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Grapes and vineyard soils as sources of microorganisms for biological control of *Botrytis cinerea*"

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In search of a biological cure against bunch rot (caused by *Botrytis cinerea*), these authors screened grape skins and vineyard soils in Uruguay in search of microorganisms naturally antagonistic of *Botrytis cinerea*.

• *Botrytis cinerea* is currently controlled with pre- and post-harvest fungicides, but biological control agents are becoming more popular due to environmental concerns. The use of antagonistic organisms to control *Botrytis* is not new. One commercial example is the product Serenade®, an organic fungicide that contains the bacteria *Bacillus* as its active ingredient.

• So for the course of 3 years (2000 to 2003), the authors isolated 223 epiphytic microorganisms (i.e. naturally occurring on the surface of grapes and soil) from the most common cultivated varieties in Uruguay, as well as from 20 vineyard soils. Once these isolates were growing on agar plates, they tested the ability of each to inhibit the growth of two strains of *Botrytis* spores.

• The authors conducted 2 types of inhibition tests. 1) **In vitro inhibition:** the authors placed spores of *Botrytis* in a well made in the center of the agar plate where the "antagonistic agent" was growing. After incubation, they measured the diameter of the *Botrytis* colony that was able to grow, called *inhibition diameter*. If the agent tested completely inhibited the growth of the fungus, the inhibition diameter was considered zero.

2) **In situ inhibition**: here the authors dipped clusters of Tannat grapes in a solution containing the most effective organisms found in 1) above. After spraying the clusters with *Botrytis* spores, and incubating them at 25°C in plastic bags for 4-5 days, they counted the number of berries with rot. The more effective the agent tested, the fewer the number of rotten berries. As a positive control (effective inhibition of *Botrytis*), the authors used the commercial product Serenade.

• **Results**. Among the 223 isolates tested, **8 yeast and 4 bacteria were selected as the best antagonists** against *Botrytis*, based on having the smallest inhibition diameters. Of these, one bacterium identified as *Bacillus sp.*, and one yeast identified as *Hanseniaspora uvarum*, were the most effective, based on a reduction of the number of infected berries of 88% and 80%, respectively. This was equivalent to the antagonism obtained with Serenade.

• **Possible mechanism**. To learn a little bit about the potential mechanism of action, the authors compared the nutrient uptake rates (mainly glucose and nitrogen) of the antagonists versus those of *Botrytis*. They also used a microscope to study what was happening to *Botrytis* spores treated with the antagonistic agent. They were able to conclude that the mechanism of action of *Hanseniaspora* was a **competition for nitrogen**. In contrast, the mechanism of action of *Bacillus sp*. was mainly the **production of antifungal substances** that disrupted the *Botrytis* spores (besides some competition for nitrogen as well).

• **Compatibility with wine yeast.** The authors made sure that the antagonistic microorganisms selected for biocontrol of *Botrytis* were compatible with the wine yeast. For that, first they fermented Chardonnay with *Saccharomyces cerevisiae* (UC Davis M522 strain). Then, they selected a panel of winemakers to determine whether the antagonist microorganisms were imparting any off-flavors to the wine. The sensory results showed that *Bacillus sp*. were not imparting any undesirable characters.

• **Preliminary vineyard applications**. A preparation of *Bacillus sp.* was heavily diluted and sprayed on a one hectare (2.5 acre) experimental block of Tannat (at a rate of 300 L/ha). (The authors do not mention the phonological stage at which the vineyard was treated). After sampling the grapes weekly to monitor the presence of the introduced organism, they were able to confirm the survival of *Bacillus sp*. in leaves and clusters 70 days after the spray treatment (10^2 cells/cm^2 of leaf).

As the authors discuss, one of the main challenges for the development of any biocontrol agent is to achieve a stable product able to retain its activity in the field, where many factors can challenge survival. The present results indicate to the authors that a reliable strategy based on the use of these natural populations can be developed to replace current synthetic fungicides. We will watch out for future communication from these authors on what remains the "ultimate test": whether the treated blocks actually developed less *Botrytis* or not.

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