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# BioProtect

## Target-specific bioprotectants for sustainable crop production in a changing climate

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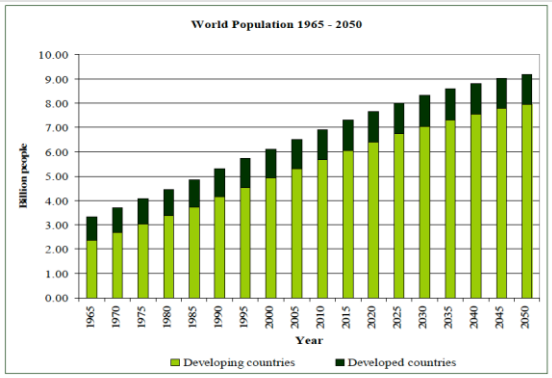
# Objectives of *BioProtect*



Source: Population Division of the Department of Economic and Social Affairs of the United Nations Secretariat (2007)


Tomato late blight (*Phytophthora infestans*) in India

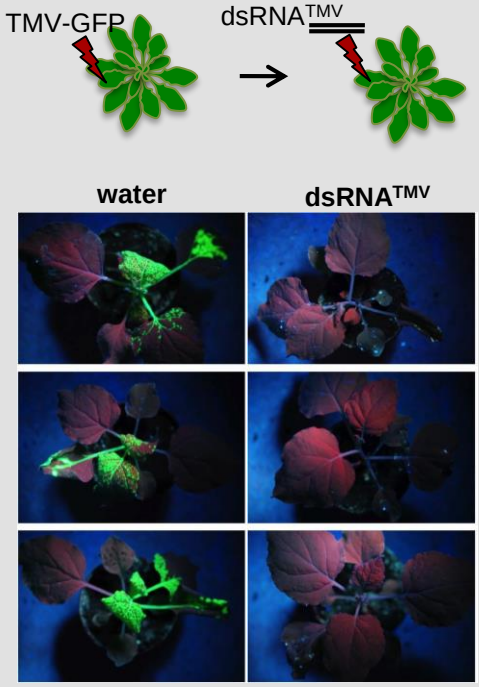
- Rapidly accelerating population growth (reaching 10 billion by 2050) → increase in 60% food production needed
- Climate change challenges the capacity to produce enough food
- Crops are threatened by pathogens (alone viruses have an impact of > $30 billion annually)
- Environmental health and safety calls for a reduction in chemical pesticides (e.g. ECOPHYTO national action)

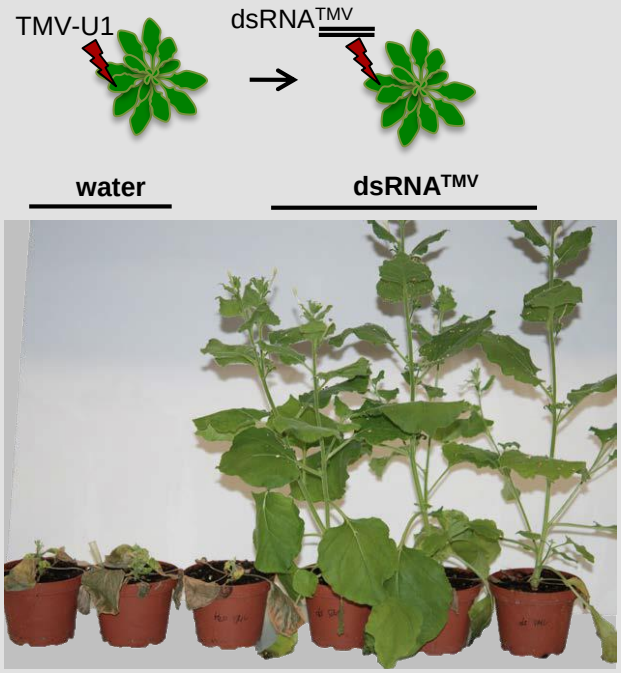
➡ **Replace contentious pesticides with safe, efficient, and cost-effective alternatives to ensure sustainable food production**

