HIGS for control of *Sphaerulina musiva* poplar leaf spot and stem canker disease: Efficacy, stability, and non-target impacts

Michael Gordon, Posy Busby, Steve Strauss, Jared LeBoldus

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Session V. Regulations and biosafety of GMOs

Oregon State University

Forests face many devastating problems without a lot of good solutions to date

Resistance is often complex and

difficult to breed for

Swiss needle cast

chestnut blight

mountain pine beetle

sudden oak death

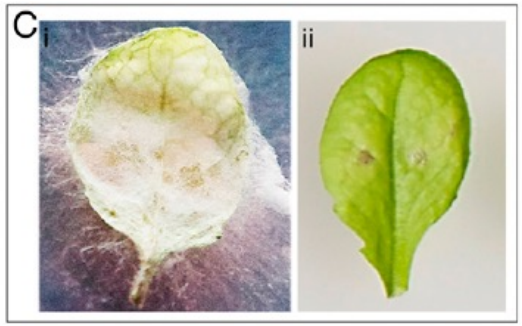
HIGS emerges as a potential solution that offers quick and powerful route to resistance

PNAS

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]

19324–19329 | PNAS | November 26, 2013 | vol. 110 | no. 48



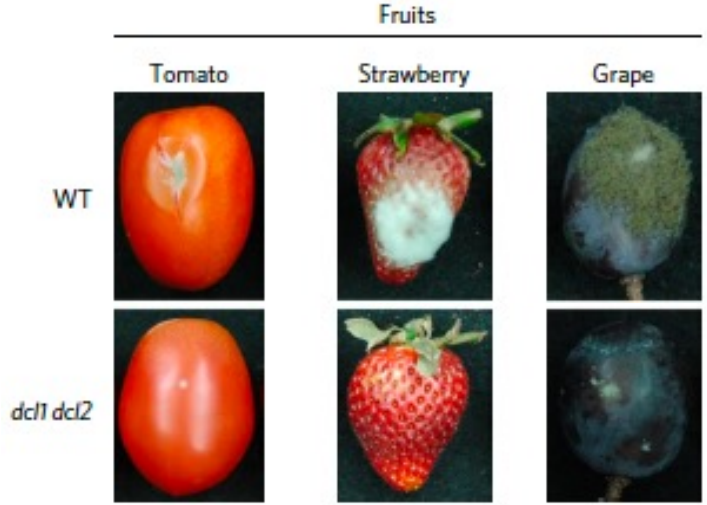
nature plants

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

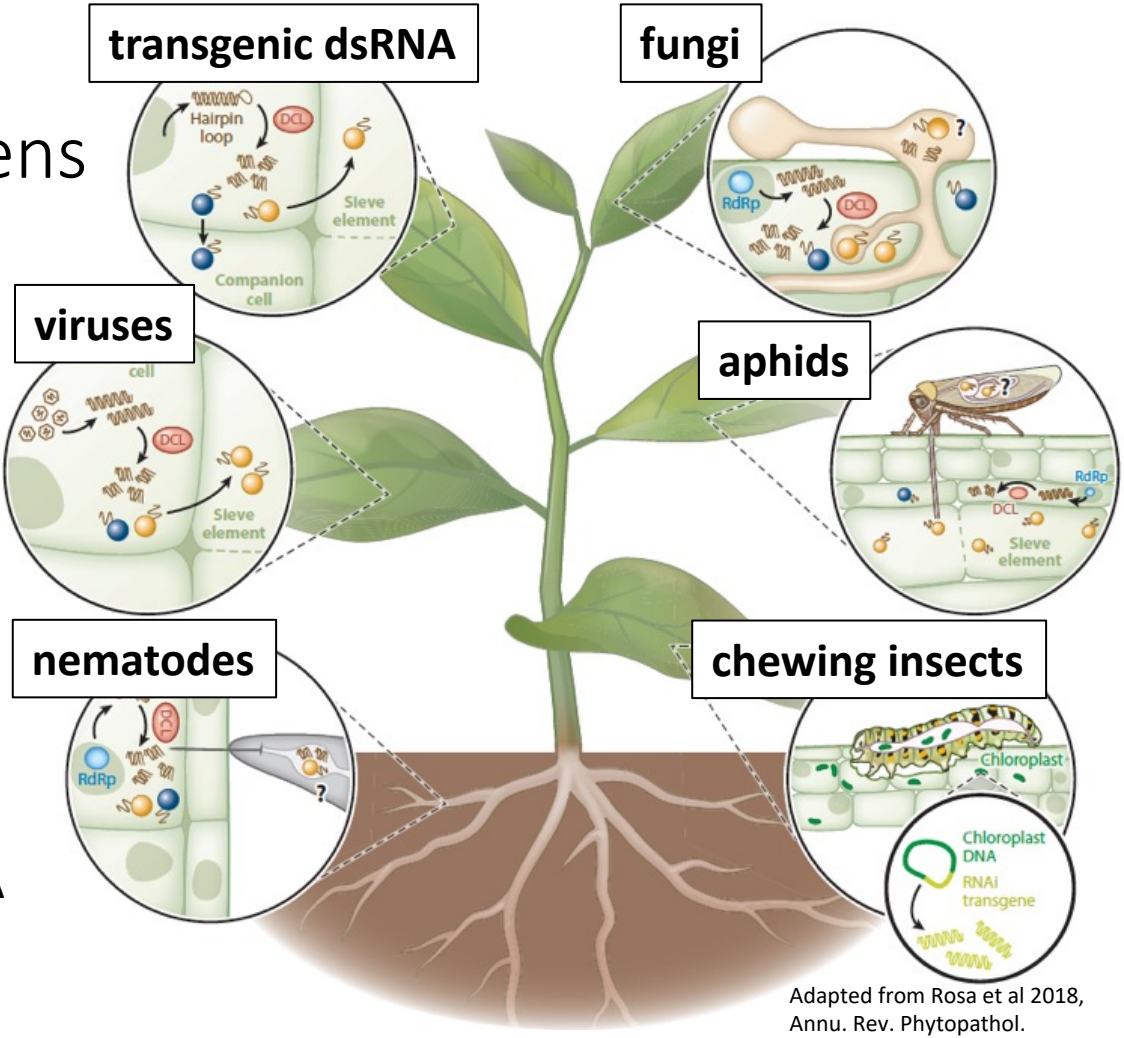
Ming Wang[1], Arne Weiberg[1†], Feng-Mao Lin[2], Bart P. H. J. Thomma[3], Hsien-Da Huang[2] and Hailing Jin[1*]



