# Grower Summary

## HNS/PO 188

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

Final 2014

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| **Project Number:** | HNS/PO 188 |
| **Project Title:** | Baiting and diagnostic techniques for monitoring Phytophthora spp. and Pythium spp. in irrigation water on ornamental nurseries |
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## *GROWER SUMMARY*

### Headline

- An apple bait bag combined with the use of a lateral flow device enables growers to conduct on-site checks for *Phytophthora* and *Pythium* spp. in stored irrigation water.
- A positive bait/LFD test indicates that further in-depth testing is required to fully determine the disease risk.

### 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* spp. 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 species of *Phytophthora* and *Pythium* in irrigation water.

In the first year of the project, laboratory tests were carried out on plant material that would be readily available to growers in order to select the plant material type and quantity that baited-out *Phytophthora* spp. and *Pythium* spp. zoospores successfully. In the second year, baits were set out at intervals throughout the year in potentially naturally infested nursery reservoirs to see if there were seasonal differences in the presence of oomycetes and if the depth in the reservoir and position around the edge of an open reservoir affected zoospore trapping success. Water was also bait tested after passing through the nursery filtration systems prior to use for irrigation. Standard water samples for laboratory plate culturing were also taken for comparison. The ability of lateral flow devices (LFDs) to indicate bait infestation was examined. Most bait bag construction and some LFD use was carried out by growers at the nurseries being sampled to check that the procedures were suitable for general use. An extension study investigated the possibility that killed oomycete material might attach to apple baits and give false positive LFD tests.

There were five specific objectives to this project:

1. To identify plant tissue baits which have the greatest sensitivity for zoospore detection.

2. To examine the sensitivity of lateral flow devices (LFDs) for detecting *Phytophthora* spp. and *Pythium* spp. in bait material in water recorded as having a range of colony forming units.

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 or 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.

Extension study objective:

1. To determine whether or not cellular debris from killed oomycete cells can attach to plant tissue baits and be detected using LFD immunodiagnostic test kits to give 'false positive' tests for live pathogen presence.

## Summary


Experimental procedures

In the first year a series of experiments was carried out in the laboratory to develop bait bags that could be used to catch *Phytophthora* spp. and *Pythium* spp. zoospores in irrigation water. Towards the end of the first year, monitoring experiments in nursery reservoirs were commenced to record seasonal and distribution patterns of zoospores using both isolation and baiting techniques. Water samples taken from three reservoirs in August 2012 resulted in the selection of one with a significant concentration of oomycetes for ongoing monitoring. Another nursery with a different design of reservoir was also included in monitoring from early 2013.

In the second year, apple bait bags were made by two growers and placed in reservoirs which collected run-off water from ornamental plants. One reservoir was open and used a reed bed to de-contaminate the inflow, with a particulate filter where water was drawn off. The second reservoir was covered and used a slow sand filter to remove pathogens from the water before use. Bait bags were also placed in samples of water post-filter. Water samples were taken at the same time as the baits were deployed in order to produce records of colony forming units of oomycetes (which include *Pythium* and *Phytophthora* species) on

culture plates. Ceanothus leaf baits were also put in the sampled water in the laboratory to become infested by oomycete pathogens. Monitoring was carried out in January, February, April, May, July, August, September and November 2013.

The baits were left in the water at the nurseries for 48 hours. The bags were then returned to the laboratory for testing with commercially available lateral flow devices (LFDs) sold to allow growers to test plant material for *Pythium* spp. or *Phytophthora* spp.. The baits were left to incubate until 6 days after they had been deployed in the reservoir to allow any *Pythium* spp. and *Phytophthora* spp. to multiply and so increase the probability that they would be detected. On a few dates the growers also tested baits with LFDs directly on bag retrieval from their reservoirs or post-filter.

Both shallow (30 mm) and deep (250 mm) floating baits were used throughout the sampling to determine if their catches might vary because of any difference in the vertical distribution of the oomycetes in the reservoir water over the year. At the open reservoir, baits were placed by the inflow and overflow. An additional three positions were baited twice around its perimeter in May to form a continuous period of baiting over 4 days to determine whether or not differences in *Pythium* spp. or *Phytophthora* spp. presence arose.

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

Water was inoculated in the laboratory with either of two species of water-mould (oomycete) found on ornamental plant nurseries, *Phytophthora cryptogea* and a zoospore-producing species of *Pythium*. Plant bait materials of pieces of either freshly picked leaves of Rhododendron, Ceanothus and Nordmann Fir and apple and carrot flesh were suspended in separate inoculated containers in replicate bait bags made from horticultural fleece. Bait infestation was recorded by isolation onto selective agar. Not all bags were recorded as infested, the greatest number (9 out of 10) was by *Pythium* sp. of carrot, with the next best (5 out of 10) being for apple. *Phytophthora* sp. was recorded from 7 out of 10 apple bait bags, but only one carrot bag. Apple (cv. Golden Delicious) was therefore selected as the bait that would attract both pathogens, and subsequently used by growers at two nurseries to bait their irrigation water.