***BioProtect*** aims to develop the production, formulation, and application of **dsRNA** as a nature-derived and environmentally friendly bioprotectant **against viruses, fungi, and insects**

- dsRNA occurs in all organisms and in food (no toxic effects if incorporated)
- dsRNA is a natural molecule that activates naturally occurring plant defense mechanisms and thus allows plants to defend themselves using their own cellular mechanisms
- dsRNA is target-specific: It acts in manner dependent on its nucleotide sequence → can be tailored to target specific pathogens
- dsRNA does not accumulate within organisms. Outside cells, dsRNA is rapidly degraded. Within cells, dsRNA is rapidly used and then degraded.

# dsRNA treatment protects against plant virus



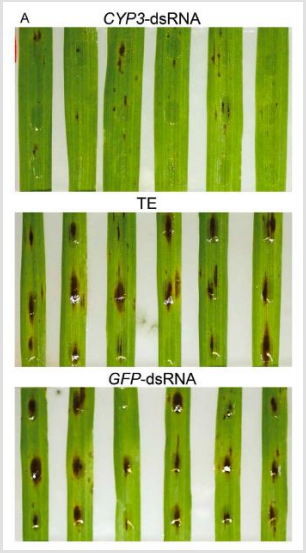

Niehl et al Plant Biotechnol J, 2018

# Topical applications of dsRNA to control pathogenic fungi

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]*

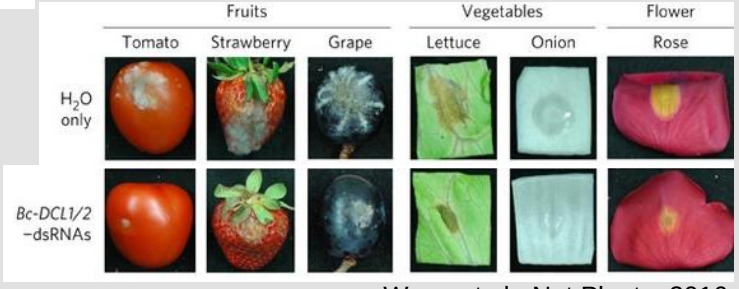



Koch et al., Plos Pathogens, 2016

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

Ming Wang, Arne Weiberg, Feng-Mao Lin, Bart P. H. J. Thomma, Hsien-Da Huang & Hailing Jin

**dsRNA-mediated protection against *B. cinerea***




Wang et al., Nat Plants, 2016

SCIENTIFIC REPORTS

OPEN

**Identification and application of exogenous dsRNA confers plant protection against *Sclerotinia sclerotiorum* and *Botrytis cinerea***

Received: 3 October 2017
Accepted: 16 April 2018
Published online: 09 May 2018

Austain G. McLoughlin[1], Nick Wytinck[1], Philip L. Walker[1], Ian J. Girard[1], Khalid Y. Rashid[2], Teresa de Kievit[2], W. G. Dilantha Fernando[2], Steve Whyard[3] & Mark F. Belmonte[1]

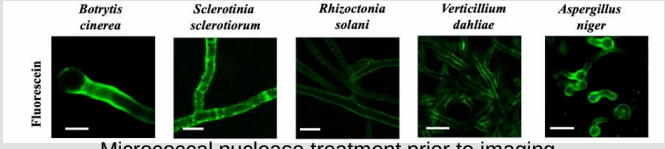

McLoughlin et al., Scientific Rep, 2018

dsRNA targeted against an essential gene of the fungus sprayed on plants

→ uptake by the plant → uptake by the fungus → enters silencing machinery of the fungus → silences the essential gene → causes growth inhibition of the fungus

