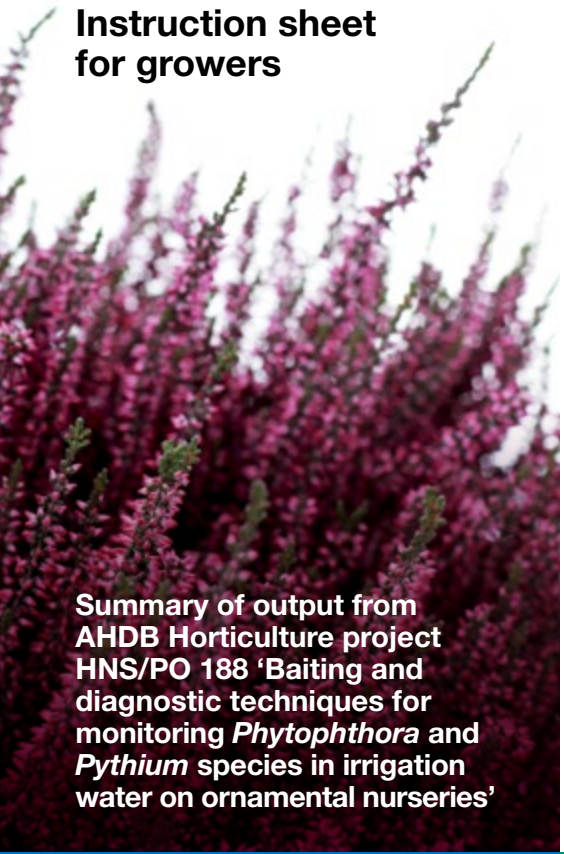
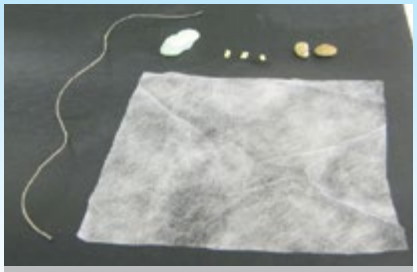
Baiting stored irrigation water to test for the presence of *Pythium* and *Phytophthora*

Instruction sheet for growers



Summary of output from AHDB Horticulture project HNS/PO 188 'Baiting and diagnostic techniques for monitoring *Phytophthora* and *Pythium* species in irrigation water on ornamental nurseries'

Pythium and *Phytophthora* water baiting – a quick guide



1 Components needed for the bait bag: 7–10g boiled stones, polystyrene, apple pieces, length of string, and fleece (size approximately 28x28cm).

2 To obtain the apple pieces, cut a slice of 'Golden Delicious' apple 7mm thick. From this cut out eight squares approximately 7x7mm from the centre of the apple slice using a clean knife.

3 Place the apple pieces in the centre of the fleece with the stones and polystyrene. Tie up with the string to produce a loose bag.

4 Place the bait bag in the reservoir. Once the fleece is wetted the bag should float below the water surface. Tether the string to the baiting location for 48 hours.

5 Untie the collected bag. With washed hands, place the apple pieces in the buffer bottle. Shake the buffer bottle vigorously for at least one minute until the buffer becomes coloured by the apple.

6 Draw up the apple solution from the buffer bottle and pipette 2–3 drops into the well on the Lateral Flow Device (LFD) test kit. A vertical line should appear next to the C (control), if the test is positive a vertical line should also appear next to the T (test) within 10 minutes.

Instructions for using a Lateral Flow Device

Store test kits at room temperature (up to 40°C), do not refrigerate or freeze.

Step 1: Plant material selection

- Undo or cut open the bait bag and find all eight apple pieces.
- Unless the pieces look soft then break up the apple pieces a little (handle with washed hands or knife) before adding to the buffer bottle (see step 2), or add to the bottle and squash the apple a little with a suitable washed item.

Step 2: Extraction in buffer

- Unscrew the extraction bottle lid and add all the plant material pieces from one bag. Replace the lid tightly. One extraction bottle per bait bag should be used.
- Label the bottle with the sample identity if there is more than one sample.
- Shake the bottle vigorously for 60 seconds so that the ball bearings break the plant cells apart. Shake until the extraction buffer is no longer colourless.
- The buffer should start to become green or brown as the tissue is broken down. If this does not happen the plant pieces may have been too big, or the shaking not vigorous enough.
- Grasping the entire bottle during the process of shaking will normally warm it to above 10°C to enable the extraction process to work.

