

Evaluation of chemical and natural resistance inducers against Downy Mildew

Harm et al.

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- The ecological harm posed by certain pesticides, coupled with the increasing development of pathogen resistant strains, have pointed out the urgent need to find alternatives to pesticides. One such approach is the use of natural substances able to stimulate the defense system of the vine, also called *resistance inducers*.

- **Resistance inducer** is a term used to describe a wide range of substances able to trigger resistance reactions in the plant similar to that caused by a pathogen. These remarkable reactions can include 1) release of *reactive oxygen compounds*, which initiate a programmed death of surrounding plant cells that inhibit further expansion of the pathogen; 2) production of what is known as *pathogenesis-related (PR) proteins* (e.g. chitinases, glucanases) that can attack the cell walls of fungus; and 3) activation of genes that cause the accumulation of various *phytoalexins* (e.g. resveratrol) toxic to the pathogen.

- The following is a list of substances known to this date to induce resistance reactions in grapevines against various pathogens:

- Benzothiadiazole (**BTH**)
- Jasmonic acid (**meJA**)
- B- aminobutiric acid (**BABA**)
- **Milsana** (=extract of the plant *Reynoutria sachalinensis*)

- In the search for a substance able to induce resistance specifically against downy mildew (*Plasmopara viticola*), some materials have shown encouraging results *in the lab or in the greenhouse*. However, when it comes to the practical application in the vineyard, **no natural substance has been detected to date that is able to induce resistance to downy mildew under field conditions**.

- The current authors wanted to change that. As a result, this study's goals were to 1) to test a wide range of natural substances for their ability to induce resistance reactions against downy mildew; and 2) to reveal the mechanism of action of these natural inducers, and compare it with the mechanism of action of well-known chemical inducers.

- **Inducers tested:** Researchers started testing 53 natural substances, belonging to various chemical categories. After a preliminary screening, they selected the following 4 substances that showed sufficient efficacy to justify further testing:

- Extract of *Solidago canadensis* (**CANG**)
- Extract of *Penidillium crysogenum* (**PEN**)
- Linoleic acid (**LIN**)
- Isolate of *Aurobasidium pullulans* (**AUREO**)

As a positive control, two well-known chemical inducers previously mentioned were added to the list (BTH, BABA); as well as two fungicides of proven effectiveness (Cuprocin, Frutogard).

- To achieve the first goal -**test for natural inducers**-, the authors used various approaches simultaneously, including:

- 1) *Leaf disc assay*: authors treated grapevine leaf discs with a solution of each candidate material, then inoculated the discs with *P. viticola*, and after incubation, measured the degree of infection (pathogen sporulation);

- 2) *Outdoor potted vines assay*: authors sprayed vines with each material until runoff, then artificially inoculated the plants with *P. viticola*, and recorded symptomatic plants;

- 3) *Zoospore inhibition assay*: each candidate material was placed in a well with a solution containing active swimming *P. viticola* zoospores. After incubation, the percentage of zoospores still alive (swimming under the microscope) was recorded.

- To achieve the second and most ambitious goal – **clarify the mechanism of action**- the authors used two approaches:

- 1) *Enzyme activity assay*: after treating leaves with each material to be tested, researchers extracted various enzymes known to increase during resistance reactions (PR proteins) and tested their enzymatic activity by providing the corresponding substrate. (See original text for specific enzymes tested).

- 2) *RNA assay*: authors isolated the plant tissue RNA and quantified the amount of messenger RNA from specific genes, to find out which genes had been particularly activated after exposure to each of the inducers. (No easy task!)

- **Results:**

- 1) **Effectiveness against downy mildew**: Of all 53 substances tested, only **CANG showed effectiveness against a wide range of pathogens, including downy mildew**. This effect was similar to that of the chemical inducers BABA and BHT. PEN, LIN, and AUREO showed unsatisfactory disease reduction of 50% both in the greenhouse and under field conditions.

- 2) **Mechanism of action**: The inhibition of zoospores suggested to the authors that the mechanism of action taking place was a *direct toxic effect* against the pathogen of some ingredient in the natural substance tested. In contrast, the elevation of PR proteins, or the activation of genes coding phytoalexins, pointed to the triggering of a general resistance reaction in the plant (obviously, needing to be further understood). The authors concluded that the mode of action of the substances studied was highly variable, involving a **combination of both mechanisms** - direct inhibition and trigger of a stress reaction.

In summary, natural substances are able to enhance the resistance of grapes to downy mildew. The liquid extract of the plant *Solidago canadensis* was the most effective of all substances tested, showing an effectiveness similar to that of chemical inducers. Still, just as is the case with chemical inducers, this level of protection is insufficient to serve as reliable protection against downy mildew. Even though the authors admit that all of these substances cannot replace fungicides to this date, they can complement their action and lower the amount of fungicide use.

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