For the reservoir monitoring, it was speculated that the chance of a small number of zoospores being detected by the LFD would be increased by leaving the bags for four days after retrieval from the water in order to encourage the growth of *Pythium* and *Phytophthora* spp. mycelium through the apple. Positive LFD results were produced more often after

warm, dark, incubation when compared with a duplicate bait bag tested straight after two days immersion.

Initial reservoir baiting in 2013 with bags containing either apple flesh or Ceanothus leaves showed that both tissues picked up *Pythium* spp. from naturally infested water, with isolation of *Pythium* spp. from a greater proportion of apple bait pieces. *Phytophthora* spp. was only isolated from an apple bait. Subsequent bait deployments using apple through the year gave positive LFDs to both *Pythium* spp. and *Phytophthora* spp. across the full range of colony forming units recorded from water sampled from a several locations.

Objective 2. Sensitivity of LFDs used on baits from water with differing colony counts

The LFDs for both *Pythium* spp. and *Phytophthora* spp. gave positive readings when used with apple baits retrieved from nursery reservoirs that were shown to be infested by isolation of colonies from water samples. The water sample colony counts (cfus) included all oomycetes, including saprophytes, with the presence of *Pythium* and/or *Phytophthora* spp. colonies noted. This gave an indication of the level of contamination rather than a quantitative assessment of *Pythium* and *Phytophthora* spp.. Detection in baits by LFDs was shown after being placed in water which containing between 20 and 3360 oomycete cfu/L and also with 20 *Phytophthora* or 26 *Pythium* cfu/L. Positive LFD results were however, also sometimes obtained from baits placed in water that had passed through a slow sand filter and for which no colonies had been detected directly from the water sample i.e. false positives. A few LFDs were negative and this was matched by the colony counts i.e. there were no false negatives. The reservoir bait LFD tests were frequently positive for both *Pythium* spp. and *Phytophthora* spp. and this was nearly always matched by these Oomycetes being detected in the water.

The LFDs produced different colour strengths of the test line in the indicator window. An index of line strength was used when recording the positive LFD readings from the baits tested a week after placement and these were compared with the Oomycete colony counts obtained from water collected when the baits were deployed. No correlation was found between the results to indicate that a stronger line on the LFD might be able to be used by growers to indicate a higher colony count in the irrigation water sampled. However, it was not known what proportion of the cfus were either *Pythium* spp. or *Phytophthora* spp. and a correlation cannot be ruled out as the LFD line strength is dependent on the amount of the correct test subject that binds to the antibodies that are labelled with coloured latex indicator material. A small concentration of the target, or debris hindering the antibodies attaching,

can both cause a faint line (Malcom Briggs, Forsite Diagnostics, pers. comm.). A small number of LFDs were carried out immediately rather than after incubation of the baits and these gave the paler test lines, suggesting that the higher amount of test subject present later was more clearly detected.

When apple baits were tested in treated water from a commercial-scale slow sand filter that had been successfully operational for over 15 years, they tested positive for both *Pythium* spp. and *Phytophthora* spp. using LFDs. Concurrent laboratory plating and baiting tests on water samples from the filter detected no colonies of either genera, whilst other oomycete and non-oomycete 'indicator' species all indicated that the sand filter was working effectively. Nevertheless, a follow-up DNA test of one of the LFDs with positive *Phytophthora* spp. confirmed the presence of *Phytophthora* spp. DNA, indicating that the apple baits were detecting material from this genus. This presented two possibilities: either the baiting/LFD procedure is more sensitive than established plating techniques, or it is giving false positive tests (possibly by detecting dead pathogen material resulting from the action of the slow sand filter). 'False positive' tests could lead to unnecessary emergency maintenance of water treatment systems and costly clean-ups of water storage tanks (costs that could amount to thousands of pounds), whilst alternatively, confidence in an increased level of testing sensitivity with an 'on-site' procedure would greatly improve disease management practice. Thus, an additional short study was completed examining the possibility that killed Oomycete pathogen material might attach to apple baits and give false positive LFD tests. This study tested two *Phytophthora* spp. LFD detection kits (Pocket Diagnostic® kits, Forsite Diagnostics and Alert LF™ kits, Adgen Phytodiagnostics) against living zoospores and zoospores of two *Phytophthora* spp. (*Phytophthora cryptogea* isolate E556 and *Phytophthora* sp. Isolate C295) killed by three different treatment types (pasteurisation, ultra-violet light and chlorine dioxide). Both LFD kit types gave good detection of zoospores in water when used with apple baits and unfortunately both gave positive tests with apple baits exposed to dead zoospores. This indicates that LFD kits used with apple baits can give false positive test results by detecting dead pathogen debris when used to assess water that has been given a disinfestation treatment.