HIGS proven against a variety of pest/pathogens

Key concepts

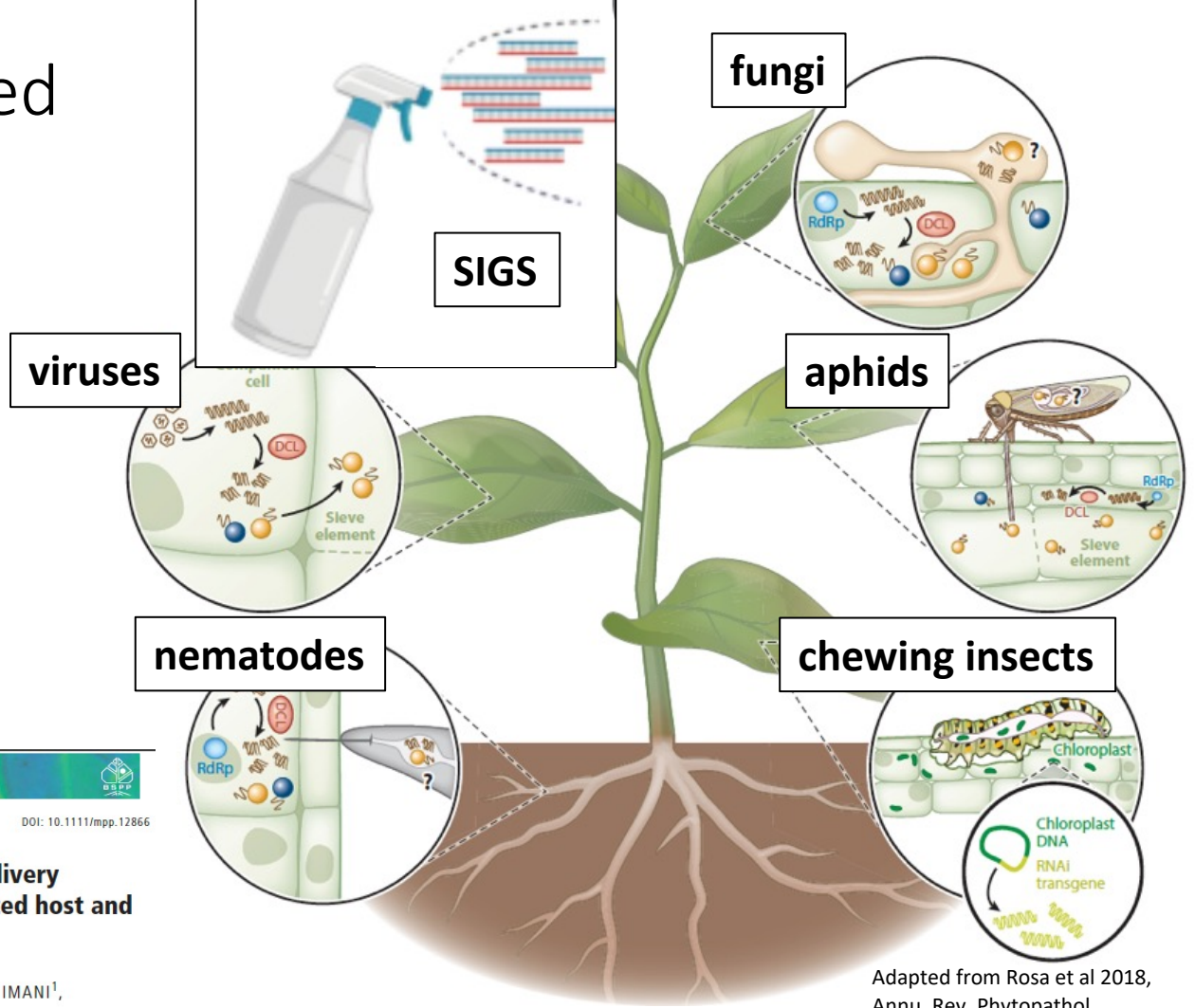
- Plants produce dsRNA
- Requires dsRNA transfer/uptake from host to pathogen/pest
- dsRNAs target critical genes that limit growth and or virulence
- Gene silencing requires native RNA interference machinery



