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Quantification of *Botrytis* in grape juice determined by a monoclonal antibody-based immunoassay

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In this paper, the authors test the ability of monoclonal antibodies to detect bunch rot in incoming loads of harvested grapes. The results have allowed the development of what we know today as the "*Botrytis* pregnancy test".

• *Botrytis cinerea* is the main cause of bunch rot in grapes, but other organisms, like *Aspergillus, Penicillium*, wild yeasts, and bacteria, can also be involved. Currently, assessment of rot in the winery is done by visual inspection at the testing stand. But this assessment has a few drawbacks: it requires training, it is time-consuming, and it becoming extremely difficult, or impossible, when the grapes are machine-harvested.

• The first attempts to create an immunoassay to detect *Botrytis* used **polyclonal antibodies**. Polyclonal antibodies are **a mixture** of proteins (immunoglobulins) secreted by different cells against different parts of a specific antigen. Because they are a heterogeneous group of molecules, they did not work for reliably quantifying the presence of *Botrytis*.

• After further attempts to find a more effective assay protocol, Dr.Dewey developed an immunoassay utilizing **monoclonal antibodies**. Monoclonal antibodies are all **identical** because they are secreted by a single B-cell line, making this approach more promising for detecting *Botrytis* using an ELISA test.

• In an **ELISA** test (**E**nzyme-**l**inked **i**mmuno-**s**orbent **a**ssay), a specific antibody is "glued" to the bottom of the wells of a microtiter plate. If the sample that is subsequently added to the wells contains the matching antigen (in this case, a specific *Botrytis* antigen), it reacts with the glued antibody, eventually developing color that can be visually or instrumentally read.

• In previous studies, the authors had developed 3 different monoclonal antibodies to detect *Botrytis* (that we will call A, B, and C). The purpose of the current research was to study how well each of these antibodies worked to detect *Botrytis* in white and red grape juices. A second goal was to evaluate how well the results correlated with the corresponding visual assessments of rot.

• **Juice collection.** During the harvest of 1998, the authors collected 41 samples of juice -including 15 different varieties- from several large wineries throughout the Central Valley of California. From each truck, the authors collected 3 subsamples: 1) one sample of juice from the incoming truckload prepared on site, 2) one sample of juice from healthy-looking berries (crushed back in the lab), and 3) one sample of juice from rotted berries. At the same time, inspectors from the California Department of Food and Agriculture were making visual inspections of rot on those same truckloads.

• **Immunoassays.** All of the frozen-and-thawed juices collected were tested by ELISA, using four replications. To do that, the wells were coated with the undiluted juice. Then they were incubated at room temperature in the presence of each of the 3 monoclonal antibodies. Finally, an enzyme was added that

resulted in color development only in those wells where an antibody/antigen complex had been formed. The authors also ran a parallel immunoassay using juice from Cabernet Sauvignon and Semillon grapes that had been artificially infected with *Botrytis*. Finally, they ran a series of previously-isolated *Botrytis* antigens at different known concentrations, so they were able to estimate the relative amount of antigen present in each unknown sample.

• **Results.** Early in the research, the authors realized that monoclonal antibody A **was much more effective for quantification of *Botrytis* than the other two**. The problem with Antibody B was that it required extremely careful dilution of the grape juice. Antibody C was not selective enough: it wasn't just detecting *Botrytis*, but also reacting with *Aspergillus* and *Penicillium* as well.

• **Visual results *versus* Immunoassay results**. The authors reported results for each of the 3 subsamples per truckload that they had collected (juice direct from the truck, juice from healthy berries, and juice from rotted berries, remember?). In the juice samples taken directly from the truckload, **there was a good correlation between the amount of visual rot and the amount of *Botrytis* as indicated by the immunoassay**. Regarding the samples from healthy berries, there was a good correlation with the exception of 2 samples. As the authors note, these false positives came from healthy berries lying in the midst of heavily-infected machine-harvested fruit. So contamination with nearby *Botrytis* antigens was the likely cause. The authors do not report any results from the samples using rotted berries.

• The authors discuss a few exceptions in which the immunoassay values of rot either overestimated or underestimated the visual values. In the first case, the explanation was that the relationship between levels of infection and absorbance values stopped being linear at infection levels of 1.5% and above. In the second case (visual rot appeared higher than the immunoassay), the authors theorized that fungi other than *Botrytis* (*Arpergillus, Penicillium*) were responsible for the visual rot. They later confirmed this using Antibody C.

In conclusion, the authors showed that one of the *Botrytis*-specific monoclonal antibodies they studied can be used as a "sensitive, rapid, and reproducible assay for detection and quantification of *Botrytis* in undiluted infected grapes". The test is effective for levels of *Botrytis* infection between 0 and 6%. As a result of these findings, Dr. Dewey developed a kit for the semi-quantitative detection of *Botrytis* in a winery setting (the "Botrytis-pregnancy kit") which is commercially available worldwide. Using this portable device **two bands of color indicate a positive result (botryzised grapes), and one band indicates a negative result (healthy grapes)**. For a lively and animated brief tutorial on how an ELISA test works, check out:
http://www.sumanasinc.com/webcontent/anisamples/molecularbiology/ELISA.html.

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