**Many fungi can take up RNAs from the environment**

Fluorescein-tagged YFP-mRNA




Micrococcal nuclease treatment prior to imaging

From: Qiao et al., Plant Biotechnol J, 2021

# Aphid control with dsRNA

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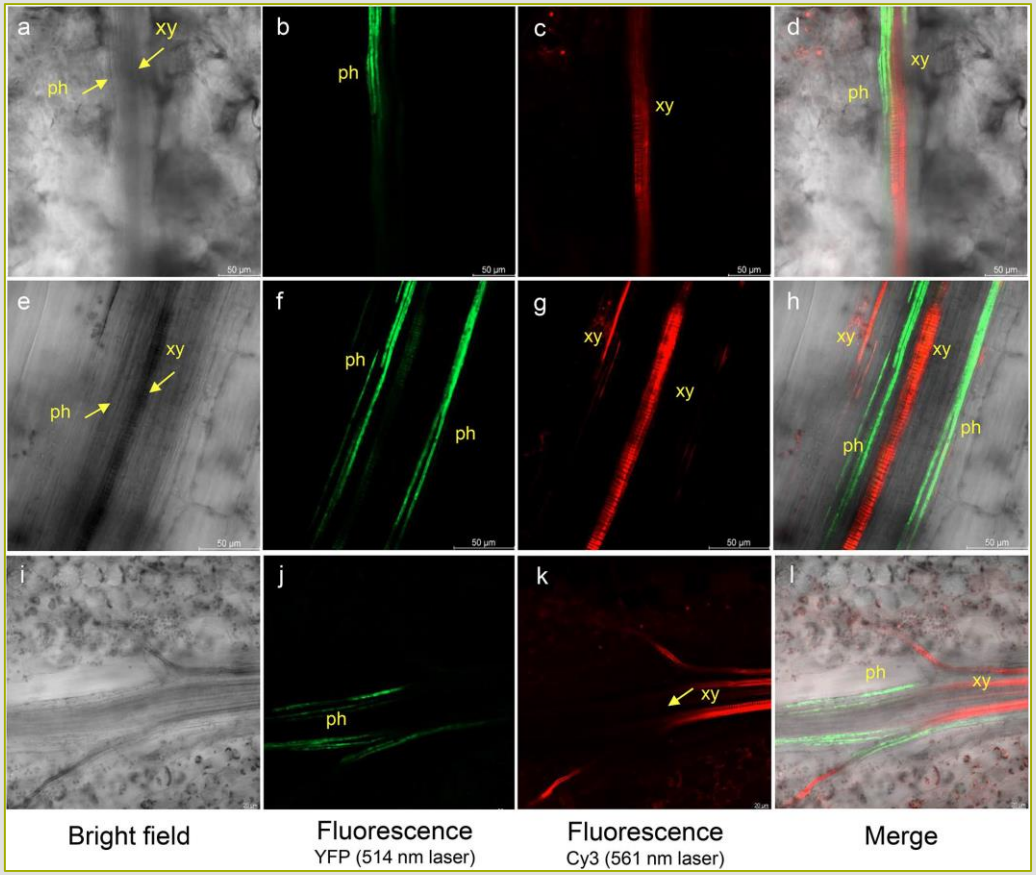
**Evaluation of dsRNA delivery methods for targeting macrophage migration inhibitory factor MIF in RNAi-based aphid control**

Shaoshuai Liu[1] · Maria Jose Ladera-Carmona[2] · Minna M. Poranen[3] · Aart J. E. van Bel[2] · Karl-Heinz Kogel[2] · Jafargholi Imani[2]

Received: 23 December 2020 / Accepted: 9 April 2021


## Experiment

YFP-labeled Arabidopsis was sprayed with cy3-labeled 21 nt dsRNA (naked dsRNA)

## Results

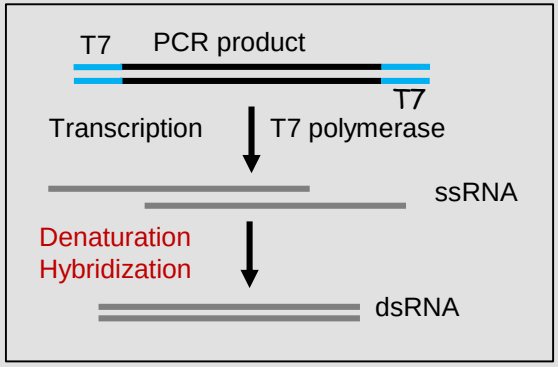
cy3-labeled 21 nt dsRNA is localized only in the xylem - not in the phloem (where aphids feed)