Adapted from Rosa et al 2018, Annu. Rev. Phytopathol.

SIGS = spray-induced gene silencing

Spraying dsRNAs onto wild type plants can be as effective as HIGS



Adapted from Rosa et al 2018, Annu. Rev. Phytopathol.

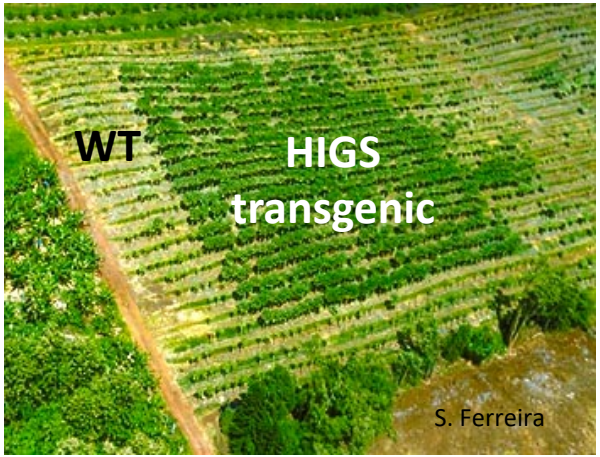
Molecular Plant Pathology

MOLECULAR PLANT PATHOLOGY (2019) 20(12), 1636–1644 DOI: 10.1111/mpp.12866

SIGS vs HIGS: a study on the efficacy of two dsRNA delivery strategies to silence *Fusarium FgCYP51* genes in infected host and non-host plants

ALINE KOCH [1,†,*], LISA HÖFLE [1,†], BERNHARD TIMO WERNER [1], JAFARGHOLI IMANI [1], ALEXANDRA SCHMIDT [1], LUKAS JELONEK [2] AND KARL-HEINZ KOGEL [1]

