 (Sammons et al., 2011)

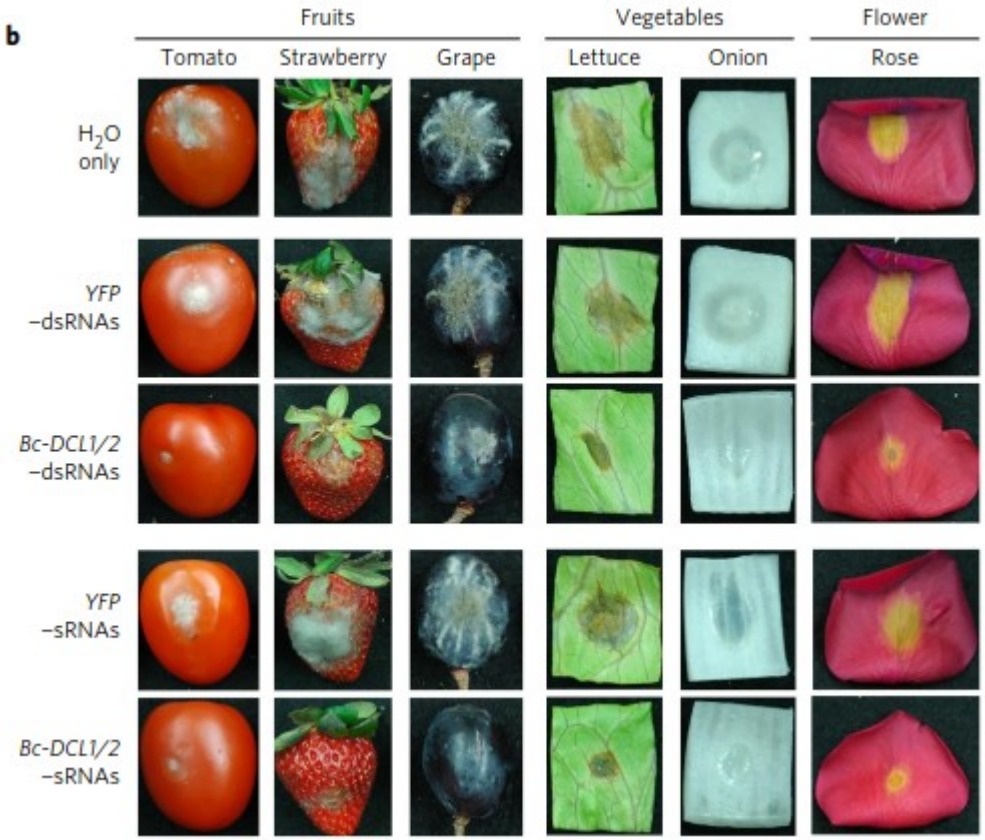
nature plants

ARTICLES

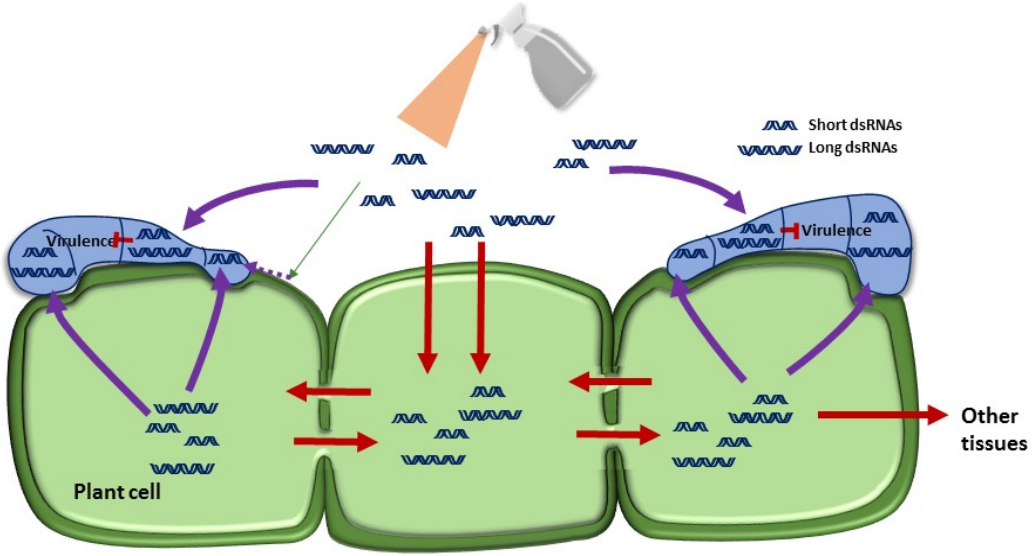
PUBLISHED: 19 SEPTEMBER 2016 | ARTICLE NUMBER: 16151 | DOI: 10.1038/NPLANTS.2016.151

