Oregon State University
Oregon Wine Research Institute

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Developing a Spray-Induced Gene Silencing (SIGS) method for the control of Grape Powdery Mildew (*Erysiphe necator*)

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Summary:

- Fungal pathogen, *Erysiphe necator,* responsible for Grape Powdery Mildew (GPM) is a significant threat to wine production
- The emergence of fungicide resistance suggests the need for the development of new strategies to control GPM.
- Recent research demonstrates that agricultural pests including fungi can be controlled through exogenous application of RNA molecules.

Spray Induced Gene Silencing (SIGS):

- dsRNA molecules **targeting plant and pathogen-related genes** were found to move between organism tissues as well as between kingdoms (**Figure 1**).
- These applications can have **systemic activity** further enhancing their utility for pest and disease control.
- We propose to develop a SIGS program aimed at targeting key endogenous genes responsible for the **susceptibility of grapevine to *Erysiphe necator* (MLO genes)** and **essential fungal genes** like those involved in the production of small-interfering RNAs (**Dicer-Like genes [DCL]**) and for its life cycle (**CYP51**).

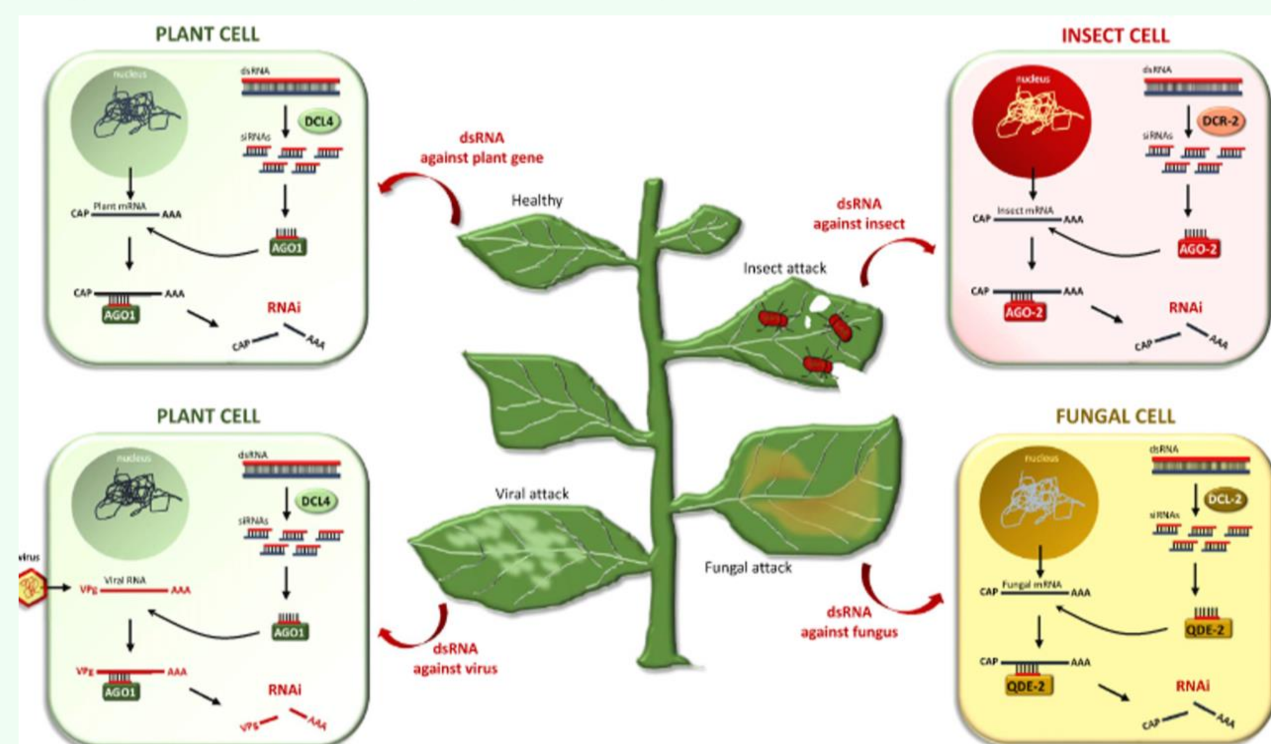


Figure 1: Cross-kingdom siRNA trafficking and dsRNA uptake by plant and pathogen cells (Dalakouras, 2020).

