To protect vineyards from pests and reduce pesticide use, CRISPR could be the answer

Wine growers face a lot of natural enemies. Drought, too much rain, insects and fungus are common problems when growing grapes for fermentation. And consumer demand for flavor and aroma also can confound an otherwise healthy winery.

Many winegrowers have turned to pesticides, including the organic farming-approved fungicide copper sulfate. But copper sulfate has presented persistent issues, like resistance, to organic and conventional wine growers alike. For some wine growers and researchers, genetics may provide a solution to the pest problems. And the new technique, CRISPR/Cas9, has shown promise (at least in the lab) against a particularly nasty wine grape pest—powdery mildew.

Powdery mildew is a fungus that can scar mature fruit, infect buds, and leave a white powdery coat over grape leaves. Once enough leaves fall off, the plant can no longer produce enough sugar to create wine-quality grapes. And, as journalist Brooke Borel pointed out in her article for NOVA, powdery mildew is found anywhere on Earth where wine grapes are grown.

Copper sulfate has been one of the ways to keep powdery mildew at bay, but plants have started showing resistance and there have been issues involving persistence of elemental sulfur in the ground. Previous articles in the Genetic Literacy Project and elsewhere have profiled organic wineries in France and the United States that have shed their organic status because of concerns about copper accumulation.

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Leaves afflicted with powdery mildew

One area that's been looking at CRISPR is the laboratory of Rong Di at Rutgers University, in New Jersey. The Garden State is the country's seventh-largest producer of wines, and has three registered growing areas, two of which totaling more than 2.5 million acres. While the soil is right, the climate is humid (the American Viticultural Area, which is the official mark of a wine growing region, terms New Jersey as "subtropical"), which is also perfect for powdery mildew.

Di's lab has isolated three genes in the grapes that appear to allow powdery mildew spores to attach and begin attacking wine grapes, particularly chardonnay. CRISPR, which is short for clustered regularly interspaced short palindromic repeats, works by precisely editing and splicing out areas where a gene may exist. This technique could excise or shut down the functions of genes that can make it easier for powdery mildew to attach and attack. If it works in the field, it could mean growers wouldn't need to use copper sulfate or a host of other fungicides.

Whether this is acceptable to consumers and oenophiles is another question. There are many, many other varieties of wine grapes besides chardonnay that can resist fungus and other pests. But nearly all of which are never used in wine production, due to perceptions of public interest particularly because people want the taste of a known variety, and not an unknown. Ironically, chardonnay itself is the product of the revered pinot variety, and a very unpopular type called gouais blanc that no well-drenched wine expert would be caught dead drinking.

Other genetic avenues are under exploration:

- At Andy Walker's lab at the University of California at Davis, researchers are looking at developing wine grapes that are resistant to Pierce's Disease, which is an infection deadly to grapevines, caused by the bacteria *Xylella fastidiosa* (which also causes problems for olive trees), and spread by sharpshooter leafhoppers. Here, they're using traditional crossing and marker-selected breeding to combine grapevines with some resistance with other, perhaps better tasting wine. One strain from northern Mexico, *Vitis arizonica*, gets its PD resistance from one dominant gene, making it easy to breed using marker assistance and get flowers and fruit within two years.
- Mark Fuchs, a researcher at Cornell University, has looking at using genetic modification to develop wine grapes resistant to grapevine fanleaf virus (GFLV), which is spread by a nematode vector and damages leaves and stalks, and prevents berries (grapes) from developing. This virus has not been susceptible to a number of other methods, Fuchs is looking at using RNAi (RNA interference) to create a new resistance to the virus.

Many grapes and mildews strain genetics

Fuchs' work illustrates one problem that's also an issue with powdery mildew. There are many strains of mildew and fan leaf virus, making specific resistance a challenge (you'd have to make sure that your new strain and genes could resist all of them).

Another issue is that it's not clear whether knocking out certain resistance genes or inserting others could have any effect on flavor. That's because the aroma and taste of wines come from a lot of sources, and not necessarily protein (and therefore not genetically) based. About 50 volatile compounds help produce the odors in wine, including hexanal, phenylethyl alcohol, guaiacol, and vanillin. Wine growers look for just the right balance of these chemicals during ripening, as grapes ripen and volumes of sugar compounds begin the increase. Exactly when that right point is reached is not an exact science.

And, as this study led by Cornell University scientist Edward Buckler showed, there is an enormous variety in wine grapes. Because of their chemical and genetic complexity, wine grapes are a tough candidate for genetic modification approaches. And while gene banks do exist that have thousands of varieties of wine grapes, few are used because of customer/expert/industry preferences. It's distinctly possible that a new, disease-resistant grape that tastes good to arise the same way the old warhorse chardonnays did—by mixing the good with the bad. Prost!

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