Bidirectional cross-kingdom RNAi and fungal uptake of external RNAs confer plant protection

Ming Wang[1], Arne Weiberg[1†], Feng-Mao Lin[2], Bart P. H. J. Thomma[3], Hsien-Da Huang[2] and Hailing Jin[1*]



HIGS and SIGS applications
Challenges and outlook



Adjusted from: Cai et al. 2018. Cross-Kingdom RNAi - nature's blueprint for modern crop protection technology.

dsRNA design strategies (target(s))?

Stability of dsRNA (formulation potential)

Off-target prediction and precautions

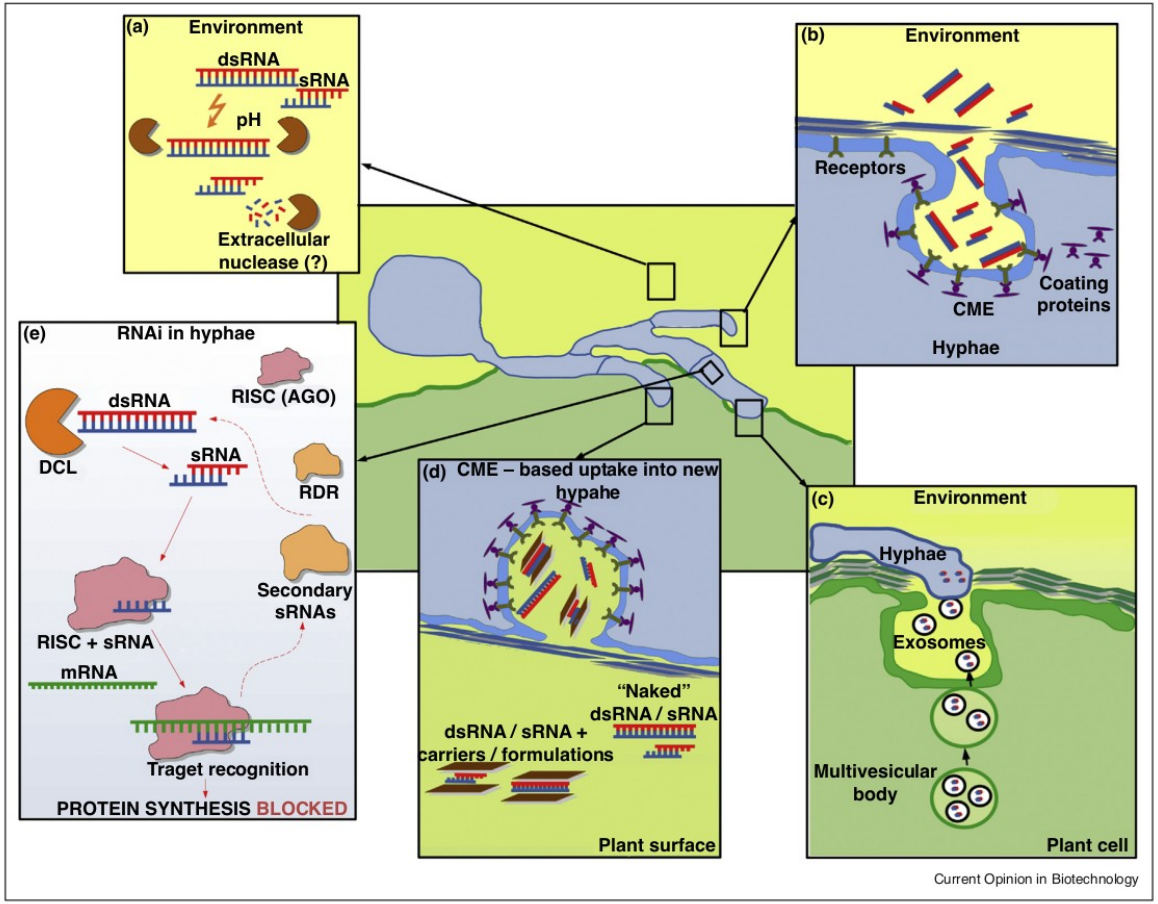
Transformation availability?

Emergence of resistant/tolerant microbes/pests?