Step 3: Using the LFD

- If the test is being performed in conditions below 10°C then warm the packaged LFD before opening.
- Remove the test device from its foil packet just before it is needed. **DO NOT TOUCH THE VIEWING WINDOW.**
- Label the front or back of the device with the sample identification and date. The same extraction bottle containing the apple bait can be used with both a *Pythium* and *Phytophthora* lateral flow device.
- Place on a level surface, or in the hand, with the viewing window upwards. Holding the device is recommended if the temperature is below 10°C.
- Allow the plant material a few seconds to settle in the extraction bottle.
- Remove the lid from the extraction bottle, tilt the bottle and draw some of the liquid into the clean pipette from above the apple bait material.
- Gently squeeze two large or three smaller drops of the sample liquid into the sample well of the test device (so the liquid is below the rim of the well). Aim to release the liquid without air bubbles as these can break the flow of the liquid across the device.
- After about 30 seconds a pale blue or pink line* will appear in the viewing window as liquid flows along the test device.
- If no line becomes visible in the viewing window after 30 seconds, another drop of sample can be added to the sample well. Using too much liquid however will flood the strip and will cause the test to run incorrectly.
- If the test still runs very slowly tap the device gently to remove any air bubbles.
- If too much debris has been added with the sample liquid the test will run slowly. It may be necessary to use a new device with clearer liquid from the extraction bottle.

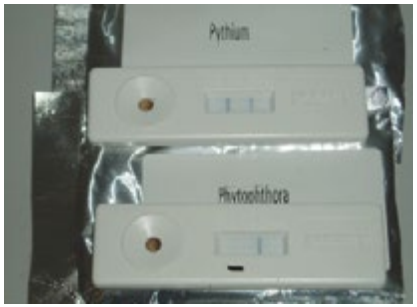
*The colour of the line depends upon the LFD used, with Forsite Pocket Diagnostic test kits the line will be blue, in the case of Neogen Alert-LF kits the line will be pink.

Step 4: Examining the results

- A vertical line (the 'Control' line) will appear next to the letter 'C' on the device. This line confirms the test is working properly.
- If the test is positive, a second line, the 'Test' line (next to the letter 'T'), will appear. Even a faint line means the result is positive and so the test should be examined in a location that is well illuminated.
- The lines can appear in *Pythium* and *Phytophthora* kits within 3–4 minutes of adding the sample to the device, but may take up to 10 minutes.
- Read the result within 10 minutes of adding the sample to the device. Ignore any changes that happen after 10 minutes. For future reference (if required), an image of the LFD can be taken, ensure the front is appropriately labelled with any sample details.
- Where comparison of the strength of the line between samples is being sought for research purposes the LFD should be placed against a similar coloured background and read under the same light level.
- After use, if a secondary confirmatory test is required on DNA extracted from the LFD, the test devices should be returned to the foil packet with the silica gel sachet provided.

Step 5: Interpretation of the results

- A positive result indicates that the plant material sampled contains the pathogen under test.
- Under some circumstances, laboratory confirmation of an on-site test result may be necessary.
- A negative result indicates that the target pathogen was not detected in the test sample. As with all diagnostic testing, a negative result does not confirm that the location is free from the pathogen under test.
- A faint or absent line may indicate a low concentration of the pathogen, uneven distribution in the host, or recent infection.



Problems with the readings

- Faint test lines are caused by either low pathogen concentration, uneven distribution, too small a sample, sample not broken up enough, or sample not shaken for long enough. If in doubt, repeat with a new device using a fresh sample, or repeat again in a few days time.
- 'T' line visible, but no 'C' line may be due to a high level of pathogen in the sample, preventing the test from working properly. Dilute the sample 1 in 10 and 1 in 100 with fresh buffer and retest with a new device.
- No 'T' line, no 'C' line can occur when too much sample material is added. Retest with a new device.

Want to know more?

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