HIGS and SIGS commercial successes

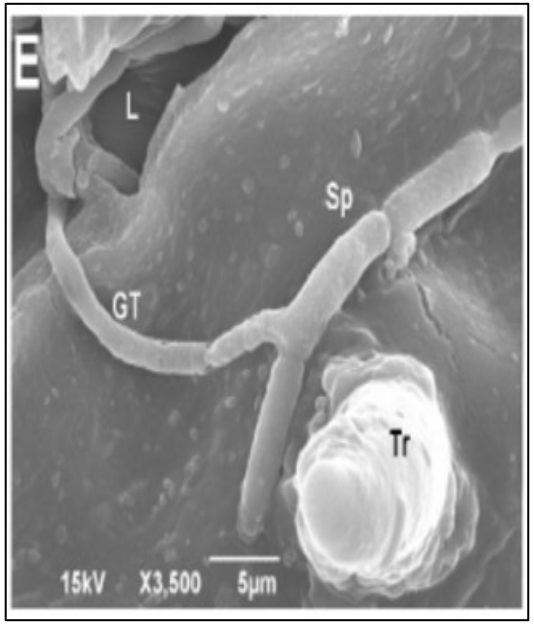


ringspot virus resistant
Rainbow Papaya

Colorado potato beetle
control by dsRNA pesticide
application

SmartStax western corn
rootworm resistant maize

Sphaerulina musiva: Septoria leaf spot and stem canker



Native to eastern U.S. but has spread west, threatening native cottonwoods

Major Research Questions

1. Does *S. musiva* take up dsRNA?

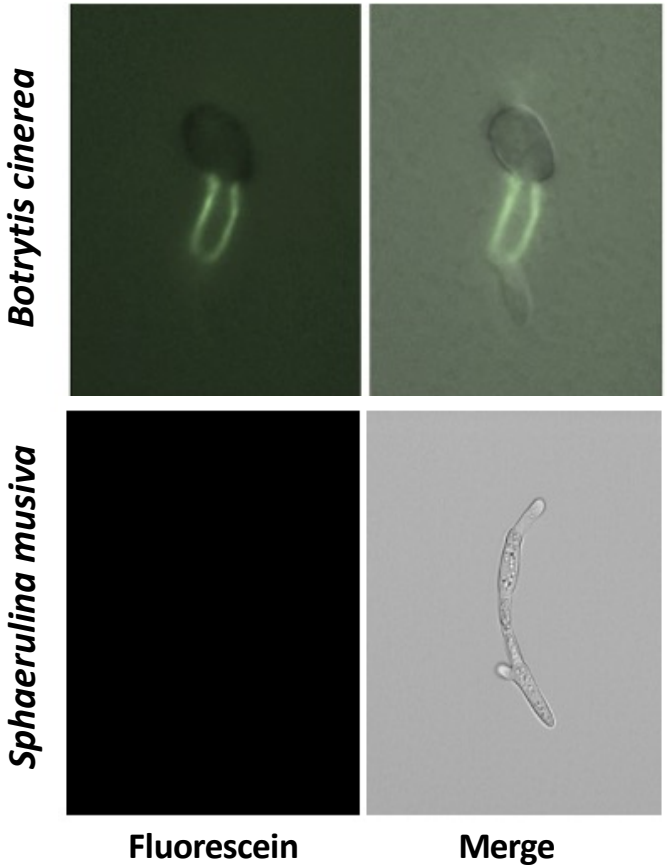
2. Would a HIGS transgenic work to limit *S. musiva* disease?

3. Are HIGS effects specific?

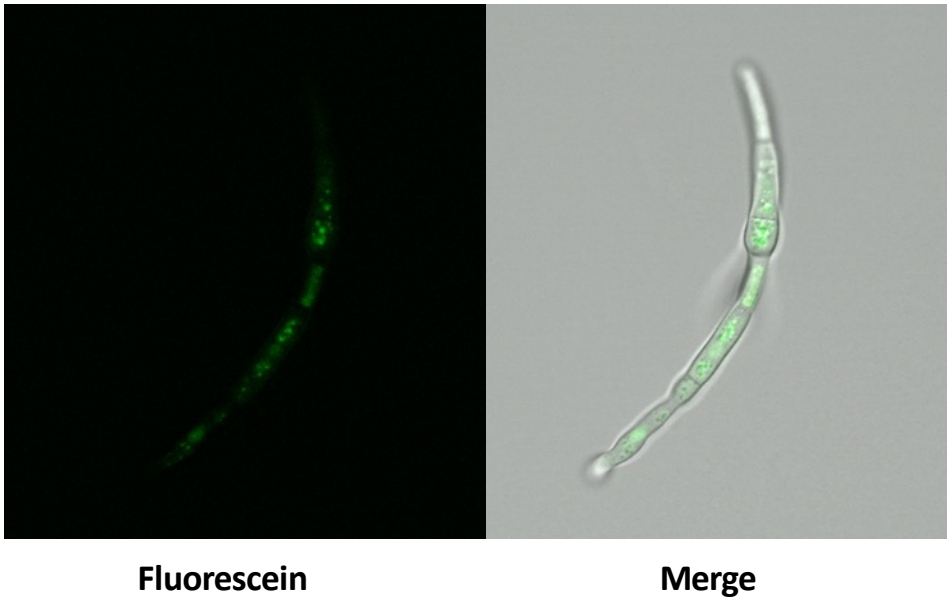
S. musiva dsRNA uptake evaluated in three ways

1. **Uptake of labeled dsRNA – visualize in cells**

Unable to detect uptake of fluorescein labeled dsRNA



Strong green autofluorescence in water treated *S. musiva*

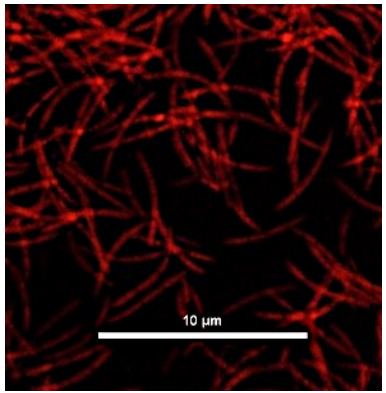


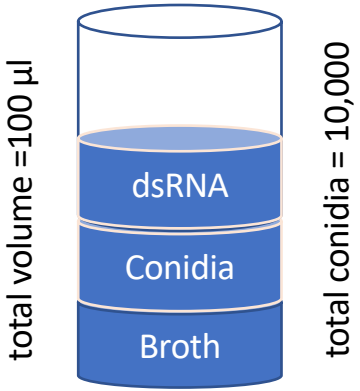
S. musiva dsRNA uptake evaluated in three ways

