More detailed information

Stage 1a: In vitro testing

In-vitro testing (in a petri dish in a laboratory) is useful as it allows a relatively quick and inexpensive way of screening a large number of products. Two *in-vitro* techniques have been used that evaluate the ability of a product to inhibit Psa growth:

1) Growth in Liquid Culture in the presence of the product
    - Psa is introduced into a liquid growth media (or broth)
    - Initially the broth is clear, but as bacteria multiply the broth becomes cloudy
    - By shining light of a particular wavelength through the broth relative bacterial cells numbers can be estimated
    - Bacterial numbers are measured in terms of 'optical density' of the broth – the higher the optical density the more bacteria are present
    - Products are introduced to the broth and if bacterial growth is inhibited (generally due to death of the bacteria) optical density will not increase (products may cause optical density to decrease if bacterial cells are destroyed)
    - Products are evaluated across the same range of concentrations to allow comparison but these concentrations don't necessarily reflect label recommendations
    - The impact of biological control agents are measured differently as they also grow in the broth, causing it to go cloudy and increase the optical density reading even if the Psa growth has been inhibited. Rather than take an optical density reading, a subsample of the broth is placed on a growth medium (agar) in a plastic dish (or plate) and the resulting number of Psa colonies counted (represented in the results as reduction in Psa population)

This method has the following limitations:

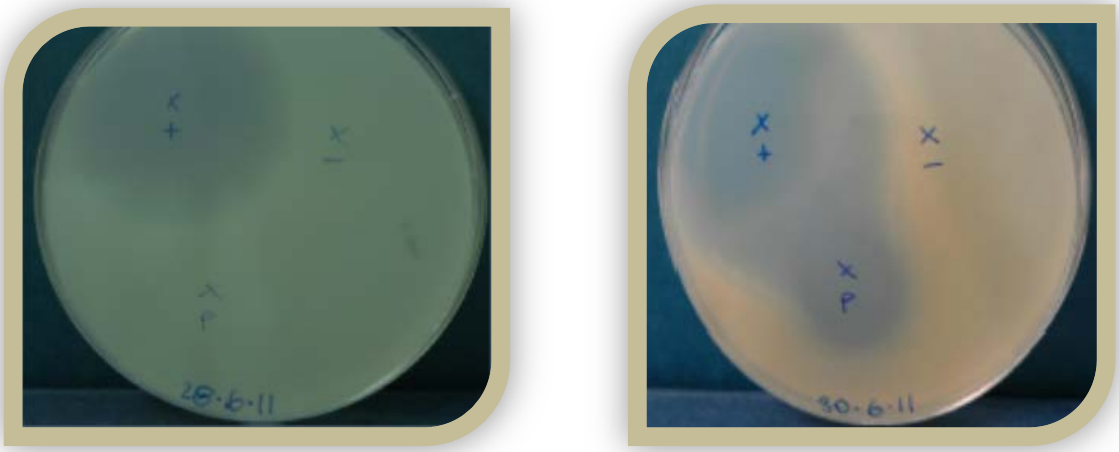
- It will not demonstrate efficacy of products that work only by stimulating the plants immune response (e.g. elicitors)
- Individual products can increase optical density (e.g. those that are cloudy, are intensely colored and/or cause precipitates in the broth)

Caption:*Set-up of Broth experiments*

2) Growth on an Agar Plate in the presence of the product
    - Psa is grown on an agar plate
    - The product is dropped in one well defined area of the plate
    - Products are evaluated at several rates
    - The plate is incubated for 24 to 48 hours which allows Psa to completely cover the plate
    - The ability of the product to inhibit Psa growth is evaluated by measuring the area not covered by Psa, this is termed the 'zone of inhibition'

This method has the following limitations:

- It cannot be used to evaluate the efficacy of biological control agents that protect plants only by occupying the sites the pathogen would normally colonise, or biological control agents which multiply faster than Psa and cover the plate before a zone of inhibition can be seen
- It will not demonstrate efficacy of products that work only by stimulating the plants defense response (e.g. elicitors)
- It will not demonstrate efficacy of products that cannot diffuse through the agar
- It will not demonstrate efficacy of products that make the agar go cloudy
- Some products will be locked-up and rendered ineffective by the agar