"mildew locus O" genes and PM susceptibility

- **Mildew resistance locus O (Mlo) genes** are susceptibility-genes which mutation confers durable and broad- spectrum resistance to some crops (barley).
- Loss of function mutations in these genes cause powdery mildew pathogenesis to terminate at the stage of cell wall penetration (Figure 2).

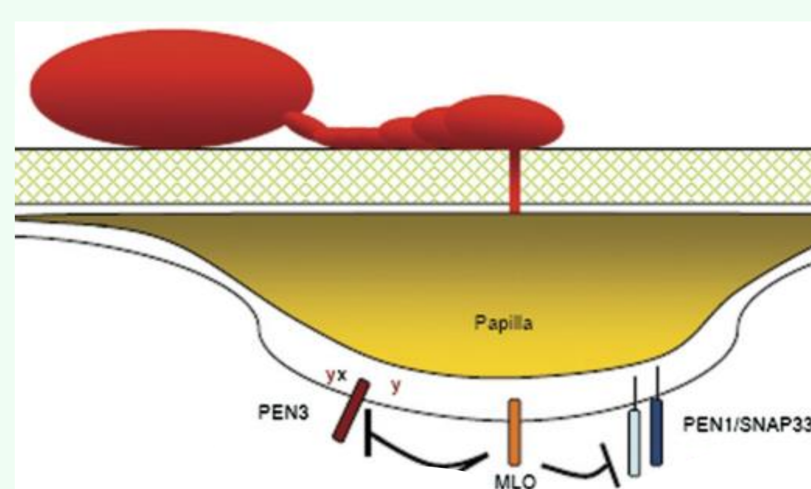
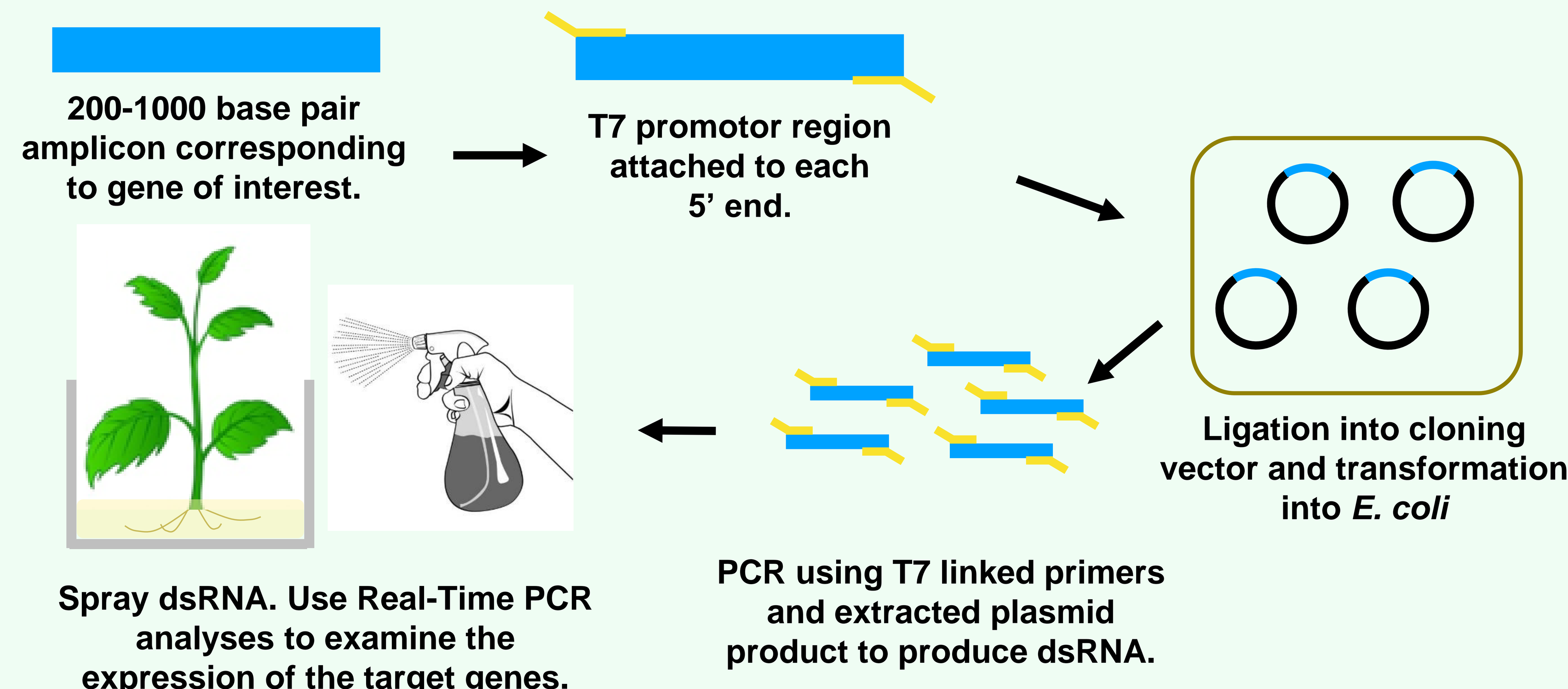