1. Uptake of labeled dsRNA – visualize in cells

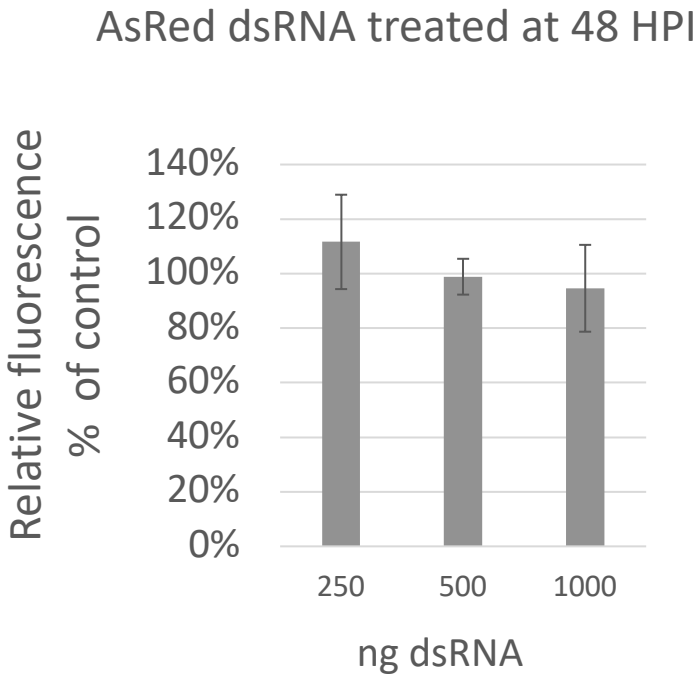
2. **Silence a fluorescent marker gene – effects on fluorescence**

Unable to detect reduced fluorescence when culturing a marked strain with dsRNA targeting the marker transcript





96-well plate: well composition



error bars = standard error of three independent experiments

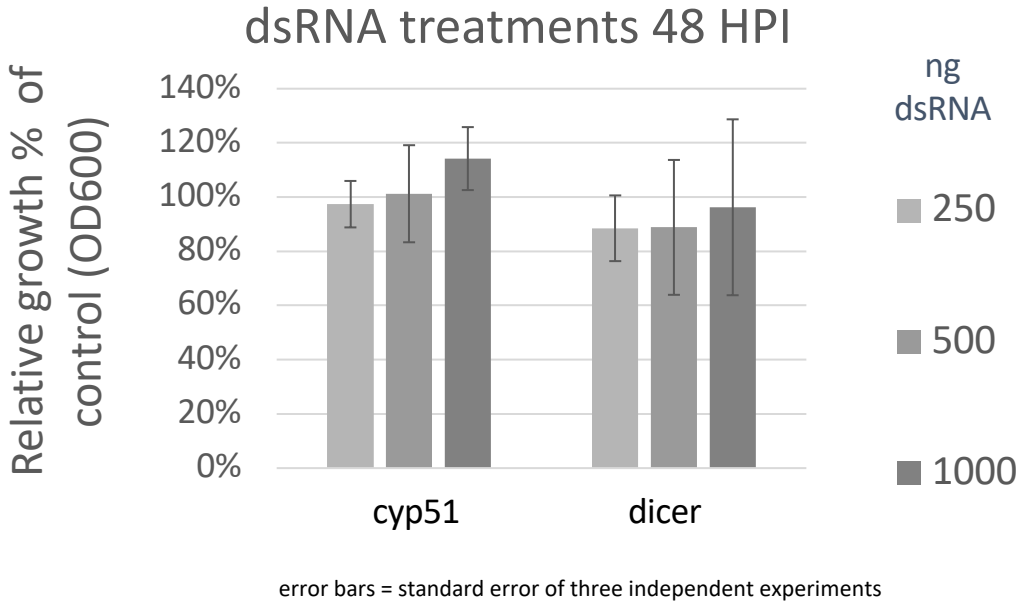
S. musiva dsRNA uptake evaluated in three ways

1. Uptake of labeled dsRNA – visualize in cells
2. Silence a fluorescent marker gene – effects on fluorescence
3. **Silence housekeeping genes – effects on growth**

In vitro culturing with dsRNAs targeting homologs of published housekeeping gene targets had no effect on growth

RT-qPCR validation shows no silencing effect

Rapid degradation of dsRNA in culture



error bars = standard error of three independent experiments

Major Research Questions

1. Does *S. musiva* take up dsRNA?

2. **Would a HIGS RNAi transgenic work to limit *S. musiva* disease?**

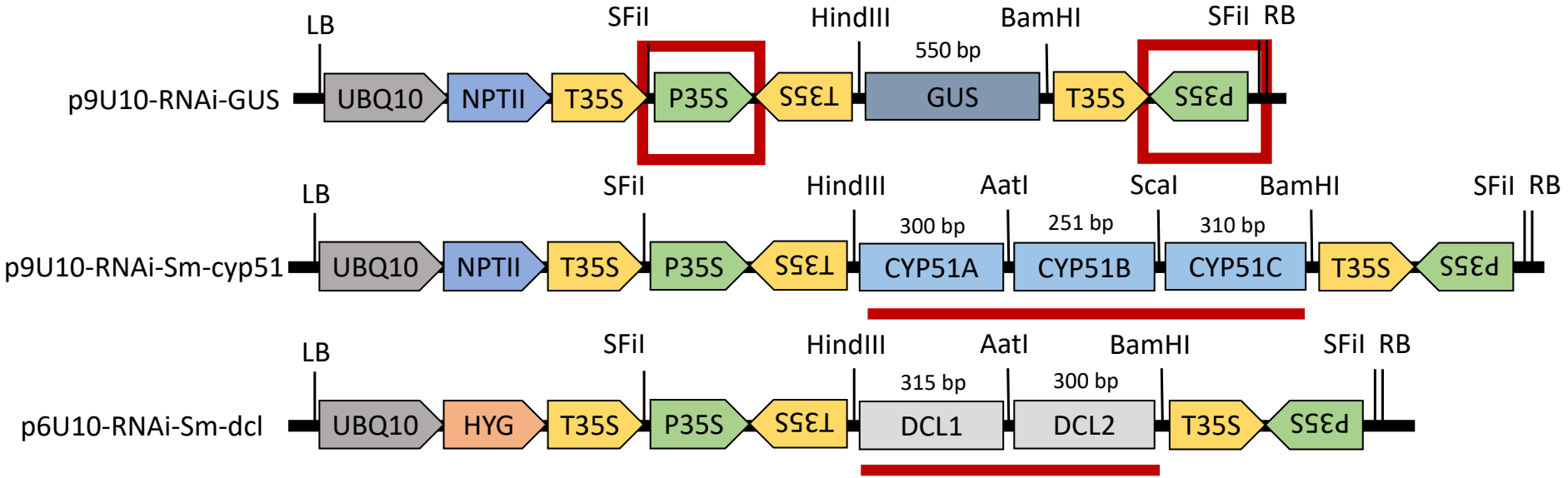
3. Are HIGS effects specific?
   - Will it create problems for symbionts?

