

RNAi in *Colletotrichum abscissum*, causal agent of citrus PFD
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Webinar 5 – RNAi-based control of fungal pathogens – February 9th 2021

Webinar 5:
 Tuesday 9 February 2021, 13:30 (GMT-3) - 13:55 (GMT-3)
 URL: <https://www.youtube.com/watch?v=8p3p3p3p3p3>

Pathogens attack

Long time until production

Breeding

Variety Selection

Plant Nutrition

Soil Conditions

Develop strategies to control pathogens

Bernard, 2018

Postbloom fruit drop (PFD)

Fundectrus

C. acutatum = *C. abscissum*

C. acutatum

Colletotrichum abscissum Pinho & D.L. Pereira, sp. nov.

Goulin, 2017

Bragança et al., 2016

How *C. abscissum* looks like?

Macro and micromorphology of *Colletotrichum abscissum*. From PDA medium. A) Macromorphology growing over the medium. B) Macromorphology reverse plate. C – E) Conidia. F – I) Conidophores. J) Conidomata. K – M) Apressoria. N) Vegetative hyphae. Scale bars = 20 µm.

PFD control = Chemical control

Targeting the Complex II of mitochondrial respiration

- Succinate Dehydrogenase inhibitors (SDHI);
- Used for control of many *Colletotrichum* species pathogenic for different crops

Targeting the Complex III of mitochondrial respiration

- Quinone-oxidase inhibitors (Qoi) and Strobilurins
- Chemical control of PFD;
- Field application each 7 – 10 day (flowering) / 10 – 14 days
- High cost and environmental damages

Resistance reports

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Alternative Control Strategies

Alternative control - RNAi

Is RNAi a functional strategy for *C. abscissum* control?

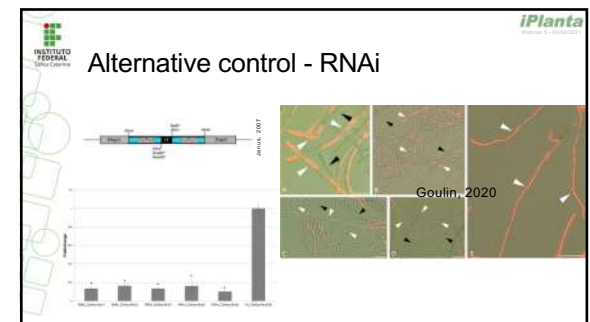
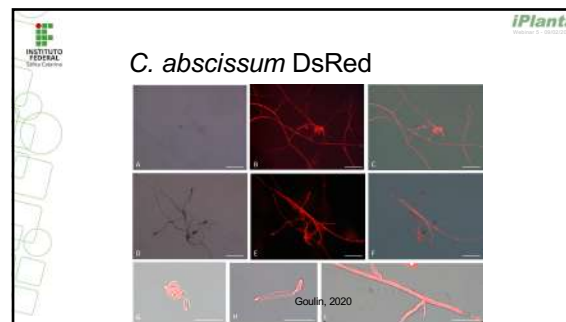
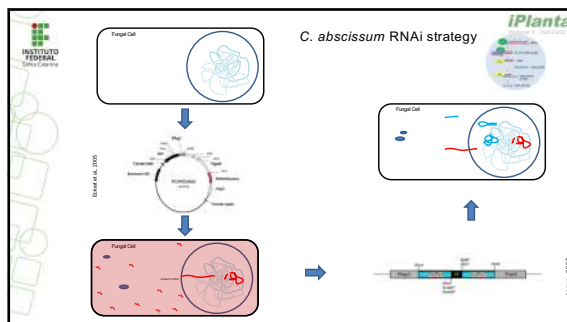
Has *C. abscissum* the canonical RNAi machinery? Is it functional?

Is *C. abscissum* able to process Double-Strand RNA and induce gene knockdown?

The RNAi can be use for fungal control in the future?

Starting the answers...

- Next Generation Sequencing – NGS Technology
 - Illumina Hi-Seq and PacBio
- Local Blast – RNAi machinery proteins related:
 - Dicer ✓
 - Argonaute ✓
 - RdRp ✓
- These proteins are present into the genome ... But, are they being expressed?
 - RT-qPCR ✓



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Is this strategy useful for endogenous genes?

How to test the effects in pathogenicity?

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Selecting RNAi targets

- Genes whose products are the targets of chemical fungicides
- Succinate Dehydrogenase subunits genes
 - sdh-a*
 - sdh-b*

Research Article
Efficacy of SDHI fungicides, including benzovindiflupyr, against *Colletotrichum* species
Hideo Ichi,^{1,2*} Zan Zhou,³ Mengjun Ma,⁴ Xingqiang Li⁵ and Guido Schvartz⁶

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Is this strategy useful for endogenous genes?

How to test the effects in pathogenicity?

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Pathogenicity Evaluation System

In vitro symptom induction of *Colletotrichum abscessum* infection in detached sweet orange flowers

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Alternative Control - RNAi

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Alternative Control - RNAi

VIGS – Virus Induced Gene Silencing
HIGS – Host Induced Gene Silencing
SIGS – Spray Induced Gene Silencing

Alternative Control - SIGS

Goulin, 2020

Merge

Alternative Control - HIGS

Inoculation, selection and regeneration

Grafting or rooting Acclimatization

R. Boscariol

Alternative Control - HIGS

Dra. R. Boscariol

***C. abscessum* genome comparison**

• Targets selection

Dr. Riccardo Baroncelli

Numbers of shared Orthologous Clusters (E-value 1e-5; Inflation value 1.5; <http://www.bioinfogenome.net/OrthoVenn/>) among available genomes belonging to the *Colletotrichum acutatum* species complex

C. abscessum RNAi functionality checked ✓

Search target genes (*C. abscessum* genome)

Select the strategy

- SIGS
- HIGS

Validate the strategy efficiency

Thank you

Prof. Dr. Elena Beraldi – Università di Bologna
 Dr. Riccardo Baroncelli – Ciale/Universidad de Salamanca
 Dr. Marcos A. Machado – IAC/CCSM
 Dra. Raquel Boscariol – IAC/CCSM
 Prof. Dr. João P. Paes – IFSC

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