



Agriculture & Horticulture
DEVELOPMENT BOARD



Grower Summary

HNS/PO 188

Baiting and diagnostic techniques for monitoring Phytophthora spp. and Pythium spp. in irrigation water on ornamental nurseries

Annual 2013

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HDC is a division of the Agriculture and Horticulture Development Board.

Project Number:	HNS/PO 188
Project Title:	Baiting and diagnostic techniques for monitoring Phytophthora spp. and Pythium spp. in irrigation water on ornamental nurseries
Project Leader:	Dr Erika Wedgwood
Contractor:	ADAS UK Ltd
Industry Representative:	Charles Carr – Lowaters Nursery Russell Woodcock – Bordon Hill Nurseries
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Previous report(s):	None
Start Date:	01 April 2012
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Project Cost:	£31,796

Headline

- A simple bait test combined with the use of a lateral flow device enables growers to conduct on-site checks for *Phytophthora* and *Pythium* species in stored irrigation water.

Background and objectives

Legislation, water quality issues and potential shortages are encouraging growers to collect and reuse irrigation water, either for field, container or hydroponically grown crops. Growers would benefit from the ability to have a rapid check that any non-mains water being used on plants was free of *Phytophthora* and *Pythium* water-moulds.

Three reservoirs from businesses producing hardy nursery stock were experimentally bait-tested as part of project HNS 181, and *Phytophthora* and *Pythium* species able to cause root rots were detected using lateral flow devices (LFDs). The current project aims to develop these techniques via laboratory testing using isolates of pathogens which can be found on ornamental plants, followed by nursery testing. The overall objective is to develop procedures and guidelines for “Do it Yourself” testing by growers for *Phytophthora* and *Pythium* in irrigation water.

Information will be sought on the type of plant material and quantity that will bait-out water-mould dispersal spores (zoospores) successfully and be readily available to growers. Whether bait position in the reservoir affects zoospore trapping success will be investigated. Once these are determined, bait monitoring of naturally infested water will be carried out at intervals over the second year of the project to see if there are periods of greater detection. The ability of lateral flow devices (LFDs) to indicate bait infestation will also be examined. These devices are available commercially to test plant infestation by *Pythium* and *Phytophthora*, showing a positive indication by producing a coloured test line. The assistance of some growers will then be sought to test out bait construction and testing procedures, with workshops then run to encourage wider uptake.

The specific objectives in the first year of this project were:

1. To identify plant tissue baits which have the greatest sensitivity for zoospore detection.
2. To examine the sensitivity of lateral flow devices for detecting *Phytophthora* and *Pythium* species to different quantities of infested bait material.
3. To determine the optimum number of bait bags, quantity of bait material and placement positions in reservoirs to maximise detection.

4. To determine whether there are any seasonal / weather related influences on zoospore release to use as guidance to maximise detection.
5. To provide step-by-step instructions for nursery staff on bait use and to provide a demonstration of the techniques at two grower events.

Summary

A series of ten experiments was carried out in the laboratory to develop bait bags that could be used to catch *Phytophthora* and *Pythium* zoospores in stored irrigation water and to determine whether or not pathogen presence could be confirmed by the use of lateral flow devices (LFDs). Monitoring experiments in nursery reservoirs were undertaken to record seasonal patterns in zoospore bait infestation and to develop baiting techniques.

Objective 1. Plant bait material with greatest sensitivity for zoospore detection

Water was inoculated in the laboratory with various concentrations of zoospores (from 25 to 6000 zoospores per litre of water) produced by *Phytophthora cryptogea* and a zoospore-producing species of *Pythium*, both of which can be found on ornamental plant nurseries.

Plant bait materials tested in the laboratory included freshly picked leaves of Rhododendron, Ceanothus and Nordmann Fir and apple and carrot flesh. After initial tests, although Ceanothus in particular had a good water-mould infestation rate, the use of leaf material was discontinued as it was found that leaves of all three species could be externally contaminated with *Pythium*, which introduces uncertainty into the results. Although external contamination of carrots was precluded by using internal tissue, it is possible that a carrot with penetrating *Pythium* cavity spot might be used and so, after discussion with an industry representative, carrot was also removed from further tests. Apple fruit flesh was the selected final choice of bait, and was successfully used by growers at two nurseries to bait their irrigation water.

Objective 2. Sensitivity of LFDs with infested bait material

In contrast to results when used in the laboratory, the LFDs gave positive readings (for both *Pythium* spp. and *Phytophthora* spp.) when used with naturally infested apple baits from nurseries. It is possible that leaving the bait pieces in the bag a few days after retrieval from the water allows growth of the water-moulds through the apple and thus increases the probability and/or strength of LFD detection.

Objective 4. Seasonal and weather influences on zoospore release

Nursery monitoring in late January and late February/early March 2013 showed that both *Pythium* and *Phytophthora* zoospores are active in collected bed effluent water at this time. The procedure has been used to test both reservoirs and the inflow and outflow of slow sand filters. Further monitoring in 2013 is intended to gain more information on the positioning of baits and if deployment should be focussed on certain times of the year.

Objective 5. Instructions of bait and LFD use

An illustrated step-by-step guide to water-baiting for *Pythium* and *Phytophthora* species, and use of the LFD test on bait tissues was devised for the nurseries taking part in the trial and will be disseminated to the wider industry during the course of the project.

Financial Benefits

A rapid on-site test has been developed which allows growers of both ornamental and edible crops to test their own irrigation water utilising readily available inexpensive materials (apple and a small piece of horticultural fleece) and commercially available relatively inexpensive diagnostic kits. An order of between 13 and 24 Pocket Diagnostic LFD kits costs £7.50 + VAT each per species (3 June 2013). A nursery sending a 1 L water sample from each of two reservoirs for water testing at a commercial laboratory would pay £115 +VAT (plus next day courier fees) for results giving the number of colony forming units of *Pythium* and *Phytophthora*. For this outlay a nursery would instead be able to test their own two reservoirs for both water-moulds on four occasions and have the results available within a few days after setting out baits in the reservoirs.

The use of the baiting test will allow growers to reduce contamination of growing areas e.g. by treating the pathogen infested water or using an alternative water source and so reduce losses to *Phytophthora* and *Pythium* root rots. Root rot pathogens can otherwise spread and cause whole crop loss (particularly in non-woody plants) or loss of vigour.

The use of baits for detection of infested water will contribute to Integrated Crop Management measures that can be utilised to fulfil the requirements of the EU Sustainable Use Directive whereby monitoring is expected to determine the need for, and justify, any chemical control measures.

Action Points

- Growers should consider using apple baits, combined with the use of lateral flow devices for *Phytophthora* and *Pythium* species, to monitor the biological safety of their irrigation water with respect to these water-mould root pathogens.
- Growers should note that work is in progress to provide more specific information on the deployment of apple baits to maximise the chance of detecting any Oomycete pathogens present in the water.