Three major HIGS constructs were transformed into *Populus trichocarpa* using standard *Agrobacterium* methods

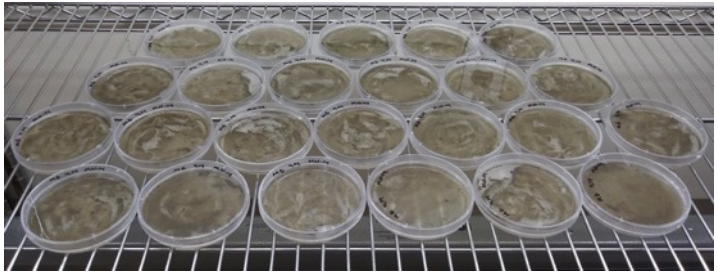
construct	n
gus	6
cyp51	42
dcl	32
cyp51 + dcl	12

inverted promoters produce dsRNA

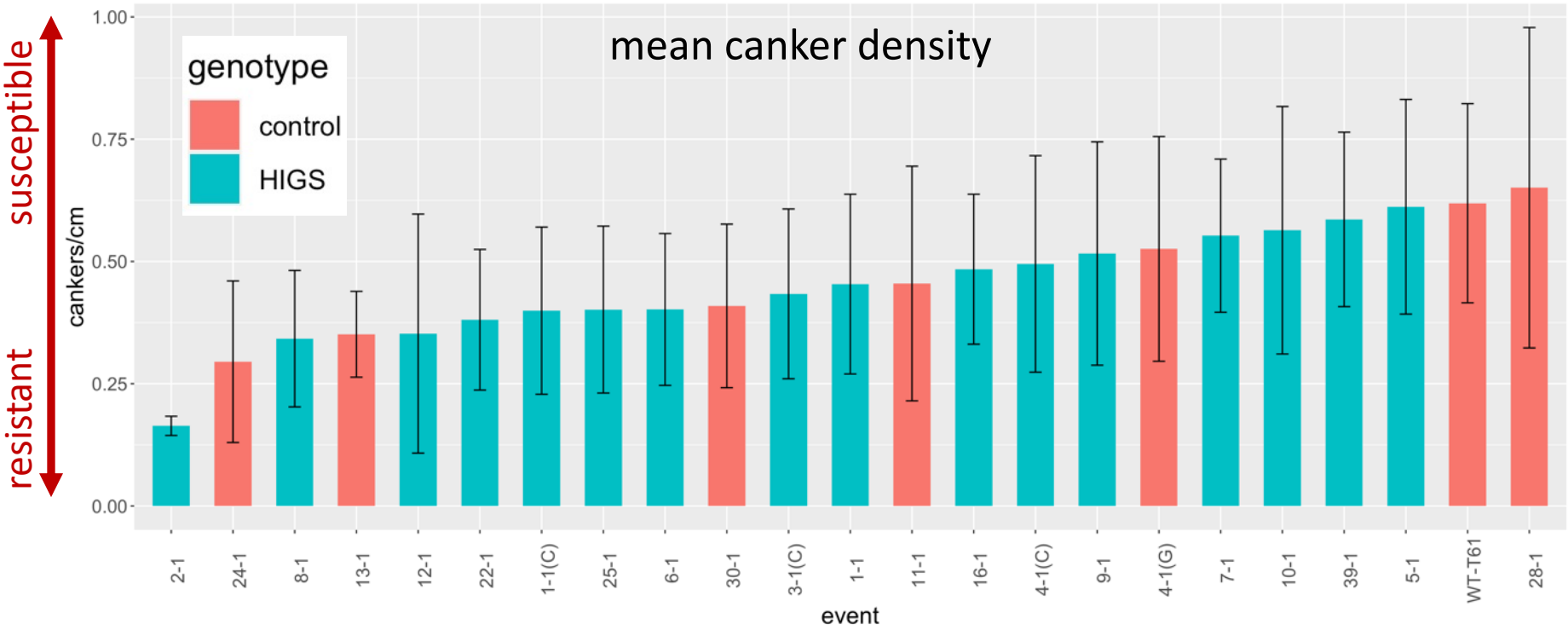
one dsRNA can target several genes



Screening HIGS transgenic lines for resistance in a greenhouse inoculation trial



No phenotypic resistance detected



error bars = standard deviation of five trees

Major Research Questions