Accessibility for application and cost considerations

Requirements for fungal uptake of dsRNA and gene silencing in RNAi-based crop protection strategies

Ena Šečić and Karl-Heinz Kogel



RNAi-based crop protection strategy against fungi – obstacles and experimental approaches

i)Stability before uptake

ii)Silencing efficiency after uptake

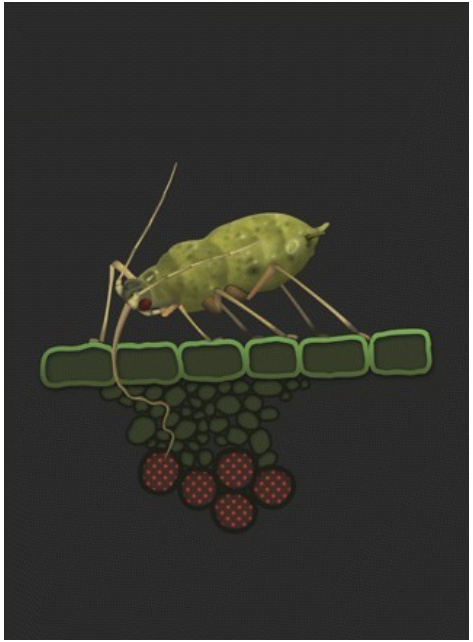
iii)Maintenance/stability of the silencing mechanism (durability)

Not all fungi contain functional RNAi machinery, nor do they (seem to) all uptake environmental RNA!

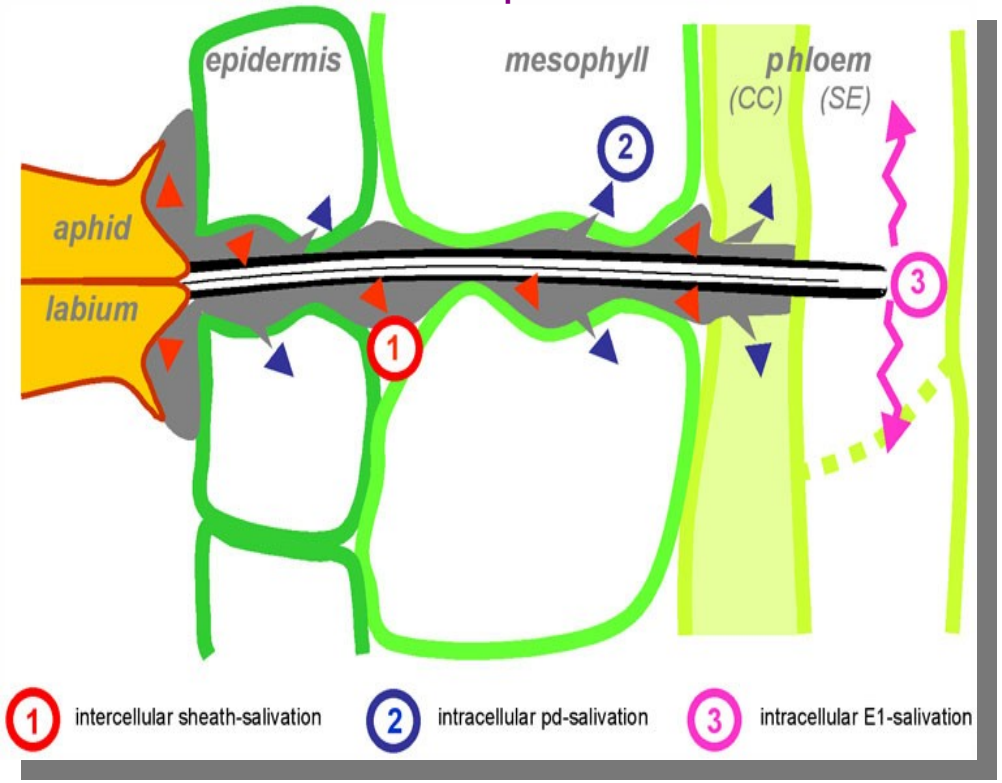
Contact: Ena.Secic@agrar.uni-giessen.de

Control of insects by dsRNA
Example: *Sitobion avenae*

Aphid target „SHEATH PROTEIN“ (shp)



from Plant Physiology, USA (2012)