→ dsRNA formulations which enable dsRNA to enter phloem are needed

# Production of dsRNA

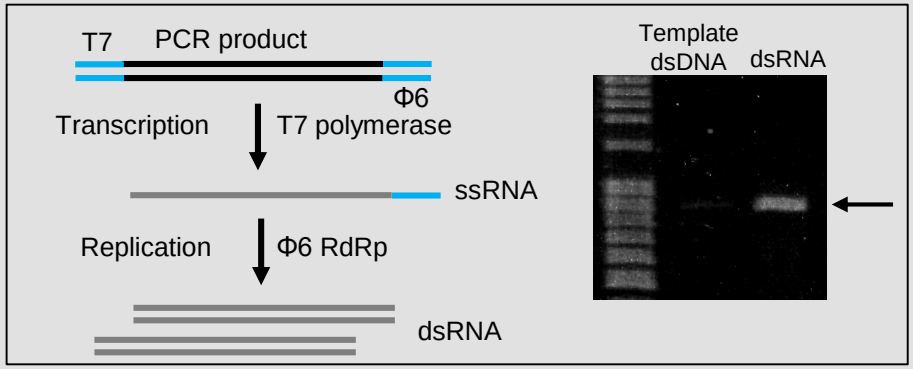
**Challenge** reliable, scalable, and cost-effective dsRNA production

### *In vitro* production of dsRNA by transcription




Post-synthetic annealing required
→ Partially aligned dsRNA (?)

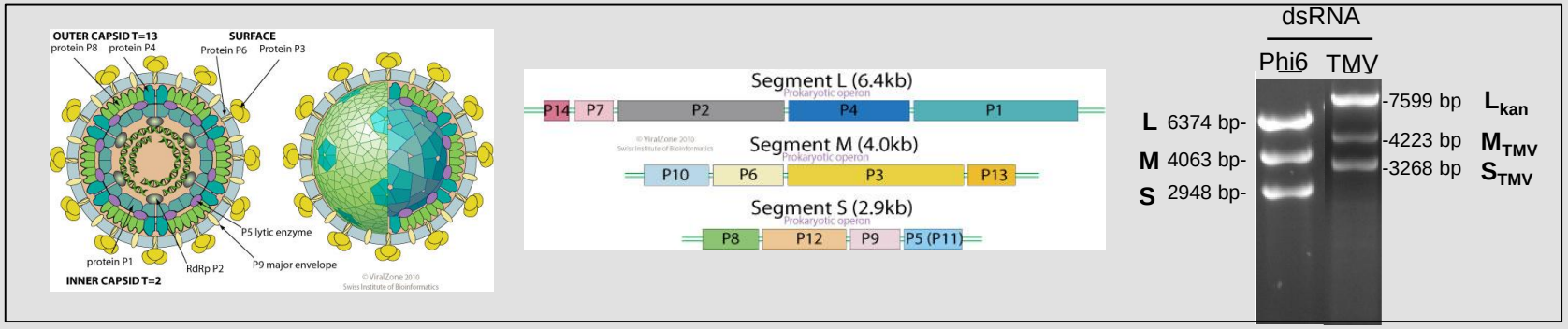
### *In vitro* production of dsRNA by replication




No post-synthetic annealing involved
→ Fully aligned dsRNA

Niehl et al 2018

### *In vivo* production of dsRNA in bacteria




Niehl et al 2018

**dsRNA-producing platform:**

- Specific dsRNAs for different targets
- Different purification protocols (purity, purification pipeline, costs)
- upscaling

# dsRNA Formulation

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## State of the Art

- Recent advances in nano-formulation of RNA towards targeted delivery[1]
- However, only few attempts in agriculture have been undertaken[2]
- All approaches mask negative charge of naked dsRNA to facilitate cell uptake: guanylate polymers[3],*"BioClay"* nanosheets[4,5], cationic oligopeptide[6], cationic oligopeptide bound to a cell-penetrating peptide[7], cationic fluorescent nanoparticle[8], complex DNA origami nanostructure[9], encapsulation in liposome complexes[10]