1. Does *S. musiva* take up dsRNA?

2. Would a HIGS RNAi transgenic work to limit *S. musiva* disease?

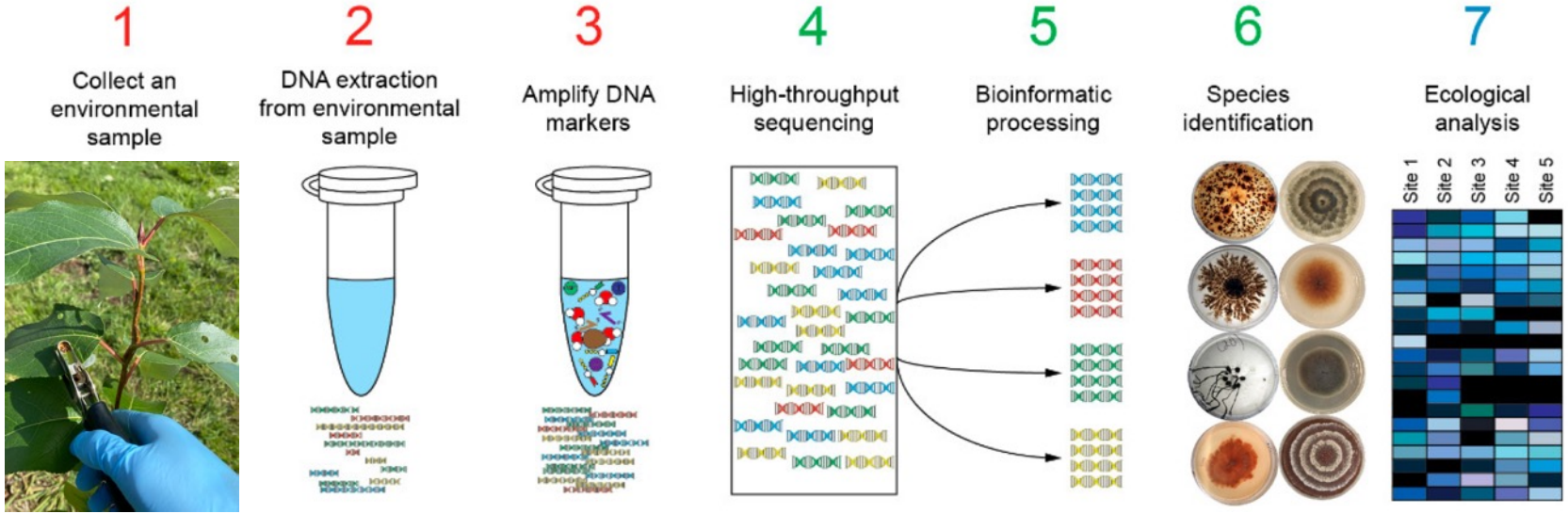
3. **Are HIGS effects specific?**
   - **Will dsRNAs create problems for symbionts?**

Field trial needed to study non-target effects

leaves nearly void of fungal endophytes after 50 days in greenhouse

326 fungal endophyte taxa detected in field leaves after one season of growth

ITS2 metabarcoding was used to characterize fungal communities in HIGS and control trees