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

Control of insects by dsRNA

Example: *Sitobion avenae*, HIGS Transgenerational Silencing

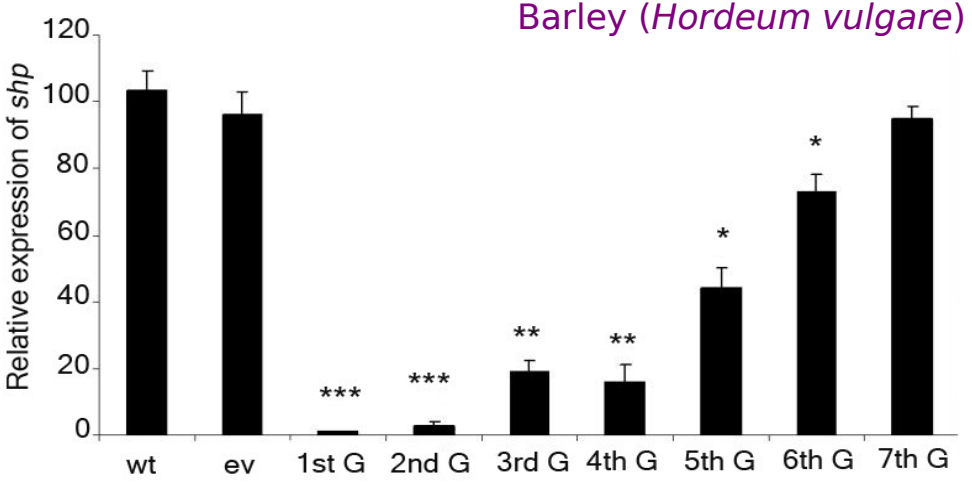
Plant Biotechnology Journal Open Access
aab Association of Applied Biologists
SEB

Research Article | Open Access

Silencing the expression of the salivary sheath protein causes transgenerational feeding suppression in the aphid *Sitobion avenae*

Eltayb Abdellatef, Torsten Will, Aline Koch, Jafargholi Imani, Andreas Vilcinskas, Karl-Heinz Kogel

Barley (*Hordeum vulgare*)

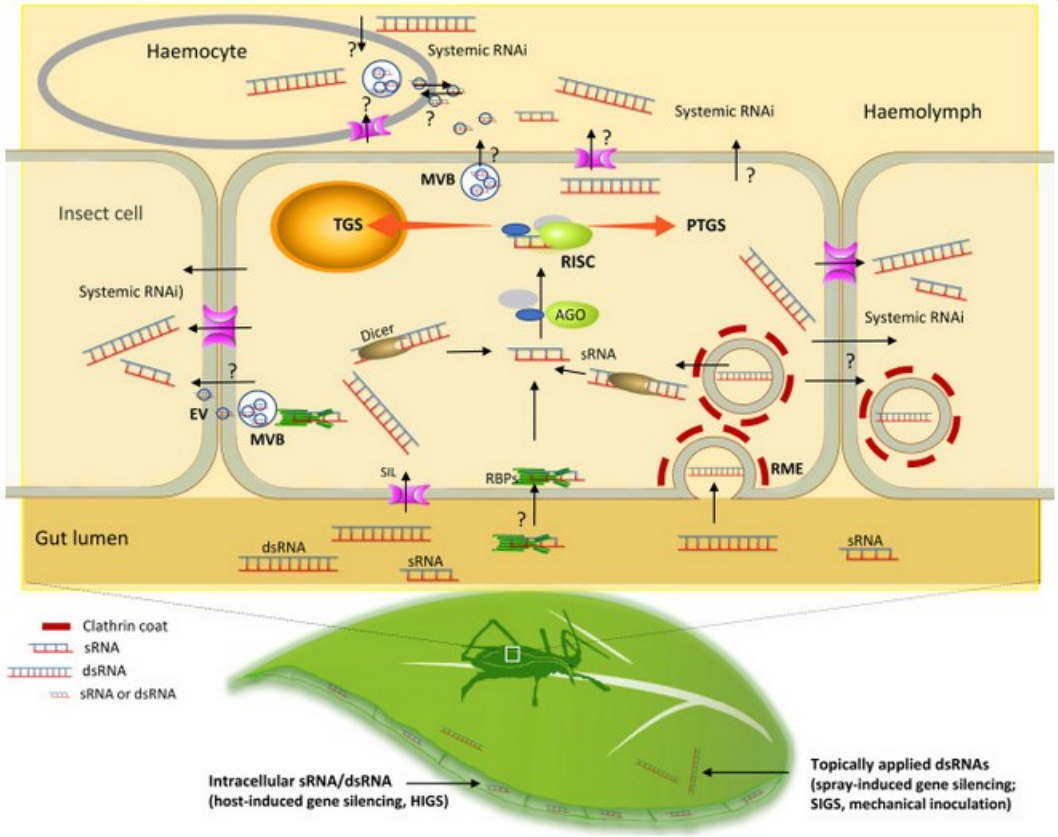


2 weeks on shp-dsRNA plants

7 generations on wt plants

RNA-based technologies for insect control in plant production

Shaoshuai Liu [a], Maelle Jaouannet [b], D'Maris Amick Dempsey [a], Jafargholi Imani [a], Christine Coustau [b], Karl-Heinz Kogel [a]