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, although at lower levels than later in the year. Monitoring continued into November in both open and lidded tank reservoirs and in the outflow of their particulate and slow sand filters,

respectively. The open reservoir had higher colony forming unit (cfu) counts in water samples taken in May and July 2013 than in the other six sample months spread through the year (**Figure 1**). The reservoir at the second nursery also had most cfu in May. The higher counts might be related to the unusually high rainfall this month, which might have flushed *Pythium* and *Phytophthora* out of pots and beds and given speedy transport into the reservoirs. The use of the water at any time of the year would lead to plant infestation regardless of the propagule concentration, therefore continual treatment and monitoring of its effectiveness would be needed at all times.

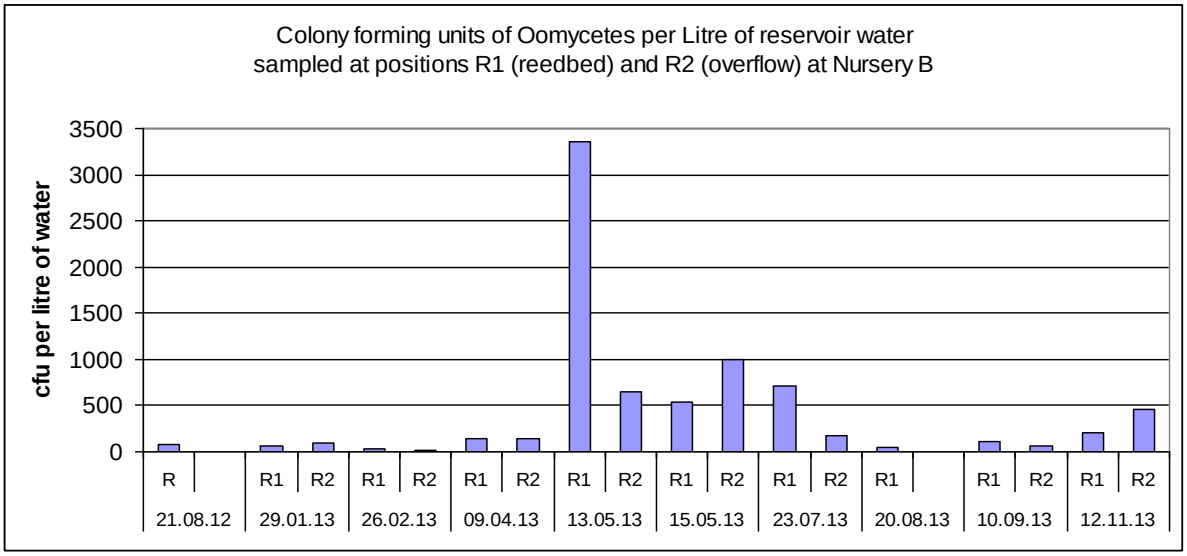


**Figure 1: Colony forming units of oomycetes in water samples taken at intervals during 2013 at positions R1 (inflow) and R2 (overflow) in an open reservoir at the time of bait bag deployment showing higher levels in May and July**

Objective 5. Instructions of bait and LFD use

An illustrated step-by-step guide to bait construction and deployment 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. Bait use was described and/or demonstrated to growers during a number of meetings in 2013 as well as growers in the current work carrying out their own bait bag construction, use and some testing.

## Financial Benefits

An on-site test has been developed which allows growers to test their own irrigation water utilising readily available materials (apple and horticultural fleece) and commercially available and relatively inexpensive diagnostic kits. Towards the end of the project, the Pocket Diagnostic LFD kits used in the work became unavailable. However, a kit for

*Phytophthora*, (but not *Pythium*), was re-introduced by Forsite Diagnostics in 2014 after validation tests. Another manufacturer, Neogen, produces Adgen kits for both pathogens which use the same antigen source as the other brand. LFDs cost in the region of £16 + VAT for the two kits to detect *Pythium* spp. and *Phytophthora* spp.. Grower use of kits for a single sample location could save around £50 + VAT plus postage on the cost of the alternative procedure of water sampling for laboratory testing (although a laboratory fee of £100 can cover the cost of five water samples) and the delay while results are returned. The latter can, however, be used to test for other pathogens and extra time is required to put together the bait bags. LFD baiting tests are useful as (a) as a supplement to laboratory procedures and (b) a quick and convenient way to get an idea of potential risks of specific pathogens, especially *Phytophthora.* Not all *Pythium* species are pathogenic.

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

- To minimise the risk of infestation of crops, growers should monitor reservoirs, or the water being drawn off from them, for *Pythium* and *Phytophthora* as these species can be found all year round in collection reservoirs.
- Growers should consider using apple baits, combined with the use of lateral flow devices (LFDs) for *Phytophthora* and *Pythium* species, to monitor the biological safety of their irrigation water with respect to these water-mould root pathogens.
- Negative bait/LFD tests give a good indication of biological safety with respect to *Phytophthora* and *Pythium* species. However, positive tests need to be interpreted with caution, especially when testing efficacy of water disinfestation treatments, as apple baits can detect dead pathogen material. With our current state of knowledge it is right to say that a positive bait/LFD test indicates that further in-depth testing is required to fully determine the disease risk.