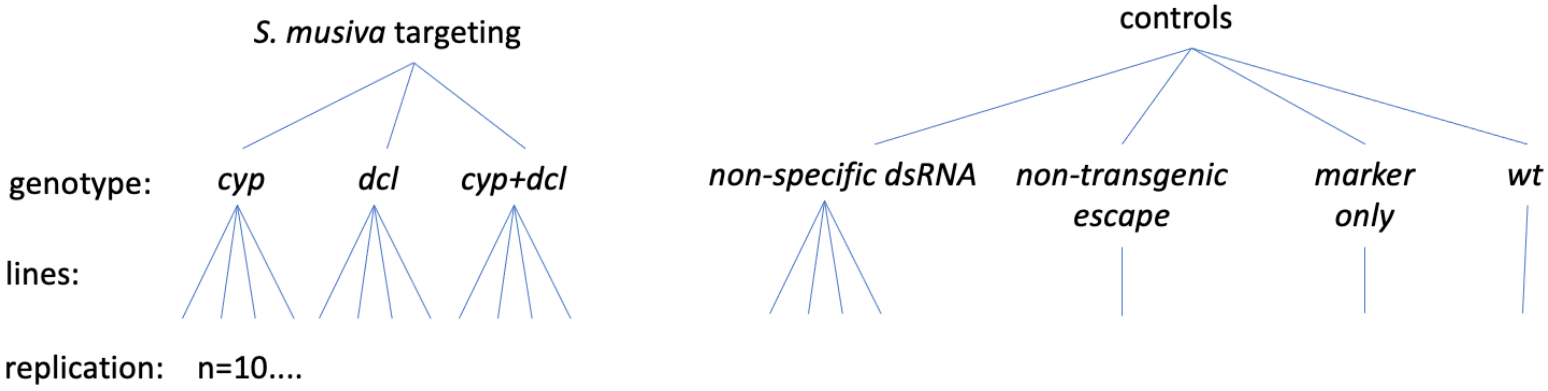


leaf discs washed to remove surface microbes

A table of sequence counts is the foundation of all downstream analysis

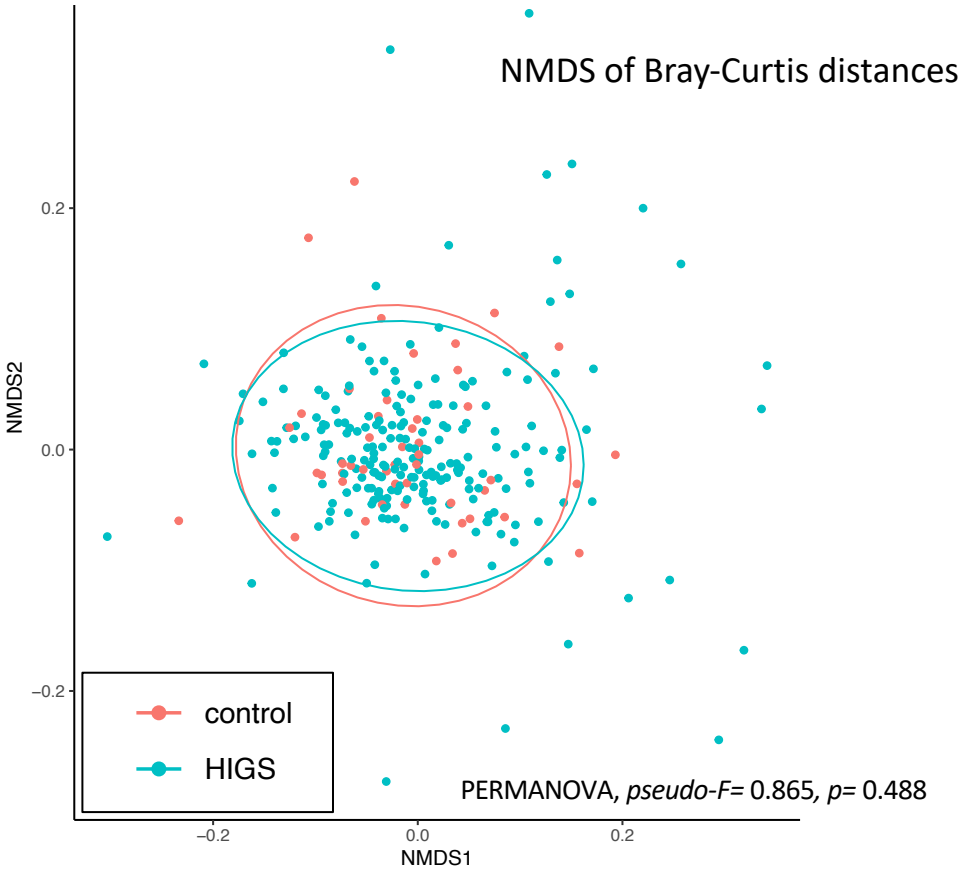
Do fungal community compositions differ between *S. musiva* targeting and control trees?

- **Hypothesis:** HIGS transgenes will have no effect on fungal community composition
- *S. musiva* is not present at this field site but closely related fungi are



No difference in fungal community composition between *S. musiva* targeting and control trees in a field trial

- Multivariate analysis of fungal OTU diversity
- Dots represent fungal communities of individual trees
- Distance between dots represents dissimilarity in fungal community composition



Conclusions

- HIGS appears ineffective in this pathosystem, in contrast to our initial hopes

- HIGS is not a cure all...
  - Other studies show HIGS/SIGS is not effective in a closely related fungus *Zymoseptoria tritici* and that not all fungi readily take up dsRNA

- However, microbiome studies suggest HIGS effects appear extraordinarily specific
  - far more so than fungicides or changing host genotypes are likely to be

Continuing work

1. Does *S. musiva* take up dsRNA?
   - uptake of red tagged dsRNA
   - assay target transcripts over time with continuous dsRNA additions

2. Would a HIGS RNAi transgenic work to limit *S. musiva* disease?
   - Greenhouse trials with highest dsRNA expression lines and higher replication

3. Are HIGS effects specific?
   - 2nd year of data collection

Acknowledgements

- Support
  - Cathleen Ma
  - Amanda Goddard
  - OSU greenhouse
  - Strauss Lab
  - LeBoldus Lab
  - Busby Lab

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  - Oregon State University Provost Fellowship

- dsRNA