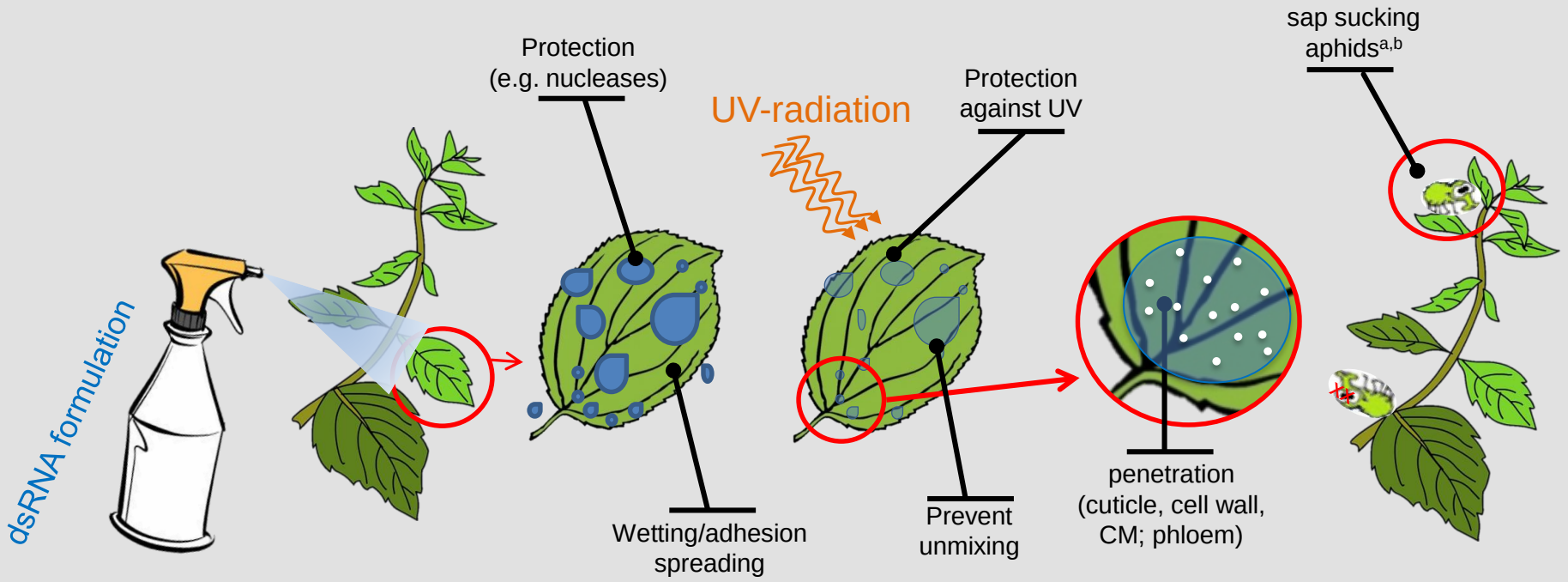
## Aim

**To develop:**

An innovative formulation based on biologically degradable ingredients that:

1. delivers the dsRNA to plant leaves
2. stabilizes and protects dsRNA on the leaves
3. supports long-lasting penetration of dsRNA into the plant cytoplasm