Figure 2: MLO negatively regulating endogenous R-genes (Underwood W. and Somerville, S. 2008).

Research Objectives of the Project:

1) Identify the stretches of RNA sequences with the **maximum interference activity** to silence fungal (***EnDCL1,2*** and ***EnCYP51***) and native genes (***VitiMLO3,4,6,9,13*** and ***17***) in grapevine. Assess resistance to GPM through inoculation of sprayed micro-vines.
2) Evaluate the **uptake and processing of double-stranded RNA** molecules by the plants and their systemic effects on the reduction of GPM using labeling of dsRNA with **fluorescent molecules and microscopy**.
3) Determine the effects of **Layered Double Hydroxide (LDH)** nanoparticles on the lifespan of sprayed dsRNA molecules on grapevine leaves.

Identifying the sequences that silence the expression of the target genes



Studying the movement of dsRNA in the plants

- dsRNA will be labelled with fluorophore Cy3 which will produce fluorescence under UV light.
- **Systemic spread** of dsRNA will show the potential for movement throughout plant tissues.

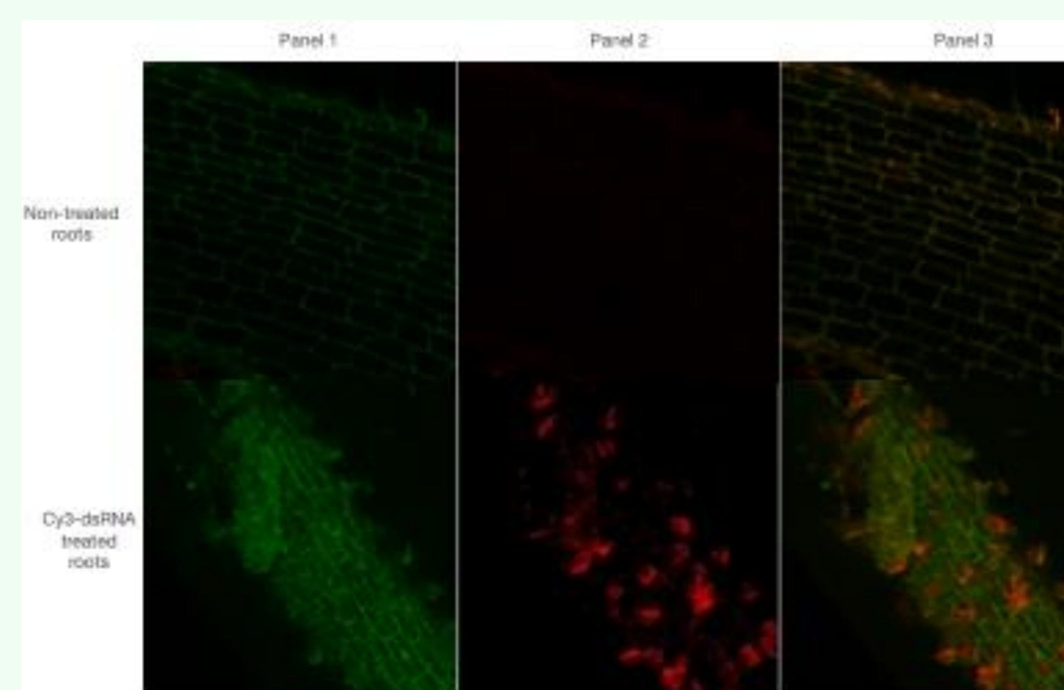


Figure 3: Fluorescent dsRNA visualized in *Vitis vinifera* roots one week post inoculation.