RNAi-based crop protection strategy against insects – obstacles

i)RNA length (>60 nt for some insects)

ii)RNA transport (formulations)

iii)RNA stability (nucleases)

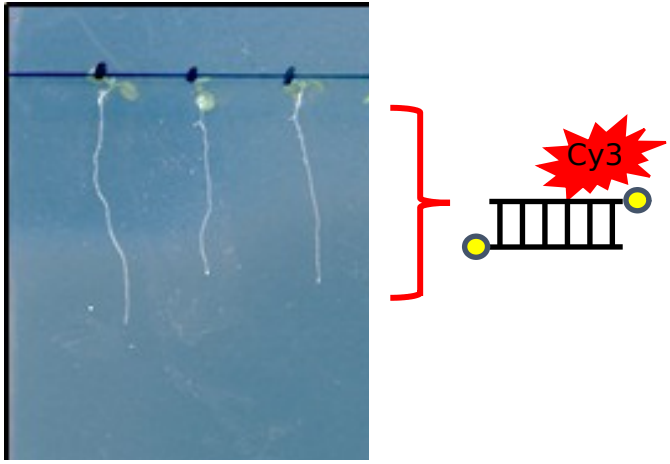
What about exogenous plant uptake? - Labeled dsRNA reaches the xylem

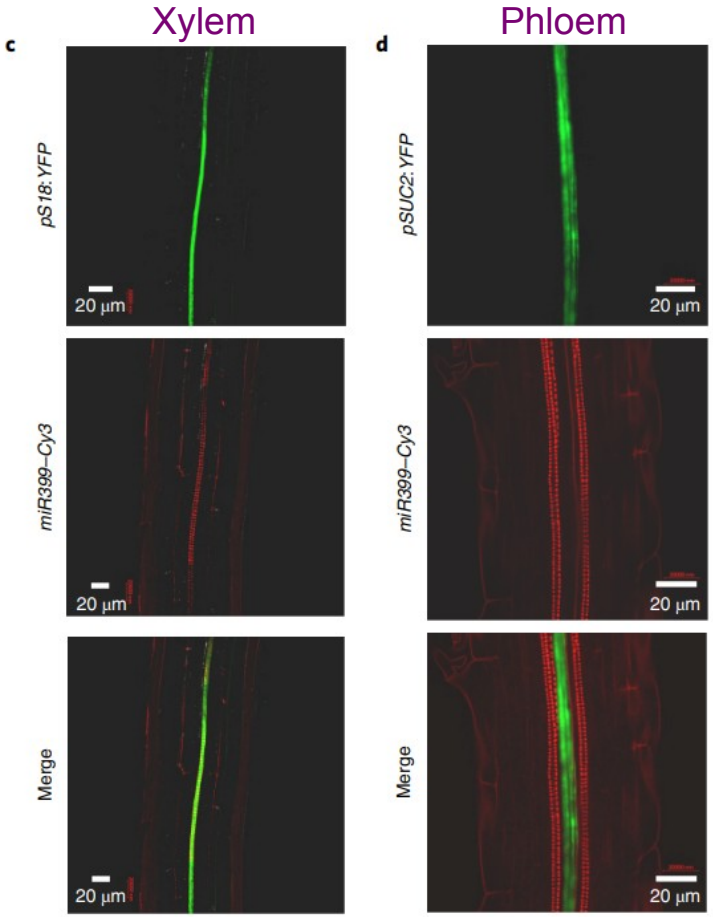
nature plants
ARTICLES
https://doi.org/10.1038/s41477-021-01005-w
Check for updates

OPEN

Exogenous miRNAs induce post-transcriptional gene silencing in plants

Federico Betti[1,6], Maria Jose Ladera-Carmona[1,6], Daan A. Weits[1], Gianmarco Ferri[2], Sergio Iacopino[3], Giacomo Novi[1], Benedetta Svezia[1], Alicja B. Kunkowska[1], Antonietta Santaniello[4], Alberto Piaggesi[4], Elena Loreti[5] and Pierdomenico Perata[1]