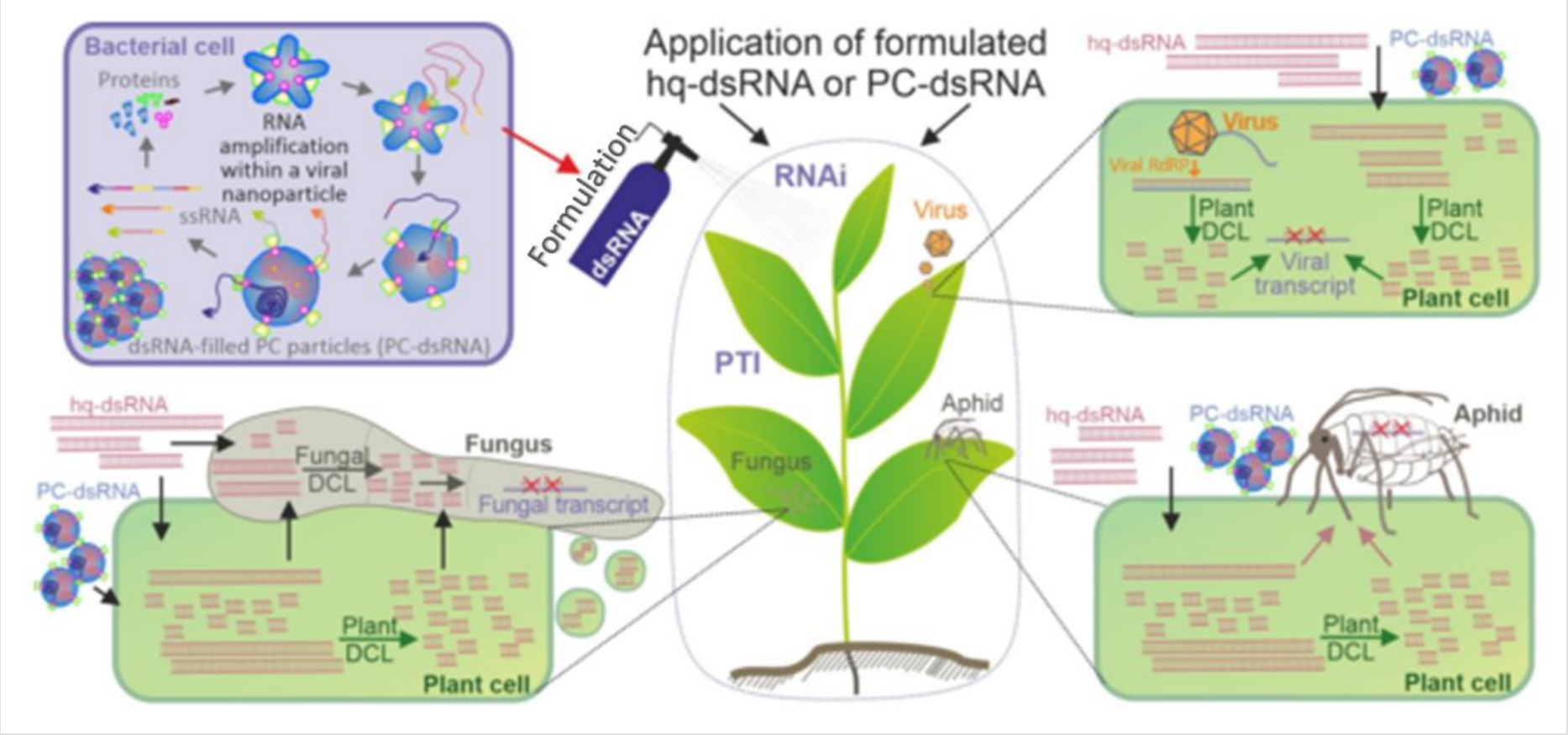
# Foliar application of formulated dsRNA




| **production storage** | **spraying** | **on the plant** | **protection** | **penetration** | **uptake and activity** |
|---|---|---|---|---|---|
| **additives** | | | | | |
| thickeners, stabilizers, fillers, antifoam, … | deposition aids | surfactants, stickers | sunscreens, humectans, protectants | penetrants | |

