Research in Plain English

A rapid, sensitive and inexpensive method for detection of grapevine red blotch virus without tissue extraction using loop-mediated isothermal amplification

Research in Plain English provides brief, non-technical summaries of journal articles by Cornell faculty, students, and staff.

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Summary by Tim Martinson.

The Takeaway.

- Grapevine red blotch disease, caused by grapevine red blotch virus (GRBV), is a newly-emerged economic disease that affects yield and quality of wine grapes.
- Current testing methods for GRBV involve either DNA extraction from leaf petioles and PCR amplification, requiring specialized laboratory equipment and expertise.
- A new modified technique using “Loop-mediated isothermal amplification” (LAMP) is a simplified testing method that doesn’t require DNA extraction, costs less than a dollar per sample, and can be completed within an hour.
- Authors report that it is up to “10,000 times more sensitive than conventional PCR methods”.
- The colorimetric test requires little specialized equipment or training, and therefore could potentially be suitable for consultants and vineyard managers to use to diagnose the extent of red blotch in vineyards.

 Red blotch virus symptoms characteristic of a red grape cultivar include darkening of the leaves. Photo courtesy of Marc Fuchs.

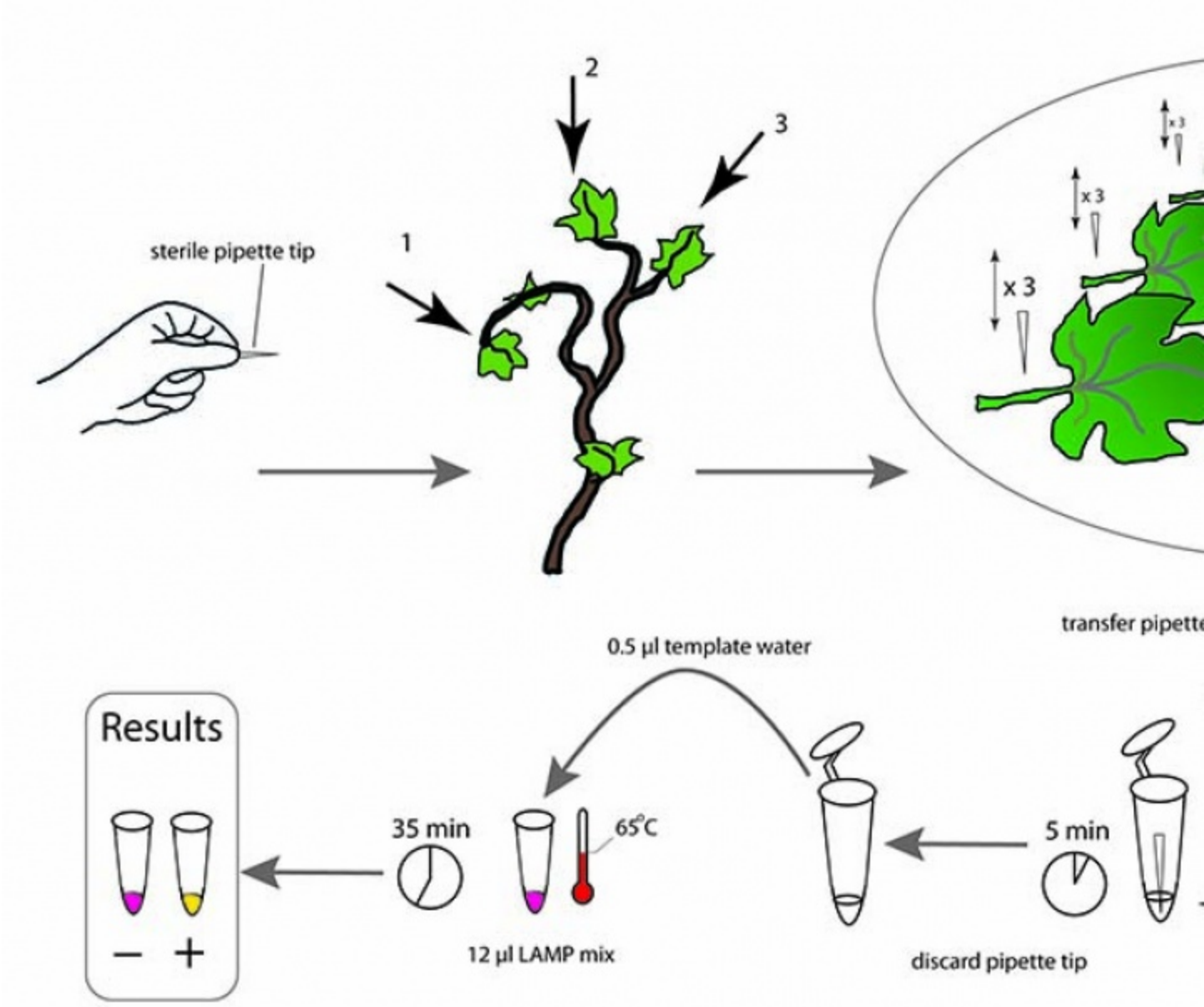
Background.

Grapevine red blotch disease emerged as a newly-recognized viral disease of grapevines in 2008. Grapevine red blotch-associated virus (GRBV) was identified in 2012, and since then the disease has been widely detected in new plantings throughout North America. In Western North America, GRBV is spread by an insect, the three-cornered alfalfa leafhopper. Management involves detecting and removing infected grapevines. Visible leaf symptoms (red blotches) appear at times on red cultivars, while white cultivars may exhibit interveinal leaf chlorosis (yellowish leaves) and marginal necrosis. Visual symptoms are not entirely reliable, so diagnostic tests are needed to establish the infection status of vines. The primary methods currently used (multiplex PCR, qPCR) involve extraction of DNA, followed by PCR amplification.

Experiment.

Loop-mediated isothermal amplification (LAMP) is a single-tube method for amplifying DNA at a constant temperature in a single tube. The authors designed a set of six different primers associated with DNA sequences coding for the viral 'coat protein'. This primer set and distilled water was used in subsequent diagnostic reactions, along with grapevine tissue obtained through the 'pin-prick' method:

- A sterile pipette tip was used to 'stab' 3 leaves in their petioles (3x) and at the base of the leaf (5x) to obtain a residue of tissue.
- The pipette tip was placed in a small sterile tube with 10 microliters of distilled water for five minutes.
- Half a microliter of the water was added to a tube with 12 microliters of the LAMP mix.
- The tube and sample were incubated for 35 minutes at 65°C.
- Solution colors of red (negative) or yellow (positive) were scored.



Flowchart of the steps involved in the GRBV LAMP assay. Photo reproduced with permission from the Archives of Virology.

Results.

The LAMP assay, when tested with serial dilutions of total DNA extraction and compared to standard PCR and qPCR testing methods, was able to detect the virus at two orders of magnitude lower concentrations than these standard methods. Because of this sensitivity, the authors were able to develop and use the 'pin-prick' method, which bypassed the 2-hour step of DNA extraction from tissues. Further testing in which the timing of collection, temperature of incubation, and length of incubation were varied showed the procedure was robust; no false negatives were observed. Due to the extreme sensitivity of the LAMP test,

contamination and false positives are a potential problem, necessitating care in handling and execution.

The cost per sample was estimated at $0.85. The procedure requires sterile pipette tips, small centrifuge vials, reagents with appropriate DNA primers, and a water bath.

Conclusions and practical considerations.

If further validated with further extensive field trials, this simplified detection test could be used in nurseries, certification programs, and by interested growers to diagnose and detect red blotch infections in samples collected from the field. It is likely this LAMP will be a useful technique for detecting other viral pathogens as well.

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