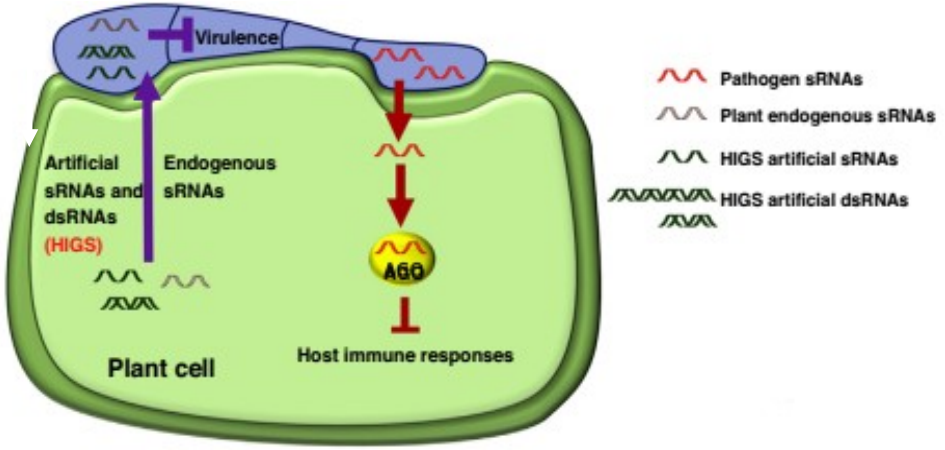
Natural RNA exchange between plants and microbes (not just pathogens!) - Cross-kingdom RNAi

Fighting pathogens and pests by means of non-coding RNA

Insect/Pest Control

Fungal Pathogens

Cross-kingdom communication – detection and implications



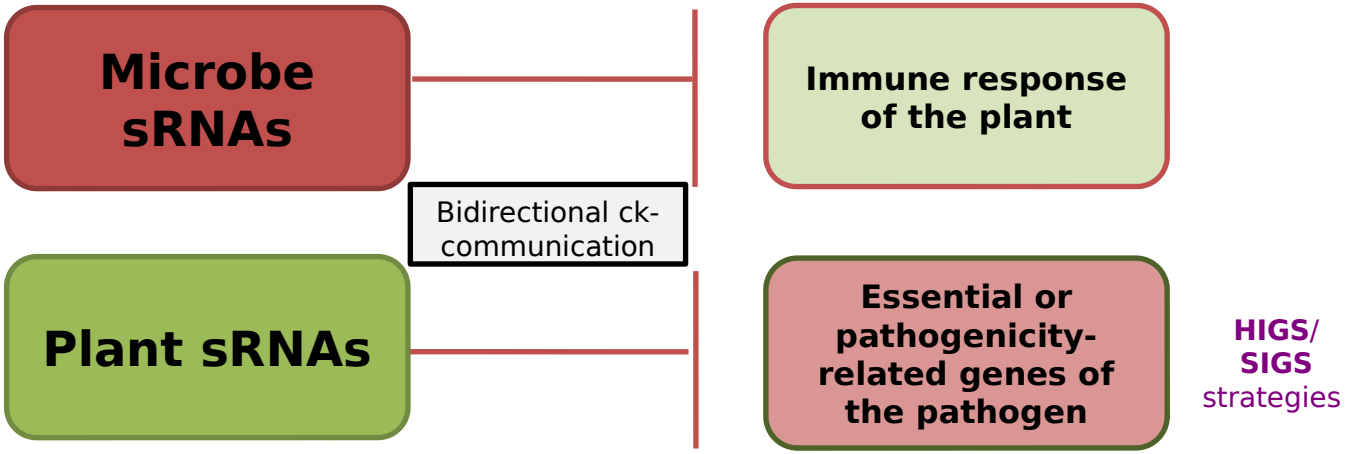
Adjusted from: Cai et al. 2018. Cross-Kingdom RNAi - nature's blueprint for modern crop protection technology.

RESEARCH ARTICLE Open Access

A novel plant-fungal association reveals fundamental sRNA and gene expression reprogramming at the onset of symbiosis

Ena Šečić[1], Silvia Zanini[1], Daniel Wibberg[2], Lukas Jelonek[3], Tobias Busche[2], Jörn Kalinowski[2], Sabrine Nasfi[1], Jennifer Thielmann[1], Jafargholi Imani[1], Jens Steinbrenner[1] and Karl-Heinz Kogel[1*]

Natural RNA exchange between plants and microbes (not just pathogens!) - Cross-kingdom RNAi



Open questions:

- Transport (vesicle and/or protein bound)
- Stability
- Detection and presence across the plant-microbe interaction spectrum

Arabidopsis apoplastic fluid contains sRNA- and circular RNA–protein complexes that are located outside extracellular vesicles

Hana Zand Karimi,[1] Patricia Baldrich,[2] Brian D. Rutter,[1] Lucía Borniego,[1] Kamil K. Zajt,[1] Blake C. Meyers[2,3] and Roger W. Innes[1,*,†]

How realistic **is** the future use of RNAi-based plant protection?