[a] *Sitobion avenae* [b] *Myzus persicae*

# Foliar application of formulated dsRNA



# Preliminary formulation results

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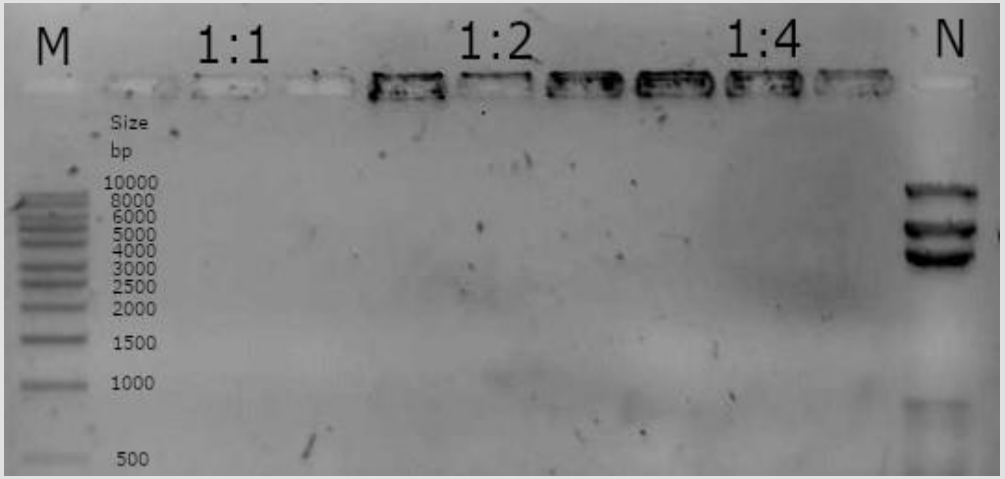
**Aim**

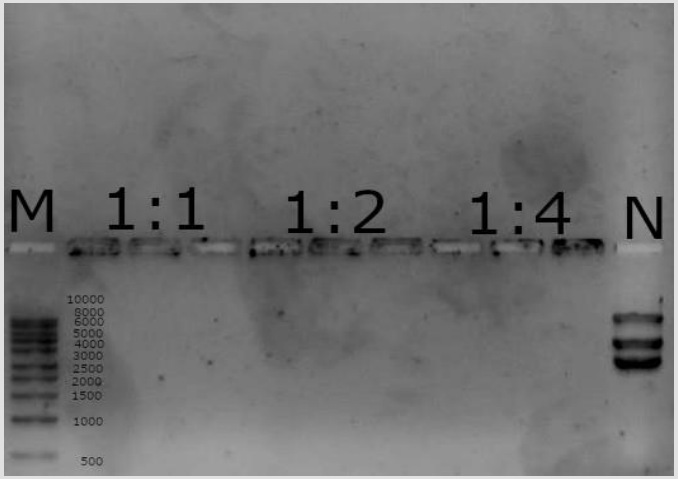
Create a formulation which masks the negative charge of dsRNA.

**Hypotheses**

1. Positively charged biopolymer chitosan[a] will electrostatically interact with negatively charged dsRNA and form nanoparticles.
2. The dsRNA-chitosan[a] formulation will be chargeless or positively charged.

**Cathode (–)**




(replicate, pockets in the centre of agarose gel)

**Anode (+)**

1 % Agarose
M = DNA Ladder (Roth 1 kbp)
N = Negative control (naked dsRNA, phi6[b])
1:1 = dsRNA : Chitosan
(w:w)

**Results**

The formulation does not migrate to the anode.

[a] Chitosan, Sigma-Aldrich
[b] high-quality dsRNA produced by Helsinki University [Niehl et al, 2018]

# Project structure and coordination

## Dr. Minna Poranen – Molecular Biology of dsRNA Viruses

- Application of *in vivo* dsRNA production platform for dsRNA synthesis
- Different purification protocols
- Upscaling of dsRNA production

## Prof. Anant Patel - Fermentation and Formulation Technology

- Formulation of dsRNA for enhanced stability and uptake by plants
- Development of a carrier liquid for dsRNA
- Upscaling of dsRNA production

## Prof. Karl-Heinz Kogel - Plant Protection and Plant Diseases (fungi and insects)

- Effect of dsRNA formulations against different fungi and aphids
- Underlying mechanisms of dsRNA uptake and activity
- Effect of temperature (changing climate)

## Prof. Manfred Heinlein – Plant Virus Movement (coordinator)

- Effect of dsRNA formulations against viruses (TMV, TuMV) in vegetable crops (tobacco, tomato, cabbage, rapeseed)
- Underlying mechanism (RNA silencing, PTI)
- Effect of temperature (changing climate)