Examining dsRNA stability with LDH nanoparticles.

- **Naked dsRNA** have shown practical efficacy for **silencing up to 7 days.**
- LDH can be used to stabilize and **assist in the delivery** of dsRNA, which can extend the effective period **up to one month** (Mitter et al., 2017)

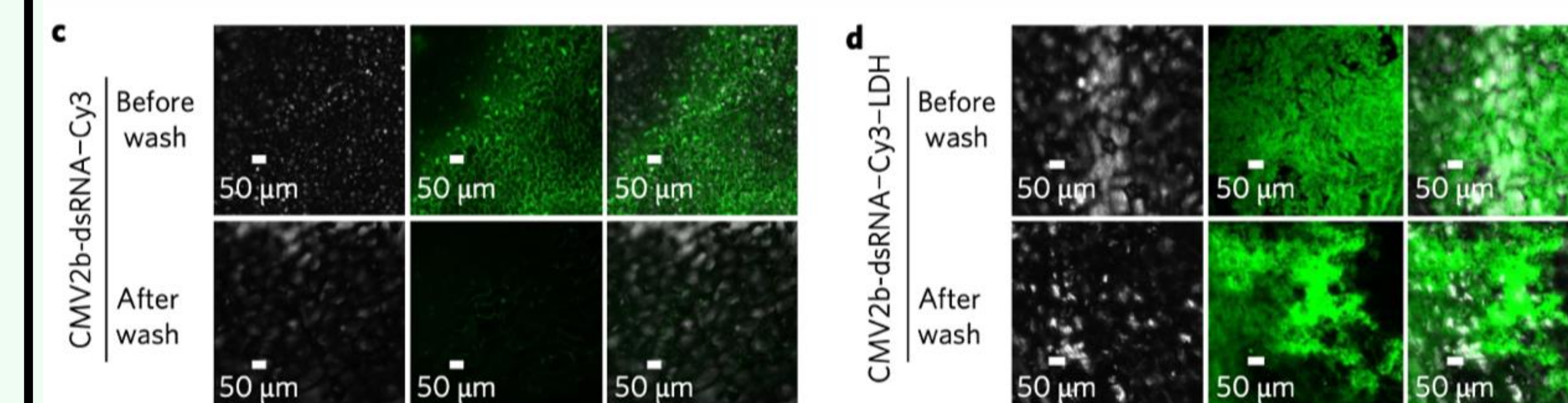


Figure 4: dsRNA-LDH protection and adhesion to leaf surfaces post-"washing" (Mitter et al. 2017).

Checkpoints:

- Test loading capacity of LDH with dsRNA.
- Test stability of dsRNA-LDH by exposing the combination to RNase enzymes (**Figure 4**).
- Compare dsRNA-LDH and naked dsRNA silencing effects over time.

Timeline and Progress on Milestones:

	2020			2021			2022		
	April	Aug	Dec	April	Aug	Dec	April	Aug	Dec
Obj. 1				rtPCR					Final Reports
Obj. 2			Optimize						
Obj. 3			Optimize						

Objective 1: Gene segments of candidate genes were identified. dsRNA synthesis for high yield is optimized, and silencing effects are being tested with real-time PCR. Fungal pathogenicity assays will take place in August 2021.

Objective 2: Initial labeling and visualization of dsRNA from tissue cultured samples was optimized. Systemic movement analysis will begin in the Summer/Autumn 2021.

Objective 3: LDH synthesis optimization and loading tests are beginning. Stability experiments using RNases will begin in late Summer 2021.

References:

Dalakouras A, Wassenegger M, Dadami E, Ganopoulos I, Pappas ML, Papadopoulou K. Genetically Modified Organism-Free RNA Interference: Exogenous Application of RNA Molecules in Plants. Plant Physiology 2019; 182: 38–50.

Mitter N, Worrall EA, Robinson KE, Li P, Jain RG, Taochy C *et al.* Clay nanosheets for topical delivery of RNAi for sustained protection against plant viruses. *Nature Plants* 2017; 3. doi:10.1038/nplants.2016.207.

Underwood W, Somerville SC. Focal accumulation of defences at sites of fungal pathogen attack. *Journal of Experimental Botany* 2008; 59: 3501–3508.