A Revolution in plant diagnostic technology: using high throughput sequencing for detection of plant disease

It has long been recognized that diseases cause production losses in perennial crops such as fruit trees and grapevines. To screen for detrimental diseases in these crops, plant quarantine and protection programs first made use of biological indicators. These indicators are specific plant species or varieties that are known to display symptoms when exposed to certain disease agents. Herbaceous indicators are used by spreading the sap of the plant being tested across the leaves of the indicator plant. This process may be completed in days or weeks. Woody indicators are used by grafting the material of the subject plant to a variety known to display symptoms if infected. After the grafting is complete, disease agents present in the subject plant are expected to infect the indicator, which would display symptoms. Indicators are only known to display symptoms for certain agents; it is possible that an indicator may show no symptoms, but a disease agent is still present. It can take many years for woody indicators to establish and show symptoms. Diagnostic technology evolved to ELISA and PCR molecular methods, often in conjunction with indicator plants. ELISA and PCR are advancements over biological indexing because they allow the determination of specific disease agents, and typically require less time to complete testing.

There are also drawbacks to these conventional disease detection methods. Biological indicator indexing is time-consuming, does not detect all pathogenic viruses, suffers from unreliable plant performance, cannot specifically identify pathogenic viruses, and demands large amounts of space and labor. Conventional molecular tests like ELISA and RT-PCR are limiting because they require prior knowledge of the pathogen and are incapable of detecting variants. In comparison to these conventional methods, the latest diagnostic tool we can employ is high throughput sequencing (HTS). HTS provides an advantage because it gives a comprehensive picture of the entire microbial profile in a sample without prior knowledge of the pathogen. Many scientists have recognized the value of HTS, and there is a growing list of published research

demonstrating its advantages over biological indicators. To replace biological indicators with HTS for regulatory testing requires thorough validation of diagnostic protocols after proving its advantages.

Foundation Plant Services (FPS), established at the University of California, Davis in Davis, California, USA, in 1958 is a source of elite propagation materials grape, fruit trees (stone and pome fruits), olives, nuts, strawberries, roses, and sweetpotatoes. FPS facilitates the introduction, quarantine, and release of imported grapes and fruit trees in the US under a Controlled Import Permit (P588) from the United States Department of Agriculture, Animal Plant Health Inspection Service, Plant Protection and Quarantine (USDA APHIS PPQ). In addition to the federal oversight, the state agency California Department of Food and Agriculture (CDFA) must also approve the release of plant materials from quarantine. USDA APHIS PPQ and CDFA regulations have required the use of conventional disease detection methods, including indexing on biological indicators, ELISA, and RT-PCR. FPS-supplied propagation materials are typically planted to create nursery increase blocks, from which the nurseries will distribute to growers. It is important that plant material distributed by FPS is thoroughly screened for viruses using a method with the lowest possible false negative rate.

FPS first began evaluating HTS for plant disease detection in 2007. Through side-by-side comparison of biological indexing and HTS, FPS determined that HTS was the superior method for disease detection. In order for USDA APHIS PPQ and CDFA to approve HTS for use in diagnostic testing, further validation was required. At FPS, the validation followed a two-phase process: first, validation of the methods used in house, then, validation in inter-laboratory trial to confirm reproducibility. FPS integrated the validation research knowledge into a protocol that employs the most reliable and efficient techniques using HTS and RT-qPCR/qPCR (Figure 1). The protocol requires informative testing on source material, followed by initial and final testing of two types.

tissue, separated by at least six months and a dormancy period. USDA-APHIS-PPQ and CDFA have approved the use of this revised diagnostic testing protocol to replace biological indexing with a combination of HTS and PCR testing for the release of plant material.

To my knowledge, the US is the first country to adopt HTS for the release of plant material from quarantine; I hope it does not stay this way for long. There are several factors that are slowing the adoption of HTS, but each may be overcome, many with collaboration:

- High cost of equipment, which may lead to a high price per sample if testing volume is low. This can be overcome by collaboration with other programs, establishing shared central laboratories, or sending samples to a third party on pay-for-service analysis.
- Bioinformatic infrastructure requiremenare for computer programs and processing power. These costs may also be reduced by sharing computing resources with others or hiring a third party for services. As HTS technology has advanced, desktop software options are now more easily accessible and affordable.
- Sample preparation. Testing requires high-quality nucleic acid, which can be challenging to generate from some plant hosts.
- Data analysis: First, one must separate the background noise from the true virus and host source sequences. Second, a trained virologist should be employed to evaluate the results and determine if infection is truly present and poses a risk.
- HTS technology is so new, most regulations predate its use and do not contemplate it as acceptable technology. This must be changed and can only be done so as more nations validate protocols that are sensitive, specific, repeatable, and reproducible.

I am confident that with the continued work of other scientists around the world to validate HTS protocols, it will soon be employed in pathogen-regulating programs worldwide.

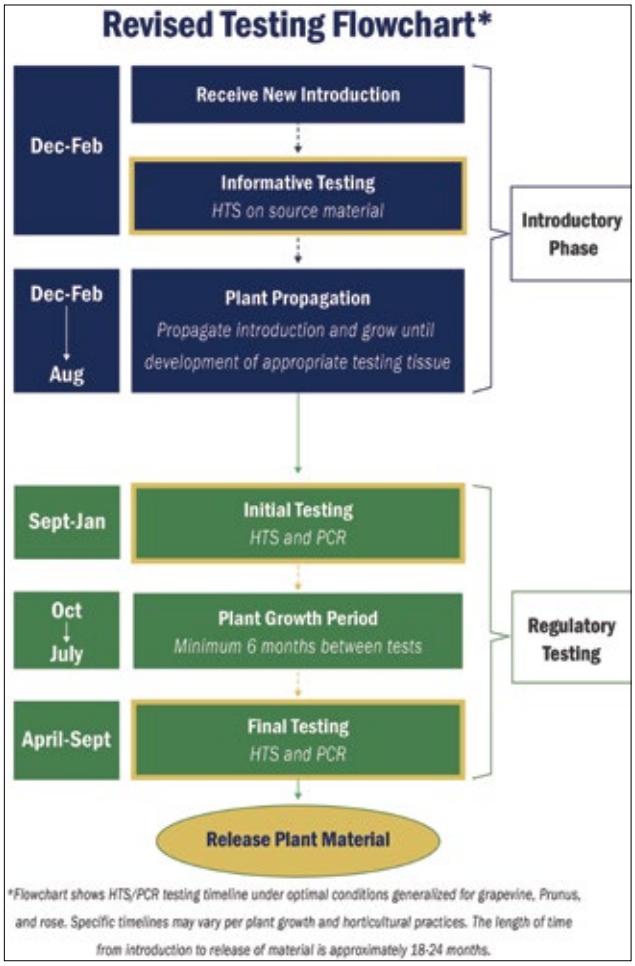

Figure 1 Flowchart showing HTS/PCR testing timeline for plant material at FPS under optimal conditions. Specific timelines may vary per plant growth and horticultural practices. The length of time from introduction to release of material is approximately 18-24 months

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