- What is the plant – pathogen system?
- Insects AND fungi?
- HIGS as an available, but not applicable strategy?
- SIGS and the delivery challenge?
- Formulations (and how far can they bring us)?
- Stability (in and on planta)?
- Extremely useful for plant-pathogen systems that don't have major chemical options
- Production cost?
- Is emergence of resistant/tolerant pests/pathogens possible?
- How wide-spread is cross-kingdom communication in the first place?

Contact: Ena.Secic@agrar.uni-giessen.de

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

PHYTO Phytopathologie JLU Gießen

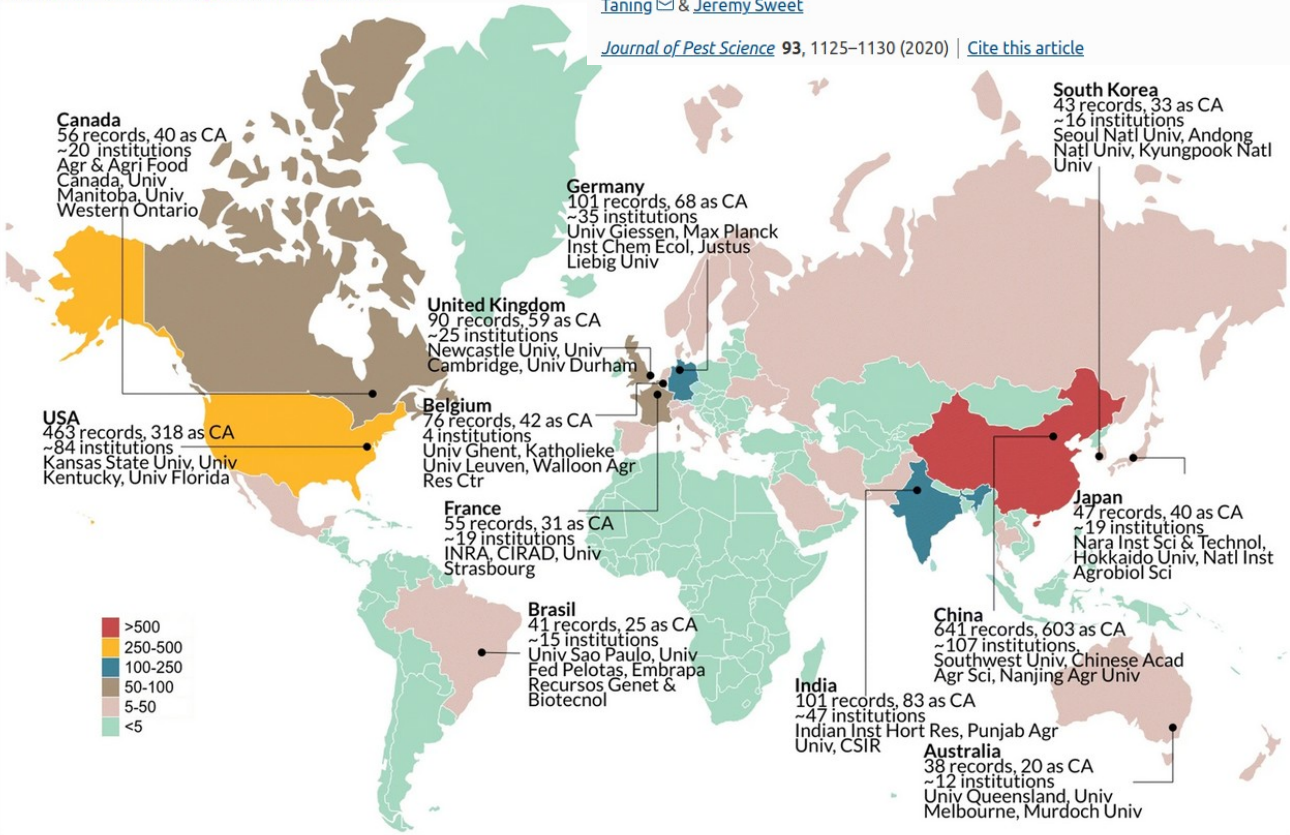
Rapid Communication | Open Access | Published: 26 May 2020

RNAi: What is its position in agriculture?

Bruno Mezzetti, Guy Smagghe, Salvatore Arpaia, Olivier Christiaens, Antje Dietz-Pfeilstetter, Huw Jones, Kaloyan Kostov, Silvia Sabbadini, Hilde-Gunn Opsahl-Sorteberg, Vera Ventura, Clauvis Nji Tizi Taning & Jeremy Sweet

Journal of Pest Science **93**, 1125–1130 (2020) | Cite this article

From: RNAi: What is its position in agriculture?