Caption: *Psa is seen here growing on an agar plates as the yellow, cloudy film. The agar plate on the left is showing no zone of inhibition where the product has been applied (x P) vs the plate on the right which is showing a clear zone of inhibition where the product has been applied (x P). x – Represents where water was applied (negative control), x + represents where an antibiotic known to inhibit Psa growth (positive control).*

Should a product be efficient at killing Psa-V then it will generally move to Stage 1b (outlined below). While the test does measure the ability of the product to kill or inhibit Psa-V growth, it **does not** measure the potential impact of the product on a kiwifruit plant.

If the product damages or kills kiwifruit vines at the same or similar concentration as that used to kill Psa-V, then it may have value as a sanitiser in other parts of the supply chain, but it would obviously not have value as a product to be applied to kiwifruit vines.

Stage 1b: In-vivo (on the plant) Glasshouse testing

Testing on plants in the greenhouse is slower and more expensive that *in vitro* testing. However it does give a better indication of the product's ability to control Psa-V on kiwifruit vines. Greenhouse trials are advantageous as they are undertaken in a controlled environment and can be completed at any time during the year. They also allow a higher throughput of testing than field trials, and plants are known to be free of Psa infection before the experiment.

Different tests are undertaken depending on the anticipated use of the product being a protectant or potential curative. Experiments are conducted in Plant & Food Research PC2 containment facilities in Hamilton and Palmerston North, using kiwifruit seedlings and cuttings. The 'greenhouses' are special climate control rooms where Psa can be safely contained and temperature, day length and humidity can be varied to provide a natural growing environment.

Protectant testing

To test for the protective value of a product, it is applied to the Psa-V-free plants. At a specified time after the product is applied to the plant, the plant is inoculated with a solution of Psa-V. All products are initially tested at their highest recommended rates. Plants are evaluated for the development of typical Psa leaf spots usually at about 8 and 14 days after inoculation of the Psa. A severity rating of 0 (lowest) to 5 (highest) is given corresponding to percent leaf area with typical Psa leaf spots.

Caption: *Psa leaf spot development on inoculation Hort16A seedlings (disease rating shown in the right-hand corner).*

Only products that show a reduction in infection severity are kept for further experimentation. Some products are trialled with, or without, the addition of an adjuvant. Combinations of products and adjuvants at various concentrations can be used to understand at what level the product is phytotoxic and the lowest level that can be applied to achieve protection.

Curative testing

For potentially curative products the plants are first inoculated with a solution of Psa-V. At a specified time after the Psa-V is applied, the tested product is applied to the plant. Symptoms are monitored in the same way as a protective screening. Again, various combinations of products and adjuvants will be trialled to find promising combinations and ratios.

Should a product show promising signs in Stage 1b it will move to Stage 2 (see below). Products that do not may be discarded, or recommended for use as a sanitiser only.

This method has the following limitations:

- The test only measures the impact of the product on the level of Psa infection, and **does not** measure any impact on yield or fruit quality, or any other commercial impacts on vine performance.
- They do not determine the impact on a mature vine, which may react differently to a cutting or seedling
- Seedlings have a natural genetic variation that may impact response to infection
- Bruno seedlings may behave differently to Hort16A (the suitability of Bruno as a proxy for Hort16A is being investigated)
- Young seedlings may respond differently to infection compared to adult plants
- Products are being evaluated in a very high disease pressure environment that may not represent the field situation - this may adversely impact the efficacy of biological control agents and some protectants that generally work better in low disease pressure environments
- Products are being evaluated in an artificial environment which may be promoting or inhibiting their effectiveness compared to the field situation (e.g. minimal impact of UV, constant temperatures and relative humidity and no rainfall)

Photograph acknowledgement - Dr Joel Vanneste