Global geographical contributions to RNAi research. Leading countries and key research institutions in RNAi are profiled, indicating a global interest in the field. Records represent the number of authored and co-authored articles. CA, corresponding author; Univ, university; Inst, institute

What is currently on the market/being developed? (and how is it regulated)?

RNAi-Based Biocontrol Products: Market Status, Regulatory Aspects, and Risk Assessment

*Kristof De Schutter[1], Clauvis Nji Tizi Taning[1], Lenny Van Daele[2], Els J. M. Van Damme[3], Peter Dubruel[2] and Guy Smagghe[1]**

HIGS
"SmartStax Pro" maize (Mon87411) - Bayer
"Vistive gold" high-oleic soybeans (Mon87705) - Bayer
"Super-High Oleic" (SHO) safflower - CSIRO and GO resources, Australia
"Innate" potatoes - Simplot GM
"HarvXtra" alfalfa - Forrage genetics

SIGS
Field trials Syngenta and Greenlight Biosciences against the Colorado potato beetle
"BioDirect" - Bayer, RNAi technology for protection of pollinators

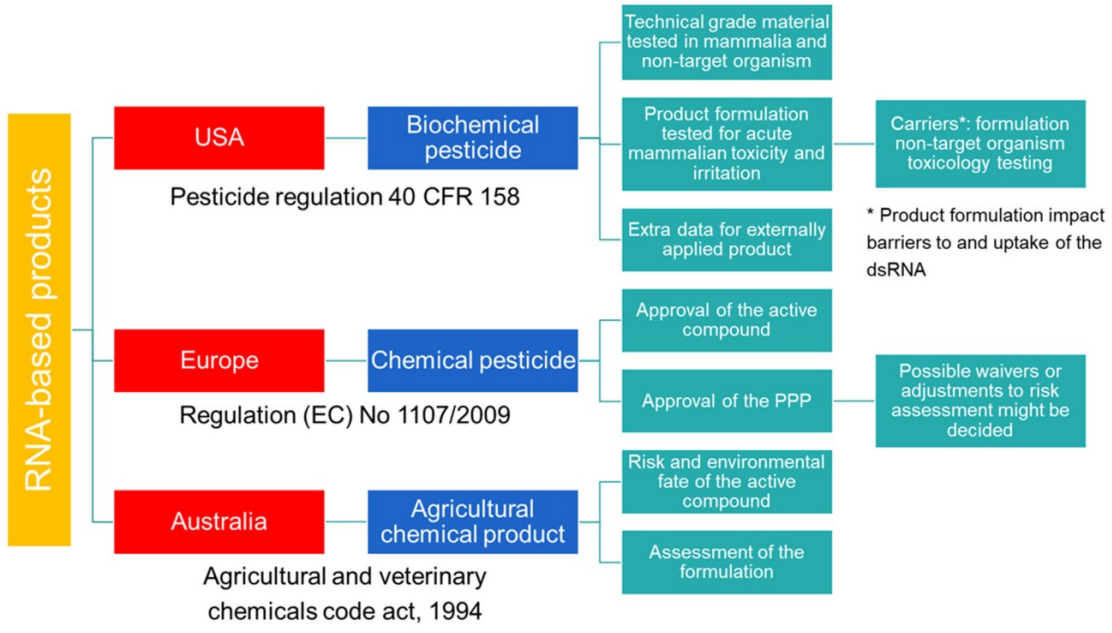


FIGURE 1 | Schematic overview of the regulatory frameworks for RNA-based products in the USA, Australia and Europe.

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

PHYTO Phytopathologie JLU Gießen

RU5116

ERA-NET BIOPROTECT

Federal Ministry of Education and Research

BASF The Chemical Company

Deutsche Forschungsgemeinschaft DFG

BAYER

Group:
Prof. Dr. Karl-Heinz Kogel
Dr. Jafargholi Imani
Dr. Jens Steinbrenner
Dr. Maria José Ladera Carmona
Dr. Silvia Zanini
Dr. Bernhard T. Werner
Jennifer Thielmann

Collaboration (s):
Prof. Dr. Alexander Goesmann (JLU)
Lukas Jelonek
Patrick Blumenkamp

Prof. Dr. Patrick Schäfer (Uni Ulm)
Dr. Ruth Schäfer

Prof. Dr. Jörn Kalinowski (CeBiTec)
Dr. Tobias Busche
Dr. Daniel Wibberg

Contact: Ena.Secic@agrar.uni-giessen.de